HORMONE TRANSPORT IN RELATION TO GEOTROPISM IN *ZEA MAYS* AND *HELIANTHUS ANNUUS* SEEDLINGS

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

by

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September

1977

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ACKNOWLEDGEMENTS

I am deeply indebted to my supervisor, Professor Malcolm B. Wilkins for his sustained and enthusiastic guidance and encouragement throughout this investigation.

I also wish to thank the following:-

Mrs. Marguerite Hall for the typing of the manuscript.

Mr. Norman Tait for the preparation of the photographs.

Science Research Council for awarding me a post-graduate studentship.

University of Glasgow for providing research facilities.

Dr. Alan Crozier for suggesting the gibberellin extraction procedure and for a gift of dwarf rice seeds.

Dr. Ian Railton for many helpful discussions.

All my friends and colleagues, in the Botany Department, both ex-Sutton Bonington and 'native' Glaswegians who are too numerous to name individually.

Last, but by no means least, I am grateful to my parents for their continual encouragement and to Kevin for his constant nagging about completing the writing of this thesis.

SUMMARY

Hormone transport in relation to geotropism in *Zea Mays* and *Helianthus annuus* seedlings - by Janet H. Webster

The possible involvement of gibberellins in the geotropic response of roots and shoots has been investigated using intact seedlings of *Zea mays* and *Helianthus annuus*.

The characteristics of the transport and metabolism of exogenously applied {^{14}C} GA_3 and {^{3}H} GA_3 within the plant, has been examined using the classical agar donor block method for segments of plant tissue and the more precise micropipette technique for whole plants.

With agar donor blocks, very little longitudinal movement of radioactivity from {^{14}C} GA_3 was found to occur in *Zea* coleoptile segments and even after transport periods of 24 h, there was virtually no movement of radioactivity into agar receivor blocks at either the apical or basal end of the segment. The application of a pulse of {^{14}C} GA_3, as an aqueous solution in a micropipette, to excised intact coleoptile apices confirmed that there was no obvious longitudinal polarity and no movement of radioactivity into receiver blocks. Similarly the application of a pulse of {^{14}C} GA_3 to geotropically stimulated roots of intact *Zea* seedlings, resulted in a very limited capacity for both acropetal and basipetal movement. Most of the radioactivity remained adjacent to the original point of application, even after 12 h transport periods.

Lateral transport studies, whereby a pulse of either {^{14}C} GA_3 or {^{3}H} GA_1 was applied to roots and shoots of horizontally and vertically orientated Zea seedlings, confirmed the minimal longitudinal movement of radioactivity. However, a significant net upward lateral movement of radioactivity from {^{14}C} GA_3 was found to occur in both roots and shoots of Zea seedlings that were geotropically stimulated. No lateral redistribution of radioactivity from {^{3}H} GA_1 was found to occur in either Zea roots or shoots.

Little metabolism of either {^{14}C} GA_3 or {^{3}H} GA_1 occurred in either Zea roots or shoots and it was apparent that the translocation pattern of radioactivity in these organs reflected that of gibberellin.

Experiments using intact Helianthus seedlings showed a very slight acropetal movement although much of the radioactivity remained in the region of application. No enhanced lateral redistribution of radioactivity was observed in either epicotyls or hypocotyls, as a result of prolonged geotropic stimulation.

Attempts were made to determine whether gibberellins were present naturally in the shoot apices of both Zea and Helianthus seedlings and if so, whether they might undergo lateral redistribution or differential synthesis or release as a result of geotropic stimulation. The classical techniques of extraction, collection in agar and

bioassay were employed. Extensive experimentation confirmed the presence of endogenous gibberellins in Helianthus shoot apices but failed to demonstrate their existence in Zea coleoptiles.

Abbreviations

The units in this thesis are based on S.I. the international system of units (1970).

min	=	minute
h	=	hour
d	=	day
m	=	metre
in	=	inch
g	=	gram
l	=	litre
ct	=	count (radioactive)
d	=	disintegration (radioactive)
Ci	=	Curie
mol	=	mole = gram molecule
pH	=	log hydrogen ion concentration
%	=	percent
w/v	=	weight/volume
d	=	relative density = specific density
W	=	Watt
lb	=	pound
^{o}C	=	degree Celsius = degree centigrade
Rf	=	$\frac{\text{distance from origin}}{\text{distance of solvent front from origin}}$
ft	=	foot
n	=	nano = 10^{-9}
μ	=	micro = 10^{-6}

m	=	milli = 10^{-3}
c	=	centi = 10^{-2}
k	=	kilo = 10^{3}
e.g.	=	example given
i.e.	=	*id est* = that is
G.M.T.	=	Greenwich Mean Time
S.E.	=	Standard Error
u.v.	=	ultra violet
T.L.C.	=	thin-layer chromatography
P.C.	=	paper chromatography
G.C.M.S.	=	gas chromatography mass spectrometry
IAA	=	indole acetic acid
ABA	=	abscisic acid
GA	=	gibberellic acid
OFN	=	oxygen-free nitrogen
PPO	=	2, 5-diphenyloxazole
c	=	*circa* = approximately
PVP	=	polyvinylpyrrolidone

INTRODUCTION

Geotropism (FRANK, 1868) is the response of a whole plant and parts of plants to gravity and is manifest as the curvature developed by a plant organ when its orientation deviates from that which it normally maintains with respect to gravity. Geotropic responses have been classified according to the orientation of the organ relative to the direction of the gravitational force. The central axes of most plants grow parallel to the direction of gravity and are said to be orthogeotropic. If their growth is in the direction of gravity (e.g. primary roots) they are said to be positively orthogeotropic; if they grow in the opposite direction they are negatively orthogeotropic (e.g. primary shoots). If a primary root or shoot is displaced from its preferred orientation with respect to gravity, it develops curvature to regain its former orientation and this curvature is the geotropic response. A lateral organ usually finds equilibrium at an angle to gravity, characteristic of its stage of development and the conditions under which it is growing, and such a response is said to be plagiogeotropic. Organs which grow horizontally, that is, at right angles to gravity are said to show diageotropic behaviour.

Research has largely been confined to the orthogeotropic responses of main axes. The majority of such organs are radially symmetrical and their geotropic response is due to a difference in the growth rate of the upper and lower halves of the stimulated organ. The unequal growth rate is maintained until the organ becomes vertical. RAWITSCHER (1932) has distinguished three separate but co-ordinated

steps in a geotropic response. Firstly, the magnitude and direction of the stimulus must be perceived by the root or shoot. Secondly this information must be transmitted from the regions of perception to the regions of response. Lastly, there is the initiation of growth processes in these regions which will give rise eventually to the geotropic curvature of the organ.

The mechanism whereby roots and shoots perceive their orientation in a gravitational field has been a matter of much speculation. Since gravity acts equally upon every cell of the organ, stimulation is uniform and therefore the most probable way for a plant organ to perceive its orientation is by the movement of one or more of its component parts. Consequently the first stage in the process of gravi-perception may involve the mass acceleration of one or more mobile cell constituents. The identity of these moveable cellular bodies is the controversial problem of gravi-perception.

NAEGLI (1858) first noted the occurrence in some plant cells of starch grains which were capable of moving under the influence of a gravitational field and BERTHOLD (1886) suggested a possible connection between this sedimentation and gravi-perception. Careful experiments by HABERLANDT (1900) and NĚMEC (1900) demonstrated a close correlation between the occurrence of geosensitivity in plant organs and the presence of sedimentable starch grains which moved to the lower side of the cell within minutes of a change in orientation. These sedimentable starch grains are almost

invariably found in and confined to the geotropically sensitive zones of plants such as root and shoot apices. HABERLANDT (1900) and NĚMEC (1900) simultaneously proposed the Starch-Statolith theory in which the sedimentation of these starch grains was thought to be responsible for gravi-perception.

In the years since the Starch-Statolith theory was originally proposed there have been many attempts to establish its validity and a large number of investigations have provided circumstantial evidence that starch grains do act as statoliths and play an important role in the gravi-perception mechanism. It is possible to detect plants responding to gravity after stimulation times of 1 - 2 minutes and for gravity to be perceived, an asymmetry must be established in less than this time. AUDUS(1962) has calculated that only starch grains are large and dense enough to sediment sufficiently quickly to account for the speed of perception.

A review of all the evidence for and against the possible involvement of sedimentable starch grains in the gravi-perception mechanism is not strictly relevant to this thesis and has been excellently reviewed by both AUDUS (1962) and WILKINS (1966). However, since the latter review, a number of important papers pertaining to the controversy have appeared. A serious objection to the Starch-Statolith theory has come from experiments in which attempts have been made to remove the statolith starch from plant

organs and to study the effect of the gravitational stimulus on the starch free organ. Unfortunately statolith starch is extremely persistent and very drastic treatments are required to remove it.

In 1966 PICKARD and THIMANN using methods that were less likely to cause serious damage to the plants, succeeded in making wheat coleoptiles completely starch free by incubation in a solution of kinetin and gibberellic acid at 30°C for 34 hours. They found that coleoptiles did not completely lose their geotropic responsiveness. Compared with the controls, treated coleoptiles developed a smaller curvature which did not begin until about 5 hours after the onset of geotropic stimulation. Although the growth of the destarched coleoptiles was retarded, the ratio of curvature to growth rate was the same for both treated and non-treated coleoptiles. Clearly these results suggested that starch grains do not form a critical part of the gravi-perception mechansim.

Further support in favour of the statolith theory came from IVERSEN (1969) who applied the technique of PICKARD and THIMANN (1966) to roots of Lepidium sativum. Incubating the roots in relatively high concentrations of kinetin and gibberellic acid at 35°C for 29 hours resulted in the complete disappearance of sedimentable starch grains from the amyloplasts of the root cap. This loss was accompanied by a total loss of geotropic responsiveness even though the roots continued to grow at a rate only slightly less than

that of untreated roots. Thus in the absence of starch grains, roots were unable to orientate themselves in a gravitational field. Subsequently when the hormone-treated, starch-free roots were illuminated, the starch grains reformed after 20 - 24 hours and geotropic responsiveness reappeared at the same time. Unfortunately IVERSEN does not present any data to substantiate this latter and most important observation.

IVERSEN (1974) has recently repeated the experiments of PICKARD and THIMANN (1966). He also found that the destarched coleoptiles exhibited a small geotropic curvature which was only 18.4% of the curvature developed by the water treated controls. Both light and electron microscopy revealed the occasional presence of residual statolith starch in the 'destarched' tissue. Therefore the small geotropic curvatures observed by both IVERSEN and PICKARD and THIMANN (1966) in supposedly 'destarched' coleoptiles could have been due to residual starch. IVERSEN (1974) tested this possibility by incubating wheat coleoptiles in a solution of kinetin and gibberellic acid at a temperature of 34°C, rather than 30°C, for 36 hours. Subsequently he was unable to detect any statolith starch in the coleoptiles and these destarched coleoptiles showed no geotropic response even after 24 hours in a horizontal position, although they were otherwise healthy and continued to elongate. Therefore for both roots and shoots there appears to be a close correlation between the disappearance of statolith starch grains as a result of hormone treatment

and the loss of geotropic responsiveness. Also, in roots there is a simultaneous reformation of starch and a restoration of geotropic sensitivitywhen the hormonal treatment is stopped and the roots exposed to light.

Recent work involving removal of the root cap has produced more evidence in favour of the Starch-Statolith theory. JUNIPER et al. (1966) reported that they had been able to remove the root cap from the apex of primary roots of Zea mays and that although the root continued to elongate, it had lost all its geotropic sensitivity. After about 20 hours, geotropic sensitivity returned as a result of the regeneration of a major part of the root cap. However detailed microscopical studies at both the light and the electron levels by BARLOW (1974a,b) have shown that roots of both Triticum vulgare and Zea mays recover their geotropic sensitivity many hours before the regeneration of the root cap. Therefore the roots regain the ability to respond to gravity before the starch grains in the new root cap have had time to develop. However, immediately after the removal of the root cap, starch grains begin to develop from proplastids in the cells of the quiescent centre, immature xylem and cortex of the root apex (BARLOW and GRUNDWAG, 1974). There is a lag period of 14 hours after cap removal, before roots regain the ability to respond to gravity, due to the time needed for development of the amyloplasts and to allow a certain number or mass to accumulate before they function as sensors of gravity. As the new root cap regenerates, the starch grains in the cells of the quiescent centre and meristem diminish but in the

regenerating root cap they persisted and increased such that the ultrastructure of the new cap was complete 3 or 4 days after decapping. Clearly the root cap has a regulatory role on the development of starch grains in the root apex and when formed these starch grains appear to function as part of the gravi-perception mechanism. Whether or not these newly formed amyloplasts in the quiescent centre sediment has yet to be determined, but presumably this must happen if they are to function as gravity sensors. These experiments are critical and the preliminary experimental evidence obtained so far has indicated that they do not sediment (WILKINS, 1976 pers. comm.). The regulatory role of the root cap is not limited only to the biosynthesis of starch grains (WILKINS, 1976). The geotropic curvature of a root arises as a result of growth inhibitors from the cap, inducing differential growth rates of cells on the upper and lower sides of the root. The occurrence of a geotropic response in a decapped root before the regeneration of the new cap, indicates that the biosynthesis, activation or release of the growth inhibitor and its transport must be initiated in the root apex. This means that when the root cap which is the normal site of gravi-perception and of growth inhibitor production is removed, the root apex has the apparent capacity to take over both these functions so that the root rapidly regains control of geotropic growth without the necessity of waiting 2 to 3 days for the development of a

complete new cap. Clearly this is a reversible role since the starch grains in the root apex diminish as the new cap is gradually regenerated.

It seems that there is little doubt that the sedimentable starch grains do play an important role in the graviperception mechanism in roots and shoots. Although there is at present no alternative explanation, it is important to remember that the evidence pertaining to the Starch-Statolith theory is entirely correlative and therefore not unequivocal. Precisely how the starch grains initiate the transport of growth regulators which are responsible, at least in part, for geotropic curvature is as yet completely unknown.

The geotropic curvature of both roots and shoots takes place in the sub-apical regions of these organs where the cells are actively growing. Growth rate measurements by SACHS (1882) established that the downward curvature of horizontally orientated roots was due to the lower half growing more slowly than the upper half. Similarly in shoots, WEBBER (1931) using Hordeum coleoptiles and NAVEZ and ROBINSON (1932) using Avena coleoptiles found that the upward curvature which developed as a result of horizontal orientation, was due to the growth rate of the lower half being increased and that of the upper half being decreased. The geotropic response of both roots and shoots could be explained if the mechanism responsible for the differential growth rates of the upper and lower sides of horizontal organs could be established.

Experiments by BOYSEN-JENSEN (1910; 1911; 1913) and PAÁL (1914; 1919) led to the idea of growth regulating substances being produced in the apices of roots and shoots and of these substances subsequently moving in a basipetal direction towards the growing region of the organ. PAÁL (1914; 1919) made the original suggestion that the curvature might arise as a result of an unequal distribution of the apically produced growth regulating substances between the upper and lower halves of the horizontal organ.

Simultaneous investigations by CHOLODNY and WENT resulted in independent papers in 1926 in which both authors attributed geotropic curvature to an asymmetry of growth regulating substances as a result of lateral transport of growth regulators from the upper to the lower half of a horizontal organ. Geotropic stimulation was thought to disturb the normal basipetal transport of hormone from the apex of the root or shoot to the growing zone such that lateral movement occurred and resulted in an accumulation of hormone on the lower side of the organ and a depletion on the upper side. The opposing geotropic responses of roots and shoots was attributed to their opposite responses to the concentration gradient of growth regulating substances established across the horizontal organ. Roots and shoots have differing sensitivities to auxins and the assumption was made that the naturally occurring auxin levels were suboptimal for shoot growth and supraoptimal for root growth.

This general idea became known as the CHOLODNY-WENT hypothesis and establishing its validity has been the subject of a large number of investigations. The validity of the hypothesis is really dependent upon establishing that auxin exists in the apices of roots and shoots and that it undergoes downward lateral transport as a result of geotropic stimulation. Recent investigations appear to have established the validity of the CHOLODNY-WENT hypothesis as an explanation for the geotropic response of shoots but the situation for roots is far from clear and there is now considerable doubt about its validity in these organs. Consequently the experimental evidence pertaining to the existence of a lateral movement of growth regulating hormones in geotropically stimulated roots and shoots will be presented separately.

The classical study by DOLK (1929) was really the first experimental attempt to test the validity of the CHOLODNY-WENT hypothesis for shoots. Detached apices of coleoptiles of Zea and Avena and subapical coleoptile segments of Avena were placed on small blocks of agar which had been bisected by a slip of mica. Agar blocks containing the natural growth regulating diffusate from coleoptile apices were placed on the apical cut surfaces of the coleoptile segments. Subsequently tissues were orientated either vertically or horizontally and after approximately 90 minutes, the amount of growth promoting activity in the agar receiver blocks was measured by the WENT Avena curvature test. It was

revealed that a gradient of growth promoting activity was established in favour of the lower of the two agar receiver blocks in contact with the basal cut surface of horizontally orientated apices. More than 60% of the total growth promoting activity extracted, was found in the lower receiver block. There was no detectable difference between the total amount of growth promoting activity diffusing out of vertical and horizontal apices. These classical experiments of DOLK with Avena and Zea have been repeated on other occasions and similar results obtained (NAVEZ and ROBINSON, 1932; GILLESPIE and BRIGGS, 1961), and DIJKMAN (1934) found more growth promoting activity in the lower receiver block than the upper one in horizontally orientated segments of Lupinus hypocotyls.

Chemical extraction of growth promoting substances using organic solvents has also shown there to be more growth promoting activity in the lower half than in the upper half of a horizontal organ (BOYSEN-JENSEN, 1936; VAN OVERBEEK, OLIVO and DE VAZQUEZ, 1945).

The experimental evidence discussed so far has shown that a lateral gradient of net growth promoting activity is established between the upper and lower halves of horizontal shoots and between agar blocks in contact with the basal cut surfaces of the upper and lower halves of horizontal shoots. However these results do not explain how such a gradient arises. Clearly the gradient may have occurred as a result of downward lateral transport of growth

promoting substances from the upper to the lower half of the tissue but it could also have arisen from the upward lateral transport of a growth inhibitory substance. The asymmetry may not be a reflection of lateral transport but may be due to differences in rates of synthesis, breakdown, activation or release or longitudinal transport of growth promoting or inhibiting substances by the upper and lower halves of the horizontal organ. Furthermore, it is only net growth promoting activity diffusing from the tissues that is measured and this may not necessarily reflect the actual amount of auxin present in the tissues since a number of growth promoting and growth inhibiting substances are now known to be present in plant tissues.

Additional evidence for the CHOLODNY-WENT hypothesis comes from experiments involving microsurgery of coleoptiles. Two such investigations suggest that the lateral transport of a growth regulating substance is involved in the geotropic response of coleoptiles. BRAUNER and APPEL (1960) inserted small pieces of mica into the apices of Zea coleoptiles which were subsequently orientated horizontally with respect to gravity. In some coleoptiles the mica plates were horizontal and in others they were vertical. Coleoptiles in which the mica plates were vertical showed a greater upward curvature than those with horizontal mica plates. The presence of the mica plates which seemed to act as a mechanical barrier to the lateral transport of a growth

regulator in the horizontal coleoptile, appeared to reduce the magnitude of the geotropic response. Clearly this implies that the lateral movement of a growth regulating substance is involved in the geotropic response of Zea coleoptiles but there is no indication from this experiment whether lateral movement was downward or upward and whether the growth regulator was a promoter or inhibitor.

Similar experiments of this type by KOCH (1934) also indicated that the lateral movement of a growth regulating substance is involved in the geotropic response of shoots and suggested further that a downward lateral movement takes place from the upper to the lower half of a horizontal shoot. KOCH (1934) excised one half of the tip of an Avena coleoptile and subsequently orientated the segments such that some were vertical and some were horizontal with the remaining half tip on the upper side. Vertically orientated tips developed curvatures in a direction away from the remaining half tip whereas in the horizontal coleoptiles, curvature was upward, i.e. in a direction toward the remaining half tip. Since total decapitation of a coleoptile completely inhibited growth, these findings suggested that a downward lateral movement of a growth promoting substance had occurred such that the growth rate of the lower half had been enhanced relative to that of the upper half. However, KOCH did not make clear the possible effect that the development of a 'physiological tip' (WENT and THIMANN, 1937) might have on the interpretation of his results.

The critical experiments in favour of the CHOLODNY-WENT hypothesis had to await the advent and ready availability of radioactively labelled indole-3-acetic acid (IAA). The first attempts to establish the existence of a lateral asymmetry of IAA in horizontal shoot apices were unsuccessful (REISENER, 1957; REISENER and SIMON, 1960; CHING and FANG, 1958) but subsequently there have been a number of successful attempts which have showed that an asymmetry of radioactivity developed both in geotropically stimulated shoot segments and in receiver blocks in contact with the basal cut ends.

GILLESPIE and THIMANN (1961) applied labelled IAA asymmetrically to the apical surface of horizontal segments of Avena coleoptiles and two receiver blocks were placed in contact with the upper and lower halves at the basal cut ends of the segments. Subsequently more radioactivity was found in the receiver block that had been in contact with the lower half than in that which had been in contact with the upper half, but no significant difference was found in the amounts of radioactivity in the upper and lower halves of the tissue.

In similar experiments with Zea coleoptiles, GILLESPIE and THIMANN (1963) found an asymmetry in the amounts of radioactivity in the lower and upper halves of the horizontal coleoptile tissue as well as the lower and upper receiver blocks. More radioactivity was recovered from the lower half of the tissue and lower receiver block as compared with the upper. Clearly these results are in close agreement

with the earlier experiments (DOLK, 1929) in which an asymmetrical distribution of natural growth promoting substances diffusing from coleoptile tips was established in coleoptile segments. In addition, these results do suggest the possibility that IAA may be the growth regulating hormone which is asymmetrically distributed.

Another technique used by both HERTEL and LEOPOLD (1963a) and GILLESPIE and THIMANN (1963) was to place agar receiver blocks in contact with the lateral cut surfaces of Zea coleoptile segments which had been divided along their longitudinal axis. The amount of radioactivity which moved from the apical donor blocks into lateral receiver blocks was determined according to the geotropic orientation of the segments and receiver blocks. Both investigations established that more radioactivity was found in the lateral receiving blocks when these were beneath the segment than in any other orientation.

None of the experiments have proved conclusively that the asymmetry of radioactivity arises as a result of lateral transport of IAA within the tissues. Unequivocal evidence for the existence of a lateral transport of auxin was provided by GOLDSMITH and WILKINS (1962; 1964) who applied radioactive IAA asymmetrically to one half of the apical ends of both vertical and horizontal Zea coleoptile segments. Uptake of IAA could therefore only occur on the one side of the coleoptile and any radioactive IAA found in the opposite non-donated half of the segment must have moved laterally within

the tissue from the half in contact with the donor block. Geotropic stimulation was found not to affect the uptake of IAA from the asymmetric donor. Thus there was no difference in uptake between vertical and horizontal coleoptile segments. However the proportion of radioactivity found in the non-donated half of the segment was found to differ when the segments were placed in different orientations with respect to gravity. Virtually twice as much IAA moved to the lower half of a horizontal segment supplied with an upper donor as moved across in a vertical segment or to the upper half of a horizontal segment supplied with a lower donor. Thus there is an enhanced downward lateral transport of IAA in horizontal coleoptile segments. Chromatographic analysis confirmed that the radioactivity extracted from both the plant tissue and the receiver blocks is still confined to the IAA molecule (HERTEL and LEOPOLD, 1963b). GOLDSMITH and WILKINS (1964) have clearly established the existence of a lateral transport of radioactive IAA in Zea coleoptile segments which occurs to a greater extent in horizontal coleoptile segments than in vertical ones. Similar experiments by DE LA FUENTE and LEOPOLD (1968) have confirmed these findings.

This gravity induced downward lateral transport of IAA is dependent upon aerobic metabolism. WILKINS and WHYTE (1968) investigated the effects of anaerobic conditions on the lateral movement of IAA in Zea coleoptile segments using the asymmetric donor technique of GOLDSMITH and WILKINS (1964). They found that although a lack of oxygen leads to a large decrease in the amount of lateral transport of IAA

in geotropically stimulated coleoptiles, it was not completely eliminated and was still greater than that in vertical segments. When segments were treated with sodium fluoride as well as being deprived of oxygen, the lateral movement of IAA was identical in vertical and horizontal segments. Treatment with the metabolic inhibitor had no effect on lateral movement in vertical segments. Therefore the enhanced lateral movement of IAA observed in horizontal coleoptiles was only reduced to the level observed in vertical coleoptiles when both aerobic and anaerobic metabolism were inhibited. This suggests that the lateral polar movement of IAA induced in coleoptile segments as a result of geotropic stimulation is largely dependent upon aerobic metabolism.

It seems well established therefore that the upward curvature of geotropically stimulated Zea coleoptile segments is brought about by the establishment of an asymmetric IAA concentration gradient between the upper and lower halves of the horizontal organ. This gradient arises as the result of a metabolic dependent downward lateral movement of IAA from the upper to the lower half of a horizontal coleoptile segment. This downward lateral movement of IAA may not be the only process which gives rise to the establishment of different amounts of IAA, and therefore different growth rates in the two halves of the horizontal coleoptile segment. There is now a considerable amount of evidence suggesting that gravity influences the longitudinal basipetal polar transport of IAA in Zea coleoptile segments.

NAQVI and GORDON (1966) observed a phenomenon which might partially contribute to the establishment of an asymmetric IAA gradient. They reported that whilst placing

a coleoptile segment in a horizontal position for 2 hours had no effect on the overall basipetal transport of IAA, measured by the amount of IAA in receiver blocks, the upper and lower halves of the horizontal segments were found to have transported different amounts of IAA. The lower half had an enhanced capacity for the basipetal movement of IAA and the upper half less of a capacity than a vertical half segment. NAQVI and GORDON (1966) went so far as to suggest that this differential change in the longitudinal basipetal movement of IAA by the upper and lower halves of the horizontal segment could be the cause of the lateral asymmetry of IAA distribution.

Those results of NAQVI and GORDON (1966) prompted CANE and WILKINS (1969) to re-investigate the basipetal transport of IAA in the upper and lower halves of Zea coleoptiles. CANE and WILKINS (1969) realised that since the coleoptile is a hollow cylinder, it could be split longitudinally and opened out into a relatively flat piece of tissue. This ensured that every cell in the coleoptile had an identical orientation with respect to gravity. Thus the pieces of tissue could be orientated such that all the cells corresponded to either the upper or lower sides of a normal horizontal coleoptile. When the 'opened-out' segments were orientated so that all cells corresponded with those at the 'bottom' of a normal horizontal coleoptile, more IAA was transported basipetally than in segments where all the cells were equivalent to those at the 'top' of the horizontal coleoptile. By orientating the 'opened-out' coleoptile tissue so that all the cells were equivalent to those at the 'side' of a horizontal coleoptile, it was established that although there was no difference in

the basipetal transport capacity of the upper and lower halves, a pronounced downward lateral transport of IAA occurred from the upper to the lower half of the tissue. The polar lateral movement of IAA can therefore occur independently of the differential basipetal transport of IAA in the upper and lower halves of horizontal Zea coleoptile segments.

The existence of a downward lateral movement of IAA in geotropically stimulated Zea coleoptile segments can therefore be accepted with a reasonable amount of certainty. However this raises two further important points. Firstly, whether or not lateral movement of IAA actually occurs in intact coleoptiles of Zea mays seedlings following geotropic stimulation. In other words, is it possible to extend to intact plants what has been established for segments and secondly, whether the results obtained from Zea mays coleoptiles reflect the mechanisms in shoots of other species. Coleoptiles of other species and other shoots may involve completely different mechanisms and therefore it is important not to overgeneralise.

The recent development of a micro-application technique has enabled very high specific activity tritiated IAA (5 - ^{3}H - IAA) to be supplied to intact organs at a predetermined point by means of fine pipettes. The pipettes are very similar to those used in neuro-physiological studies and they enable a pulse of IAA to be applied to a precise predetermined point in a tissue by acting as a point source

for diffusion into a highly localised area of plant tissue. The tip of the pipette penetrates the plant tissue and remains in position for a few seconds before being withdrawn. This advanced technique allowed the transport of IAA to be investigated in relatively undamaged coleoptiles which had an endogenous supply of growth regulators and therefore had the capacity to undergo normal geotropic curvature. Using this micro-application technique, SHAW, GARDNER and WILKINS (1973) demonstrated a marked downward lateral transport of IAA in coleoptiles of intact Zea mays seedlings and a less marked but significant downward lateral movement of IAA in coleoptiles of intact Avena sativa seedlings. Chromatographic analysis revealed that the distribution of radioactivity within the plant tissue reflected the distribution of IAA. Use of the pipettes had no effect on the normal negative geotropic response of the coleoptiles and the tissue damage was minimal.

The validity of the CHOLODY-WENT hypothesis for coleoptiles is not only dependent upon establishing that a downward lateral transport of a growth promoting hormone occurs in geotropically stimulated intact undamaged coleoptiles but also that the growth promoting hormone exists in the apex and growing regions of coleoptiles. Unequivocal evidence that IAA is present in and diffuses from coleoptile tips of Zea mays was obtained recently using high-resolution mass spectroscopy (GREENWOOD et al., 1972).

On the basis of present evidence the validity of the CHOLODNY-WENT hypothesis for shoots seems well established. However it may well be that the hypothesis is an over simplification and other processes may well be involved in the establishment of the differential growth rates of the upper and lower sides of a horizontal shoot.

The growth regulating mechanism involved in the positive geotropic response of roots was much less well understood than that in coleoptiles until recently. The downward geotropic curvature of a horizontal root is due to the growth rate of the lower half being decreased to a greater extent than that of the upper half (SACHS, 1882; AUDUS and BROWNBRIDGE, 1957; RUFELT, 1961). The CHOLODNY-WENT hypothesis attributes this differential growth of the two halves of the horizontal root to the establishment of an auxin concentration gradient across the root by means of a downward lateral transport of auxin from the upper to the lower half. The theory supposes that auxin is produced at the apex of the root and is present at either optimal or supraoptimal concentration; so that any increase in the auxin concentration on the lower side of the root would lead to a reduction in the growth rate on that side and therefore downward curvature. The validity of the hypothesis as an explanation of root geotropism clearly depends upon establishing that auxin is produced by the root apex, present at supraoptimal concentrations and transported laterally on geotropic stimulation.

Early investigations concerning these points involved experiments which were principally designed to achieve independent geotropic stimulation of the extreme apex and growing zone of the root. It was found that the region of highest sensitivity was confined to one millimetre of the apex (CZAPEK, 1895; DARWIN, 1899; HABERLANDT, 1908) and this suggested that the root apex was the site of either the gravi-perception mechanism or the production of growth regulators which control the geotropic response.

These suggestions found support from experiments involving the removal of the root apex. Observations by DARWIN (1880) and CIESIELSKI (1872) showed that decapitated roots did not react to a geotropic stimulus. Contrary to this however, have been several reports indicating that decapitated roots can respond to gravity (GORTER, 1932; VON GUTTENBERG, 1933; SPURNY, 1968). Since it is not clear precisely how much tissue was removed during the decapitation procedure and since no account was made of the possible regeneration of the root apex, these conflicting data are of little value.

Experiments by JOST (1912) and SYRE (1938) showed that roots were able to regain their geotropic sensitivity following decapitation. JOST (1912) found that removal of 0.3 - 0.7 mm from the apex of a Lupinus albus root was sufficient to abolish geotropic curvature for 5 hours only, after which time the roots showed a return of geotropic

responsiveness. Cutting 0.7 - 0.9 mm of the apex prevented curvature for at least 24 hours. SYRE (1938) showed that removal of 0.5 mm from the apex of Zea roots was required to prevent curvature completely but after removal of 0.7 mm from the root apex of Lupinus albus, 65% of the roots had curved after 12 hours.

Several independent studies have shown that the growth rate of a root is increased when the apex is cut off (WIESNER, 1884; CHOLODNY, 1926; BÜNNING, 1928). CHOLODNY (1926; 1931) and KEEBLE, NELSON and SNOW (1931) have shown that the lower growth rate could be restored by replacing the apex of the root or by applying the apex of a coleoptile. In addition, several investigations showed that both the normal growth rate and geotropic responsiveness could be restored to decapitated roots by placing the excised tip of either a root or coleoptile onto the cut root apex (CHOLODNY, 1924; 1928; SNOW, 1923; BOYSEN-JENSEN, 1933; KEEBLE, NELSON and SNOW, 1931). CHOLODNY (1926; 1928; 1929) showed that root tips were able to restore geotropic responsiveness to coleoptiles of Avena and hypocotyls of Lupinus and to induce curvatures in vertically orientated decapitated coleoptiles when placed asymmetrically on their apices (CHOLODNY, 1934).

Early work of KÖGL, HAAGEN-SMIT and ERXLEBEN (1934) showed that low concentrations of IAA which promote the growth of shoots, inhibit root extension and THIMANN (1937) showed that there was an optimum type of relationship between auxin concentration and growth rate for both

roots and shoots. The concentration of auxin which gave the maximum growth rate was about 10^{-4} ppm in roots and 5 - 10 ppm in shoots. As the IAA concentration was increased above these values, the growth rates of both roots and shoots progressively decreased. Therefore at some concentrations, shoot growth is promoted and root growth is inhibited.

CHOLODNY (1926) proposed that the apices of roots and shoots produce an identical substance which inhibits root growth and promotes shoot growth and the lateral redistribution of this substance in horizontal organs would account for the upward curvature of shoots and the downward curvature of roots. There are several ways in which a substance might act as an inhibitor of root growth and a promoter of shoot growth. Firstly it might act specifically in inhibiting the growth of roots and promoting the growth of shoots. Alternatively, whether it acts as a growth inhibitor and promoter of either roots or shoots may depend entirely upon its concentration. The decapitation experimentswith roots could be explained on the basis of auxin normally being supraoptimal. The effectiveness of a coleoptile in reducing the growth rate of and restoring geotropic responsiveness to decapitated roots could be due to the production of auxin at the cut surface of the apex at concentrations inhibitory to root growth. The CHOLODNY-WENT hypothesis for roots therefore was that the normal concentration of auxin in roots is supraoptimal and geotropic stimulation leads to a downward lateral transport of auxin such that the

concentration of auxin in the lower half increased further thereby reducing the growth rate which resulted in downward curvature.

Attempts to ascertain whether an asymmetric distribution of growth regulating substances occurred in the apices of horizontal roots was established by experiments similar to those carried out for shoots by DOLK (1929) using Avena coleoptiles. HAWKER (1932) geotropically stimulated Vicia roots for 3 hours and then removed their apices. The detached apices were bisected into upper and lower halves and placed with their basal cut ends in contact with gelatin blocks. After a period of diffusion the agar blocks were applied asymmetrically to the apices of vertical decapitated roots. Agar blocks which had been in contact with the lower half tip caused a much greater curvature towards the side of application (inhibition of growth) than those which had been in contact with the upper half. BOYSEN-JENSEN (1933) bioassayed the growth activity present in agar receiver blocks which had been in contact with the upper and lower halves of the apices of horizontal Vicia roots. More growth promoting activity was found in the lower than the upper receiver block as measured by the Avena curvature test using de-tipped Avena coleoptiles. Similar results were later obtained using an auxin extraction technique (BOYSEN-JENSEN, 1936).

The results of these experiments by HAWKER (1932) and BOYSEN-JENSEN (1933; 1936) showed that root apices produce at least one growth regulating substance which inhibited root growth and promoted shoot growth and that an asymmetric concentration gradient of this substance is established in geotropically stimulated root apices, with a greater amount of activity present in the lower as compared with the upper half. For the reasons discussed previously for shoot geotropism, no conclusions can be made about the mechanisms involved in the establishment of the asymmetry.

Experiments carried out by SNOW (1923; 1924); KEEBLE, NELSON and SNOW (1931) and KEEBLE and NELSON (1935) also suggested that the root apex is a source of growth regulators which have an effectiveness in the growing zone. These experiments involved the insertion of small pieces of mica into transverse incisions made just behind the extreme apex of *Vicia* and *Zea* roots. If the mica barriers were inserted into incisions made one millimetre behind the apex, thus preventing longitudinal transport of growth regulators between the apex and the growing zone on one side of the root, curvatures developed in a direction away from the barrier. This suggests that a substance with an inhibitory influence is produced in the apex and moves basipetally into the growing zone of the root. KEEBLE, NELSON and SNOW (1931) also showed that

when one half of the root apex of a vertical root is cut away, curvatures developed in a direction towards the remaining half tip which suggested that the root apex is the source of a growth inhibiting influence. Another important finding was that when Vicia roots were placed horizontally, the geotropic curvature of those with a barrier in the upper side was greater than the curvature of roots with a barrier in the lower side. Since a double mica barrier on both sides of the root prevented curvature completely, it was concluded that an inhibitory substance was produced in the apex of the roots and that more of this inhibitory stimulus moved to the growing zone on the lower side of the root than the upper side. Again, from these experiments, it is impossible to distinguish between any of the possible number of ways in which an asymmetrical gradient of the inhibitory influence could occur in the growing zone of roots.

However KEBBLE and NELSON (1935) described some experiments which may suggest that lateral transport of a growth regulator could occur in roots. They compared the curvature developed by horizontal roots which had a mica barrier inserted into a horizontal incision made into the apex of the root, with that developed by roots without a barrier. It was found that the curvature of roots in which the barriers were positioned so as to prevent lateral movement of growth regulators was much smaller than the

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curvatures of roots with no barriers. Unfortunately they did not determine the curvature developed by horizontal roots with a vertical barrier inserted into their apices as a control, as this would have produced clear evidence of whether or not lateral transport occurred in the apex of roots. As some curvature took place in roots with horizontal barriers, KEEBLE and NELSON (1935) decided that a gradient was established in the growing zone which was a result of a difference in transport capacity between the upper and lower halves of the root. They attributed the reduced curvature of the treated roots, compared to control roots, to a wounding effect caused by the insertion of the barrier.

The early evidence upon which the CHOLODNY-WENT hypothesis for roots is based scarcely allows a critical appraisal of the theory to be made. A number of more recent investigations have given results which are not consistent with the theory. YOUNIS (1954) excised exactly 0.5 mm from the apex of Vicia roots which he claimed was just sufficient to remove the root cap and found that neither the growth rate nor the geotropic response was affected. If the apical meristem of the root was cut away, root growth was retarded and the geotropic response eliminated. Removal of the root cap and therefore the inhibitory influence should have resulted in an acceleration of the growth of the root and this is not consistent

therefore with the CHOLODNY-WENT hypothesis. The results of YOUNIS (1954) are similar to those of SPALDING (1894) who also claimed removing portions of root cap of Vicia roots had no effect on root growth. Both authors claimed therefore that the growth regulatory substances involved in geotropism must be produced by the root meristem rather than the root cap.

On the other hand, KONINGS (1968) working with Pisum and CERCHK (1970) working with Hordeum showed that removal of exactly 0.5 mm from the root apex was sufficient to remove all the differentiated root cap tissues and abolished the geotropic response. CERCHK (1970) also showed that the regeneration of the root cap was accompanied by a return of geotropic responsiveness. The data is conflicting and appears to depend upon the degree of precision with which the root cap is removed from the root. The results obtained by SPALDING (1894) and YOUNIS (1954) suggest that the root cap of Vicia is not indispensable for geotropism.

In 1966, an important series of experiments which showed that removal of the root cap of a Zea mays root completely eliminated the geotropic response (JUNIPER, GROVES, LANDAU-SCHACHAR and AUDUS, 1966) initiated the most recent phase of work on the geotropic response mechanism of primary roots. Removal of the cap had no effect on the growth of the root, a finding which led to the suggestion that the root cap was not the source of any growth regulators (JUNIPER et al., 1966; AUDUS, 1969; 1971). This suggestion is consistent with that of YOUNIS (1954) and SPALDING (1894). The easiest way to determine whether

or not the apex of an organ is the source of growth regulating substances and whether they promote or inhibit growth is to remove one half of the apex. In a series of experiments which were dependent upon the determination of curvatures rather than straight growth measurements, GIBBONS and WILKINS (1970) confirmed that removal of the entire root cap of a primary root of Zea mays eliminated the geotropic response and reported that when one half of the root cap was removed, the root developed a large curvature towards the side upon which the remaining half cap was located, regardless of the geotropic orientation of the root. The cap has therefore a significant effect on the growth of the root and GIBBONS and WILKINS (1970) and WILKINS, GIBBONS and SHAW (1971) suggested that the cap is the source of at least one substance which is inhibitory to root growth.

The conflicting evidence based upon straight growth measurements may be due to the possibility that only minute amounts of inhibitor are produced by the cap and although such small amounts would be sufficient to induce curvatures when supplied to one side of the root, they may not be at a sufficient level to be detected by straight growth measurements.

SHAW and WILKINS (1973) made a detailed study of the transport of the growth hormone in the root cap of primary roots of both Zea mays and Pisum sativum seedlings. Removing one half of the root cap always resulted in the

root developing curvature towards the remaining half cap. When one half of the extreme apex of a decapped root was excised, the root developed no curvature which indicated that the root apex, as opposed to the root cap, is not the source of any growth regulating substance. By inserting barriers which prevented longitudinal transport of growth regulating substances on one side of the root, it was found that the only growth regulating substances involved in the geotropic response of the root are transported basipetally from the cap into the growing zone. When barriers were inserted on the apical side of the extension zone, roots with intact caps developed large curvatures but decapped roots did not develop any curvature. Similar barriers inserted into the side of the root behind the growing zone had no effect on the curvature developed by horizontal roots with an intact cap and did not induce curvature in decapped roots or in vertical roots with intact caps.

If a growth inhibitor is produced in the root cap which is responsible for the geotropic curvature of a horizontally orientated root, by inducing the differential growth rates of the two sides of the organ, then either an asymmetric distribution has to be established between the two halves of the root or changes in the sensitivity of cells in the two halves of the root to the inhibitor must arise. As mentioned previously there are a number

of ways in which this differential growth rate could arise in a horizontal root.

Experiments by SHAW and WILKINS (1973) revealed that a downward lateral transport of a growth inhibitor from the upper to the lower half does occur in a horizontal root and that this lateral transport of inhibitor is involved in the geotropic mechanism. A comparison was made of the curvatures developed by roots which had either one half of their caps removed or their caps left intact but a barrier to longitudinal transport of inhibitor inserted into either the upper or lower half of the root between the root cap and the extension zone. Roots with barriers always developed greater curvatures than half decapped roots. Roots with intact caps and a barrier inserted into the upper side developed greater downward curvature than roots with the upper half of the cap removed. This suggests that more inhibitor must reach the lower half of the growing zone of the roots with an intact cap than the root with only one half of a cap and presumably the extra inhibitor must have come from the upper half of the cap. Roots with an intact cap and a barrier inserted on the lower side, developed less curvature than a root with the lower half of the cap removed. Lateral transport of the inhibitor occurred in the root with the barrier but the inhibitor accumulated on the apical side of the barrier and was therefore ineffectual in retarding the growth of cells in the

growing zone. But when roots had had the lower half cap removed, no inhibitor could be transported laterally downward and therefore the inhibitor would be transported basipetally into the extending zone on the upper side of the root thereby resulting in curvature.

PILET (1972) reported that the growth rate of a Zea mays root increased following the removal of the entire root cap thus confirming the results of GIBBONS and WILKINS (1970) and their suggestion that the root cap is the source of growth inhibitory substances. Further, PILET (1972) found that the activity of the growth inhibitor is not specific to Zea roots since placing Zea root caps on the tips of intact Lens roots decreased their growth rate. Confirmation that half decapped roots developed curvature towards the side on which the remaining half cap was located was also made by PILET (1973). When the detached half caps were replaced onto the original roots using Ringer's solution, no curvature occurred indicating that the inhibitor was able to pass from the cap through the Ringer's solution into the root apex. Replacing the half cap with oil instead of Ringer's resulted in curvature suggesting that the inhibitor could not pass the oil barrier. Thus the growth inhibitory substance produced by the root cap is most probably water soluble (PILET, 1971).

The geotropic response of a root therefore involves the principle of the CHOLODNY-WENT hypothesis in that a growth inhibitor is produced in the root cap and undergoes downward lateral transport from the upper to the lower side of a horizontal root, resulting in a differential

growth rate of the two sides of the root and thus curvature. However, although the occurrence of a lateral movement of a growth inhibitor has been established as being in part responsible for the geotropic response of roots, other processes may well be involved.

There have been several recent attempts to identify the chemical nature of the inhibitory substances in the root cap. According to the CHOLODNY-WENT hypothesis, the inhibitor involved in the geotropic response of roots was IAA but evidence seems to be growing opposing this view. Unequivocal evidence for the presence of IAA in roots has only just recently been established by means of combined gas chromatograhy and mass spectometry (BRIDGES, HILLMAN and WILKINS, 1973; ELLIOTT and GREENWOOD, 1974). BRIDGES et al. (1973) found that IAA occurred predominantly in the stele with very little in the cortex and root apex. Recently RIVIER and PILET (1974) successfully demonstrated the presence of IAA in root caps of Zea mays by quantitative mass fragmentography. Several other studies of growth hormones present in root caps have been unable to detect IAA in the cap or apex of a Zea mays root using bioassay techniques (KUNDU and AUDUS, 1974; WILKINS and WAIN, 1974).

The transport of IAA in Zea mays roots has been shown unequivocally to be highly polarised in the acropetal direction (SCOTT and WILKINS, 1968) and is confined to the stele (BOWEN et al. 1972; SHAW and WILKINS, 1974) and dependent upon aerobic metabolism (WILKINS and SCOTT, 1968).

How this acropetal polarity of IAA regulates cell extension and therefore the growth rate of a root is as yet unknown. It would appear that IAA is transported in the wrong direction for it to be the hormone responsible for the geotropic response of roots and SHAW and WILKINS (1973) were unable to detect any downward lateral transport of IAA in geotropically stimulated Zea mays roots.

Recent investigations have shown that there are in fact two and possibly three growth inhibitory substances present in the root cap. KUNDU and AUDUS (1974) extracted large numbers of Zea root caps and found a root growth inhibiting substance (measured by a Zea root segment bioassay) which had chromatographic properties similar to those of ABA. This inhibitory substance was certainly not IAA and in addition had the capacity to close stomata of Commelina leaves, again suggesting an ABA-like substance. Traces of this cap inhibitor were found in the root apex which is in accordance with the view that the cap is the site of synthesis, followed by basipetal transport into the root apex. A second inhibitor was present in the root apex which was not found in the cap. It has not yet been identified and its chromatographic properties do not correspond to those of any known growth regulator.

WILKINS and WAIN (1974) also found ABA and two other unidentified growth inhibiting substances to be present in the root caps of light grown but not dark grown Zea

seedlings. About 2.5-3 hours was needed for the synthesis and transport of the inhibitors from the root cap into the root apex following the exposure of dark grown roots to white light.

If ABA is to fulfill the role of the inhibitor of cell extension responsible for the geotropic response of roots, it is necessary to show that ABA is basipetally transported from the root cap to the root apex and that it undergoes downward lateral transport from the upper to the lower half of the cap or root apex as a result of geotropic stimulation. A recent investigation by PILET (1975) based on growth and curvature experiments, suggested that ABA can move both basipetally and laterally in horizontal Zea roots. ABA was applied asymmetrically in agar blocks to one half of the apical cut end of a sub-apical Zea root segment and impermeable barriers were inserted in various waysinto the apical ends or sides of the root segments. ABA must have moved laterally downward since the roots developed downward curvature, most probably caused by an increase in the ABA concentration in the lower half of the horizontal root.

The critical experiment necessary to provide unequivocal evidence that ABA undergoes downward lateral transport is to employ the technique of micro-application of high specific activity labelled ABA. HARTUNG (1976) found that application of {2-^{14}C} ABA to the upper side of geotropically stimulated root apices of intact seedlings of Phaseolus coccineus resulted in a weak downward lateral movement of

radioactivity. However a significant upward lateral movement of radioactivity was found to occur as a result of application to the lower side of the root tip. Unfortunately HARTUNG (1976) neglected to establish that the radioactivity was still confined to the ABA molecule. It is also possible to criticise that the ABA was applied to the plant at toxic concentrations (1 mg ml^{-1}) at a point 1 - 1.5 mm behind the root apex, that is, behind the root cap. HARTUNG was therefore investigating the movement of ABA in that region of root immediately behind the root cap and not within the root cap itself.

Additional evidence for the involvement of ABA in the geotropic response mechanism arises from the finding that Zea roots require exposure to light before they will exhibit a positive geotropic response (SCOTT and WILKINS, 1968). SHAW (1972) reported that Zea roots grown and placed horizontally in darkness developed greater curvature after being exposed to light than those roots which had been continuously maintained in darkness. In contrast roots of Pisum appeared not to possess this light requirement since the curvature developed was equal in the light and in the dark.

A series of experiments by WILKINS and WAIN (1974) have shown that light inhibits the growth rate of primary roots of Zea mays and that the root cap was the site responsible for the perception of the light stimulus by the root. Detached root caps and decapped roots were exposed separately to either white light or else kept in darkness.

A light-exposed cap placed on a dark-exposed root inhibited the growth rate of the root whereas growth was not inhibited when a dark-exposed cap was placed on a light-exposed root. Therefore the decapped root was not able to perceive the light stimulus which inhibited growth and its rate of elongation was greater than that of roots with intact root caps that had been exposed to the light. The capacity to perceive the light stimulus and therefore to be inhibited by it was restored to decapped roots 5 hours after the removal of the root cap. This suggested that light initiates the synthesis or release of a growth inhibitor from the root cap and as mentioned previously, ABA and two other inhibitors have been found to be present in the root caps of light grown but not dark grown Zea seedlings (WILKINS and WAIN, 1974).

In another paper WILKINS and WAIN (1975) investigated the relationship between the light-induced inhibition of root elongation and the geotropic response of primary roots of Zea mays and revealed a close similarity between the growth inhibitors produced in response to both stimuli. Placing root caps which had been removed from roots grown vertically in darkness on the apices of decapped roots, also grown vertically in darkness, resulted in little geotropic curvature when the roots were subsequently placed horizontally in darkness. Appreciable curvature was detected, however, when light-exposed tissue was used instead of dark-exposed. A positive geotropic response was also induced when caps removed from roots grown vertically in white light were attached to decapped roots which had been grown vertically in darkness and subsequently orientated horizontally.

However attachment of caps from roots grown in darkness, to decapped roots, which had been grown vertically in white light, induced little curvature upon orientating the roots horizontally in darkness. It appeared therefore that a dark-exposed decapped root can be induced to bend by attaching a light-exposed root cap to its apex, followed by horizontal orientation of the root in darkness despite neither the root cap nor the decapped root receiving previous geotropic stimulation.

Very recent experiments by WILKINS and WAIN (1975) provide further evidence of ABA being involved in the geotropic response mechanism of Zea roots (WILKINS, 1976). Application of ABA at concentrations of 10^{-9} M to 10^{-4} M to horizontally orientated intact dark grown Zea roots resulted in the development of downward curvature. Water controls showed little curvature. ABA did not induce any downward curvature in horizontal decapped roots. These results seem to establish, firstly, that the gravi-perception mechanism is operative in the darkened root cap and not dependent upon previous exposure to light, and, secondly that the root cap is the main site of the lateral transport of the inhibitor.

The mechanism responsible for the geotropic response of roots seems partially understood and there seems little doubt that it involves the synthesis or release and downward lateral transport of ABA in the root cap and the subsequent basipetal transport of different amounts of ABA in the upper and lower halves of the horizontal root (WILKINS, 1976).

The asymmetry in ABA concentration leads to unequal growth rates of the two halves of the root and ultimately to downward curvature. The demonstration of a downward lateral transport of ABA in horizontally orientated root caps has still to be unequivocally established but the main features of the mechanism concur with those originally proposed by the CHOLODNY-WENT hypothesis.

The possibility of other growth regulating substances being involved in the geotropic response of both shoots and roots has only just recently begun to receive attention. PHILLIPS (1972) investigated the gibberellin-like activity which diffused from apical buds of Helianthus hypocotyls in relation to geotropic stimulation. Approximately ten times as much gibberellin-like activity was obtained from agar receiver blocks in contact with the lower half of the horizontal shoot tissue than from receiver blocks in contact with the upper half. Approximately equal quantities were obtained from the two halves of vertically orientated shoot apices. It would appear that a horizontal Helianthus hypocotyl apex transports approximately ten times as much gibberellin-like activity through the lower side than through the upper side. Although these results do not allow a decision to be made as to how the differential occurs, PHILLIPS (1972) suggested that changes in gibberellin transport and/or rates of gibberellin synthesis may be part of the geotropic response of sunflower shoots.

Somewhat similar findings were reported by RAILTON and PHILLIPS (1973) for detached Zea coleoptile apices. The amount of gibberellin-like activity diffusing out of a horizontal coleoptile apex was approximately 5 times greater than that from a vertical shoot. With horizontally orientated apices, the ratio of gibberellin-like activity recovered from the lower half compared to the upper half was 4 : 1. Again these results suggest that the release, synthesis, activation or interconversion of gibberellin may be initiated as a result of geotropic stimulation. There is no evidence to suggest that a downward lateral transport of endogenous gibberellins occurs in horizontal Zea coleoptile apices. Also, growth inhibitory activity was apparently greater in extracts from upper receiver blocks than lower receiver blocks which suggested that geotropic stimulation may affect the concentration or synthesis of inhibitors between the upper and lower halves of horizontal coleoptile segments.

In an attempt to elucidate the mechanism whereby this gradient of gibberellin-like activity is established in shoots, WILKINS and NASH (1974) investigated the possible lateral transport of radioactivity in horizontal and vertical sub-apical segments of Zea mays coleoptiles supplied asymmetrically at their apical ends with agar donor blocks containing [^{3}H]GA_1. They did not observe

any polarity of movement within the segment and no evidence of any radioactivity emerging from the segment into a basal receiver block could be detected, even after a transport period of 24 hours. No downward lateral transport was observed even after 24 hours in a horizontal position.

More recently EL-ANTABLY and LARSEN (1974a) extracted the upper and lower halves of horizontal primary roots of Vicia faba and found that the upper half of the root contained more gibberellin-like activity than the lower half as measured by the lettuce hypocotyl bioassay. The results suggested that gibberellin might assist in the positive geotropic response of Vicia roots by stimulating elongation of the upper half of the root. This suggestion does of course depend upon gibberellin being a promoter of root growth. Consequently EL-ANTABLY and LARSEN (1974a) applied low concentrations of GA_3 to germinating Vicia faba seeds just after their radicle had broken the seed coat. It was found that GA_3 stimulated root elongation in Vicia faba within 24 hours and Lepidium within 36 hours. There seems therefore to be a definite possibility that gibberellins participate in the development of the positive geotropic curvature.

Unlike IAA, transport of the gibberellins has not been a topic of great research interest. Our limited knowledge of gibberellin movement in plants comes almost entirely from studies involving the application of exogenous

gibberellins and transport has been detected and measured either by observations of growth responses in parts of the plant remote from the point of application or by using radioactively labelled gibberellic acid and directly measuring the radioactive content of appropriate portions of tissue and agar receiver blocks applied to cut surfaces of segments.

The data on gibberellin movement in isolated segments is limited but in the majority of cases tested so far, gibberellins have been found to move in a non-polar manner. KATO (1958) observed no difference between acropetal and basipetal transport of GA (structure not stated) through 5 mm segments of etiolated pea epicotyls as measured by the extractable gibberellin in receiver blocks. Although probably compelled to do so because of the insensitivity of the spectrophotomic method of assay, KATO (1958) used extremely high non-physiological concentrations of GA in the donors (1.4g l^{-1}) and it is well established that the polarity of IAA movement which is so striking at physiological concentrations, decreases as the donor concentration is increased.

These results were confirmed by CLOR (1967) who used (^{3}H)GA (unstated purity, concentration and structure) to investigate further the possible polarity through 3 cm segments cut from the second internodes of dark grown pea epicotyls. Radioactivity present in agar receiver blocks was assayed by scintillation counting and the transport

pattern was found to be the same for both normal and inverted pea stem segments, i.e. no significant difference between acropetal and basipetal transport. Radioactivity present within the tissue itself was not assayed and no evidence was presented that the radioactivity was still confined to the GA molecule. The rate of transport was found to be the same for both directions.

Similarly {^{3}H}GA_1 did not show any longitudinal polar transport through 2 mm segments of Zea mays coleoptiles after transport times of ~ 1.5 hours (HERTEL et al., 1969). Radioactivity was assayed by scintillation counting and only that present in receiver blocks was assayed. There was no check that the radioactivity counted was still in the form of GA_1.

The nonpolar transport of {^{3}H}GA_1 through segments was confirmed by SCOTT and MOST (1972). The quantity of {^{3}H}GA_1 transported was low and it was found to move at the same rate through 8 mm sections of nodes and internodes of immature stems from sugar cane. Only radioactivity in the agar donor and receiver blocks was assayed and a chromatographic analysis revealed that the radioactivity was still associated with GA_1. Simultaneous application of IAA and GA_1 resulted in GA_1 moving with a basipetal polarity. The presence of GA_1 did not change the basipetal transport of IAA in any way.

As mentioned previously, WILKINS and NASH (1974) studied the movement of radioactivity from {^{3}H}GA_3 in sub-

apical Zea coleoptile segments and found no evidence of a longitudinal polarity within the segment and no radioactivity above background was detected in the receiver blocks. PHILLIPS and HARTUNG (1974) reported that transport of {^{3}H}GA_1 was essentially non-polar through stem segments of Phaseolus coccineus and little metabolism of {^{3}H}GA_1 seemed to occur during 16 hour transport periods. An apparent acropetal polarity was observed in young 8 cm segments but was explained in terms of gibberellin being withdrawn from the transport system into growing regions.

Evidence for non-polar transport of exogenously applied gibberellin also comes from whole plant studies. Autoradiographs reveal that {^{14}C}GA applied in ethanol to the stems, primary leaves and terminal buds of 14 day old Phaseolus vulgaris seedlings is rapidly absorbed and that the GA and/or its metabolic products moved in a non-polar manner, although primarily downward from the site of application (WATANABE and SCULLY, 1957). Application to the stem seemed to give the widest distribution to the various plant organs whilst application to the bud gave the least. This apparent distribution difference could be due not only to uptake and transport differences peculiar to stems, leaves and buds but also to differences in surface area at the different sites.

Similar results were obtained by ZWEIG et al. (1961) who applied labelled gibberellin as a lanolin paste to the leaves of Zea mays and to both leaves and cotyledon nodes of red kidney beans. Transport was studied by auto-

radiography and growth responses with a time course from 1 - 216 hours. Foliar application to beans resulted in low uptake and very little radioactivity distributed throughout the rest of the plant. This was confirmed by the growth response because even 4 days after GA application, there was no visible elongation of the internode. Application to the cotyledon node resulted in a greater absorption than foliar application, but still most of the radioactivity remained at the point of application. However 8 hours after application, the first internode had elongated reflecting some upward GA movement. Radioactivity was found to be distributed throughout the plant after only 3 hours and by 24 hours could be detected all the way down into the roots. GA was found to accumulate mainly in the growing regions of the stem tip. Autoradiographs of Zea plants also showed limited adsorption but sufficient was taken up to give observable growth responses. In contrast to beans, there was no evidence for any movement to the roots but rather a light distribution to all developing leaves. As shown in beans, radioactivity was found to accumulate in growing regions.

Further confirmation of this non-polar pattern of transport came from translocation experiments using intact plants of Phaseolus vulgaris L. (WHITE, 1973). {^{14}C}GA_3 was applied in agar blocks to fully grown and rapidly expanding trifoliate leaves of 3 week old plants by abrasion

of the leaf surface with a carborundum-water paste (method of HOCKING, HILLMAN and WILKINS, 1972). Following treatment, plants were maintained for 24 hours in constant light at 25°C prior to being cut into segments. The distribution of radioactivity was assayed by scintillation counting and the results revealed that a large proportion of the radioactivity originally applied remained at the site of application. However a small proportion was present in the laminar around the donor area and even smaller amounts in the mid rib and petiole of the donor leaf. There was no transport either acropetally into the upper shoot or basipetally into the roots.

However, some auxin-gibberellin synergism experiments (GALSTON, 1958; GALSTON and WARBURG, 1959) suggested evidence for an acropetal polarity of gibberellin movement in segments. GALSTON (1958) investigated gibberellin transport as a basis for auxin-gibberellin synergism and found that the effect of gibberellic acid applied to the base of 10 cm long sections of etiolated pea epicotyls, could be noted at the apex 1 minute after application, indicating a rate of acropetal transport of 10 cms/minute. GALSTON and WARBURG (1959) also observed, and it was confirmed by PURVES and HILLMAN (1959), that basally applied GA_3 moved acropetally to the apex of pea epicotyl sections. Since growth effects were evident within 3 - 5 minutes of application, the GA seemed to be transported at a rate of 20 - 30 mm/minute.

Observations by WEAVER and McCURR (1959) indicated that when gibberellin was applied to young shoots of vine, it was not translocated to other shoots when judged by its effect on shoot elongation and that more gibberellin reached the growing apex of the shoot when basal leaves were sprayed, than when apical leaves were (i.e. acropetal transport).

Gibberellin was applied as a liquid to the terminal buds of Jerusalem artichokes and was also sprayed onto the plants (EVTUSHENKO, 1961). Growth of the axillary buds was stimulated and only that part of the stem above the site of application, thickened. Axillary buds below the site of application were not stimulated. The effect of the gibberellin did not seem to pass downwards only acropetally. Thus, when the apical leaves are sprayed, the gibberellin is absorbed and transferred to the stem from where it is transported acropetally to the axillary and terminal buds.

McCOMB (1964) studied translocation and metabolism of $\{^{14}C\}GA_3$ applied as a solution to fully expanded leaflets of light grown Pisum seedlings. An increase in the growth rate of the apical region of the shoot was used as an indication that gibberellin had been transported from the point of application. The rate of transport was estimated to be approximately 5 cms/hour. Autoradiography which was used to study the distribution of radioactivity as a function of time, showed that most of the radioactivity remained where it was applied. Little radioactivity

moved into leaves which were fully expanded at the time of treatment or into the roots, but movement did take place into young immature leaves which were expanding and into immature regions of the stem. Chromatographic analysis revealed that the radioactivity extracted was still confined to the GA molecule and therefore the pattern and distribution of {^{14}C} reflected that of {^{14}C}GA_3. McCOMB (1964) concluded that the GA_3 moved acropetally into regions of growth, based on the observations that GA_3 was transported from mature to immature leaves.

McCOMB (1964) suggested that the acropetal transport of GA is in the phloem and more direct confirmation of this was provided by CHIN and LOCKHART (1965) who studied the direction and extent of gibberellin transport in Phaseolus vulgaris. Again transport was measured by an increase in the growth rate of the stem apex. The results showed that a similar increase in stem growth occurred when GA was applied to the first trifoliate leaf or to the shoot apex but considerably less elongation resulted when GA was applied to the primary leaves. When leaves were treated with GA after remaining in darkness for extended times, no increase in stem elongation was observed. However growth was promoted as a result of acropetal transport when plants were returned to the light. These results of CHIN and LOCKHART seemed to demonstrate that applied GA moved with the carbohydrate stream.

Indirect evidence for an acropetal polarity was obtained through the use of topical application (either by

injection or surface application in ethanol) of GA_3 to conifer plants that had been treated with growth retardants (PHARIS et al., 1970). The results suggested that plants not treated with retardants may have directed the exogenously applied GA_3 to move acropetally whereas plants treated with retardants showed little or no polarity of GA_3 movement (as evidenced by the flowering response). Further evidence for an acropetal polarity in non-treated plants comes from experiments whereby [^{3}H]GA_1 was injected and the distribution of radioactivity measured after 24 and 72 hours. There are two criticisms which even the authors themselves mentioned. Observation of a flowering response is not synonymous with transport of the injected GA_3 and no verification was made that the movement of radioactivity was still in the form of GA_1.

In contrast there is an increasing amount of evidence that GA moves with a basipetal polarity. Foliar application of gibberellin was found to induce foliar sprouting of subsequently harvested tubers with the implication that gibberellin is transported basipetally in potato plants (LIPPERT, RAPPAPORT and TIMM, 1958).

KENTZER and LIBBERT (1961) reported a significant amount of gibberellin present in basal receiver blocks 3 hours after the application of GA_3 in agar donor blocks to the apical ends of Helianthus hypocotyl segments. However since the gibberellin present in receiver blocks was measured by a bioassay which was not calibrated, an estimate of the actual quantity of GA recovered was impossible.

GREENBLATT and JACOBS (1966) did some similar experiments whereby GA_3 was applied in agar donor blocks to internode sections of Coleus and at the end of the transport period (unknown) the receiver blocks were assayed with the barley endosperm test. A basipetal polarity was reported and was of the same order of magnitude as reported for IAA in the same internode.

1 µg of gibberellin applied to the apical end of a 6 mm section of pea epicotyl was found to be transported with a slight basipetal polarity into receiver blocks after 12 hours (COHEN, ROBINSON and PALEG, 1966). Similarly JONES and LACEY (1968) applied 0.5 µg of either GA_1 or GA_5 in agar donor blocks to 15 mm sections cut from pea stems and found gibberellin activity in extracts from the basal receivers.

After making a critical appraisal of the rather controversial literature on gibberellin transport, JACOBS and KALDEWEY (1970) decided that they would be more likely to meet with success on the problem if they were to use physiological concentrations of GA_3 and an experimental system which had already been established for auxin transport studies (JACOBS and KALDEWEY, 1970) thereby enabling a direct comparison to be made with properties of other hormone transport systems. Consequently they applied agar blocks containing 0.1 µg GA_3 to one end of 5 mm petiole segments of Coleus and receiver blocks were assayed by the very sensitive barley endosperm bioassay after transport periods of 3 hours. The results revealed that at

least ten times as much GA_3 moved basipetally as acropetally. Therefore when GA_3 is tested under the same conditions as IAA, it moves with a similar polarity, time course and velocity. Thin layer chromatography revealed that the GA_3 was recovered unchanged.

Confirmation that the time course of GA_3 movement through sections of young fast growing petioles of Coleus into basal receiver blocks is very much like that of IAA, was given by JACOBS and PRUETT (1971). However, experiments on the time course of polar movement of GA_3 through segments of Zea roots (JACOBS and PRUETT, 1973) revealed that the transport was away from the root tip (basipetal) and therefore in the opposite direction to the polarity of auxin through similar root sections. In addition, the time course of basipetal movement was dissimilar to that of IAA in roots, in contrast to the striking similarity of GA_3 and IAA in their polarity and time course in petioles.

Results presented by HARTUNG and PHILLIPS (1974) confirm the existence of a basipetal polarity for the transport of both {^{3}H}GA_1 and {^{14}C}GA_3 through root segments from Phaseolus coccineus seedlings. The magnitude of this polarity was greater in the more apical elongating tissue than in the more basal, non-elongating root segments. Furthermore, a comparison of transport revealed that the polarity of gibberellin transport was restricted to the stele and absent from the cortex.

Little evidence exists relating to the movement of endogenous gibberellins in plants but it seems likely that they move in a non-polar manner. In Pisum and Helianthus, endogenous gibberellins are synthesised in the young leaves and stem tips (LOCKHART, 1957; JONES and PHILLIPS, 1966) and from their stimulatory effect on the growth of the internodes, it appears that they move basipetally. It is apparent that they also move acropetally in Helianthus since excising the pair of leaves immediately below the fourth internode, reduced the growth of this internode by half (JONES and PHILLIPS, 1966). Certainly in the case of sunflowers, there is a correlation between sites of gibberellin synthesis and the extent to which the elongation of the internode is stimulated. Since gibberellins synthesised in the leaves influenced the growth of immediately adjacent internodes, the distance they need to be transported is not very far. Gibberellins are also known to be synthesised and/or interconverted in root tip tissue and translocated acropetally into the shoot system (PHILLIPS, 1964; PHILLIPS and JONES, 1964; CARR, REID and SKENE, 1964; JONES and PHILLIPS, 1966; 1967; KENDE and SITTON, 1967; SKENE, 1967; SITTON et al., 1967; CROZIER and REID, 1971).

Gibberellins are transported both in the phloem and xylem. Evidence for phloem transport is twofold. Firstly movement of gibberellins in the phloem is indicated by the presence of GA-like substances in the sieve tube sap

of a number of both woody and herbaceous plants (KLUGE et al., 1964). The second line of evidence for phloem transport comes from the fact that application of gibberellins to mature leaves of other higher plants (CHIN and LOCKHART, 1965; McCOMB, 1964) has resulted in a general distribution throughout the plant (ZWEIG et al., 1961; McCOMB, 1964; CHIN and LOCKHART, 1965). The distribution pattern was found to be very typical of assimilates moving into the phloem and would have been exactly the pattern expected if labelled sugar had been applied to the leaves. The rate was also estimated to be the same as that of phloem movement (5 cms/hour).

The evidence for xylem transport rests mainly on the presence of GA-like substances in xylem exudates which has been repeatedly shown in a wide variety of plants (JONES and PHILLIPS, 1966; KENDE and SITTON, 1967; SITTON et al., 1967; SKENE, 1967; CROZIER and REID, 1971).

If gibberellins are moving in both the xylem and phloem then it is important to ascertain whether or not there is an interchange between the two systems. Experiments by BOWEN and WARDING (1969) showed that considerable two-way interchange of label derived from {^{14}C}GA$_3$ and {^{14}C} kinetin does occur between the xylem and extra cambial tissue of Salix viminalis. Radial translocation of both growth substances from the transpiration steam into the sieve tube sap was demonstrated by the use of the aphid technique.

There is an additional possibility that GA may be transported by an active transport mechanism independent of the vascular system and passive diffusion. The lateral transport of GA in segments excised from Helianthus hypocotyls and Pisum epicotyls was blocked by application of TIBA to plants before the isolation of the segments (LIBBERT and GERDES, 1964). Likewise the basipetal polarity of GA found in sunflower hypocotyls (KENTZER and LIBBERT, 1961) was considerably reduced by TIBA application.

It is evident therefore that very little is really known about the transport of gibberellins especially in the intact plant and an explanation of the nature of the gibberellin transport system may give insight into the part, if any, played by gibberellins in geotropism. The primary aim of this thesis was to assess the role of gibberellin transport in the geotropic responses of roots and shoots of Zea mays seedlings. This involved several lines of investigation. The first part was designed to make a detailed study of the transport and metabolism of {^{14}C}GA$_1$ and {^{3}H}GA$_1$ in both roots and shoots of Zea mays and in seedlings of Helianthus annuus, using both the classical agar donor block technique of WENT(1928) and the more precise micropipette technique developed by SHAW et al., (1973). The second part was an attempt to quantify and qualify the endogenous gibberellins in the shoot apices of both Zea mays and Helianthus annuus seedlings, using a variety of both diffusion and extraction techniques.

MATERIALS AND METHODS

1. PLANT MATERIAL

Fruits of Zea mays L. variety Giant White Horsetooth were obtained from Martin's Seeds Ltd., Goat Lane, Norwich, U.K. and stored at 10°C in darkness. As a general procedure, fruits were soaked for 8 h in flowing tap water in the light before planting using the following procedures:-

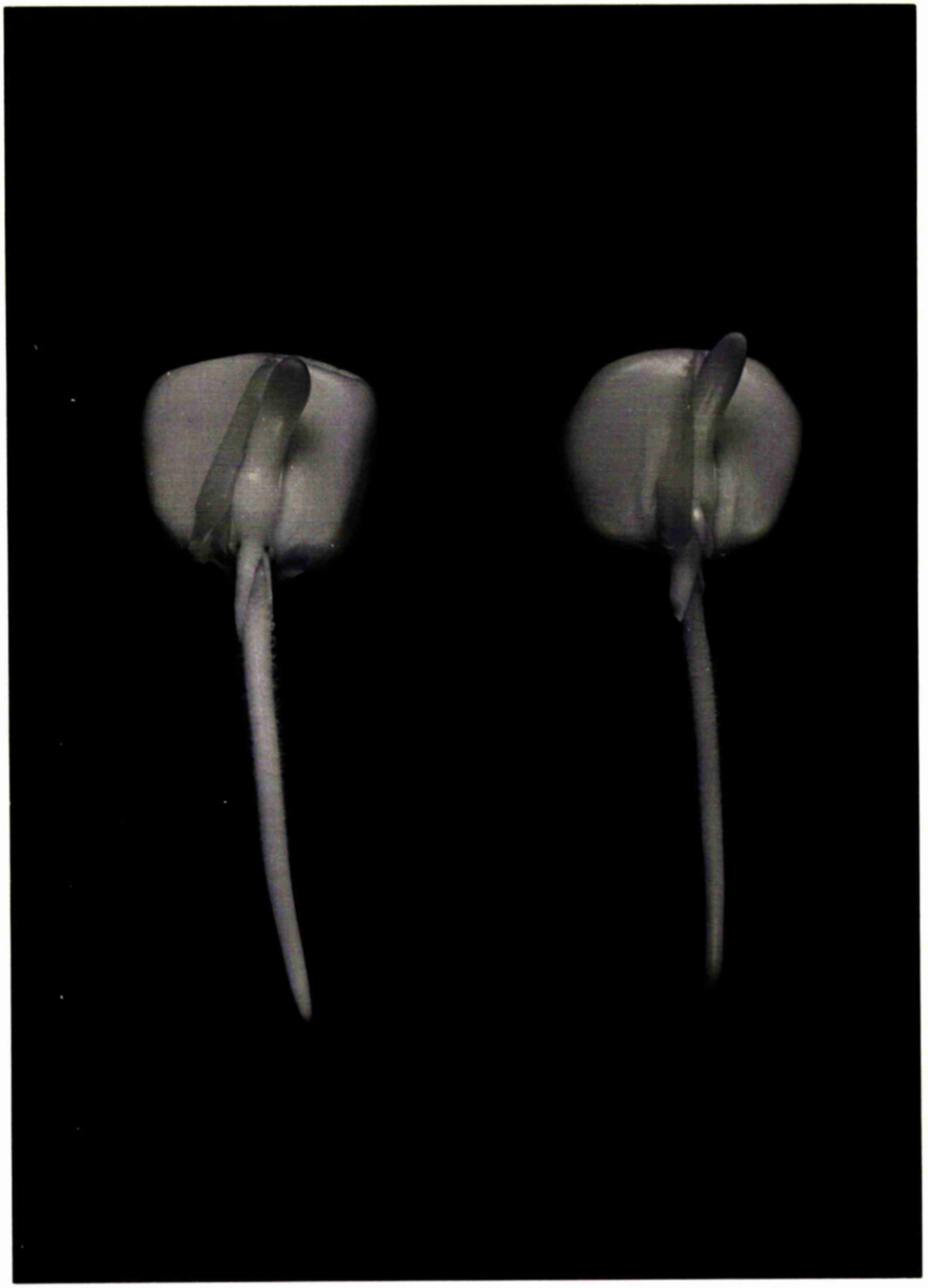
For experiments using subapical segments and apices of coleoptiles, the fruits were planted in moist vermiculite (J. and W. Henderson, 130, Salkeld Street, Glasgow, U.K.) in open polythene boxes (200 mm x 270 mm x 80 mm) and grown for 5 d in darkness at 25°C, at which time the coleoptiles were approximately 30 mm in length (Plate 1).

For experiments using roots and coleoptiles of intact seedlings, the fruits were planted out, embryo uppermost, on moist paper towels in covered polythene boxes which were placed in darkness at 25°C. Under these conditions, the primary roots grew out horizontally along the surface of the paper.

For the experiments with roots, the seedlings were used approximately 68 h after soaking, when the primary roots had attained an average length of approximately 30 mm (Plate 2). Immediately before an experiment, the seedlings were brought into white fluorescent light supplied by a bank of Mazda 65/80 W 'Warm White' 'Universal' fluorescent tubes, under which they were maintained for the duration of the experiment. For the experiments with coleoptiles,

PLATE 1: A 5 D OLD SEEDLING OF ZEA MAYS

PLATE 2: A $2\frac{1}{2}$ D OLD SEEDLING OF ZEA MAYS



seedlings were transferred individually, 56 h after soaking, to Pyrex vials which had been filled previously with 1% (w/v) agar ("Purified", Oxoid Ltd., London). The seedlings were maintained in darkness at 25°C except for the transfer and experimental procedures, which were carried out under dim green light. The seedlings were used when they had attained an age of 6 d, at which stage the coleoptiles were between 30 mm and 40 mm in length (Plate 3).

Achenes of Helianthus annuus variety Tall Single were obtained from Asmer Seeds Ltd., Ash Street, Leicester, U.K. and stored at 10°C in darkness. For the agar collection experiments, sunflower plants were grown in plastic trays (60 cm x 32 cm x 9 cm) containing a standard compost mixture of top soil : 'Humax' Sphagnum moss peat : coarse sand :: 1 : 1 : 1.

Plants for the hormone transport experiments (Plates 4 and 5) were grown singly in plastic pots (8 cm diameter, 7.5 cm depth) in the standard compost mixture. Plants were grown in a heated glasshouse. Between the months of October and May inclusively, supplementary lighting was supplied daily from 0300 h to 1900 h G.M.T. by 400 W 'GES MBFR Kolorlux' high pressure mercury vapour reflector lamps, to maintain a 16 h photoperiod throughout the year. During the rest of the year similar supplementary lighting was supplied daily between 0300 h and 0700 h G.M.T.

Plants were watered twice daily.

PLATE 3: A 6 D OLD SEEDLING OF ZEA MAYS GROWN IN AN AGAR SLOPE

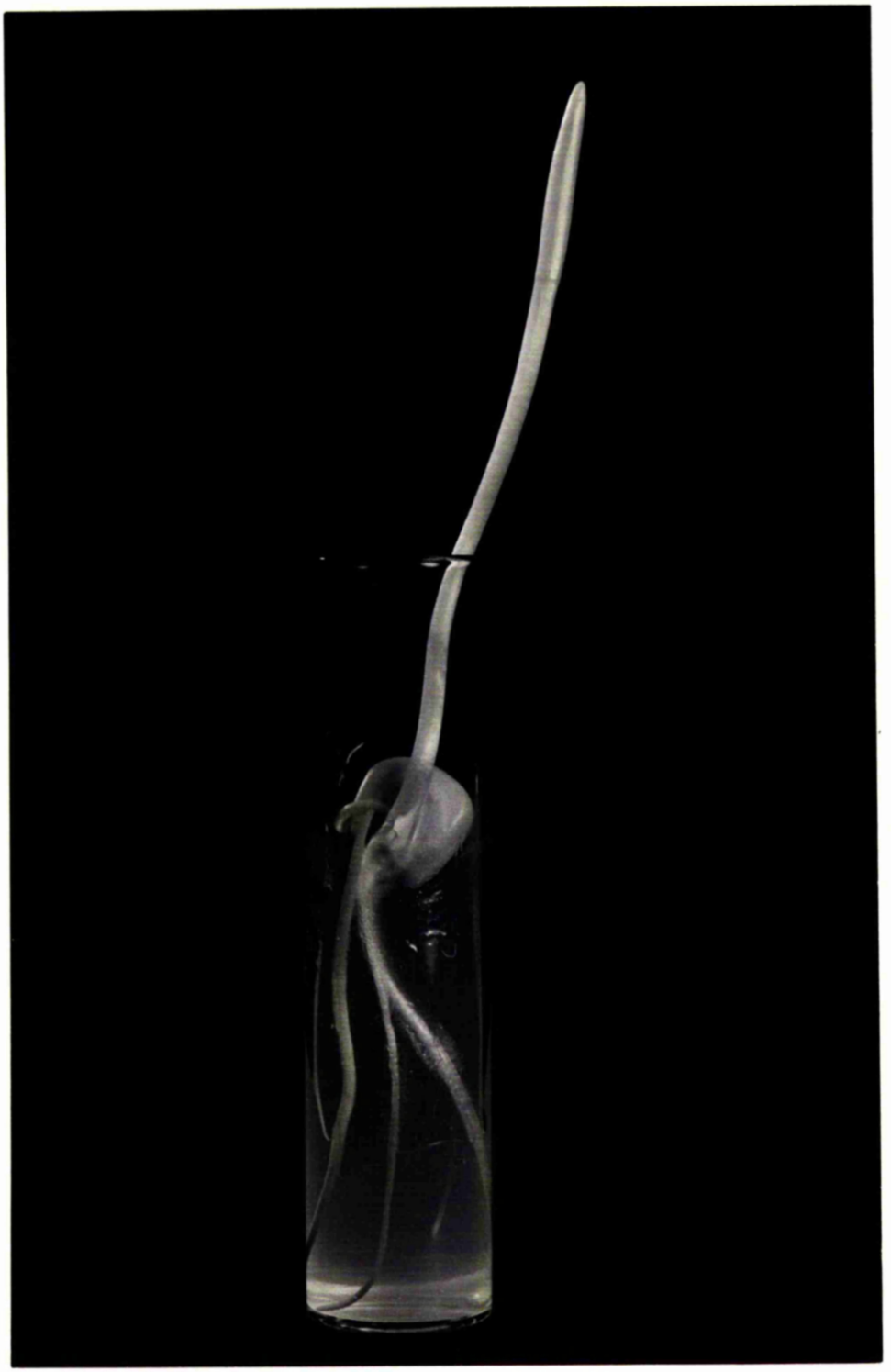


PLATE 4: A 10 D OLD SEEDLING OF *HELIANTHUS ANNUUS*

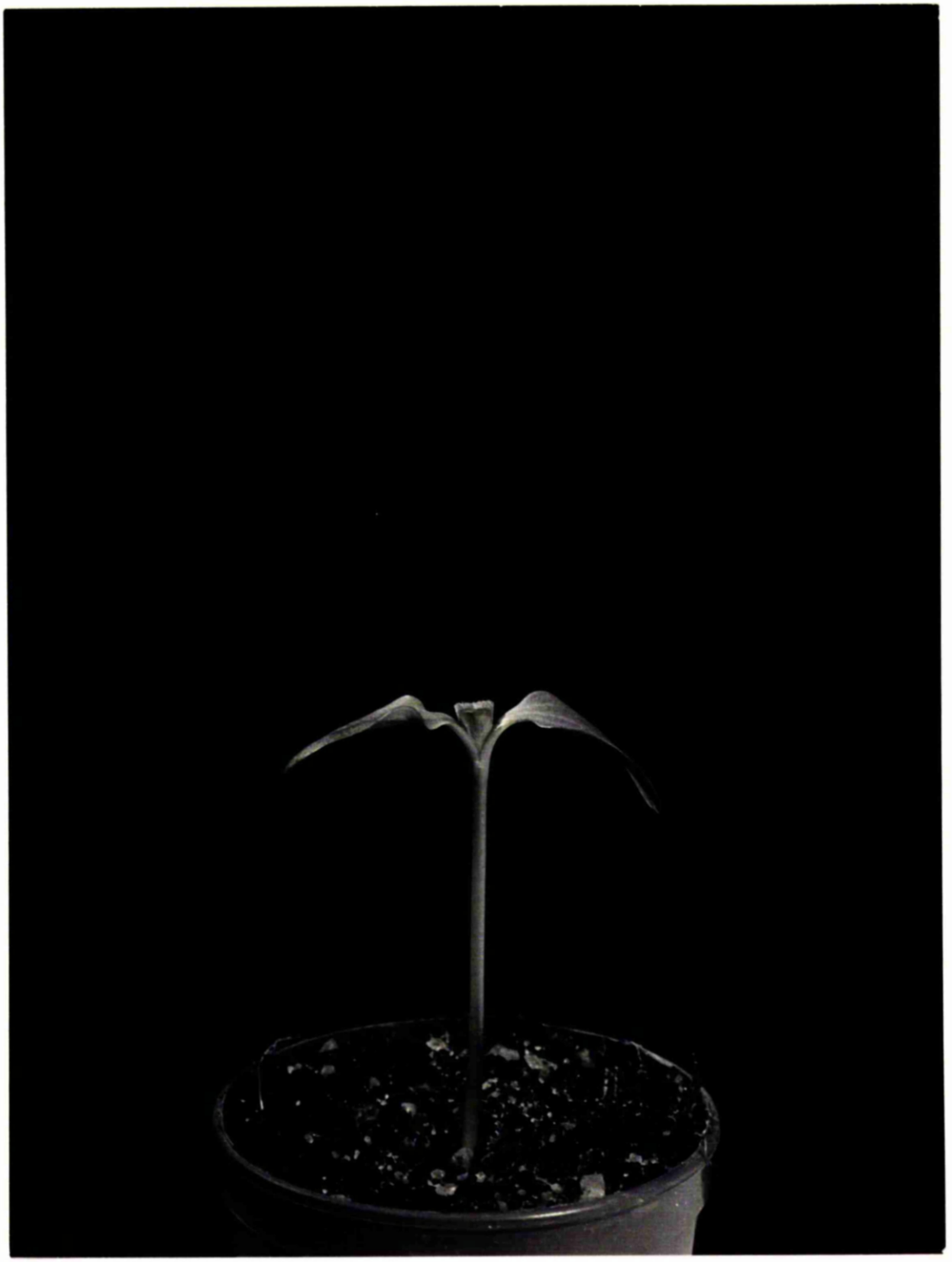
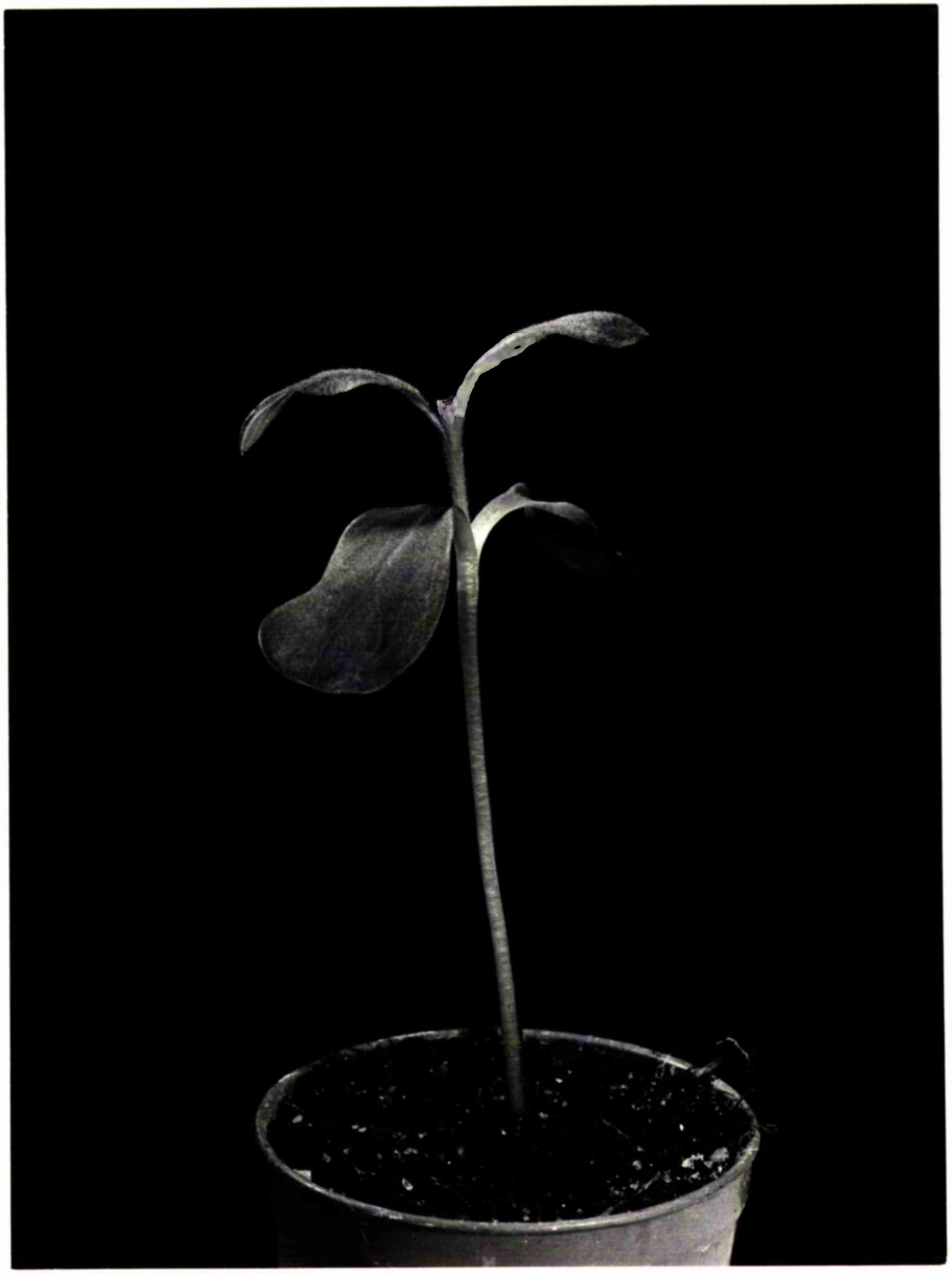


PLATE 5: A 14 D OLD SEEDLING OF HELIANTHUS ANNUUS



All the experiments using Helianthus plants were carried out under continuous white fluorescent light (see above) at 25°C and plants were moved into these conditions at least 12 h before the beginning of an experiment.

2. HORMONE TRANSPORT EXPERIMENTS

A. Radioactive Chemicals

{1-^{14}C} Indole-3-acetic acid ({^{14}C} IAA) of specific activity 33Ci M^{-1} and {17-methylene-^{14}C} gibberellic acid ({^{14}C} GA_3) of specific activity 6.1Ci M^{-1} were obtained from the Radiochemical Centre, Amersham, Bucks., U.K. The {1,2-^{3}H} gibberellin A_1 ({^{3}H} GA_1) of specific activity 31,000Ci M^{-1} was supplied by the New England Nuclear Corporation, 575, Albany Street, Boston, Mass. 02118, U.S.A.

B. Preparation

The {^{14}C} IAA was supplied in benzene : acetone :: 9 : 1 in sealed glass vials. The laboratory stock solution was made up by evaporating the solvent to dryness at room temperature under a stream of dry oxygen-free nitrogen (OFN). The residue was taken up in one drop of absolute ethanol before dilution with distilled water to give an aqueous stock solution of 2×10^{-6}M.

The {^{14}C} GA_3 was supplied in solid form and was dissolved directly in distilled water to give an aqueous stock solution of 2×10^{-6}M. For the experiments involving the application of aqueous hormonal solutions in micro-pipettes, a more concentrated solution of {^{14}C} GA_3 was

prepared, by dissolving the solid {^{14}C} GA_3 in a smaller volume of distilled water to give a stock solution of 2×10^{-3}M. The {^{3}H} GA_1 was supplied in ethyl acetate : ethanol :: 5 : 1 in sealed glass vials. These solvents were removed under a stream of dry OFN and taken up in 0.1 ml of absolute ethanol before dilution with distilled water to give an aqueous stock solution of 10^{-3}M. All the stock solutions were stored in darkness at -15°C.

C. Application of Radioactive Hormones to Seedlings

Both {^{14}C} IAA and {^{14}C} GA_3 were supplied to coleoptile segments in agar blocks. {^{14}C} GA_3 and {^{3}H} GA_1 were supplied to intact Zea and Helianthus seedlings in aqueous solutions.

Agar Blocks Ionagar No. 2 (Oxoid Ltd.) was incorporated into distilled water to give a 30 g l^{-1} agar solution which was autoclaved at a pressure of 15 lb in^{-2} for 15 min and was stored at 4°C until required. The 30 g l^{-1} agar stock solution was melted in a water bath and diluted to 15 g l^{-1} with an equal volume of either distilled water (receiver blocks) or with an aliquot of the appropriate hormonal solution (donor blocks). The molten agar was cast in brass moulds between clean glass slides. The donor blocks (25 mm x 22.5 mm x 3 mm) were subdivided into 8 smaller units each measuring 6.25 mm x 11.25 mm x 3 mm. Plain agar receiver blocks (25 mm x 21 mm x 1 mm) were subdivided into 8 units of 6.25 mm x 10.5 mm x 1 mm.

The agar blocks were made not more than 24 h before use and were stored on clean glass slides in petri dishes lined with damp filter paper, at 4°C until required.

Aqueous solution

{^{14}C} GA_3 (2×10^{-3}M) and {^{3}H} GA_1 (10^{-5}M) were supplied to seedlings as aqueous solutions, taken up in glass micropipettes similar to those used previously by Shaw and Wilkins (1974), and Shaw et al. (1973). The micropipettes were made on a Narishege P.E.2 vertical, glass electrode puller (Plate 6) made by the Narishege Scientific Instrument Laboratory, 1754-6, Karasuyama-Cho, Setagaya-Ku, Tokyo, Japan, and supplied by Eastern Scientific Ltd., Norwich, Norfolk, U.K. High tolerance glass electrode tubes (6" in length, 1 - 1.25 mm bore) supplied by Jencons (Scientific) Ltd., Mark Road, Hemel Hempstead, Herts., U.K. were used to make the micropipettes. The fine tip of the micropipette was removed by driving it into the ground edge of a glass slide. The end diameter of the pipette was measured with a calibrated binocular microscope. With practice, pipettes could be obtained with the required end diameter of about 100 μ and wedge-shaped. The pipette containing the radioactive GA was placed on the surface of the coleoptile, root, hypocotyl or epicotyl so that the sharp tip just penetrated the plant tissues for a few seconds before being withdrawn (Plate 7). No change in the volume of solution in the pipette was detected as a result of this

PLATE 6: NARISHIGE P.E. 2. VERTICAL GLASS ELECTRODE PULLER

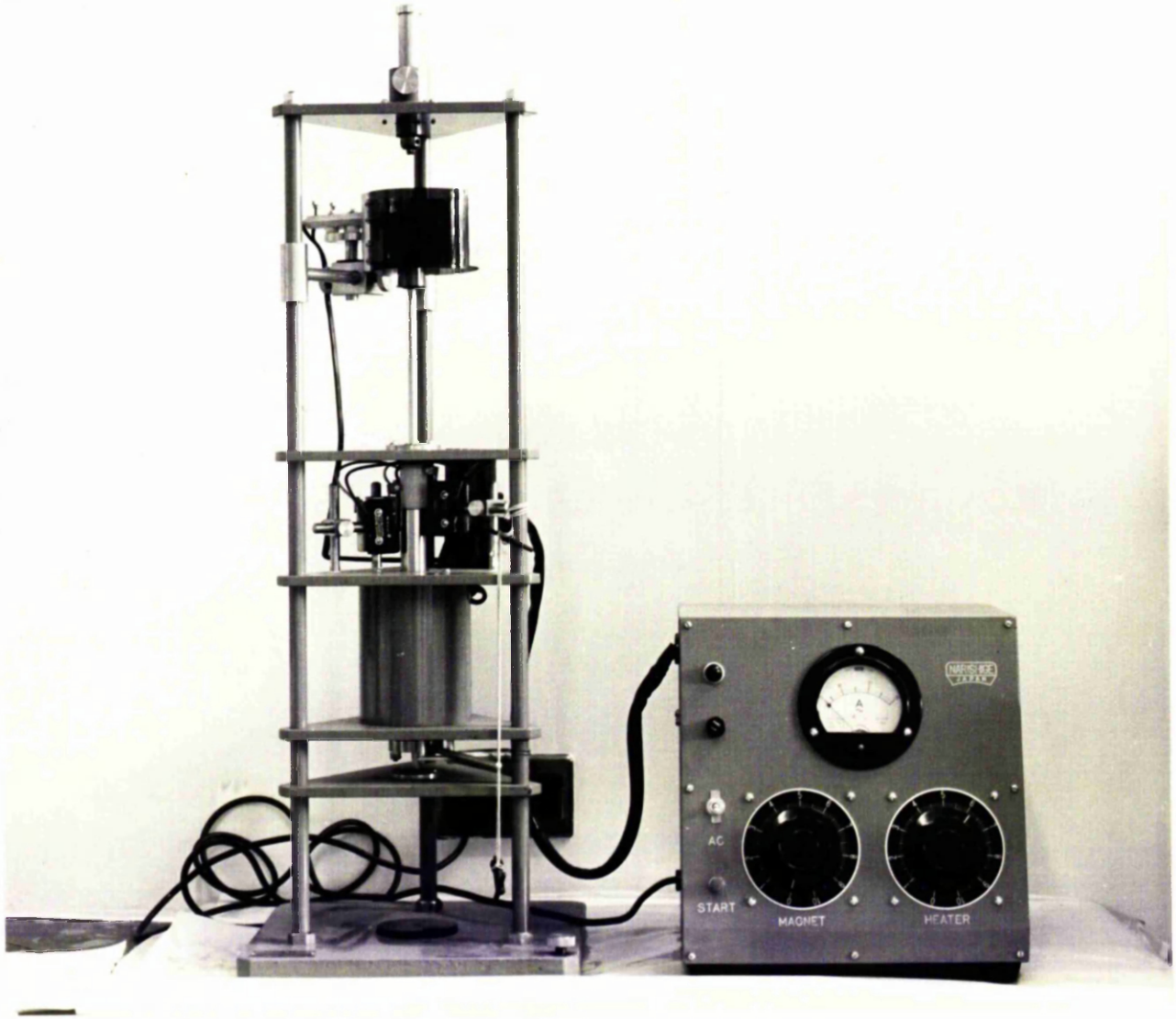

AC
START
MAGNET
HEATER

PLATE 7: APPLICATION OF A GLASS MICROPIPETTE CONTAINING RADIOACTIVE GIBBERELLIC ACID TO THE INTACT COLEOPTILE APEX OF A 6 D OLD *ZEA MAYS* SEEDLING

procedure and the pipette thus provides effectively a point source for diffusionof the GA into a highly localised region of the plant organ.

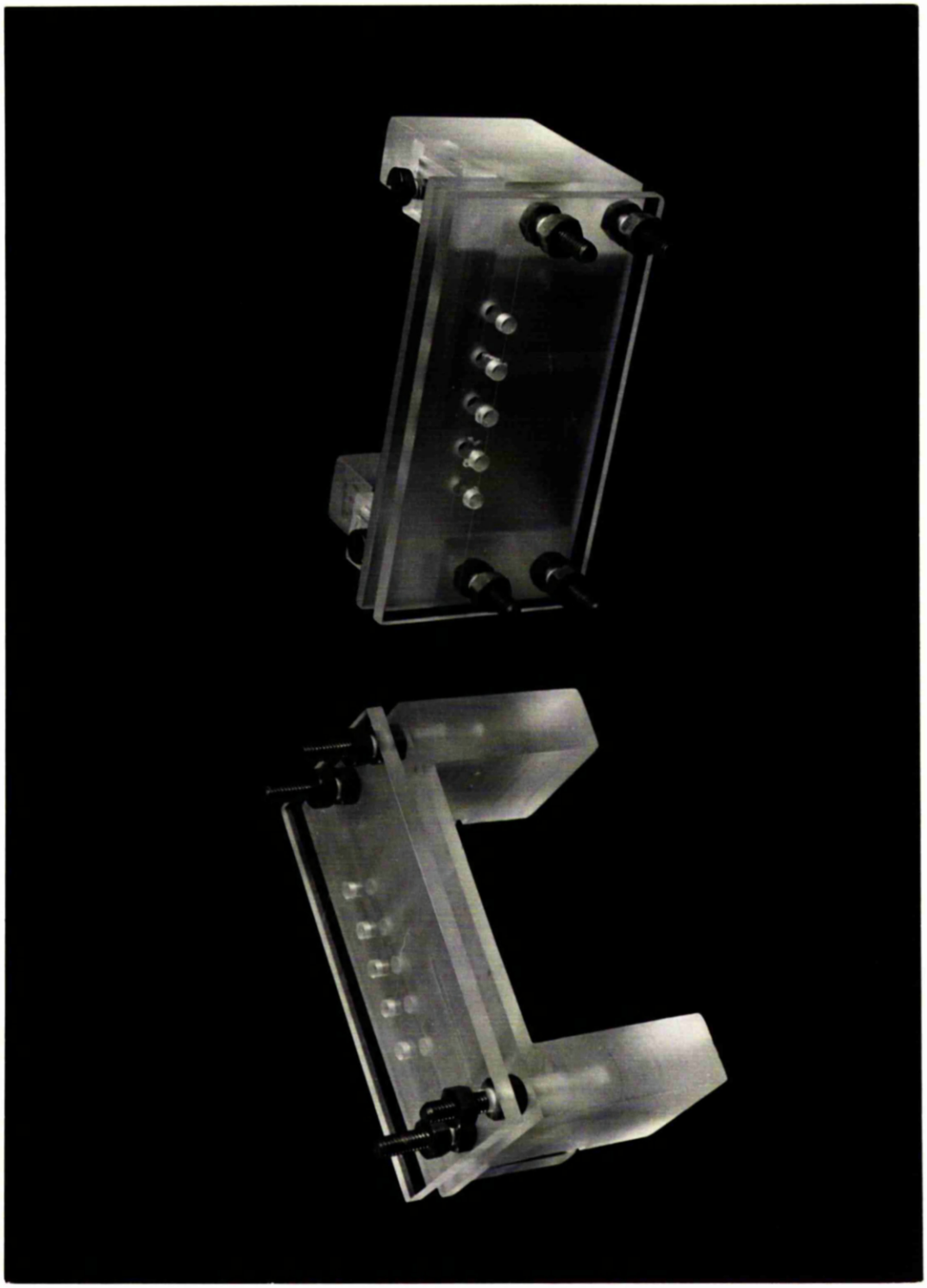
D. Tissue support

Both the segments and the intact seedlings used for the transport experiments required mechanical support in order to maintain the tissue in precise orientations with respect to gravity. This was achieved by using perspex supporting holders of various designs. Experiments which investigated polar transport in subapical coleoptile segments utilised holders as illustrated in Plate 8. Two rectangular pieces of perspex $\frac{1''}{16}$ in thickness were assembled such that 4 segments could be supported vertically in four holes. The holder was arranged on a self-locating perspex base $\frac{3''}{16}$ in thickness which had 4 slight depressions for the location of either donor or receiver agar blocks at the end of each segment.

Lateral transport studies in subapical coleoptile segments used perspex holders as illustrated in Plate 9. Two rectangular pieces of perspex, $\frac{2''}{16}$ in thickness, were assembled to support coleoptile segments in a way such that the apical cut end of the segment was mounted on a stainless steel razor blade with the cutting edge of the blade horizontally bisecting the apical cut surface of the segment to a depth of approximately 2mm. The razor blade was fixed in the perspex holder by metal screws. Donor or receiver

PLATE 8: PERSPEX HOLDER USED TO SUPPORT SUB-APICAL *ZEA* COLEOPTILE SEGMENTS DURING LONGITUDINAL TRANSPORT EXPERIMENTS

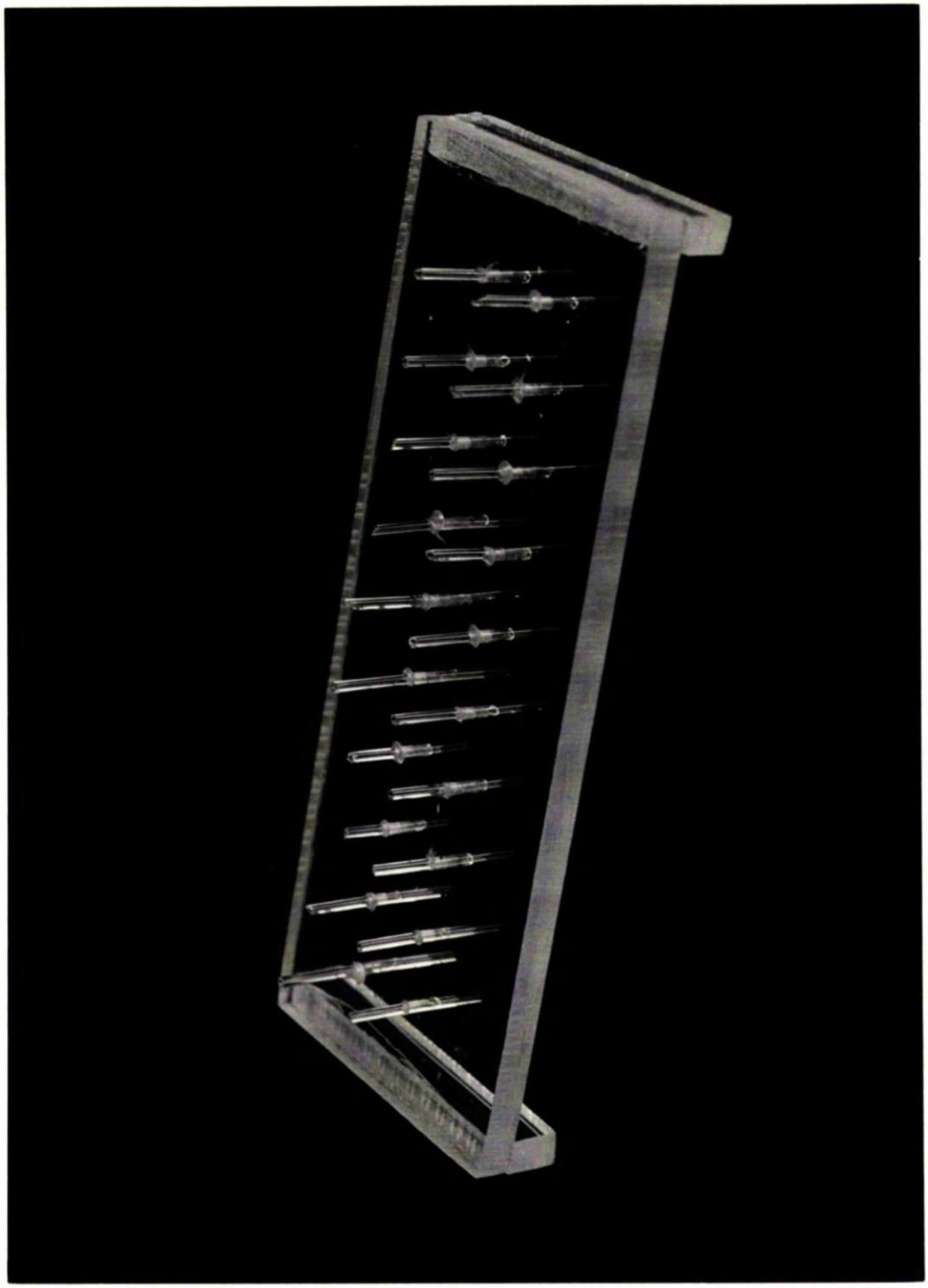
PLATE 9: PERSPEX HOLDER USED TO SUPPORT SUB-APICAL *ZEA* COLEOPTILE SEGMENTS DURING LATERAL TRANSPORT EXPERIMENTS



blocks could be placed on either side of the razor blade such that they were in contact with either half of the apical cut end of the segment. The perspex holders could be orientated so that segments were either in the vertical or horizontal position with respect to gravity. Up to 5 coleoptile segments could be mounted on each razor blade assembly.

The perspex holder illustrated in Plate 10 was used to support intact coleoptile apices in the vertical position with respect to gravity. Small holes (less than 1 mm diameter) were drilled in a rectangular piece of perspex (7.5 cm x 2.9 cm) and lengths of very fine glass tubing (approximately 1 cm in length, 0.5 mm bore) was inserted into the holes so that about 0.75 cm of the tubing protruded from the upper surface of the perspex holder. The pieces of glass tubing were glued into position. Intact coleoptile apices could therefore be supported vertically on the pieces of glass tubing. Plain agar receiver blocks could be applied to the basal cut surface of the apices by inserting blocks onto the tubing. Up to 20 apices could be supported on one perspex holder. During the experiments carried out under an anaerobic atmosphere, the glass tubes proved invaluable in allowing air present in the hollow central cylinder of the apices to be evacuated and released to an atmosphere of O_2N_2.

PLATE 10: PERSPEX HOLDER USED TO SUPPORT INTACT *ZEA* COLEOPTILE APICES DURING LONGITUDINAL TRANSPORT EXPERIMENTS



During the course of all transport experiments, conditions of high humidity were maintained to prevent dehydration of the tissue. The perspex supporting holders were placed in polythene boxes with fitted lids, which had been lined with moist paper towels.

E. Anaerobic atmosphere

Ananaerobic atmosphere was achieved using a simple technique, similar to the method described by Wilkins and Martin (1967). Coleoptile segments were mounted in perspex holders in the normal way, with the donor agar block in contact with one end and the receiver block at the opposite end of the segment. The entire assembly (holder, segments plus agar blocks) was then placed in a humidified vacuum desiccator. The desiccator was then evacuated to approximately 70 cm of mercury using a high vacuum pump (Edwards High Vacuum Ltd., Manor Royal, Crawley, Sussex, U.K.), and subsequently released to an atmosphere of OFN. This sequence of evacuation and subsequent flushing with high purity nitrogen was repeated six times within 15 minutes and ensured that most of the air trapped in the tissues of the coleoptile segments was removed. The gas flow rate was then adjusted such that a slow rate of nitrogen continued to bubble through the desiccator for the duration of the transport period. The gas stream was humidified prior to passing into the desiccators, by bubbling through a Dreschel bottle filled with distilled water and situated between the nitrogen gas cylinder and the vacuum desiccator.

As a control, a similar system was set up using compressed air instead of OFN.

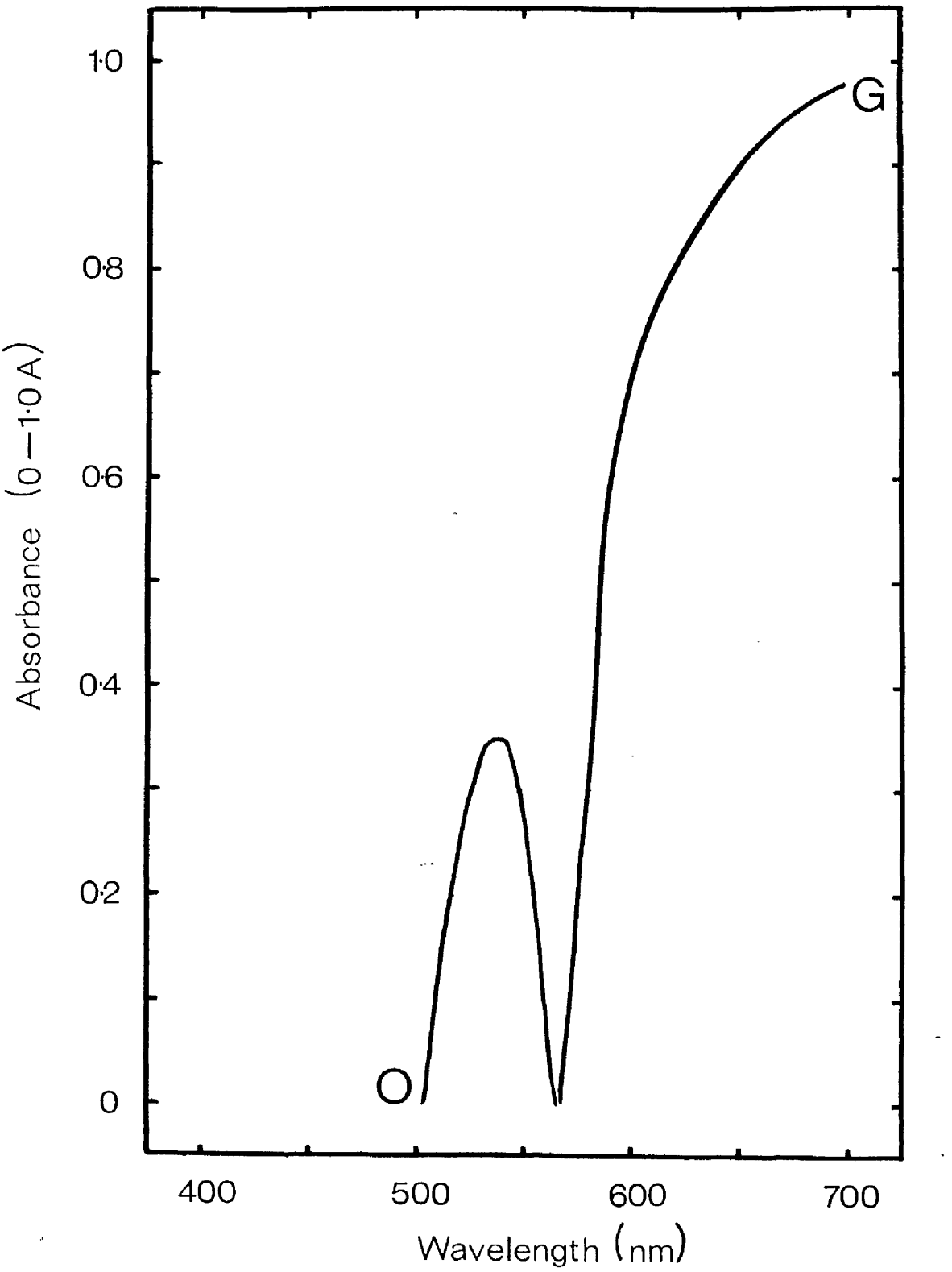
F. Safelights

During the transport experiments, some of the experimental manipulations could not be performed efficiently in total darkness and consequently dim green safe lights were used. Two light sources were used:- a 2 ft. 30 W Atlas 'Warm White' fluorescent tube and a Phillips 60 W tungsten filament bulb in a Kodak beehive lamp. Both these lights were filtered through 2 layers of Primary Green (No. 39) and 2 layers of Deep Orange (No. 5) Cinemoid acetate filters (Rank Strand Electric Company, London, U.K.). A visual examination, using a hand Spectroscope (Carl Zeiss, 31-36, Foley Street, London, U.K.) of the light transmitted by these filtered lights revealed a band of green light.

The transmission spectra (Figure 1) of the filters used in the construction of the safe lights were determined using a Unicam SP 8000 Ultraviolet Recording Spectrophotometer (Pye Unicam Ltd., Cambridge, U.K.).

During the experimental manipulations, both the safe lights were positioned approximately 30 cm from the plant material. The radiant flux density at the plant level was 0.749 W m^{-2} using a Pyroelectric Radiometer Model PR 200 (Molectron Corporation, 177, North Wolfe Road, Sunnyvale, California 94086, U.S.A.).

Figure 1: Spectral distribution of illumination from the cinemoid acetate filters used in the construction of the experimental green safe lights. The spectra were measured with a Unicam SP 8000 Ultraviolet Recording Spectrophotometer.


1·0
0·8
0·6
0·4
0·2
0
Absorbance (0—1·0A)
G
O
400
500
600
700
Wavelength (nm)

G. Experimental Procedure

Continuous application of {^{14}C} IAA and {^{14}C} GA_3 to subapical coleoptile segments

Polar transport

Individual subapical coleoptile segments, 10 mm in length and excised 1 mm behind the apex, were orientated vertically in perspex holders (described above) to observe polar transport through the segment. Segments were orientated such that the morphological apex was always uppermost. To observe basipetal transport, donor blocks were placed on the uppermost cut surface of each segment and receiver blocks were placed on the lowermost cut surface, so that transport was always downwards with respect to gravity. For acropetal transport studies, donor blocks were placed on the lower cut surface and receiver blocks on the upper surface of the segment so that transport was upwards with respect to gravity. For {^{14}C} IAA experiments, 4 segments formed 1 replicate. All 4 segments shared 1 donor block but had individual receiver blocks. There were 3 replicates (each of 4 segments) for each transport time, for both acropetal and basipetal transport. At the end of each transport period, each segment was divided horizontally into 2 for radioassay. Each segment piece and receiver block was counted individually. During the longer transport periods the segments grew. Consequently the 10 mm segments were divided from the donor block end such that the segment piece furthermost from the donor

block (i.e. nearest to the receiver block) was often slightly longer than the 5 mm segment piece closest to the donor block.

Subapical segments for the {^{14}C} GA_3 polarity experiments were orientated in the same holders as for the {^{14}C} IAA except that each 10 mm segment had individual donor and receiver blocks. At the end of each transport time, each 10 mm segment was cut horizontally into 5 x 2 mm pieces, starting from the donor block end of the segment, plus 'x' which refers to the piece of segment due to growth during the transport period. Because of the low specific activity of the {^{14}C} GA_3, it was found necessary to combine equivalent pieces of tissue from 18 coleoptile segments in order to obtain workable levels of radioactivity. Receiver blocks were also combined in groups of 18 for counting. There were 2 replicates (i.e. each of 18 coleoptiles) for each transport time, for both acropetal and basipetal transport.

Lateral transport

Lateral transport of {^{14}C} IAA was studied in subapical coleoptile segments using the asymmetric donor block technique (Goldsmith and Wilkins, 1964). Coleoptile segments, 15 mm in length, were excised 1 mm behind the apex and orientated in either the vertical or horizontal position with respect to gravity. The apical cut end of the coleoptile segment was divided horizontally by a razor

blade (described above) and agar blocks (approximately 2 mm x 2 mm x 2 mm) were placed on either side of the razor blade in contact with either half of the cut end of the segment. For examining the possibility of downward lateral transport of {^{14}C} IAA, asymmetric donor blocks were applied to the upper half of the apical cut surface of the coleoptile segment and a plain agar receiver block applied to the lower half. Conversely, for examining the possibility of upward lateral transport of {^{14}C} IAA, donor blocks were applied asymmetrically to the lower half of the apical cut surface and a plain receiver block applied to the upper half. After transport periods of 4 hours, receiver blocks were removed for counting and segments were bisected longitudinally and then divided transversely into 5 x 5 mm pieces in order to determine the lateral distribution of radioactivity within the tissue. Each piece of tissue and each receiver block was assayed individually. 8 coleoptile segments constituted 1 replicate and there were 2 replicates (i.e. 16 coleoptiles) for each of the orientations with respect to gravity.

An investigation to compare the lateral transport of {^{14}C} IAA in 10 mm segments excised 1 mm and 11 mm behind the apex was made using a similar technique as described above. 5 coleoptile segments constituted 1 replicate and there were 2 replicates (i.e.10 coleoptiles) for both types of tissue, for each of the 3 orientations with

respect to gravity. At the end of the transport period coleoptile segments were divided up into 3 pieces, as described above. Segment pieces and receiver blocks were assayed individually.

Aqueous application of a pulse of {^{14}C} GA_3 to coleoptile apices

Polar transport

A pulse of {^{14}C} GA_3, as an aqueous solution, was applied to either the apex or base of 10 mm intact coleoptile apices which were supported vertically as described previously. In order to investigate basipetal transport, the pulse was applied to the apex of the apical segment and a plain agar receiver block was placed on the basal cut surface. To investigate acropetal transport, apices were injected with {^{14}C} GA_3, 1 mm up from the basal cut surface of the coleoptile apex. At the end of each transport time, each apical segment was divided horizontally into 2; a 3 mm segment piece nearest to the point of injection and a 7 mm segment piece farthest from the point of injection. 10 intact coleoptile apices represented 1 replicate and there were 4 replicates for each transport time, in both the acropetal and basipetal direction.

Aqueous application of a pulse of {^{14}C} GA_3 to roots of intact seedlings

Polar transport

To investigate the longitudinal polar transport of {^{14}C} GA_3 in roots of intact seedlings, a pulse of {^{14}C} GA_3 was applied as an aqueous solution to pre-determined points

either 1 mm or 15 mm behind the root apex. In order to investigate basipetal transport the pulse was applied to a point 1 mm behind the root apex of seedlings which were subsequently orientated either vertically or horizontally with respect to gravity. At the end of each transport time, the roots were horizontally divided into 3 mm sections from the root apex.

Similarly, to investigate acropetal transport the {^{14}C} GA_3 was applied to a point 15 mm behind the root apex and the roots were orientated in the vertical position only. At the end of each transport time, the roots were divided horizontally into 3 mm pieces on either side of the point of application. For both basipetal and acropetal transport, equivalent pieces of tissue from 12 roots were combined for radioactive assay and there were 4 replicates (i.e. each of 12 roots) for each transport time with respect to each orientation.

Aqueous application of {^{14}C} GA_3 and {^{3}H} GA_1 to roots and coleoptiles of intact seedlings

Roots and coleoptiles of intact Zea seedlings were used to investigate the lateral transport of {^{14}C} GA_3 and {^{3}H} GA_1. The gibberellic acid was applied as described previously to one side of the root or coleoptile at a point approximately 1 mm behind the apex. During the hormone application, seedlings were orientated vertically. They were subsequently orientated vertically or horizontally with

the point of application on either the upper or lower side of the seedling. The coleoptiles were growing in agar in Pyrex vials as described previously and these tubes were supported in wooden racks which were subsequently placed in the different orientations with respect to gravity. The roots were supported in either the horizontal or vertical position as described above. At the end of each transport period, each coleoptile or root was bisected longitudinally and then divided transversely into several portions. In order to obtain workable levels of radioactivity from {^{14}C} GA_9 it was necessary to combine equivalent pieces of tissue from 12 seedlings. There were either 2 or 3 replicates, each of 12 seedlings, for each transport time, for each of the 3 orientations with respect to gravity. The specific activity of the {^{3}H} GA_1 was higher and consequently it was found necessary to combine equivalent pieces of tissue from only 4 coleoptiles or roots. There were 4 replicates, each of 4 coleoptiles or roots, for each of the 3 orientations with respect to gravity.

Aqueous application of {^{3}H} GA_1 to hypocotyls and epicotyls of intact Helianthus seedlings

To investigate lateral transport of {^{3}H} GA_1 in *Helianthus* seedlings, a pulse of {^{3}H} GA_1 was applied to one side of either the hypocotyl or epicotyl of intact seedlings at pre-determined points below the nodes of the cotyledons or the first foliage leaves. After application,

the seedlings were orientated vertically or horizontally with the point of application on either the upper or lower side of the seedling. At the end of each transport period each seedling was bisected longitudinally and then divided transversely. There were 8 seedlings for each orientation with respect to gravity and since the specific activity of the {^{3}H} GA_1 was higher, individual pieces of tissue were assayed.

Chromatographic analysis of radioactivity in the plant tissues

It seemed essential to establish that the movement of radioactivity within the plant tissue was a true representation of the movement of the growth hormone under investigation, i.e. was the radioactivity still confined to the gibberellin molecule.

Consequently approximately 200 either 2½ or 6 d old seedlings were injected with either {^{14}C} GA_3 or {^{3}H} GA_1 at a point approximately 1 mm behind the apex of either the root or coleoptile, as described previously. The seedlings were allowed to transport the gibberellic acid for a transport period of either 3 h {GA_1} or 6 h {GA_3} at 25°C in either the dark (coleoptiles) or white fluorescent light (roots). At the end of this time, in the case of coleoptiles, the apical 25 mm was removed from each seedling and the 200 apices were combined for extraction in 100 ml of redistilled methanol. In the case of roots, the entire roots were excised from the

seedlings and combined for extraction in 50 ml of redistilled methanol. The extraction was allowed to take place in the dark at 4°C and the methanol was changed 3 times at intervals of 24 h. Methanolic extracts were filtered through a Buchner funnel with sintered glass filter before being reduced to an aqueous phase (approximately 10 ml) under vacuum on a rotary evaporator at 40°C (Rotavapor - R, Buchi, Switzerland). The aqueous residue was made up to a volume of 50 ml with distilled water and adjusted to pH 2.5 with 50 g l^{-1} with HCl. The acidified fraction was partitioned 4 times with equal volumes of redistilled ethyl acetate and the resulting acidic ethyl acetate fraction was stored overnight at -15°C to freeze out any remaining water in the fraction. The resulting ice was filtered off and washed 3 times with chilled ethyl acetate in order to remove any remaining radioactivity from the ice. The ethyl acetate fraction was evaporated to a small volume (approximately 0.5 ml) and strip loaded onto either a 5 cm wide strip of Watman 3MM chromatography paper or 5 x 20 cm plastic sheets precoated with 0.25 mm silica gel without gypsum (Polygram Sil G, Macherey-Nagel and Co., 516, Düren, West Germany). Extracts and marker spots of stock solution of {^{14}C} GA_3 and {^{3}H} GA_1 were strip loaded under a stream of cold air onto a line 5 cm from the base of a sheet or 7 cm from the base of the paper.

Paper chromatograms were developed for 30 cm beyond the origin in the machine direction, with a descending solvent, in darkness at room temperature. The solvent used was either propanol-2-ol : ammonia ($\underline{d}$ = 0.88) : distilled water :: 10 : 1 : 1 (basic solvent) or di-iso-propyl ether : acetic acid :: 95 : 5 (acidic solvent).

Thin layer plates were devloped for 10 cm beyond the origin with an ascending solvent, in darkness at room temperature. The solvent used was either propanol-2-ol : ammonia ($\underline{d}$ = 0.88) : distilled water :: 10 : 1 : 1 (basic solvent), di-iso-propyl ether : acetic acid :: 95 : 5 or methyl ethyl ketone : acetic acid :: 95 : 5 (acidic solvent).

All solvents used were of the 'Analar'quality.

Assay procedure

Ultraviolet scanning

After development and air drying, both thin layer and paper chromatograms were sprayed with either dichloro-fluorescein (1 g l^{-1}) B.D.H. Chemicals Ltd., Poole, Dorset, in redistilled methanol or sulphuric acid, (Hopkin and Williams, Chadwell Heath, Romford, Essex) (1.8 g/ml) : redistilled ethanol :: 4 : 1. Subsequently chromatograms were viewed under both 254 nm and 350 nm wavelength ultra-violet light from a Universal U.V. Lamp Type TL-900 (Camag, Muttonz, Switzerland) and the positions of fluorescent areas noted.

Scintillation counting

Chromatograms were divided into 10 equal sized zones between the origin and the solvent front. Each Rf zone was

placed in a scintillation vial and assayed for radioactivity as described in the next section.

H. Radioactive Assay

Radioactivity in the plant tissue, agar blocks and chromatogram sections was assayed by one of the following two methods:-

Liquid Scintillation Counting

Samples of plant tissue segments and agar blocks from transport experiments or chromatograph sections from metabolism experiments were placed in scintillation vials and left to extract in 2 ml of either redistilled ethanol or methanol for at least 24 h in darkness at 4°C. The ethanol or methanol was subsequently removed by evaporation under reduced pressure at room temperature and filled with 10 ml of toluene (Asschem Ltd., Falkirk, Stirlingshire, U.K., May and Baker Ltd., Dagenham, Essex, or Fisons Scientific Apparatus, Loughborough, Leics., U.K.) containing 4 g l^{-1} 2,5-diphenyloxazole, PPO, (Fisons Scientific Apparatus). Vials were stored at 4°C in darkness for 0 - 3 d before assay.

The samples were assayed in one of the following systems:-

1) A Tri-Carb Liquid Scintillation Spectrometer (Model 3380) with an absolute activity analyser (Model 544) manufactured by the Packard Instrument Co. Inc., Illinois, U.S.A.

2) A Packard Tri-Carb Liquid Scintillation Spectrometer (Model 3380) without the automatic activity analyser.

3) A Corumatic 200 (Tracerlab G.B. Ltd., Weybridge, Surrey, U.K) Liquid Scintillation Spectrometer.

Each sample was counted once for either 10 min or 10,000 counts. Background radioactivity was determined by assaying clear vials containing only 10 ml of scintillation fluid and the background values obtained were subtracted automatically by the spectrometers. The Packard with the automatic activity analyser uses an external standard as a reference to calculate quenching. The automatic activity analyser corrects for both the quenching and background and expresses radioactivity as disintegrations per minute (dpm). Both the Packard without the automatic activity analyser and the Corumatic tracerlab calculate quenching but only correct for background radioactivity, expressing radioactivity as counts per minute (cpm).

All radioactive data is presented as disintegrations per minute.

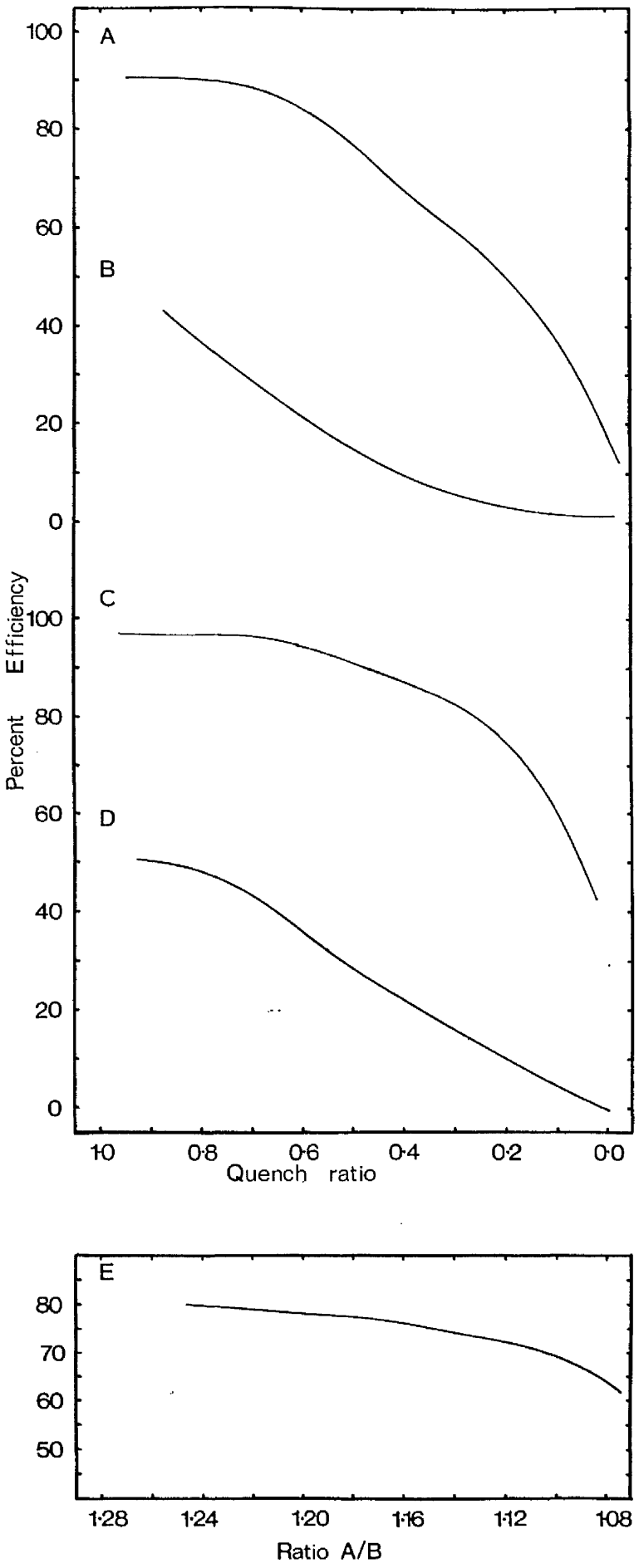
All the spectrometers were calibrated for efficiency at different quench levels by counting samples quenched with varying amounts of chloroform and chlorophyll which contained known quantities of {1-^{14}C} n-hexadecane (specific activity 1.1 μCi g^{-1}) or {1,2(n)^{3}H} n-hexadecane (specific activity 2.0 μCi g^{-1}). The calibration curves are shown in Figure 2.

Figure 2: Calibration curves for efficiency of A {^{14}C} B {^{3}H} Packard Tri-Carb (with absolute activity analyser), C {^{14}C} D {^{3}H} Packard Tri-Carb (without absolute activity analyser), E {^{14}C} Tracerlab Comumatic 200 liquid scintillation spectrometers at different levels of quenching in samples.

$$\text{Efficiency} = \frac{\text{Counts min}^{-1} \text{ detected in sample}}{\text{Disintegrations min}^{-1} \text{ supplied in sample}} \times 100$$

$$\text{Quench ratio} = \frac{\text{Counts min}^{-1} \text{ detected in external standard}}{\text{Disintegrations min}^{-1} \text{ supplied in external standard}}$$

$$\text{Ratio A/B} = \frac{\text{Counts min}^{-1} \text{ detected in external standard in Channel A}}{\text{Counts min}^{-1} \text{ detected in external standard in Channel B}}$$


A
B
C
D
E
100
80
60
40
20
0
Percent Efficiency
1·0
0·8
0·6
0·4
0·2
0·0
Quench ratio
70
50
1·28
1·24
1·20
1·16
1·12
1·08
Ratio A/B

The scintillation vials were 20 ml low potassium glass disposable vials with double screw caps manufactured by Johnsen and Jorgensen and obtained from R & J. Wood, 10 Hunter Street, Paisley, Renfrewshire, U.K. Between experiments they were soaked for 10 min in running hot tap water before being boiled twice in water with 'Pyroneg' detergent (Diversey Ltd., Barnet, Herts., U.K.) for at least 2 h each time. Subsequently they were rinsed 3 times with cold water, left soaking overnight in cold water and then rinsed individually 3 times with distilled water. Plastic caps were left soaking overnight in 'Pyroneg' and warm water, rinsed with cold water and rinsed finally with distilled water.

Chromatogram Scanning

Radioactivity present on intact chromatograms was analysed using a Panax Radio-chromatogram Scanner (Panax Equipment Ltd., Redhill, Surrey, U.K.). A flow of argon : propane :: 49 : 1 (British Oxygen Co. Ltd., London, U.K.) was the carrier gas and passed through the detection chamber at a pressure of 5 lb in^{-2}. The chromatogram passed through at a speed of 60 cm h^{-1}. The aperture was set at 2 mm width. No compensation was made for quenching or background and a trace of the percentage radio-activity was produced on a 'Servoscribe' RE511.20 flat-bed chart recorder.

3. GIBBERELLIN ISOLATION EXPERIMENTS

A. Chemicals

GA_3 was supplied by BDH Chemicals Ltd., and contained not less than 90% of GA_3. The c. 10% impurity consisted mainly of GA_4 and GA_7.

Sodium dihydrogen orthophosphate ($NaH_2PO_4.2H_2O$) and di-sodium hydrogen orthophosphate (Na_2HPO_4) were supplied as 'Analar' reagents by BDH Ltd.

The chemicals were stored at 4°C in darkness.

B. Preparation

Typically 1 mg of GA_3 was dissolved in 1 drop of liquid ammonia to form the soluble ammonium gibberellin salt. The ammonia was evaporated under a stream of OFN and the dried residue was dissolved in distilled water to give a stock solution of 10 µg/ml. A series of working solutions were prepared by serial dilution of the stock solution.

All solutions were stored at 4°C in darkness and were renewed monthly.

Molar solutions of both $NaH_2PO_4.2H_2O$ and Na_2HPO_4 were prepared by dissolving the molecular weight of each compound in 1 litre of distilled water. Molar Na_2HPO_4 solution was added to the molar $NaH_2PO_4.2H_2O$ solution until the mixed solution was pH 8.0. This resulting stock solution of 0.5 M pH 8.0 phosphate buffer was stored at room temperature.

C. Isolation of endogenous gibberellins from Zea coleoptiles

Collection in Agar

Attempts were made to collect endogenous gibberellins from Zea coleoptile apices in agar according to the method of Railton and Phillips (1973). Ion agar No. 2 was dissolved in boiling distilled water to give a 10 g l^{-1} solution of agar which was poured into 9 cm diameter disposable plastic petri dishes (Sterilin Ltd., Teddington, Middlesex, U.K.) to a depth of 6 mm. The apical 4 mm of coleoptiles were excised and placed such that the cut end of the apex was just inserted into the agar. The petri dish plus agar and coleoptile apices was orientated in either the vertical or horizontal position with respect to gravity and placed in a closed polythene sandwich box lined with moist absorbant paper towel and maintained in darkness at 25°C for 24 h. At the end of the diffusion period, the coleoptile apices were removed and the petri dishes plus agar were frozen at -15°C for 24 h. The agar plates were subsequently thawed at room temperature and the aqueous phase of the agar, together with dissolved substances was decanted. The residual agar was extracted 4 times at room temperature with sufficient redistilled methanol to cover the agar. Each extraction was approximately 12 h. The combine aqueous and methanolic extracts were filtered through a sintered glass Buchner funnel, to

remove any traces of agar and then reduced to an aqueous phase by rotary evaporation at 35°C. The residue (approximately 10 ml) was made up to 50 ml with distilled water, adjusted to pH 9.0 with 50 g l^{-1} $NaHCO_3$ and partitioned 3 times with equal volumes of redistilled ethyl acetate. The pooled basic plus neutral ethyl acetate fractions were retained for thin layer chromatography and bioassay. The aqueous fraction was adjusted to pH 2.5 with 50 g l^{-1} HCl and partitioned 3 times with equal volumes of redistilled ethyl acetate to give an acidic ethyl acetate soluble fraction. Each ethyl acetate fraction was frozen overnight at -15°C to freeze out any remaining water, as described previously. Each fraction was evaporated to dryness under vacuum at 35°C by rotary evaporation. The dried residue was redissolved in 0.15 ml redistilled ethyl acetate ready for thin layer chromatography.

In later trials of this nature, the final dried residues from the ethyl acetate fractions were divided into 2 for further purification.

One half was redissolved in 2 ml 80% redistilled acetone and then added to the top of a 12.5 x 2.5 cm charcoal : celite column (1 : 2 W/W) in a Quickfit CR32/20 preparation column with an integral sintered glass filter, overlaid by a layer of glass beads surmounted by a layer of glass wool with a top layer of glass beads. 1.5 g of

"Darco" Activated Charcoal (Atlas Chemical Industries Inc., Chemical Division, Wilmington, Delaware 19899, U.S.A.) and 3.0 g of "Celite 254" (Hopkin and Williams Ltd.) were mixed together and then suspended in 100 ml of 80% redistilled acetone, poured into the column and allowed to settle under its own weight. The column was washed through with 20 ml of 80% redistilled acetone before allowing the extract to be absorbed. The columnwas eluted successively 3 times with 20 ml of 80% redistilled acetone. The successive eluates were combined and evaporated to dryness by rotary evaporation before being redissolved in 0.15 ml of redistilled ethyl acetate prior to thin layer chromatography. The remaining half of the extract was chromatographed on a thin layer sheet as normal. The thin layer sheet was air dried and then divided up into 10 Rf values. Each Rf value was scraped from the plastic sheet and run individually through a 10 x 1 cm preparation column with an integral sintered glass filter overlaid by a layer of glass beads, a layer of glass wool and surmounted by a final upper layer of glass beads. The column was prewashed initially with 20 ml of water-saturated ethyl acetate. Subsequently each Rf value, dissolved in 125 ml of water-saturated ethyl acetate, was added to the top of the column. Each extract was washed through with another 50 ml of water-saturated ethyl acetate. Successive eluates were

combined for each extract and evaporated to dryness by rotary evaporation. The dried residues were redissolved in 0.1 ml of redistilled ethyl acetate. This was designated the original concentration and serial dilutions were prepared to give diluted fractions of $\frac{1}{10}$ and $\frac{1}{50}$ of the original concentration. Each diluted fraction was evaporated to dryness and redissolved in 2 ml of distilled water prior to bioassay.

Extraction and Partitioning

The extraction procedure was adapted from A. Crozier (personal communication).

Coleoptiles were excised from 4 d old dark grown Zea seedlings and the primary leaf was removed from the coleoptile. The deleaved coleoptiles were extracted 4 times in redistilled methanol in darkness at 4°C. Each extraction was for 12 h. The combined methanolic extracts were evaporated under vacuum at 35°C on a rotary evaporator until no more methanol distilled over. The aqueous residue (approximately 25 ml was made up to a volume of 50 ml with 0.5 M pH 8.0 phosphate buffer adjusted to pH 9.0 with 50 g l^{-1} $NaHCO_3$ and partitioned 5 times with half volumes of redistilled diethyl ether until there was no more colour appearing in the organic phase. The ether phase was discarded and the aqueous phase was acidified to pH 2.5 with 50 g l^{-1} HCl and partitioned 3 times with equal volumes of redistilled ethyl acetate. The resulting

acidic ethyl acetate soluble fraction was stored overnight at -15°C to remove residual water from the fraction, as described previously. The acidic fraction was evaporated to dryness under vacuum at 35°C and the residue redissolved in 2 - 3 ml of 0.5 M pH 8.0 phosphate buffer in preparation for loading onto the top of a 12.5 cm x 2.5 cm polyvinyl pyrrolidone (PVP) column ('Polyclar AT' obtained from BDH Chemicals Ltd.) contained in a Quickfit preparation column. 20 g of PVP were mixed with 100 ml of 0.5 M pH 8.0 phosphate buffer, poured into the column and allowed to settle under its own weight. The column was washed through with 20 ml of phosphate buffer before allowing the extract to be absorbed onto the top of the column. The column was eluted successively 3 times with 20 ml of phosphate buffer. Successive eluates were combined, adjusted to pH 2.5 with 50 g l^{-1} HCl and partitioned 3 times with equal volumes of redistilled ethyl acetate. The acidic ethyl acetate soluble fraction was stored overnight at -15°C to freeze out any remaining water, as described previously. The ethyl acetate fraction was evaporated to dryness under vacuum at 35°C by rotary evaporation. The residue was taken up in 0.15 ml of redistilled ethyl acetate ready for thin layer chromatography.

Thin layer chromatography

Extracts and marker spots of GA_3 were loaded under a stream of cold air onto a line 3 cm from the base of 5 x 20 cm plastic sheets. The chromatograms were developed in

ascending solvent to a distance of 10 cm from the origin. The solvent was propanol-2-ol : ammonia (d = 0.88) : water :: 10 : 1 : 1.

Bioassays

After development each thin layer sheet was air dried in a fume cupboard for 2 - 3 h and then divided transversely into 10 equal strips between the origin and the solvent front. Each 1 cm strip of chromatogram was scraped from the plastic sheet and bioassayed using either the lettuce hypocotyl or the dwarf rice bioassay.

Lettuce Hypocotyl Bioassay (Frankland and Wareing, 1960)

Seeds of Lactuca sativa variety 'Arctic King', supplied by Suttons Seeds Ltd., Reading, Berkshire, U.K. were placed on a circle of Whatman No. 1 filter paper moistened with distilled water, in a 9 cm diameter petri dish and left in darkness at 25°C. After between 20 - 24 h radicle emergence had taken place. Seedlings were selected with radicles of between 0.5 - 1 mm in length and were placed in 5 cm diameter plastic petri dishes lined with a 4.5 cm diameter circle of Whatman No. 1 filter paper. Rf values from the chromatogram were scraped into the petri dishes and moistened with 2 ml of distilled water. Twelve lettuce seedlings were placed in each dish and they were distributed such that they were approximately equidistant from each other and from the edge of the filter paper. As a control standard quantities of GA_3 were introduced

to a set of petri dishes and an equivalent quantity of silica gel was scraped into them from a blank developed chromatogram. All petri dishes were placed under white fluorescent light at 25°C. After 72 h, the length of the hypocotyls were measured to the nearest 0.5 mm.

Dwarf Rice Bioassay (Murakami, 1968)

Seeds of Oryzae sativa variety 'Tan-Ginbozu' were germinated in deionised water for 56 h in the dark at 32°C. The deionised water was changed every 12 h to minimise microbial contamination. After 56 h, seedlings were selected with coleoptiles that were between 1 and 2 mm long and transferred to glass vials (50 mm x 25 mm) which had been filled previously with a solution of 'purified' agar in water at a concentration of 9 g l^{-1}. Seedlings were planted such that the grain was half immersed in the agar. The glass vials were placed subsequently under fluorescent light, supplied by 4 Phillips L8 W/35 4J 26 cm tubes in an incubator at 32°C. The vials were contained in a perspex chamber which had been lined with paper towels to maintain a humid atmosphere. Water was added to the surface of the agar such that grains were immersed in 3mm depth of water.

Test solutions were prepared by eluting the silica gel from each Rf zone in redistilled methanol in 12.5 cm glass centrifuge tubes, centrifuging the silica gel 3 times and decanting the supernatant each time. The combined supernatant was reduced to dryness by rotary evaporation at 35°C.

Dried residues were taken up in 20 µl of 50% redistilled ethanol ready for application to the plants.

48 h after being transferred to the light, 1.0 µl of test solution was applied, using a 10 µl Terumo UMSN-10 Micro-syringe to the angle formed between the coleoptile and the second leaf sheath. Vials were returned to the light at 32°C. Three days after applying the test solution the second leaf sheath was excised and the length measured.

D. Isolation of endogenous gibberellins from Helianthus seedlings

Collection in Agar

Attempts were made to collect endogenous gibberellins from Helianthus apical buds using a technique adapted from that described by I.D.J. Phillips (1972). Shoot tips were excised in the elongating first internode (epicotyl), 10 mm below the apical bud, from 14 - 18 d old plants. Subsequently these will be referred to as 'apical buds'.

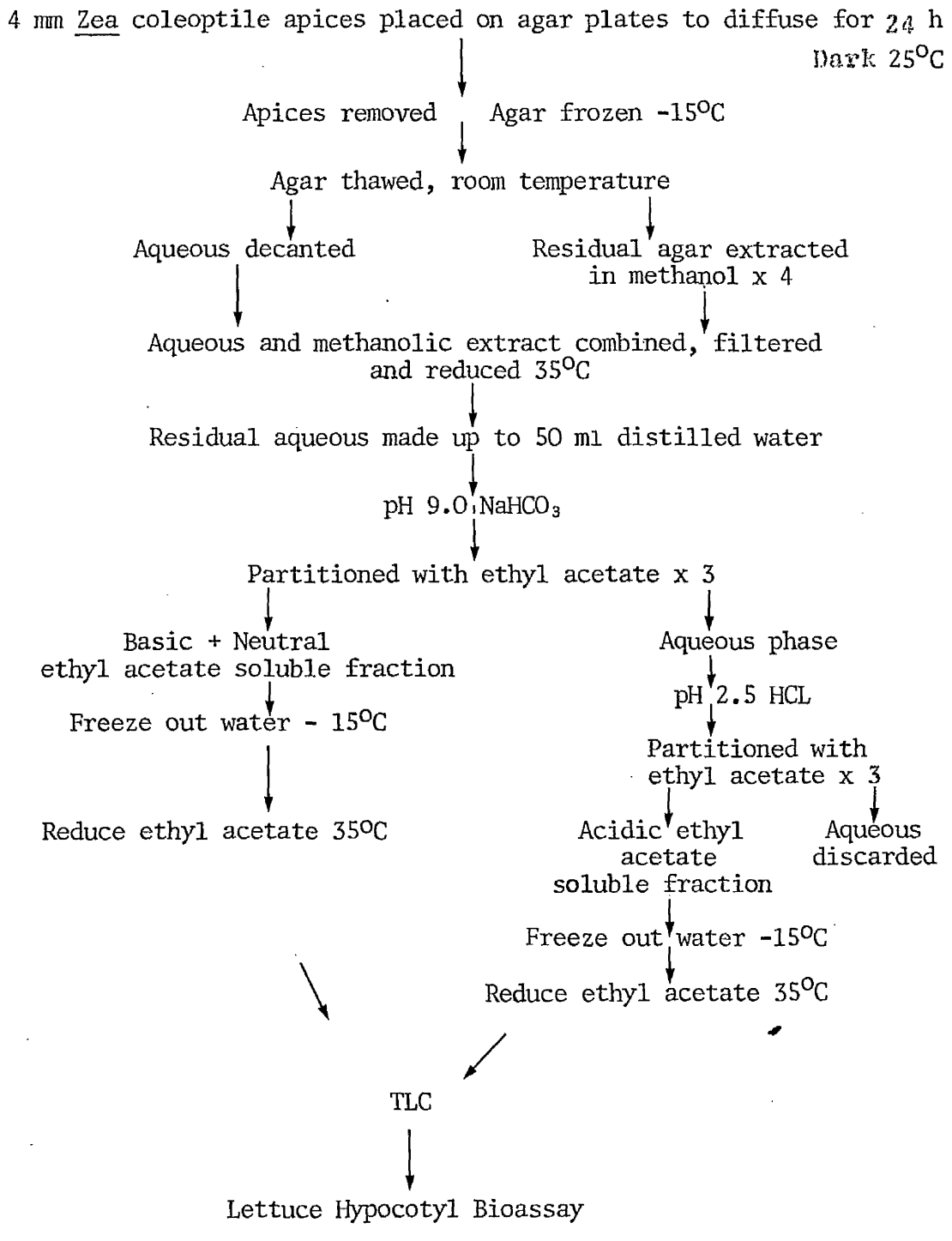
Agar plates were prepared as described previously and the apicalbuds were placed with the cut end of the internode just inserted into the agar. Approximately 500 'apical buds' were used for each experiment and they were incubated for 24 h at 25°C under white fluorescent light (described previously) and maintained in damp chambers to ensure high humidity.

After a 20 h diffusion time, the apical buds were removed and weighed. The agar plates were stored overnight at -15°C and subsequently thawed as described

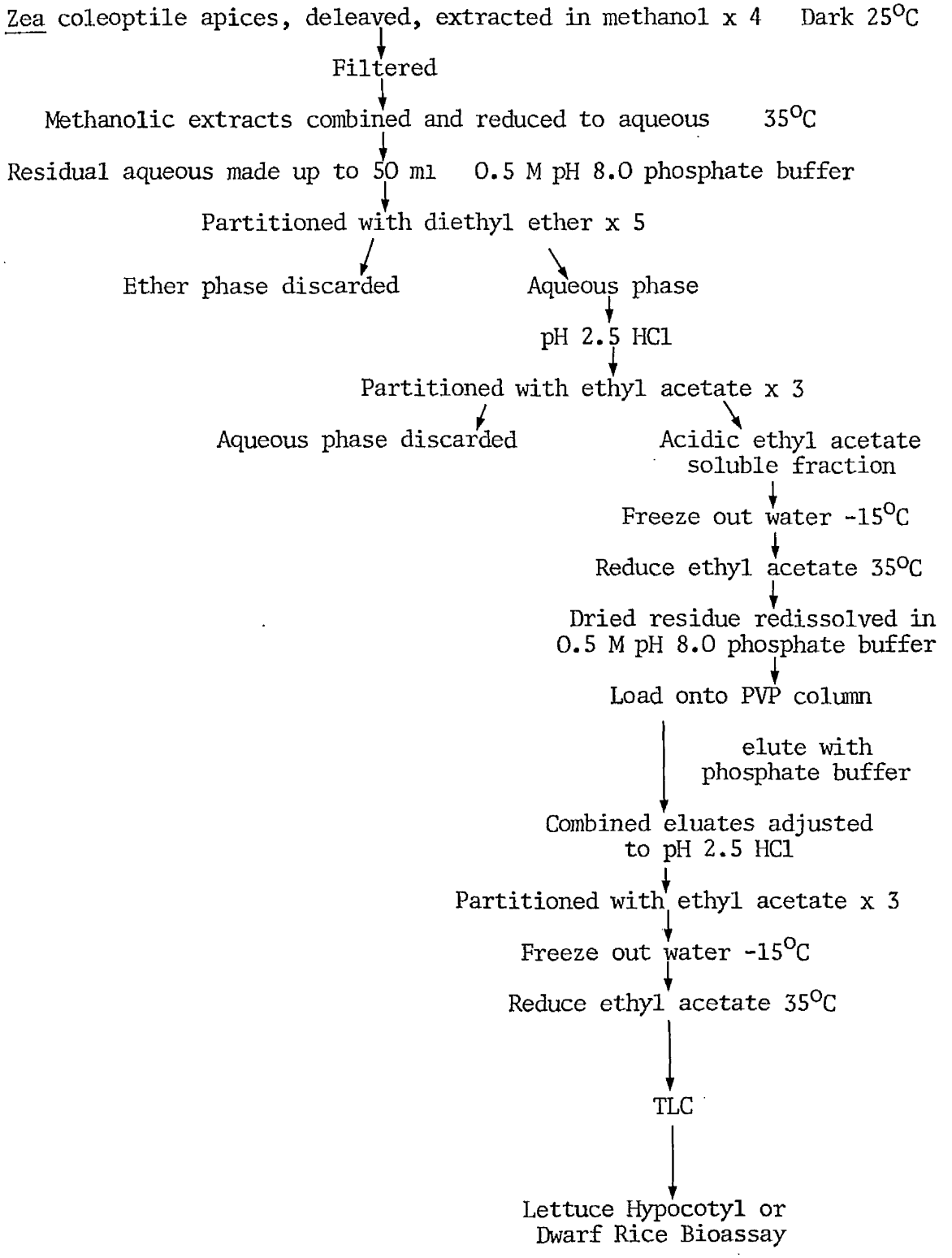
previously. The aqueous phase was decanted and the residual agar extracted 4 times with sufficient re-distilled methanol to cover the agar. Each extraction was for 12 h. The combined aqueous and methanolic extracts were reduced under vacuum at 35°C until no more methanol distilled over. The residual aqueous phase (approximately 20 - 30 ml) was adjusted to pH with 50 g l^{-1} HCl and partitioned 3 times with equal volumes of re-distilled ethyl acetate. The acidic ethyl acetate phase was stored overnight at -15°C to remove any residual water as described previously. The acidic ethyl acetate fraction was subsequently reduced to dryness under vacuum at 35°C and the dried residue dissolved in 0.15 ml of redistilled ethyl acetate and strip loaded onto a 5 cm wide strip of Whatman 3MM chromatography paper. Chromatograms were developed for 30 cm beyond the origin in the machine direction with a descending solvent in darkness at room temperature. The solvent used was propanol-2-ol : ammonia (d = 0.88) : distilled water :: 10 : 1 : 1. Developed chromatograms were air dried in a fume cupboard for 2 - 3 h and then divided transversely into 10 equal sized strips between the origin and the solvent front. Each 3 cm strip was eluted with 3 ml of distilled water and bioassayed with the lettuce hypocotyl elongation test as described previously.

The procedures used for the collection in agar and extraction experiments are summarised in the accompanying flow sheets 1, 2 and 3.

Flow Sheet 1: Collection in Agar of Endogenous Gibberellins from Zea Mays Coleoptile Apices



Flow Sheet 2: Extraction of Endogenous Gibberellins from *Zea Mays* Coleoptile Apices



Flow Sheet 3: Collection in Agar of Endogenous Gibberellins from Helianthus Seedlings

'Apical buds' from Helianthus seedlings placed on agar plates to diffuse for 24 h — Light 25°C

↓

Apical buds removed. — Agar frozen -15°C

↓

Agar thawed room temperature

↙ ↘

Aqueous decanted — Residual agar extracted in methanol x 4

↘ ↙

Aqueous and methanolic extract combined, filtered and reduced 35°C

↓

Residual aqueous pH 2.5 HCl

↓

Partitioned with ethyl acetate x 3

↓

Acidic ethyl acetate soluble fraction

↓

Freeze out water -15°C

↓

Reduce ethyl acetate 35°C

↓

Paper chromatography

↓

Lettuce Hypocotyl or Dwarf Rice Bioassay

4. BIOMETRY

An Olivetti programma 101 desk top computer was used to calculate the standard error of the mean value for a series of observations, from the formula:

$$\text{Standard error} = \sqrt{\frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n\,(n-1)}}$$

where x = value of each individual observation

n = number of observations

The student's 't' test was used to determine whether the mean value of two samples taken from the same population were significantly different using the formula:-

$$m_1 = \frac{\Sigma x}{n_1} \qquad m_2 = \frac{\Sigma y}{n_2}$$

$$s_1^2 = \frac{n_1 \Sigma x^2 - (\Sigma x)^2}{n_1\,(n_1 - 1)} \qquad s_2^2 = \frac{n_2 \Sigma y^2 - (\Sigma y)^2}{n_2\,(n_2 - 1)}$$

$$\sigma = \sqrt{\frac{(n_1 - 1)\,s_1^2 + (n_2 - 1)\,s_2^2}{n_1 + n_2 - 2}}$$

$$t = \frac{m_1 - m_2}{\sigma\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

where m_1 = mean of the first sample

m_2 = mean of the second sample

s_1^2 = variance of the first sample

s_2^2 = variance of the second sample

n_1 = number of observations in the first sample

n_2 = number of observations in the second sample

σ = standard deviation

The level of significance for each 't' value was obtained from the values quoted by Fisher and Yates in Statistical Tables (6th edition, Oliver and Boyd, Edinburgh, 1963).

RESULTS

A. HORMONE TRANSPORT STUDIES

1. The Longitudinal Movement of Radioactivity from {^{14}C} IAA through Coleoptile Segments of Zea Mays.

The acropetal and basipetal movement of radioactivity from {^{14}C} IAA through 10 mm subapical segments of Zea coleoptiles into receiver blocks was investigated as a function of time. Segments were supplied at either their apical or basal ends with donor blocks containing {^{14}C} IAA and with receiver blocks in contact with their opposite ends. The amounts and distribution of radioactivity in the apical and basal halves of the coleoptile segments and in the receiver blocks are shown in Figure 3. Figure 4A shows the total uptake of radioactivity by the intact segment plus the receiver block as a function of time and Figure 4B the amount of radioactivity in the receiver blocks expressed as a function of time. All data are the means of 4 independent experiments.

When the donors were applied to the apical end of the segments (Figure 4B) the amount of radioactivity reaching receiver blocks increased with time for the first 8 hours, from 50 dpm after only 1 hour to 1022 dpm after 8 hours and then remained relatively uniform at approximately 900 dpm for the next 4 hours. When the segments were supplied with donor blocks at their basal ends, the total amount of radioactivity found in the apical receiver blocks did not

Figure 3: Time course for the basipetal and acropetal movement of radioactivity through 10 mm segments of Zea coleoptiles supplied continuously with donor blocks containing {^{14}C} IAA at either their apical or basal ends. Distribution of radioactivity in the apical and basal halves of the coleoptile segments and in the receiver blocks is shown as a fraction of time .1 = segment half nearest to the donor block; 2 = segment half furthest from the donor block; R = receiver block. These data are the mean of four separate experiments.

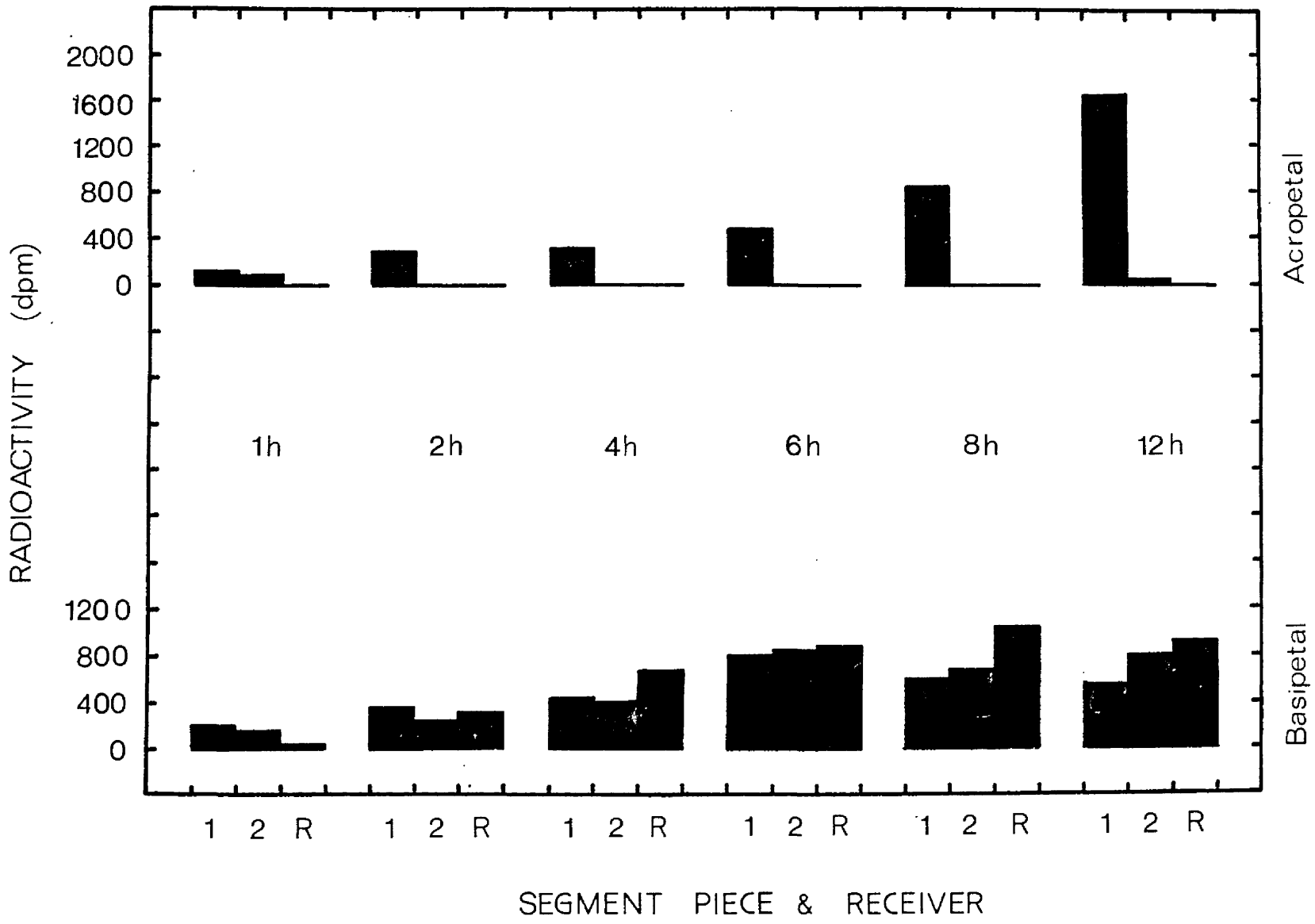
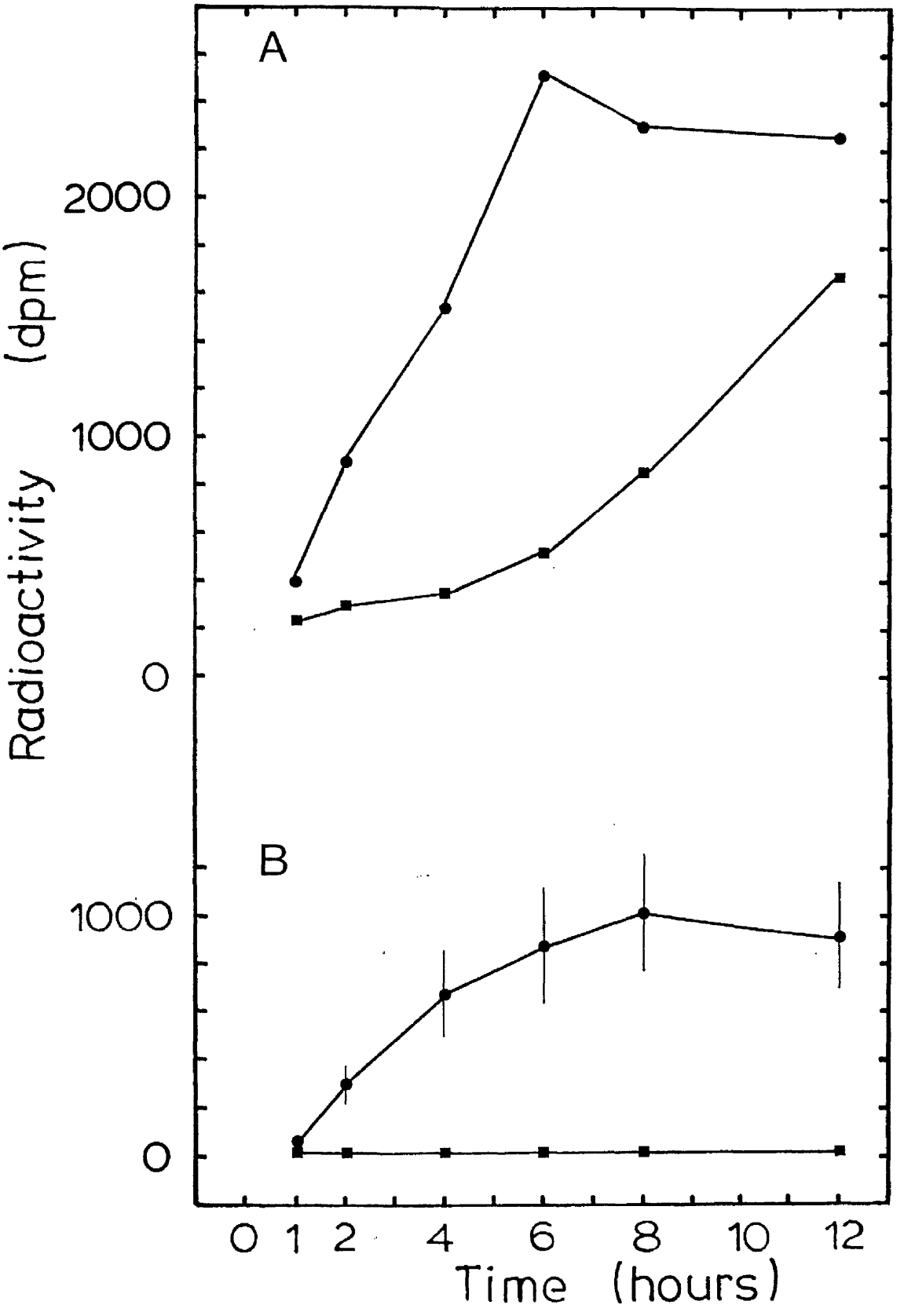

RADIOACTIVITY (dpm)
2000
1600
1200
800
400
0
Acropetal
1h
2h
4h
6h
8h
12h
1200
800
400
0
Basipetal
1 2 R 1 2 R 1 2 R 1 2 R 1 2 R 1 2 R
SEGMENT PIECE & RECEIVER

Figure 4: A: Total uptake of radioactivity by coleoptile segments and receiver blocks as a function of time when segments are supplied with donor blocks containing {^{14}C}IAA at either their apical (●) or basal ends (■).

B: The basipetal and acropetal movement of radioactivity into receiver blocks as a function of time following the application of donor blocks containing {^{14}C} IAA to Zea coleoptile segments at either their apical (●) or basal ends (■).


A
B
2000
1000
0
1000
0
Radioactivity (dpm)
0 1 2 4 6 8 10 12
Time (hours)

exceed 20 dpm even after transport periods of 12 hours. Since more radioactivity moved into a basal receiver block than into an apical one, it is indicative that radioactivity from (^{14}C) IAA moves with a basipetal polarity. The very small amounts of radioactivity which were present in the apical receiver blocks were probably due to diffusion.

The total uptake of radioactivity (Figure 4A) was calculated by the amount of radioactivity present in the tissue and receiver block at the end of the transport period and was found to increase with time for all segments whether they were supplied with apical or basal donor blocks. For segments supplied with apical donor blocks the total uptake of radioactivity increased from 408 dpm after 1 hour to reach a maximum of 2514 dpm after 6 h. After transport periods of eight hours, the total uptake fell slightly to 2302 dpm and after 12 hours this had fallen slightly further to 2260 dpm. Similarly, for segments supplied with basal donors the total uptake increased with time throughout the entire transport period from 242 dpm after 1 hour to 1670 dpm after twelve hours. Throughout the entire transport period, uptake at the apical end of the segment was always greater than at the basal end.

The distribution of radioactivity within the two halves of the coleoptile segments and the receiver blocks is shown in Figure 3. When supplied with a basal donor, all the radioactivity was confined to the lower half of the segment (i.e.segment piece No. 1) which had been next to the donor

block. The amount of radioactivity in this lower half increased with time from 138 dpm after 1 hour transport time to 1623 dpm after 12 hours. The amount of radioactivity present in the upper half of the segment (segment piece No. 2) was minimal and did not exceed 85 dpm throughout the 12 hours transport time. In contrast to the situation with basal donors, when segments were supplied with apical donors there was more movement of radioactivity down the coleoptile segment and the distribution of radioactivity within the entire segment was quite different. Both the segment half next to the donor block and the distal half of the segment contained large amounts of radioactivity. After 1 hour transport time segment piece No. 1 contained 189 dpm and segment piece No. 2 170 dpm and this had increased after 2 hours to 344 dpm in segment piece No. 1 and 242 dpm in segment piece No. 2 and after 4 hours to 435 dpm and 414 dpm respectively. So therefore for the first 4 hours there were approximately equal amounts of radioactivity in both halves of the coleoptile segment but thereafter, for the next 8 hours, radioactivity in the segment half furthest away from the donor block (segment piece No. 2) increased more than in segment piece No. 1. Maximum uptake had occurred after 6 hours when segment piece No. 1 contained 785 dpm and segment piece No. 2 549 dpm. After 8 hours both halves of the coleoptile segment contained approximately equal amounts of radioactivity; 606 dpm

in segment piece No. 1 and 674 dpm in segment piece No. 2 but by 12 hours segment piece No. 2 contained a higher amount of radioactivity (785 dpm) compared to segment piece No. 1 (564 dpm). These results clearly show the existence of a basipetal polarity of radioactivity from {^{14}C} IAA through sub-apical segments of Zea coleoptiles.

2. The Longitudinal Movement of Radioactivity from {^{14}C} IAA through Coleoptile Segments of Zea Mays under both Aerobic and Anaerobic Conditions.

The amounts of radioactivity from {^{14}C} IAA moving both acropetally and basipetally through 10 mm sub-apical segments of Zea coleoptiles into receiver blocks was determined as a function of time under both aerobic and anaerobic conditions. The distribution of radioactivity within the upper and lower halves of the coleoptile segments and in the receiver blocks is shown in Figure 5 and the total uptake of radioactivity by the tissue and the receiver blocks is shown in Figure 6. All data are the means of 3 independent experiments.

For all segments whether supplied with apical or basal agar donor blocks, anaerobic conditions markedly reduced the total amount of radioactivity taken up from the donors throughout the entire transport period. Under aerobic conditions, uptake by coleoptiles supplied with apical donors was 1473 dpm after 4 hours and increased with time to 2585 dpm after 12 hours. However, in contrast, under anaerobic

Figure 5: Time course for the basipetal and acropetal movement of radioactivity through 10 mm segments of Zea coleoptiles under both aerobic (white columns) and anaerobic (black columns) conditions. Segments were supplied continuously with donor blocks containing {^{14}C} IAA at either their apical or basal ends. Distribution of radioactivity in the apical and basal halves of the coleoptile segments and in the receiver blocks is shown as a function of time. 1 = segment half nearest to the donor block; 2 = segment half furthest from the donor block; R = receiver block. These data are the mean of three separate experiments.

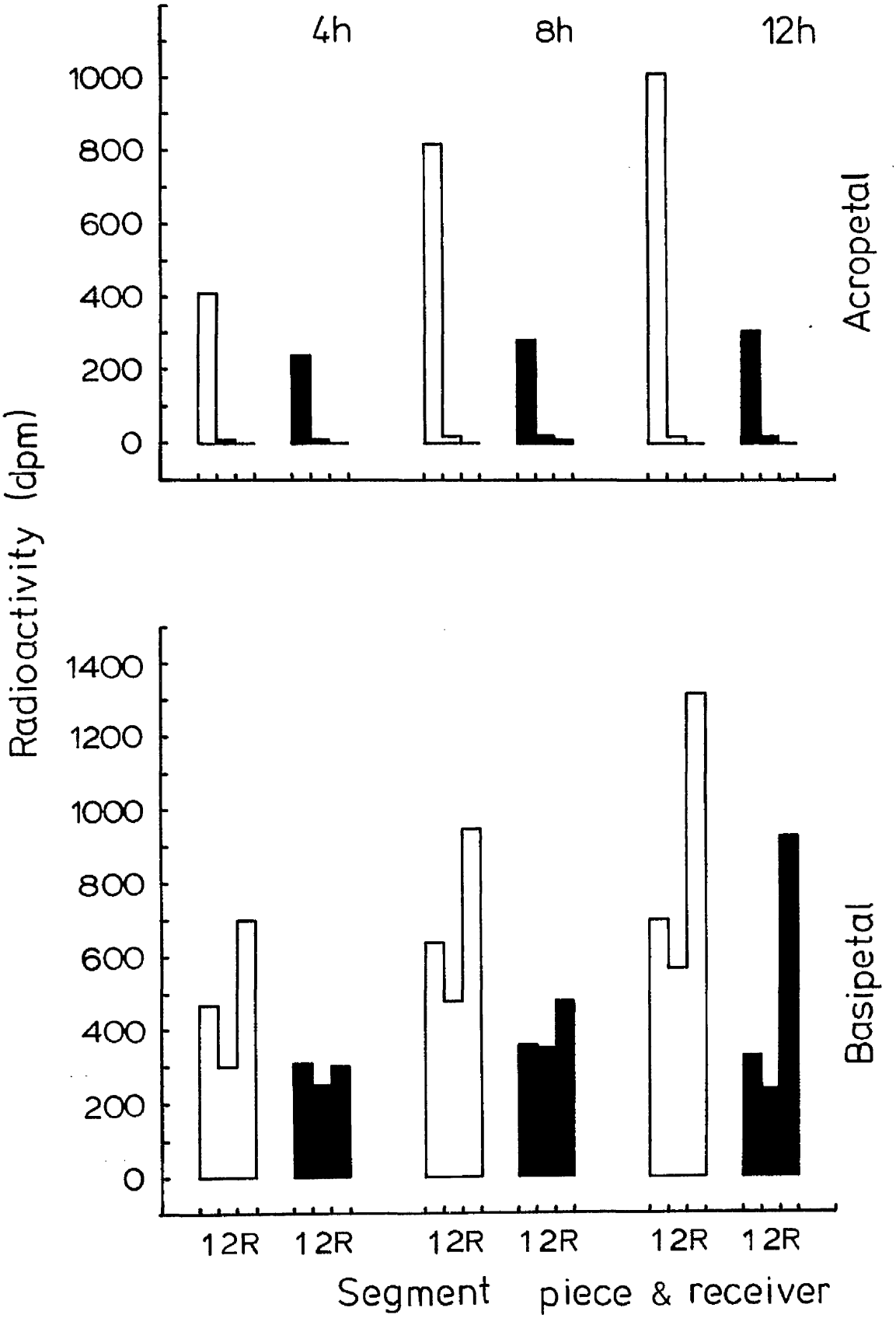
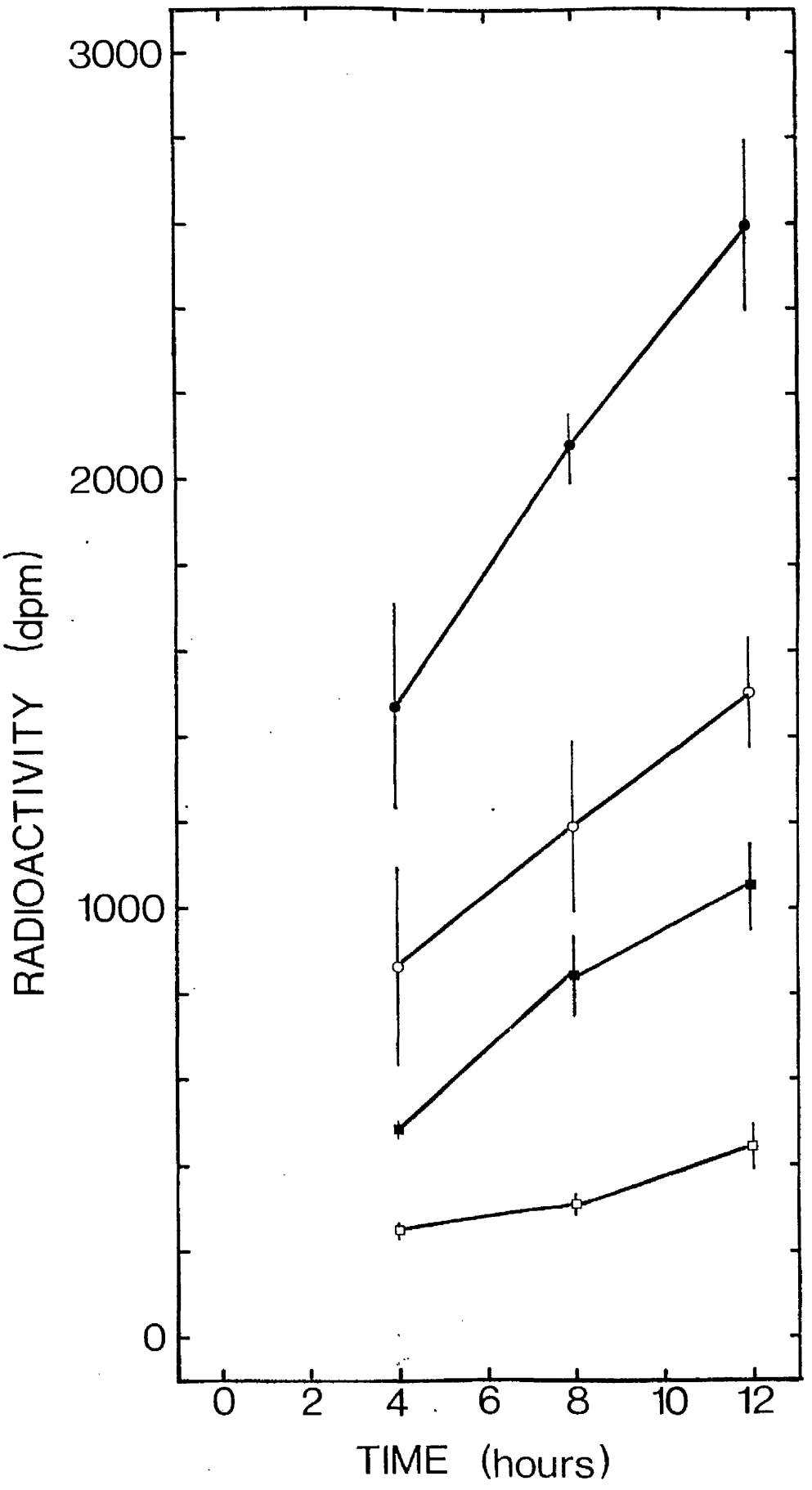

4h
8h
12h
1000
800
600
400
200
0
Acropetal
Radioactivity (dpm)
1400
1200
1000
800
600
400
200
0
Basipetal
12R
12R
12R
12R
12R
12R
Segment piece & receiver

Figure 6: Total uptake of radioactivity by coleoptile segments and receiver blocks as a function of time when segments are supplied with donor blocks containing {^{14}C} IAA at either their apical (● ○) or basal ends (■ □) under both aerobic (closed symbols) and anaerobic conditions (open symbols).


3000
2000
1000
0
RADIOACTIVITY (dpm)
0
2
4
6
8
10
12
TIME (hours)

conditions, uptake was drastically reduced although it still increased with time. After 4 hours uptake was only 861 dpm (reduction of 42%) and after 12 hours was 1507 dpm (reduction of 42%). Similarly, when segments were supplied with basal donors, under aerobic conditions uptake was 421 dpm after 4 hours and had increased to 1053 dpm after 12 hours. The corresponding values under anaerobic conditions were 246 dpm after 4 hours and 339 dpm after 12 hours which represented reductions of 42% and 68% respectively. These facts suggest that uptake of radioactivity at both the apical and basal ends of the coleoptile segment is partially dependent upon aerobic metabolism. With apical donors, the amount of radioactivity which reached receiver blocks under aerobic conditions increased with time from 705 dpm after 4 hours to 1316 dpm after 12 hours. Corresponding values under anaerobic conditions were 302 dpm after 4 hours (reduction of 57%) and 931 dpm after 12 hours (reduction of 30%). In contrast, when segments were supplied with basal donors, the amount of radioactivity in the receiver blocks never exceeded 10 dpm even after 12 hours under both aerobic and anaerobic conditions. Thus even under anaerobic conditions the amount of radioactivity found in basal receiver blocks is greater than in apical receivers. It seems apparent that the basipetal polarity of radioactivity observed under aerobic conditions, persists

even under anaerobic conditions but at a much reduced level. This finding is supported by the distribution of radioactivity within the coleoptile segment.

When segments were supplied with apical donors, in air the amount of radioactivity found in the segment piece next to the donor block increased with time from 471 dpm after 4 hours to 642 dpm after 8 hours and 704 dpm after 12 hours. Similarly, the radioactivity found in the segment piece furthest away from the donor block increased with time from 298 dpm after 4 hours to 566 dpm after 12 hours. This pattern of distribution of radioactivity within the segment persisted under anaerobic conditions but at reduced levels. After 4 hours segment piece No. 1 contained 313 dpm and this had increased to 334 dpm after 12 hours and segment piece No. 2 contained approximately 240 dpm after 4 and 12 hours with a slight increase to 350 dpm after 8 hours. Whereas, with basal donors, all the radioactivity was confined to the segment piece nearest to the donor block under both aerobic and anaerobic conditions. Although in air the amount of radioactivity present in the segment piece next to the donor block increased with time from 413 dpm after 4 hours to 1029 dpm after 12 hours, in nitrogen, the radioactivity was only 239 dpm after 4 hours and 315 dpm after 12 hours. And in contrast to segments supplied with apical donors, the segment piece furthest away from the donor, contained minimal amounts of radioactivity which did not exceed 23 dpm throughout the time course regardless of aerobic or anaerobic conditions.

The results of these experiments suggest that even under anaerobic conditions, the longitudinal basipetal movement of radioactivity from {^{14}C} IAA although occurring at a much reduced rate to that observed in aerobic conditions, is slightly greater than acropetal movement.

3. The Effect of the Metabolic Inhibitor Sodium Fluoride on the Longitudinal Polarity of Radioactivity from {^{14}C} IAA through Coleoptile Segments of Zea Mays under both Aerobic and Anaerobic Conditions.

The persistence of a basipetal polar flux of radioactivity from {^{14}C} IAA under anaerobic conditions could be due either to aerobic metabolism occurring as the result of oxygen remaining within the tissue or to anaerobic metabolism. The first possibility seems unlikely since strict evacuation procedures were employed to ensure the exclusion of oxygen from the coleoptile tissue. The second possibility can be tested by using metabolic inhibitors which are known to block certain stages in the Embden-Meyerhof pathway.

Consequently coleoptile segments 10 mm in length were soaked in a 1.0 m M sodium fluoride solution for 2 hours. Control segments were soaked in water for the same time. Subsequently, both batches of treated and control segments were divided further into two batches; one being transferred to anaerobic conditions and the other retained for aerobic conditions. Within each of these two batches, half of the segments were supplied with apical donors and

the other half with basal donors. Uptake and movement of radioactivity through the segments into receiver blocks was compared for all treatments. The results are shown in Figures 7 and 8.

When segments were supplied with basal donors under aerobic conditions, the total uptake and movement of radioactivity within the segment was similar for the first 4 hours, regardless of whether the segments had been treated with the metabolic inhibitor or not but by 8 hours, treatment with sodium fluoride had decreased the uptake by 42% from 1173 dpm to 678 dpm. Similarly, under anaerobic conditions, the total uptake of radioactivity was closely similar for both control and treated segments throughout the time course. Under nitrogen the total uptake was reduced for the entire 8 hours transport period thus confirming results from the previous experiment.

For both treated and control segments no significant amounts of radioactivity reached the receiver blocks in either air or nitrogen, even after transport periods of 8 hours and almost all the radioactivity within the segment was confined to the segment half nearest to the donor block throughout the time course. It seems apparent that treating coleoptile segments with the metabolic inhibitor sodium fluoride had little effect upon the acropetal movement of radioactivity from {^{14}C} IAA under either aerobic or anaerobic conditions until after 8 hours when under aerobic

Figure 7: The effect of the metabolic inhibitor sodium fluoride on the uptake and movement of radioactivity through 10 mm segments of Zea coleoptiles supplied continuously with donor blocks containing {^{14}C} IAA at either their apical or basal ends as a function of time. Distribution of radioactivity in the apical and basal halves of the coleoptile segments and in the receiver blocks is shown for the control tissue by the white columns and for the treated tissue by the black columns. 1 = segment half nearest to the donor block; 2 = segment half furthest from the donor block; R = receiver block. These data are the mean of three separate experiments.

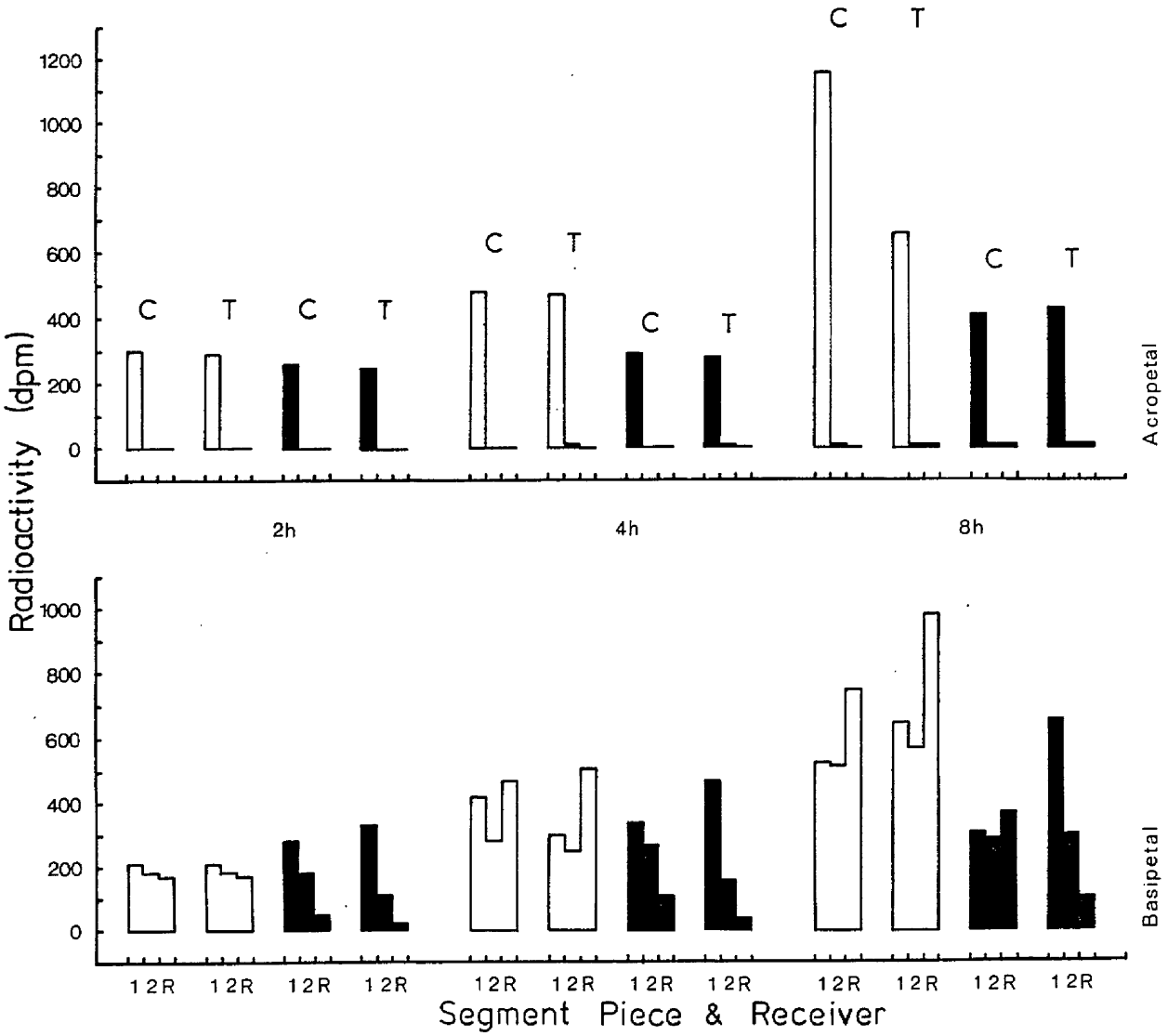
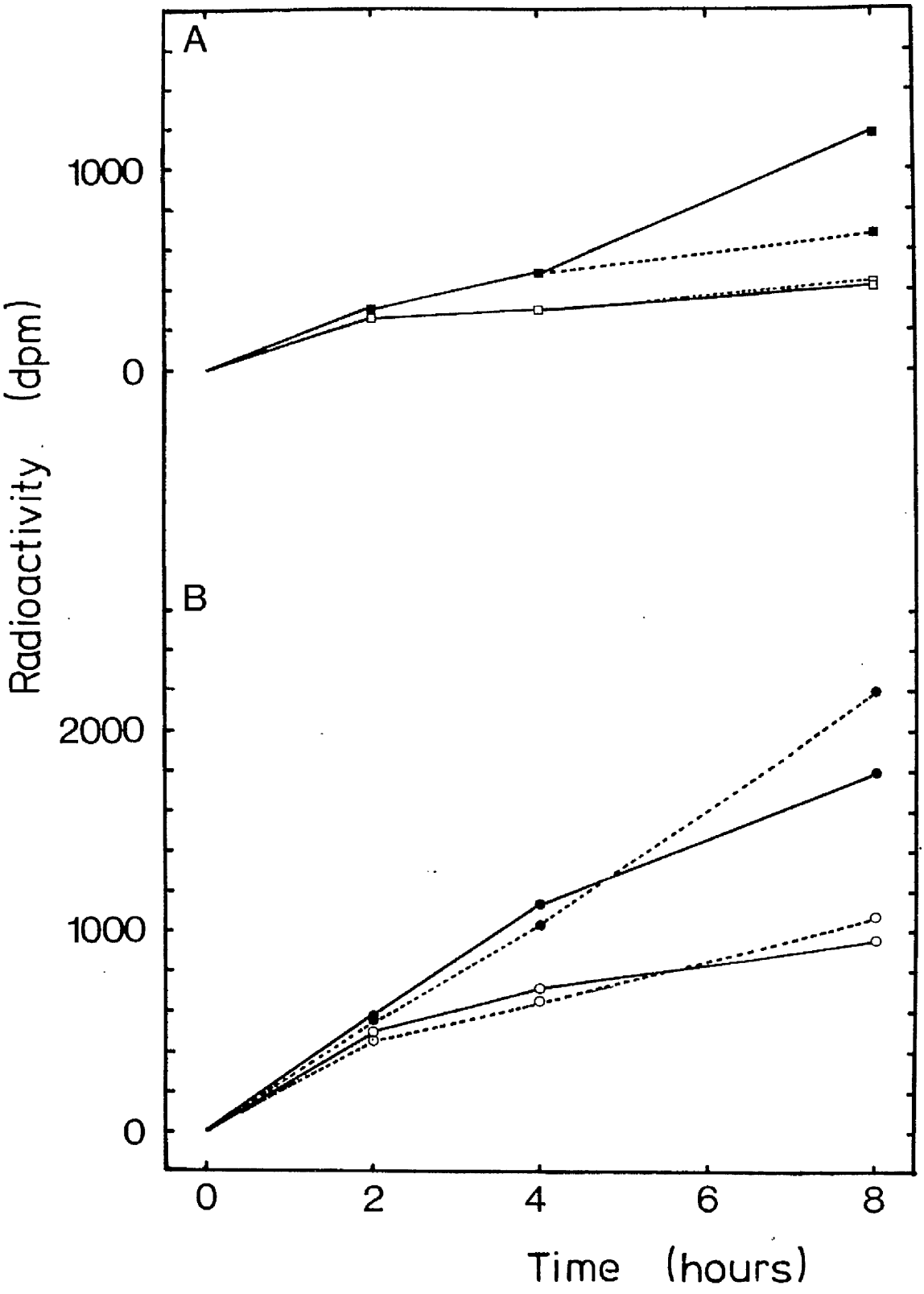

C T
1200
1000
800
600
400
200
0
C T C T C T C T C T
Radioactivity (dpm)
Acropetal
2h
4h
8h
1000
800
600
400
200
0
Basipetal
12R 12R 12R 12R 12R 12R 12R 12R 12R 12R 12R 12R
Segment Piece & Receiver

Figure 8: A: The effect of the metabolic inhibitor sodium fluoride on the total uptake of radioactivity by coleoptile segments and receiver blocks supplied continuously with {^{14}C} IAA at their basal ends under both aerobic and anaerobic conditions as a function of time:

■——■ = control segments under aerobic conditions
■----■ = treated segments under aerobic conditions
□——□ = control segments under anaerobic conditions
□----□ = treated segments under anaerobic conditions

B: The effect of the metabolic inhibitor sodium fluoride on the total uptake of radioactivity by coleoptile segments and receiver blocks supplied continuously with {^{14}C}IAA at their apical ends under both aerobic and anaerobic conditions as a function of time:

●——● = control segments under aerobic conditions
●----● = treated segments under aerobic conditions
○——○ = control segments under anaerobic conditions
○----○ = treated segments under anaerobic conditions


A
B
1000
0
2000
1000
0
Radioactivity (dpm)
0
2
4
6
8
Time (hours)

conditions uptake by treated segments was reduced. With apical donors, in both air and nitrogen, treatment with sodium fluoride solution had little effect on the total uptake of radioactivity by the segments, i.e. uptake was closely similar regardless of pretreatment throughout the time course. In fact, after 8 hours, in both air and nitrogen, uptake by the treated segments was slightly higher than the water controls. Under aerobic conditions the distribution of radioactivity within the tissue was similar regardless of whether the segments had been pretreated with an inhibitor. For the first 2 hours, there were similar amounts of radioactivity present in each half of the segment and receiver block but by 4 hours the amount of radioactivity present in the receiver block had increased slightly above that in each segment half and by 8 hours the differential was even greater. Therefore in air, sodium fluoride apparently had little effect on the basipetal movement of radioactivity within the tissue and receiver block.

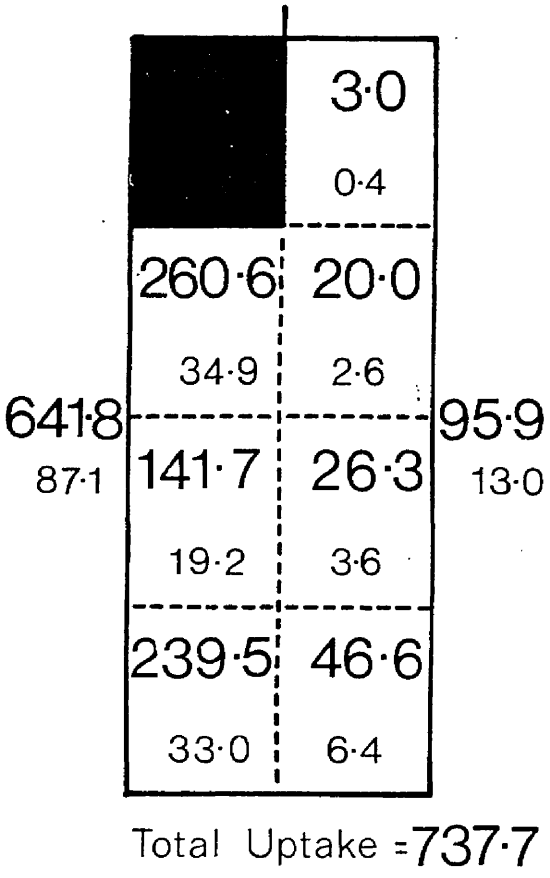
However in nitrogen, the inhibitor decreased the movement of radioactivity within the segment and into the receiver block. In treated segments radioactivity accumulated in the half nearest to the donor block and less moved into the more basal half of the segment and the receiver block than in control segments. This was the case after 2, 4 and 8 hours.

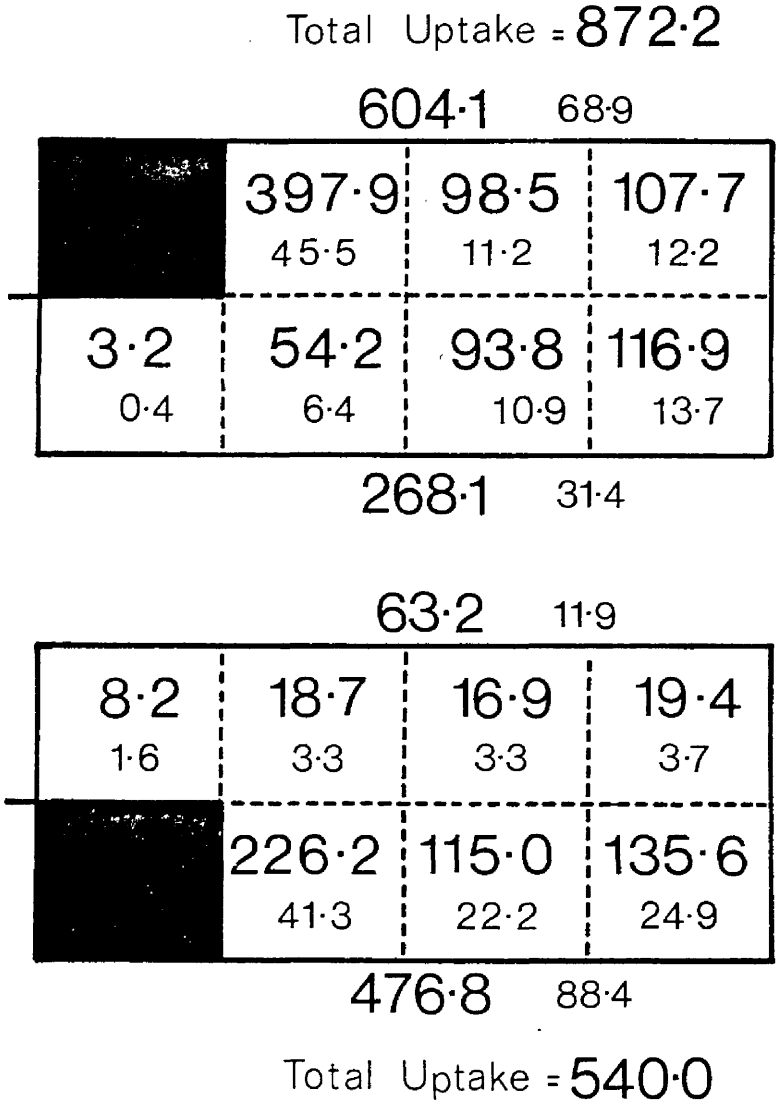
After 2 hours the amount of radioactivity found in receiver blocks of treated segments was reduced by 59% and after 4 hours the reduction had increased to 61% and after 8 hours to 73%. Treatment with sodium fluoride seems therefore to reduce even further, the already reduced basipetal polarity observed under anaerobic conditions.

4. The Lateral Movement of Radioactivity from {^{14}C} IAA Applied Asymmetrically to Horizontal and Vertical Coleoptile Segments of Zea Mays.

Donor blocks containing {^{14}C} IAA were applied asymmetrically to one half of the apical cut surfaces of 15 mm subapical coleoptile segments. Plain agar blocks were applied to the other halves of the apical cut surfaces and barriers of aluminium foil separated the donor and plain agar blocks. During the application of the agar blocks segments were maintained in the vertical position with respect to gravity. They were subsequently orientated vertically or horizontally with the donor blocks on either the upper or the lower side. Transport was allowed to take place in total darkness for 4 hours after which time the segments were bisected longitudinally and then divided transversely into 3 x 5 mm pieces as described previously, in order to determine the distribution of radioactivity within the tissue. The results shown in Figure 9 are the

Figure 9: Lateral movement of radioactivity from {^{14}C} IAA applied asymmetrically to horizontal and vertical coleoptile segments of Zea mays. Transport time was 4 h. Donor blocks are shown in black at the apical ends of the segments. Segments were bisected longitudinally and then divided transversely into 5 mm portions (dotted lines). The actual dpm (upper values) and the percent distribution of the total radioactivity in the tissue (lower values) are shown in each portion of the half-segments. These data are the mean of two separate experiments.


3·0
0·4
260·6
20·0
34·9
2·6
641·8
87·1
95·9
13·0
141·7
26·3
19·2
3·6
239·5
46·6
33·0
6·4
Total Uptake = 737·7


Total Uptake = 872·2
604·1
68·9
397·9
98·5
107·7
45·5
11·2
12·2
3·2
54·2
93·8
116·9
0·4
6·4
10·9
13·7
268·1
31·4
63·2
11·9
8·2
18·7
16·9
19·4
1·6
3·3
3·3
3·7
226·2
115·0
135·6
41·3
22·2
24·9
476·8
88·4
Total Uptake = 540·0

mean of 2 independent experiments in each of which there were 7 replicates for each of the 3 orientations with respect to gravity.

The total radioactivity taken up by the segments was similar regardless of the position of the donor source and the orientation of the segment with respect to gravity. However the lateral distribution of radioactivity within the tissue was quite different and very dependent on the geotropic orientation and position of the donor block. When donor blocks were supplied to the upper side of horizontal coleoptile segments, the percentage of the total uptake found in the half segment opposite the donor was 31.4%. Whereas in coleoptile segments which had been supplied with donor blocks on the lower side, the percentage radioactivity found in the upper nondonated half was 11.9% of the total uptake. The difference between these two values is 19.5% and represents a significant net downward movement of radioactivity in horizontal Zea coleoptile segments. In vertical segments 13% of the total radioactivity was found in the nondonated segment half which was closely similar to the value found in the nondonated upper half of horizontal segments supplied with lower donors (11.9%).

The pattern of distribution of radioactivity within the segment is similar for all orientations with respect to gravity and regardless of position of the donor blocks in that in both horizontal and vertical coleoptiles there is

a gradual decline of radioactivity with increasing distance from the donor in the donated side of the coleoptile segment. In contrast, there is a gradual increase in radioactivity with increasing distance from the donor source in the nondonated side of the segment for all orientations with respect to gravity.

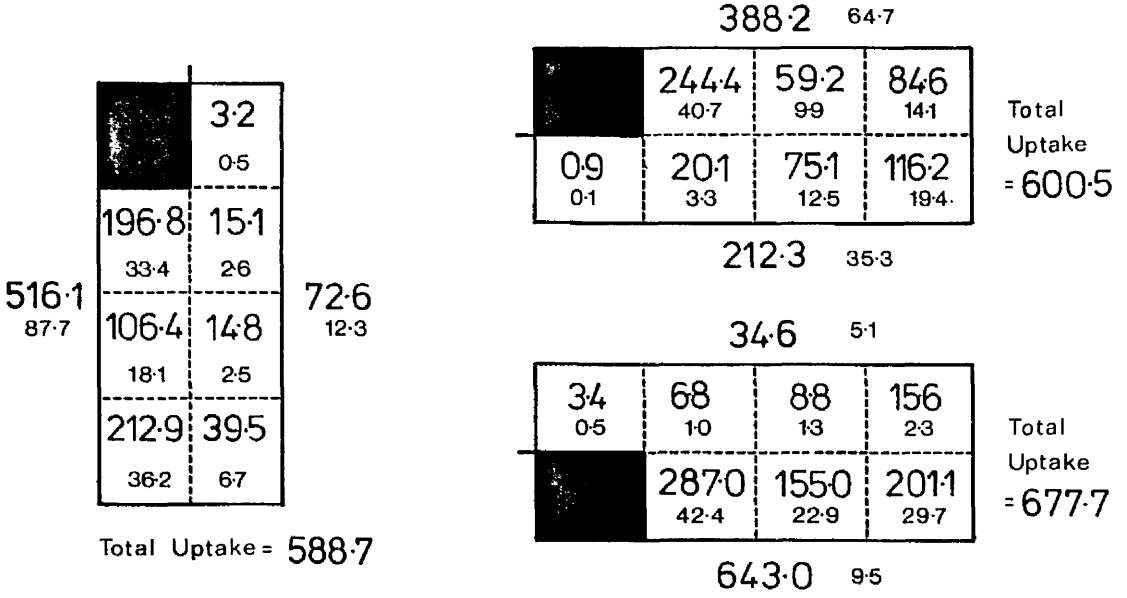
Since the asymmetrically applied agar donor blocks contained closely similar amounts of {^{14}C} IAA and since the uptake of radioactivity by the geotropically orientated segments was fairly similar, any radioactivity recovered from the segment half opposite the asymmetric source must have undergone a lateral redistribution.

5. The Lateral Movement of Radioactivity from {^{14}C} IAA Applied Asymmetrically to 10 mm Segments excised from Different Regions of Zea Mays Coleoptiles.

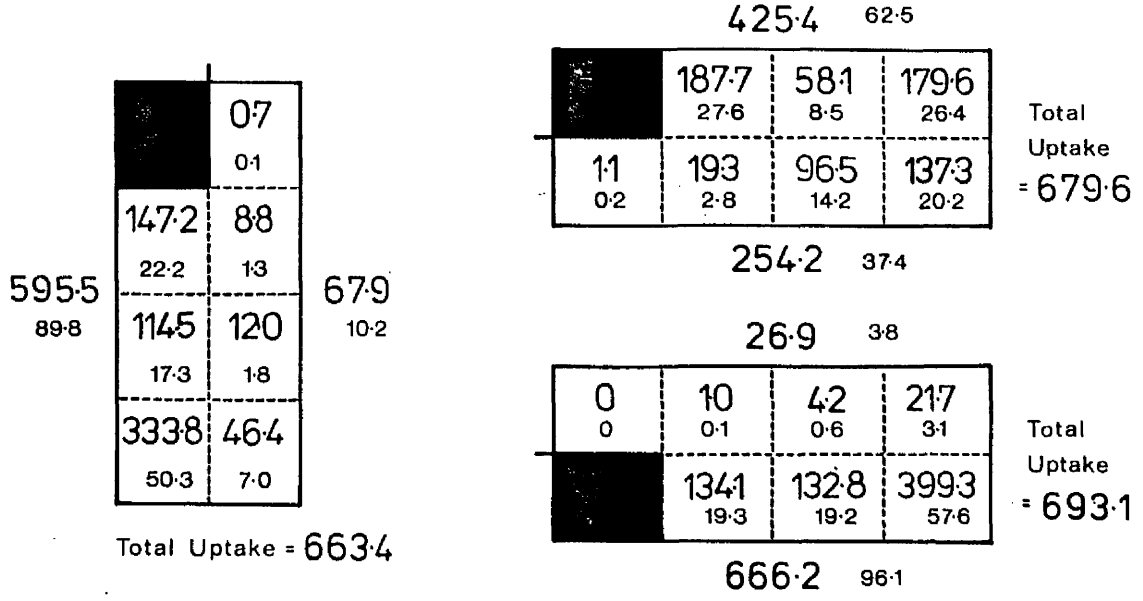
The lateral movement and distribution of radioactivity from {^{14}C} IAA was compared in 10 mm segments of Zea mays coleoptiles excised 1 mm behind the apex (upper segment = one) and 11 mm behind the apex (lower segment = two) during a 4 hour transport period in total darkness. The experiment was performed using the procedures described for the previous experiments and segments from both regions of the coleoptiles were orientated in the previously described positions with respect to gravity. The results are shown in Figure 10 in which the data are the means of 2 independent experiments.

Figure 10: Lateral movement of radioactivity from {^{14}C} IAA applied asymmetrically to horizontal and vertical segments excised from different regions of Zea mays coleoptiles. Transport time was 4 h. Tissue 1 refers to segments excised 1 mm behind the coleoptile apex and tissue 2 to those segments excised 11 mm behind the coleoptile apex. Donor blocks are shown in black at the apical ends of the segments. Segments were bisected longitudinally and then divided transversely into 3 portions (dotted lines). The actual dpm (upper values) and the percent distribution of the total radioactivity in the tissue (lower values) are shown in each portion of the half-segments. These data are the mean of two separate experiments.

TISSUE 1



TISSUE 2



For both segments one and two, the total uptake of radioactivity by the segments was not significantly different with respect to position of donor blocks and orientation of the segments with respect to gravity. However, the distribution of radioactivity within the segment depended very much on both geotropic orientation and the position of the radioactive source. Both segments one and two showed evidence of a lateral redistribution of radioactivity. When asymmetric donor blocks were applied to vertical segments from both regions of the coleoptile, the proportion of the total uptake of radioactivity found in the half opposite the donor block was 12.3% for segment one and 10.2% for segment two. When horizontal segments were supplied with donor blocks applied to their lower sides, there was scarcely any lateral redistribution of radioactivity in segments excised from both zones of the coleoptile; 5.1% moved laterally from the lower into the upper half of segment one and 3.8% moved laterally upward in segment two. In contrast, for both segments one and two, a significantly higher proportion of radioactivity moved laterally from the upper into the lower half of horizontal segments that had been supplied with donor blocks on the upper side; 35.3% of the total radioactivity moved downward in segment one and 37.4% moved downward in segment two. Therefore in both segments one and two placed in the horizontal position with asymmetric donor blocks applied to

the upper side, a downward lateral movement of radioactivity was shown to have occurred since three times as much radioactivity was found to be present in the nondonated side as in the equivalent side of vertical segments.

The distribution of radioactivity within the segments is similar in that for both segments one and two, in all of the orientations with respect to gravity, there is a gradual increase of radioactivity on the nondonated side with increasing distance from the donor block.

Clearly these results show evidence of a lateral movement of radioactivity in both segments one and two in all three of the orientations with respect to gravity but in horizontally orientated segments with asymmetric donor blocks on their upper side, the effect is greatly enhanced.

6. The Longitudinal Movement of Radioactivity from {^{14}C} GA_3 through Coleoptile Segments of Zea Mays.

The acropetal and basipetal movement of radioactivity from {^{14}C} GA_3 through 10 mm subapical segments of Zea coleoptiles into receiver blocks was investigated as a function of time. Segments were placed vertically with either their apical or basal ends on a donor block containing {^{14}C} GA_3 and their opposite ends were placed in contact with receiver blocks. At the end of each transport period, each coleoptile segment was cut into 5 x 2 mm pieces

plus 'X' and combined for radioassay as described previously. The total amount of radioactivity taken up by the intact segment plus the receiver block and the distribution of radioactivity within each piece of the coleoptile segment and the receiver block are shown in Figures 11, 12 and 13, in which the data are the means of 6 independent experiments. In all segments, whether supplied with apical or basal donors, there was virtually no movement of radioactivity into the receiver blocks, (Figure 12) since the amount of radioactivity found in receivers did not exceed 2% of the total uptake, even after segments had been supplied with donors for 24 hours. The distribution of radioactivity within the segments seemed to follow a similar pattern regardless of the position of the donor blocks or lengths of the transport time. Whether segments were supplied with apical or basal donor blocks, the segment piece nearest to the donor block (No. 1) always contained the highest amount of radioactivity and thereafter the radioactivity present in each segment piece declined with increasing distance from the donor block. Calculated on a percentage basis, segment piece No. 1 contained between 40 - 54%, segment piece No. 2 20 - 25%, No. 3 12 - 21%, No. 4 6 - 11%, No. 5 3 - 5% and 'X' from 1 - 3%

From Figure 13 it is evident that the percentage of the total radioactivity present in each segment piece calculated on a logarithmic scale is virtually linear with time; the radioactive content declines exponentially with

Figure 11: Time course for the basipetal (●) and acropetal (■) movement of radioactivity from {^{14}C} GA$_3$ through 10 mm segments of Zea coleoptiles. Total uptake of radioactivity by coleoptile segments and receiver blocks as a function of time when segments are supplied with donor blocks containing {^{14}C} GA$_3$ at either their apical or basal ends. These data are the mean of 6 separate experiments.

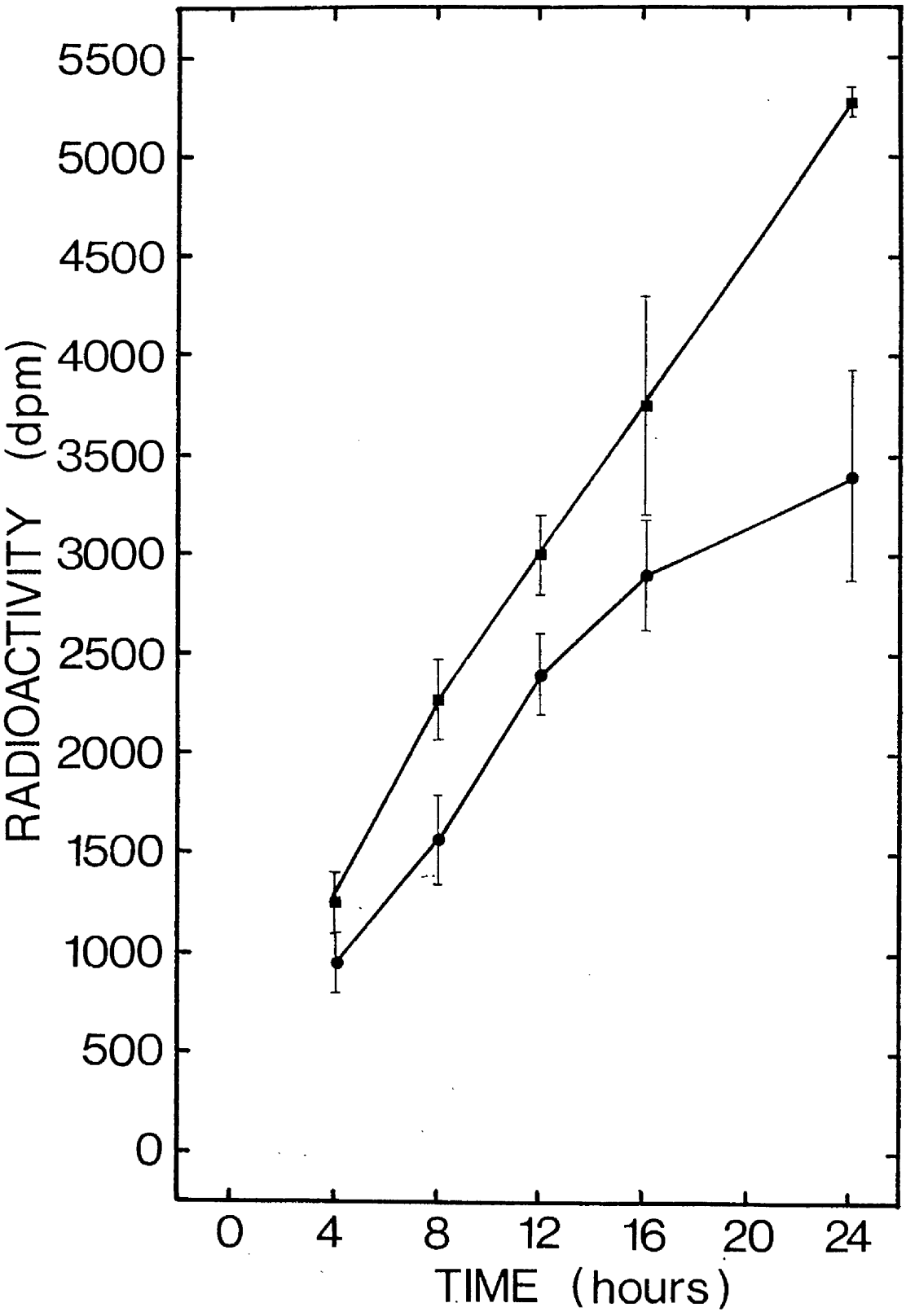

5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
RADIOACTIVITY (dpm)
0
4
8
12
16
20
24
TIME (hours)

Figure 12: Time course for the basipetal and acropetal movement of radioactivity from {^{14}C} GA_3 through 10 mm segments of Zea coleoptiles. Percent distribution of radioactivity within the coleoptile segments and receiver blocks is shown as a function of time. 1, 2, 3, 4, 5 = 2 mm zones of coleoptile segment; x = extra coleoptile tissue due to growth; R = receiver block.

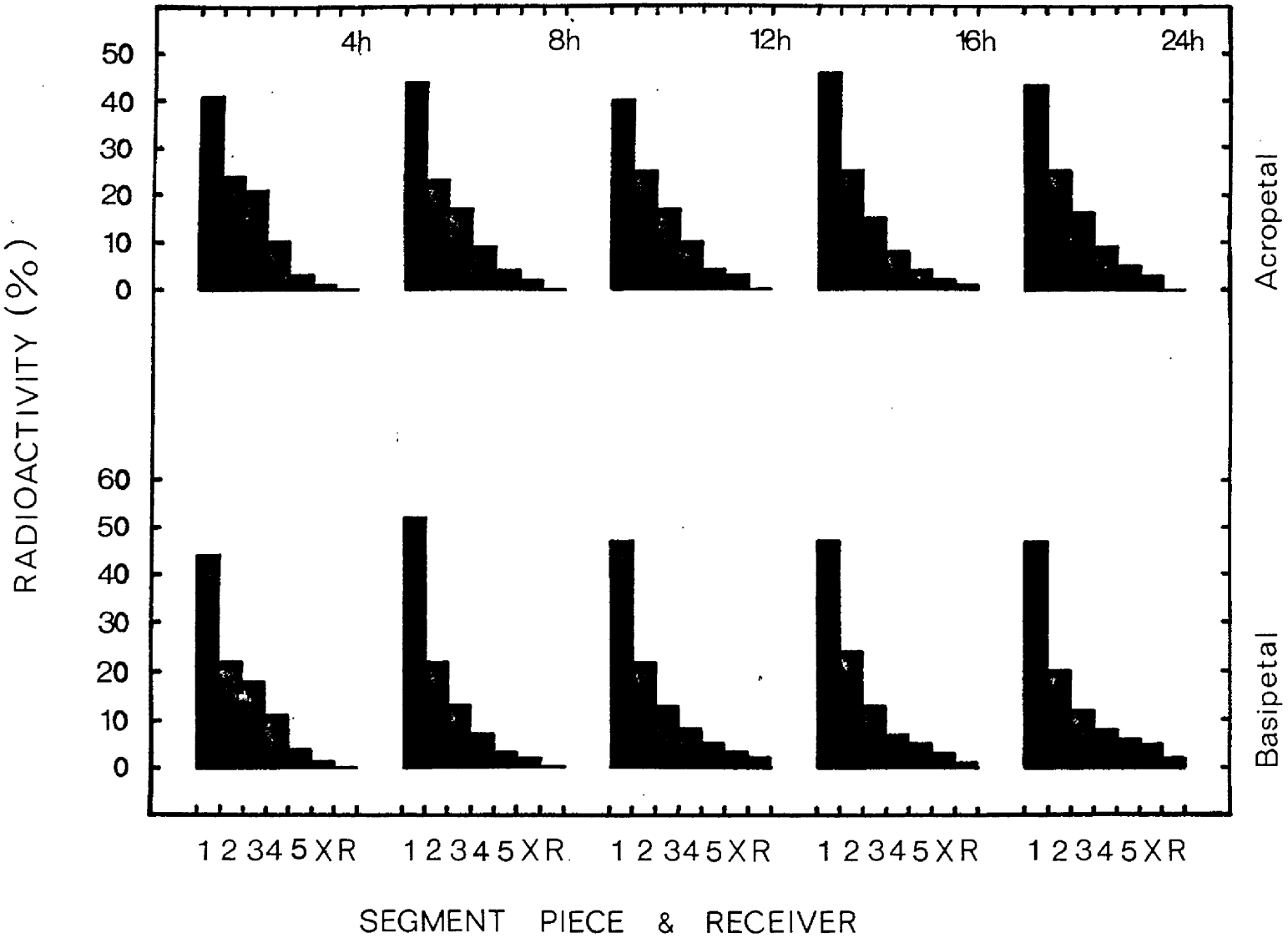
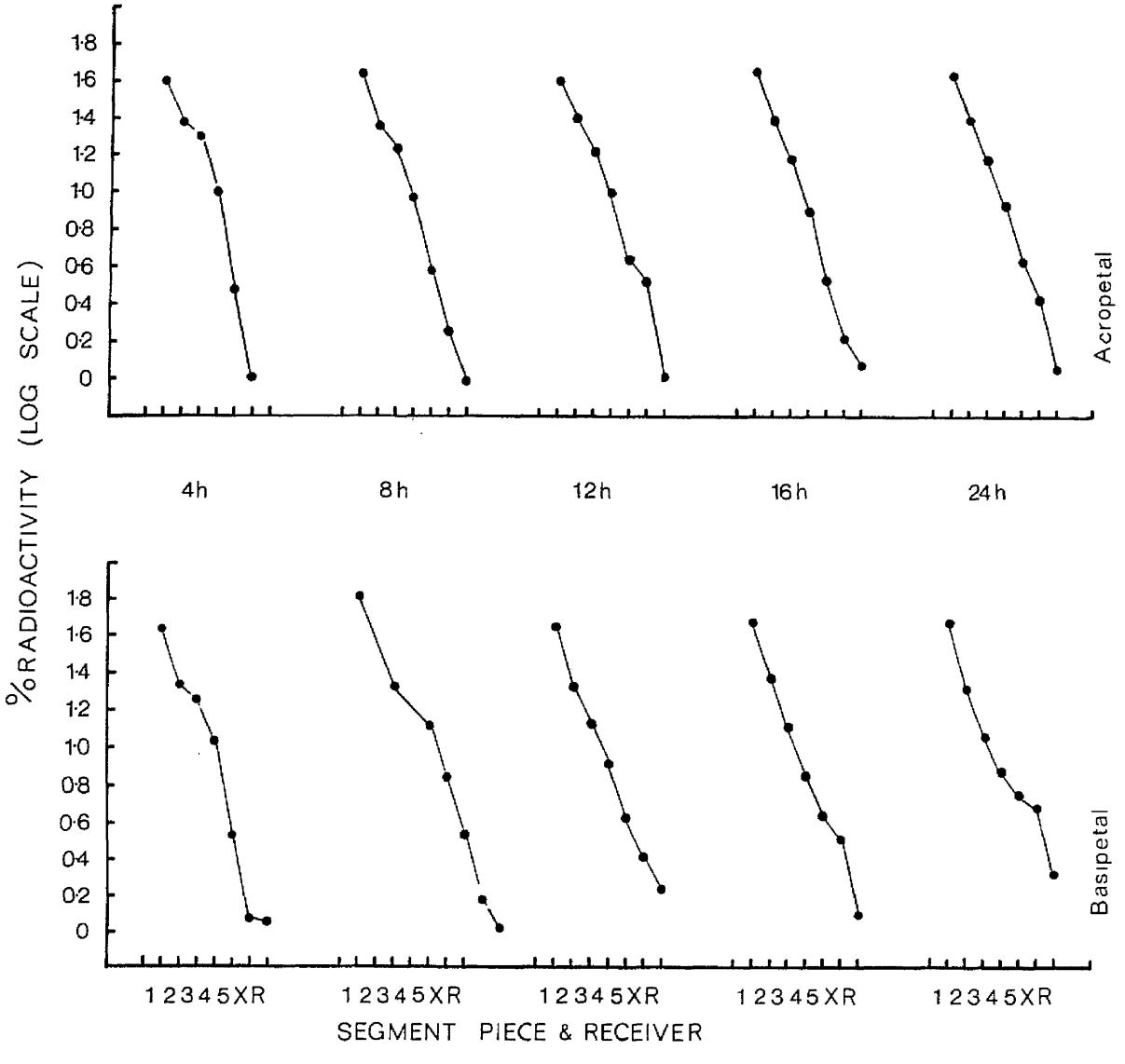

4h
8h
12h
16h
24h
50
40
30
20
10
0
60
50
40
30
20
10
0
RADIOACTIVITY (%)
Acropetal
Basipetal
1 2 3 4 5 X R
1 2 3 4 5 X R
1 2 3 4 5 X R
1 2 3 4 5 X R
1 2 3 4 5 X R
SEGMENT PIECE & RECEIVER

Figure 13: Time course for the basipetal and acropetal movement of radioactivity from {^{14}C} GA_3 through 10 mm segments of Zea coleoptiles. Percent distribution of radioactivity, on a logarithmic scale, within the coleoptile segments and receiver blocks is shown as a function of time. 1, 2, 3, 4, 5 = 2 mm zones of coleoptile segment; x = extra coleoptile tissue due to growth; R = receiver block.


1·8
1·6
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0
% RADIOACTIVITY (LOG SCALE)
Acropetal
4h
8h
12h
16h
24h
Basipetal
1 2 3 4 5 X R
SEGMENT PIECE & RECEIVER

with increasing distance from the donor block. The total uptake of radioactivity as a function of time is shown in Figure 11. When segments were supplied with apical donors uptake after 4 hours was 957 dpm and this had increased with time to a value of 3410 dpm after 24 hours. Similarly with basal donors the corresponding values for uptake were 1253 dpm after 4 hours and 5279 dpm after 24 hours. Therefore with both apical and basal donors, uptake of radioactivity increases with time. Throughout the 24 hours of the time course uptake at the basal end of the segment was consistently greater than at the apical end.

No evidence of a longitudinal polarity of radioactivity from {^{14}C} GA_3 has therefore been found, either on the basis of the distribution of radioactivity within the tissues of the coleoptile segment, or the amounts of radioactivity recovered from the receiver blocks.

7. The Effect of Anaerobic Conditions on the Basipetal Movement of Radioactivity from {^{14}C} GA_3 through Coleoptile Segments of Zea Mays.

The uptake, distribution and movement into basal receiver blocks, of radioactivity from {^{14}C} GA_3 through 10 mm subapical segments of Zea coleoptiles supplied with apical donor blocks was determined as a function of time under both aerobic and anaerobic conditions. Segments were harvested and combined for radioassay as described

previously after transport periods of 8, 12 and 24 hours. The results are shown in Figures 14 and 15 in which the data are the means of 4 independent experiments. In air (Figure 14) the total uptake of radioactivity increased with time from 1390 dpm after 8 hours to 1984 dpm after 24 hours. However in contrast, in nitrogen, uptake was much reduced throughout the 24 hours. After 8 hours the amount of radioactivity in the tissue plus receiver block was only 350 dpm (reduction of 75%) and this had decreased slightly to 221 dpm after 12 hours (reduction of 87.3%) but increased again to 350 dpm (reduction of 82.4%) after 24 hours. Therefore uptake of radioactivity from apical donor blocks by coleoptile segments seemed to be dependent upon aerobic metabolism.

Under aerobic conditions (Figure 15)no significant amounts of radioactivity were present in the receiver blocks. Radioactivity was taken up from the donor block and moved along the segment but only 3 - 5% of the total uptake of radioactivity moved out of the segment into agar receiver blocks. The pattern of distribution of radioactivity within the segment, based on the percentage of the total uptake is similar regardless of the length of the transport time. Segment piece No. 1 nearest to the donor block always contained the highest percentage of radioactivity. Segment piece No. 3 contained more radioactivity than segment piece No. 2 and this was as much if not more than in segment piece No. 1. This small plateau of radioactivity in segment pieces Nos. 1, 2 and 3 may be due to the evacuation procedure which may result in the donor blocks being 'sucked' onto the segment. The radioactivity present in segments 4, 5 and 'X' decreased with increasing distance from the donor block.

Figure 14: Time course for the basipetal movement of radioactivity from {^{14}C} GA_3 through 10 mm segments of Zea coleoptiles under aerobic (●) and anaerobic (○) conditions. Total uptake of radioactivity by coleoptile segments and receiver blocks is shown as a function of time. These data are the mean of 4 separate experiments.

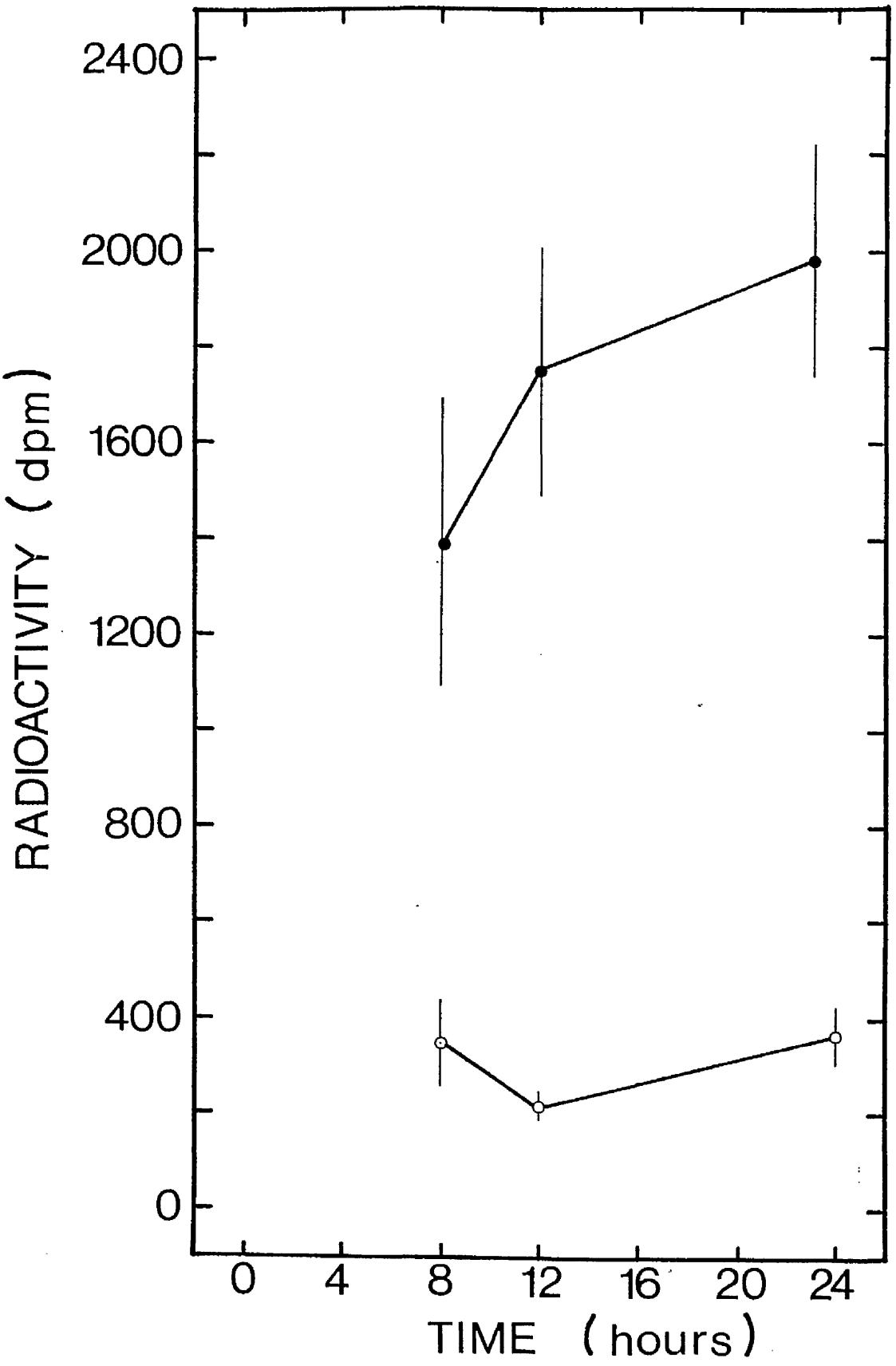
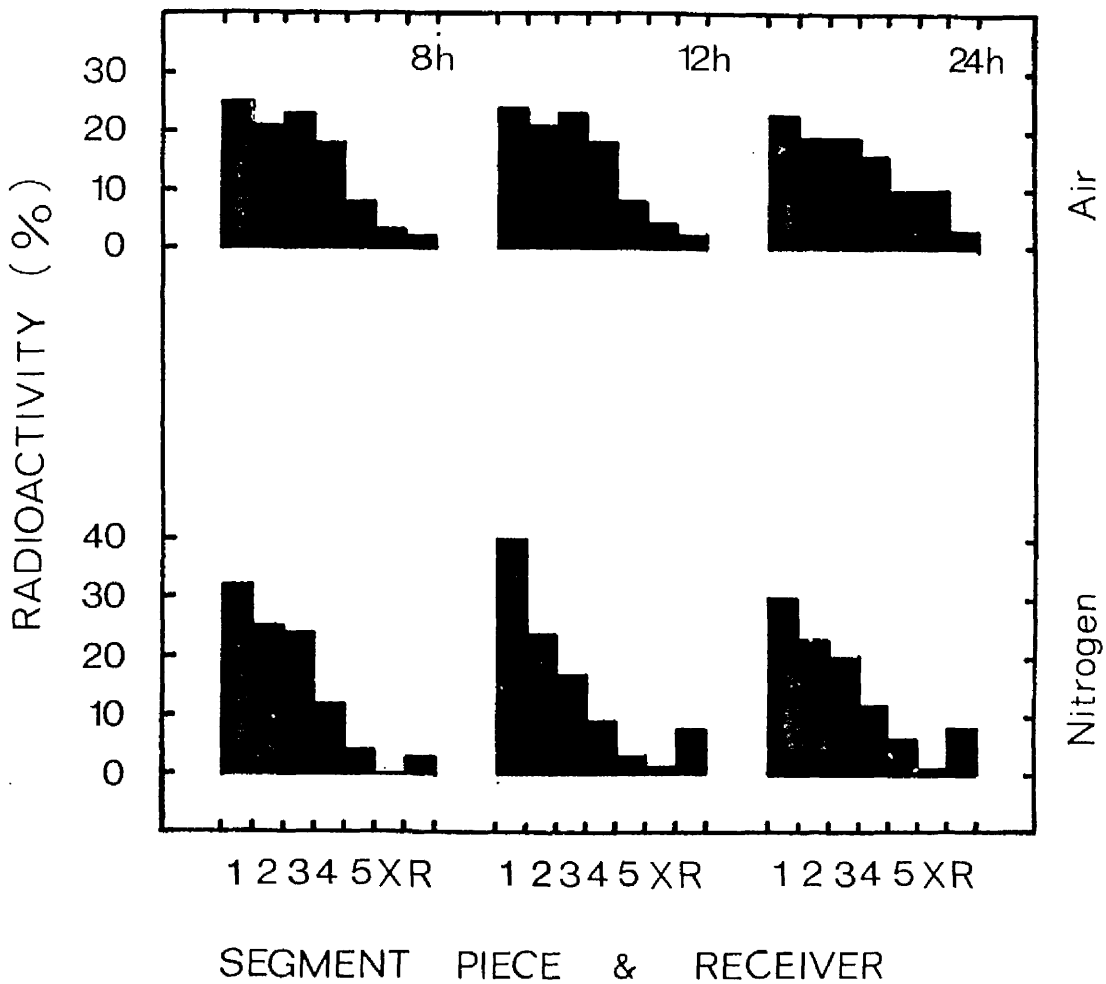

2400
2000
1600
1200
800
400
0
RADIOACTIVITY (dpm)
0
4
8
12
16
20
24
TIME (hours)

Figure 15: Time course for the basipetal movement of radioactivity from {^{14}C} CA_3 through 10 mm segments of Zea coleoptiles under both aerobic and anaerobic conditions. Percent distribution of radioactivity within the coleoptile segments and receiver blocks is shown as a function of time. 1, 2, 3, 4, 5 = 2 mm zones of coleoptile segment; x = extra coleoptile tissue due to growth; R = receiver block.


8h
12h
24h
30
20
10
0
40
30
20
10
0
RADIOACTIVITY (%)
Air
Nitrogen
1 2 3 4 5 X R
1 2 3 4 5 X R
1 2 3 4 5 X R
SEGMENT PIECE & RECEIVER

activity. Segment piece No. 3 contained more radioactivity than segment piece No. 2 and this was as much if not more than in segment piece No. 1. This small plateau of radioactivity in segment pieces Nos. 1, 2 and 3 may be due to the evacuation procedure which may result in the donor blocks being 'sucked' onto the segment. The radioactivity present in segments 4, 5 and 'X' decreased with increasing distance from the donor block.

Likewise, in nitrogen the pattern of distribution of radioactivity within the segment is similar regardless of transport time. As occurred in air, segment piece No. 1 had the highest content of radioactivity and thereafter there was a decrease in radioactive content along the segment with increasing distance from the donor block. No 'plateau' effect occurred. And, similarly to results under aerobic conditions, a slight movement of radioactivity into the receiver blocks occurred in nitrogen. However, the amount of radioactivity present in the receiver blocks did not exceed 8% of the total uptake throughout the 24 hour transport time.

8. The Effect of Anaerobic Conditions on the Acropetal Movement of Radioactivity from {^{14}C} GA_3 through Coleoptile Segments of Zea Mays.

The uptake, movement and distribution of radioactivity into apical receiver blocks through 10 mm segments of Zea coleoptiles supplied with basal donor blocks, was

investigated as a function of time under both aerobic and anaerobic conditions. Segments and receiver blocks were harvested and combined for radioassay after transport periods of 8, 12 and 24 hours. The results are shown in Figures 16 and 17. The data are the means of 3 independent experiments. The results obtained are similar to those obtained in the previous experiment for basipetal movement. The total uptake of radioactivity increased under aerobic conditions from 2383 dpm after 8 hours to 4446 dpm after 12 hours. Under anaerobic conditions the uptake was reduced throughout the entire 24 hours transport time. After 8 hours the uptake was only 492 dpm (reduction of 80%). Again, as for the previous experiment, there was a slight decrease after 12 hours to 376 dpm (reduction of 86%) but this increased to 632 dpm after 24 hours (reduction of 86%). Uptake of radioactivity at the basal end of the segment appeared to be dependent upon aerobic metabolism.

Figure 17 shows the distribution of radioactivity within the tissue and receiver blocks and again the results are closely similar to those obtained for the previous experiments i.e. the distribution is similar regardless of the length of the transport time. In air, radioactivity moved along the coleoptile segment but did not move out from the tissue into receiver blocks even after 24 hours. This is in contrast to the results of the previous experiment, obtained for basipetal transport, where there was a slight, but not significant movement of radioactivity into basal receiver blocks in air. Segment piece No.1 contained the highest amount of radioactivity on a percentage basis (26 - 29%) and there was a decline in the radioactive content of the tissue with increasing

Figure 16: Time course for the acropetal movement of radioactivity from {^{14}C} GA_3 through 10 mm segments of Zea coleoptiles under both aerobic (■) and anaerobic (□) conditions. Total uptake of radioactivity by coleoptile segments and receiver blocks is shown as a function of time. These data are the mean of 3 separate experiments.

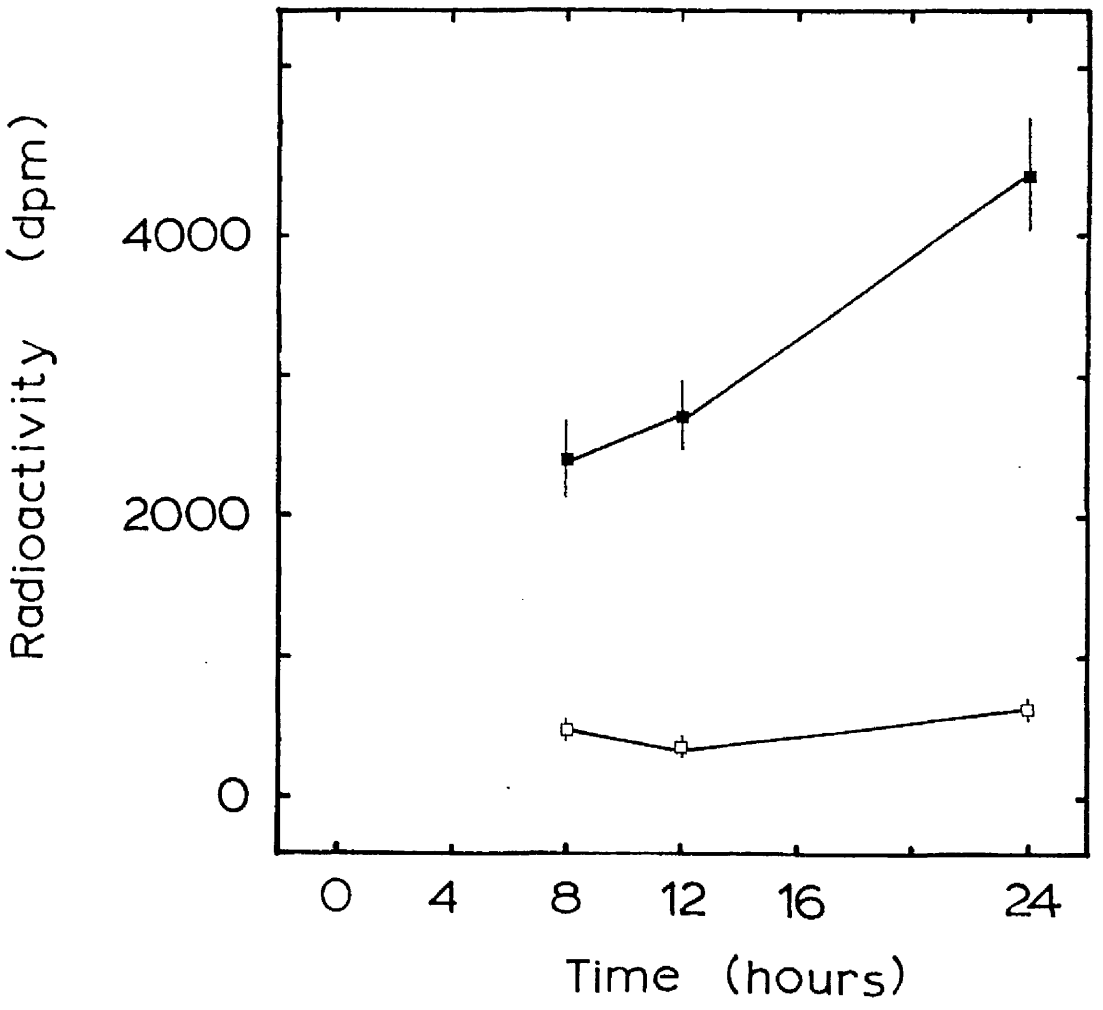
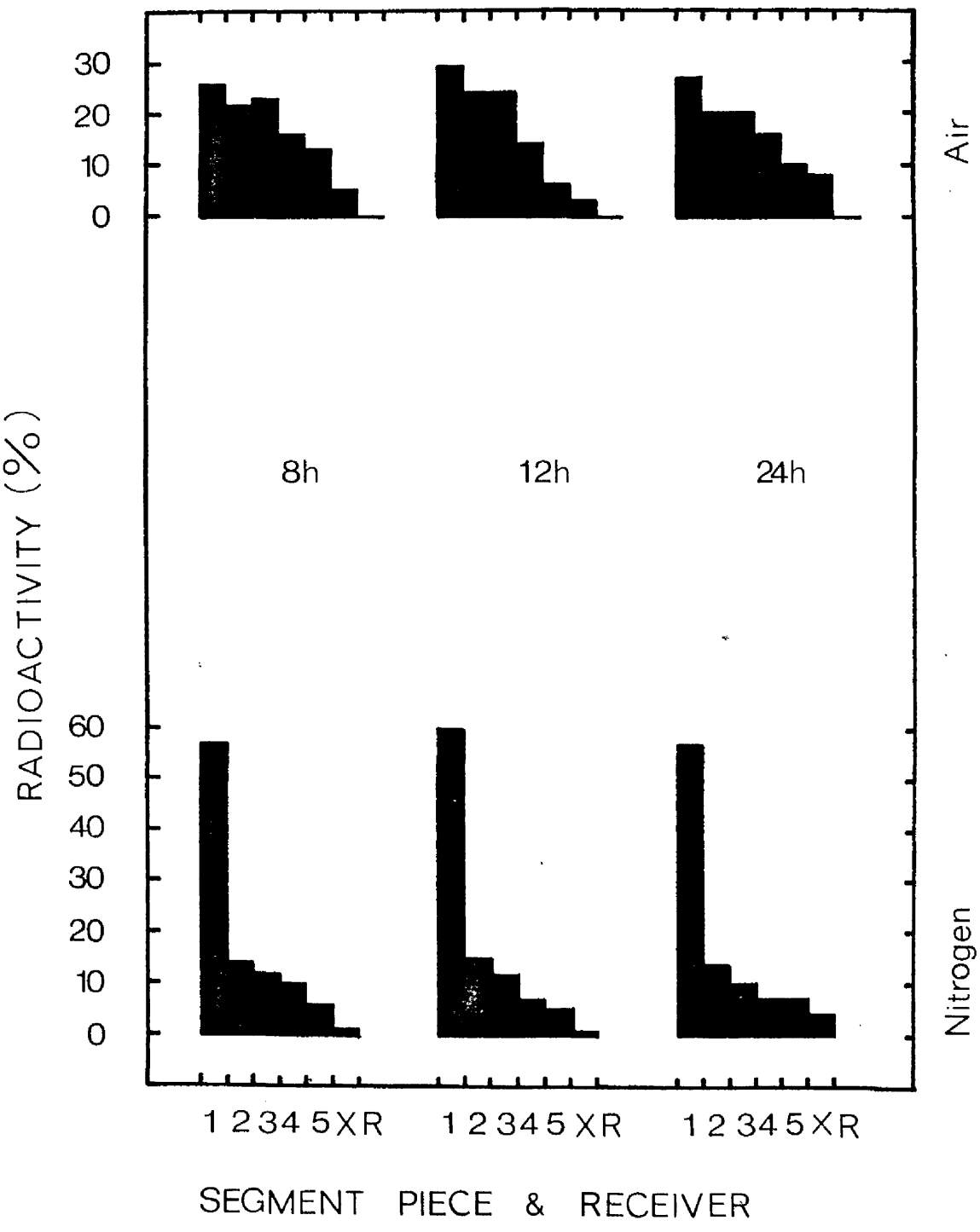

Radioactivity (dpm)
4000
2000
0
0
4
8
12
16
24
Time (hours)

Figure 17: Time course for the acropetal movement of radioactivity from {^{14}C} GA_3 through 10 mm segments of Zea coleoptiles under both aerobic and anaerobic conditions. Percent distribution of radioactivity within the coleoptile segments and receiver blocks is shown as a function of time. 1, 2, 3, 4, 5 = 2 mm zones of coleoptile segment; x = extra coleoptile tissue due to growth; R = receiver block.


30
20
10
0
Air
8h
12h
24h
RADIOACTIVITY (%)
60
50
40
30
20
10
0
Nitrogen
1 2 3 4 5 X R
1 2 3 4 5 X R
1 2 3 4 5 X R
SEGMENT PIECE & RECEIVER

distance from the donor. A plateau of radioactivity in segment pieces Nos 2 and 3 (20 - 24%) occurred again.

In nitrogen, segment piece No. 1 contained a very high proportion of radioactivity (57 - 60%). The radioactive content of segment piece No. 2 was between 14 - 15% and thereafter there was a decline in radioactivity with increasing distance from the donor. As in the previous experiment there was a limited movement of radioactivity into the receiver blocks (1 - 4%).

9. A Comparison of the Effect of Anaerobic Conditions on both Basipetal and Acropetal Movement of Radioactivity from [^{14}C] GA_3 through Coleoptile Segments of Zea Mays.

As a check on the previous two experiments, it was decided to compare both the basipetal and acropetal movement of radioactivity under anaerobic conditions, simultaneously. The experimental procedure used was largely the same as for the previous experiments. The total uptake by and distribution of radioactivity within the coleoptile segments are shown in Figures 18 and 19 in which the data are the means of 2 independent experiments. The results are essentially what would be expected from the previous investigations. Throughout the entire transport period, uptake of radioactivity was greater when segments were supplied with basal than with apical donors (Figure 18). There was a decrease in uptake

Figure 18: Time course for the basipetal (●) and acropetal (■) movement of radioactivity from [^{14}C] GA_3 through 10 mm segments of Zea coleoptiles under anaerobic conditions. Total uptake of radioactivity by coleoptile segments and receiver blocks is shown as a function of time. These data are the mean of 2 separate experiments.

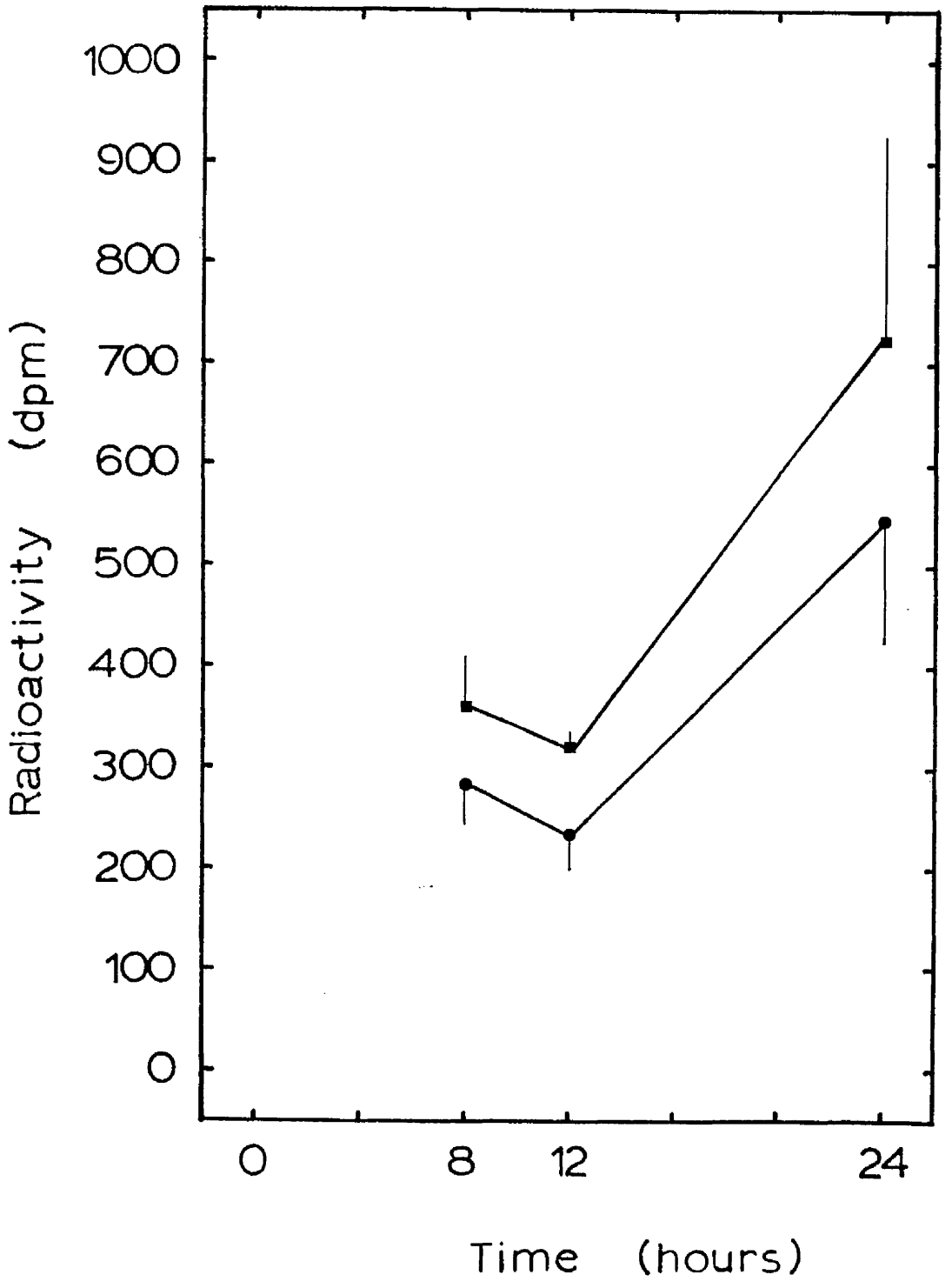
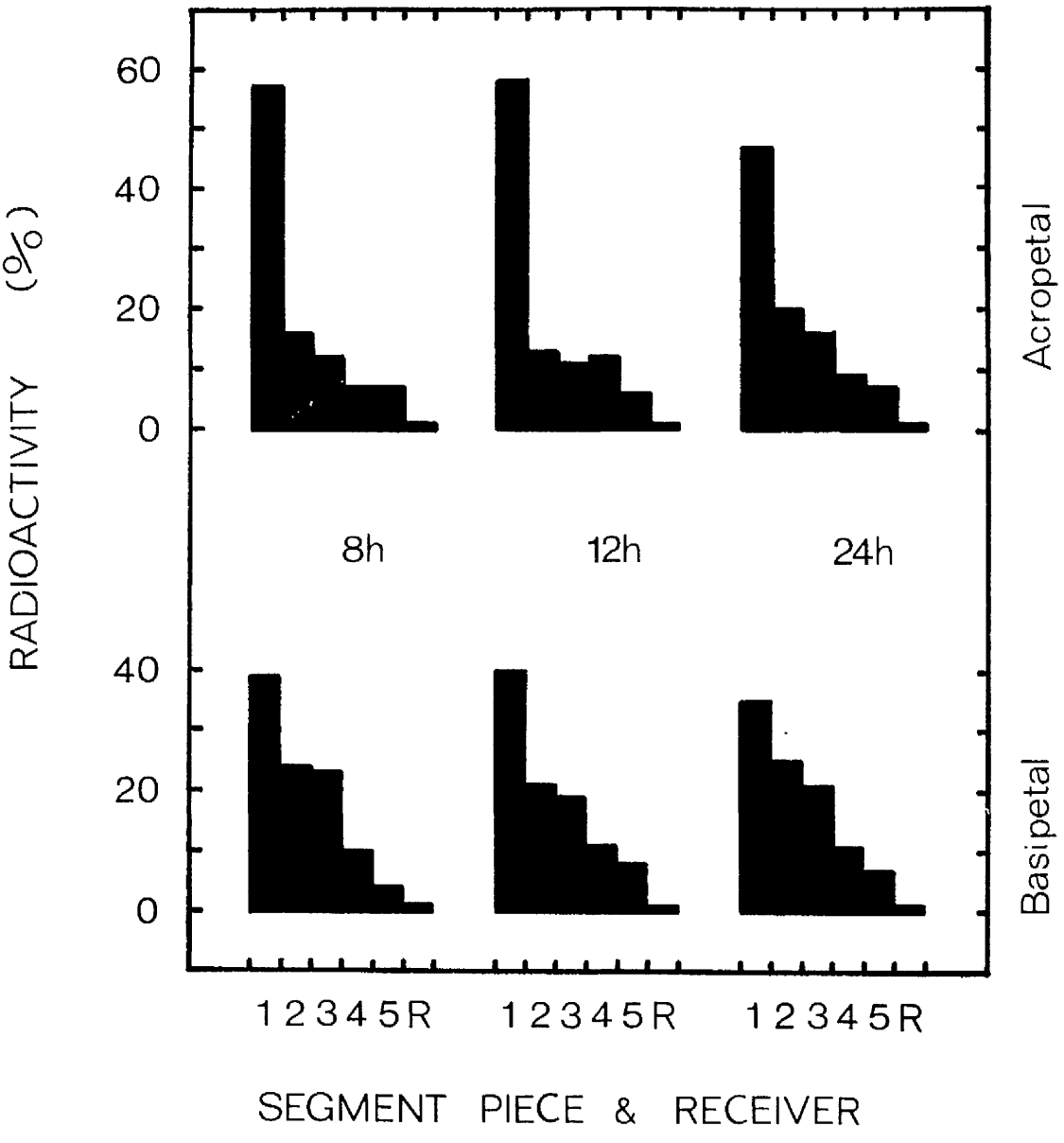

1000
900
800
700
600
500
400
300
200
100
0
Radioactivity (dpm)
0
8
12
24
Time (hours)

Figure 19: Time course for the basipetal and acropetal movement of radioactivity from {^{14}C} GA_3 through 10 mm segments of Zea coleoptiles under anaerobic conditions. Percent distribution of radioactivity within the coleoptile segments and receiver blocks is shown as a function of time. 1, 2, 3, 4, 5 = 2 mm zones of coleoptile segment; x = extra coleoptile tissue due to growth; R = receiver block.


RADIOACTIVITY (%)
60
40
20
0
8h
12h
24h
Acropetal
40
20
0
Basipetal
1 2 3 4 5 R
1 2 3 4 5 R
1 2 3 4 5 R
SEGMENT PIECE & RECEIVER

at 12 hours by segments supplied with either apical or basal donor sources. The pattern of distribution of radioactivity within all segments was independent of the transport time for both acropetal and basipetal movement (Figure 19). In all segments, whether supplied with apical or basal donor blocks, segment piece No. 1 contained the highest proportion of radioactivity and thereafter there was a decline in radioactive content with increasing distance from the donor source. There was a slight movement into both apical and basal receiver blocks.

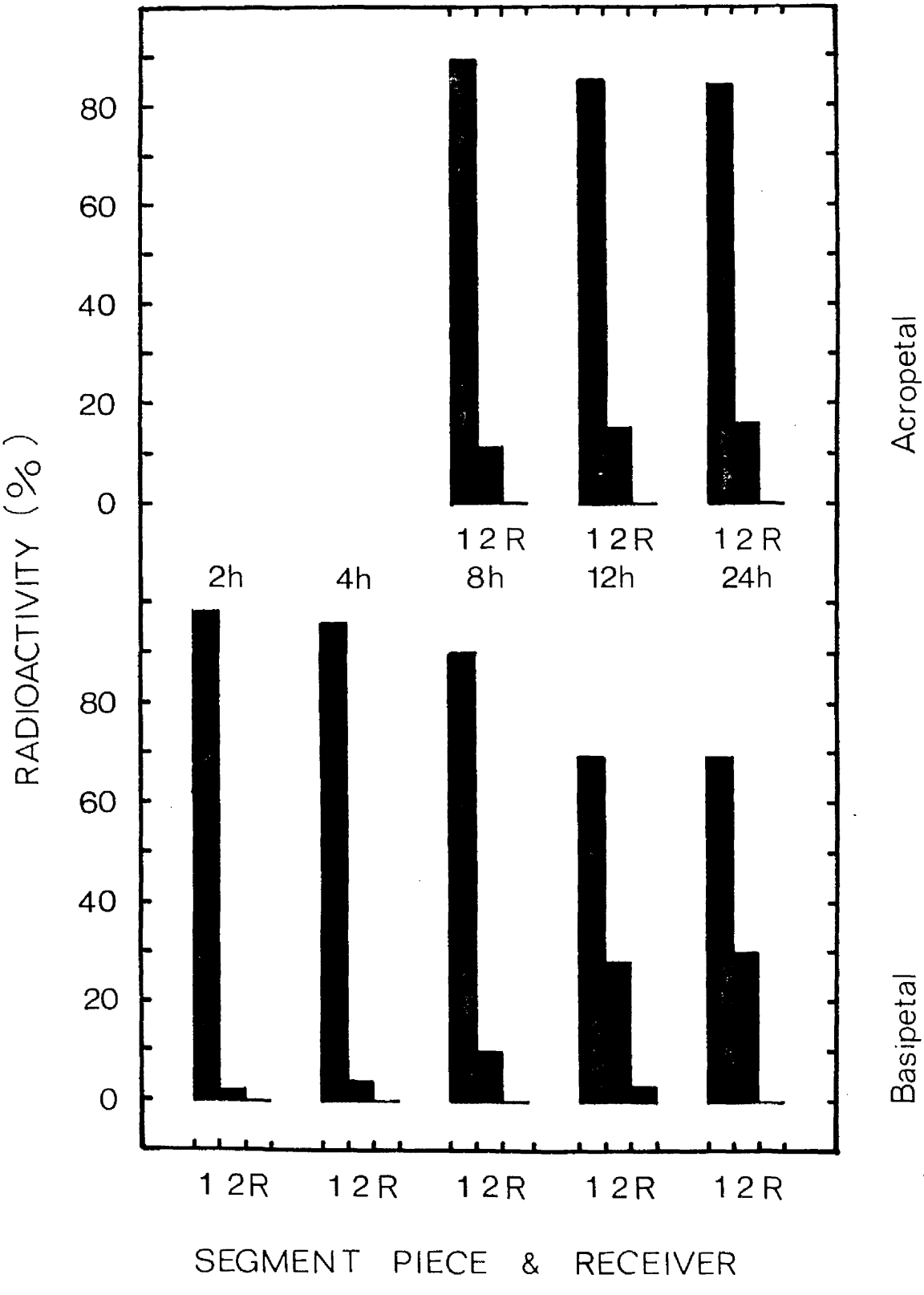
10. The Longitudinal Movement of Radioactivity from {^{14}C} GA$_3$ through excised Intact Coleoptile Apices of Zea Mays.

Since experiments with subapical coleoptile segments had produced no evidence of a longitudinal polarity these next experiments were designed to determine whether there was a longitudinal polarity of movement of radioactivity following the application of a pulse of {^{14}C} GA$_3$ to excised intact 10 mm coleoptile apices. To investigate basipetal movement the pulse was applied to a point 1 mm behind the apical extremity of the intact apex and an agar receiver block was applied to the cut basal surface at the other end of the apex. To investigate acropetal movement the pulse was applied to a point 1 mm up from the cut basal surface. There were no apical receiver blocks. Micropipettes were

used to apply the pulse and apices were bisected horizontally and pooled for radioassay as described previously, after transport times of 2, 4, 8, 12 and 24 hours (basipetal movement) and 8, 12 and 24 hours (acropetal movement). The data are shown in Figure 20 and are the means of 4 independent experiments for each direction of movement. When the {^{14}C} GA_3 was applied to the extreme apex there was very little movement of radioactivity for the first 4 hours. After 2 hours, 98% of the total radioactivity was still confined to the 3 mm of the apex nearest to the point of application; very little radioactivity had moved into the distal 7 mm region. After 4 hours 96% of the radioactivity was confined to the apical 3 mm and only 4% had moved into the more basal region. 10% of the total applied radioactivity was present in the basal region of the apex after 8 hours and after a 24 hour transport time this had increased to 30%. Throughout the entire experiment there was no movement of radioactivity out of the tissue into the basal receiver blocks except after 12 hours but even this only represented 3% of the total radioactivity originally applied.

Similar results were obtained when the {^{14}C} GA_3 was applied to a point 1 mm up from the basal cut surface. Most of the radioactivity remained in the basal 3 mm nearest to the point of application. However there was less acropetal movement from the point of application into the more

Figure 20: Time course for the basipetal and acropetal movement of radioactivity following the application of a pulse of {^{14}C} GA_3 to either the extreme apex or base of 10 mm intact coleoptile apices of Zea mays seedlings. Percent distribution of radioactivity in the apical and basal halves of the coleoptile apices and in the receiver blocks is shown as a function of time. 1 = apical half nearest to the point of {^{14}C} GA_3 application; 2 = apical half furthest from the point of {^{14}C} GA_3 application; R = receiver block. These data are the mean of 4 separate experiments.


RADIOACTIVITY (%)
80
60
40
20
0
2h
4h
8h
12h
24h
1 2 R
Acropetal
Basipetal
SEGMENT PIECE & RECEIVER

apical regions of the coleoptile than when {^{14}C} GA$_3$ was applied to the apex and moved downwards. After transport times of 8 hours, 90% of the total applied radioactivity was retained in the basal 3 mm as was the case for basipetal movement after 8 hours. By 12 hours the percentage radioactivity found in the distal apical 7 mm piece was 15% and this had only increased to 16% after 24 hours.

11. The Basipetal Movement of Radioactivity from {^{14}C} GA$_3$ through Roots of Intact Zea Mays Seedlings During Geotropic Stimulation.

The basipetal movement of radioactivity from {^{14}C} GA$_3$ through primary roots of intact Zea seedlings was investigated as a function of time. A pulse of {^{14}C} GA$_3$ was applied to a point 1 mm behind the root apex and roots were orientated subsequently in either the vertical or horizontal position with respect to gravity. The distribution of radioactivity within the roots expressed as a function of time is shown in Figures 21 and 22 in which the data are the means of 4 (vertical) and 3 (horizontal) independent experiments.

When roots were orientated vertically (Figure 21) a high level of radioactivity remained near to the point of application throughout the 12 hours of the time course. After 3 hours 98% of the applied radioactivity was still confined to the apical 3 mm of the root and only 2% had moved into the next 3 mm section (i.e. 6 mm from the apical extremity

Figure 21: Time course for the basipetal movement of radioactivity following the application of a pulse of {^{14}C} GA_3 to the apex of vertically orientated roots of intact Zea mays seedlings. Percent distribution of radioactivity in 3 mm zones of the root is shown as a function of time. Amounts of radioactivity in the more basal regions of the roots are shown by the columns labelled (+). These data are the mean of 4 separate experiments.

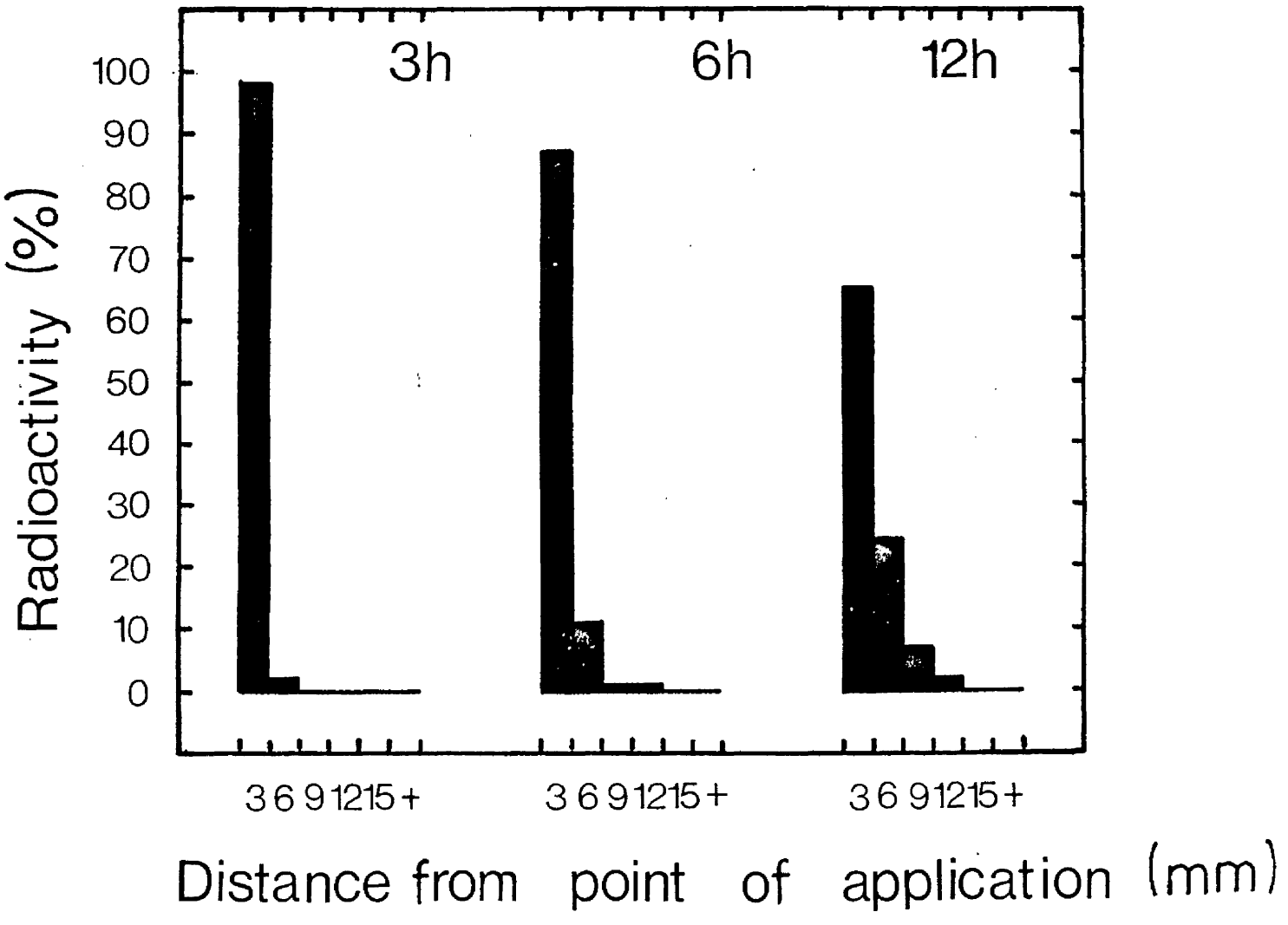
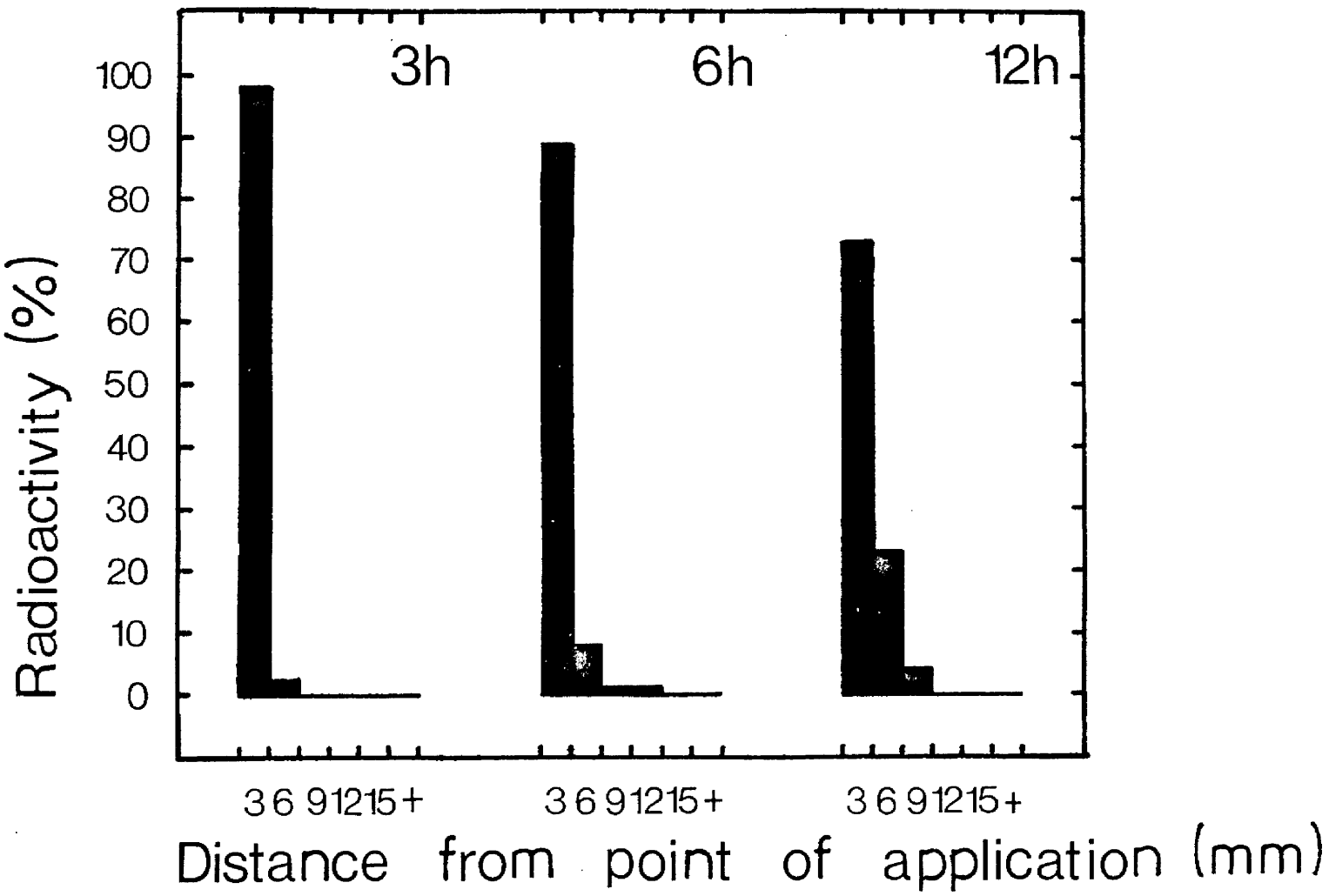

3h
6h
12h
100
90
80
70
60
50
40
30
20
10
0
Radioactivity (%)
3 6 9 12 15 +
3 6 9 12 15 +
3 6 9 12 15 +
Distance from point of application (mm)

Figure 22: Time course for the basipetal movement of radioactivity following the application of a pulse of {^{14}C} GA_3 to the apex of horizontally orientated roots of intact Zea mays seedlings. Percent distribution of radioactivity in 3 mm zones of the root is shown as a function of time. Amounts of radioactivity in the more basal regions of the roots are shown by the columns labelled (+). These data are the mean of 3 separate experiments.


3h
6h
12h
100
90
80
70
60
50
40
30
20
10
0
Radioactivity (%)
3 6 9 12 15+
3 6 9 12 15+
3 6 9 12 15+
Distance from point of application (mm)

of the root). After 6 hours, 87% still remained in the apical 3 mm and 11% had moved as far as 6 mm from the root apex. There were very small amounts of radioactivity found as far as 12 mm from the application point. After 12 hours transport time, 35% of the applied radioactivity had moved out of the apical 3 mm region :- 24% was present in the region 3 - 6 mm behind the root apex, 7% in the 6 - 9 mm region and 2% as far as the 9 - 12 mm region. Even after 12 hours the radioactive front had not reached further than 12 mm from the initial point of application. Similar results were obtained when the roots were orientated in the horizontal position with respect to gravity (Figure 22). There was a decrease in the amount of radioactivity in the root tissue with increasing distance from the point of application and the 3 mm segment piece nearest to the point of application always contained the highest amount of radioactivity even after transport times of 12 hours. Again, as was the case for vertical roots, after 3 hours 98% of the radioactivity was retained in the apical 3 mm and by 6 hours this had decreased to 89%, and 8% could be found up to 6 mm away from the apex, with minimal amounts in the region 6 - 12 mm from the point of application. After a 12 hour transport period 27% of the applied radioactivity had moved out of the apical 3 mm and of this 27%, 23% was present in the region 3 - 6 mm from the apex and the remaining 4% in 6 - 9 mm region. It is apparent from these results that in both vertically and horizontally orientated

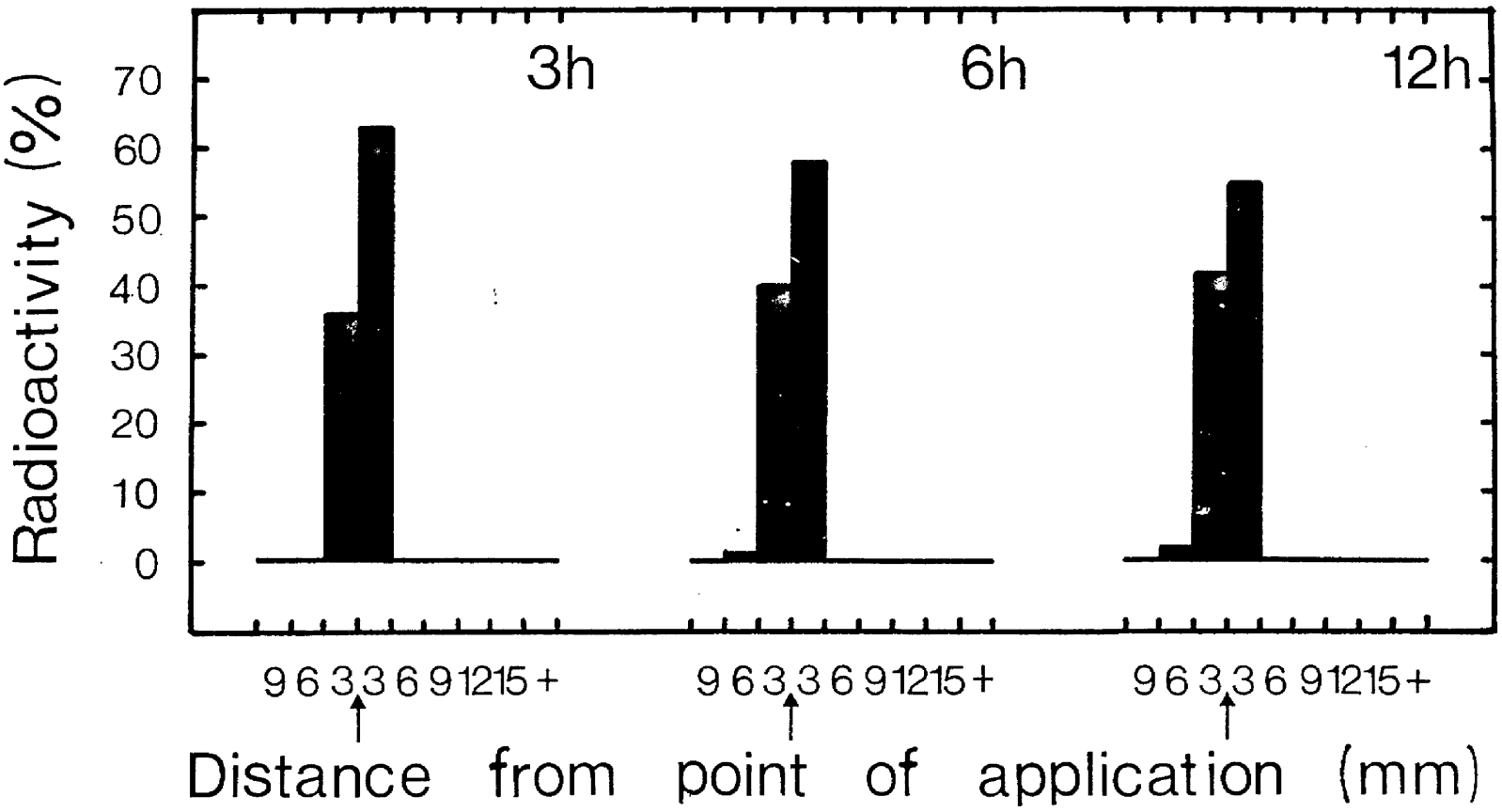
roots, there is a limited capacity for the longitudinal movement of radioactivity in the basipetal direction and that after a transport time of 12 hours the radioactivity front had moved only as far as 12 mm from the original point of application which was 1 mm behind the apex.

12. The Acropetal Movement of Radioactivity from {^{14}C} GA, through Roots of Intact Zea Mays Seedlings.

The acropetal movement of radioactivity from a pulse of {^{14}C} GA, applied to a point 15 mm behind the apex of primary roots of intact Zea seedlings was followed as a function of time. Roots were orientated in the vertical position only and the distribution of radioactivity within the roots expressed as a function of time, is illustrated in Figure 23 in which the data are the means of 3 independent experiments.

As was the case for roots injected 1 mm behind the apex, most of the radioactivity was confined to that region of the root tissue nearest to the point of application. Throughout the duration of the time course, even after the longer transport period of 12 hours, 98% or more of the applied radioactivity was present in the 3 mm regions on either side of the point of application. Moreover the 3 mm region of tissue next to the point of application and nearest to the root cap, consistently contained a higher percentage of radioactivity throughout the time course,

Figure 23: Time course for the acropetal movement of radioactivity following the application of a pulse of {^{14}C} GA_3 to the apex of vertically orientated roots of intact Zea mays seedlings. Percent distribution of radioactivity in 5 mm zones of the root is shown as a function of time. Amounts of radioactivity in the more basal regions of the roots are shown by the columns labelled (+). These data are the mean of 3 separate experiments.


3h
6h
12h
Radioactivity (%)
70
60
50
40
30
20
10
0
9 6 3 3 6 9 12 15 +
9 6 3 3 6 9 12 15 +
9 6 3 3 6 9 12 15 +
Distance from point of application (mm)

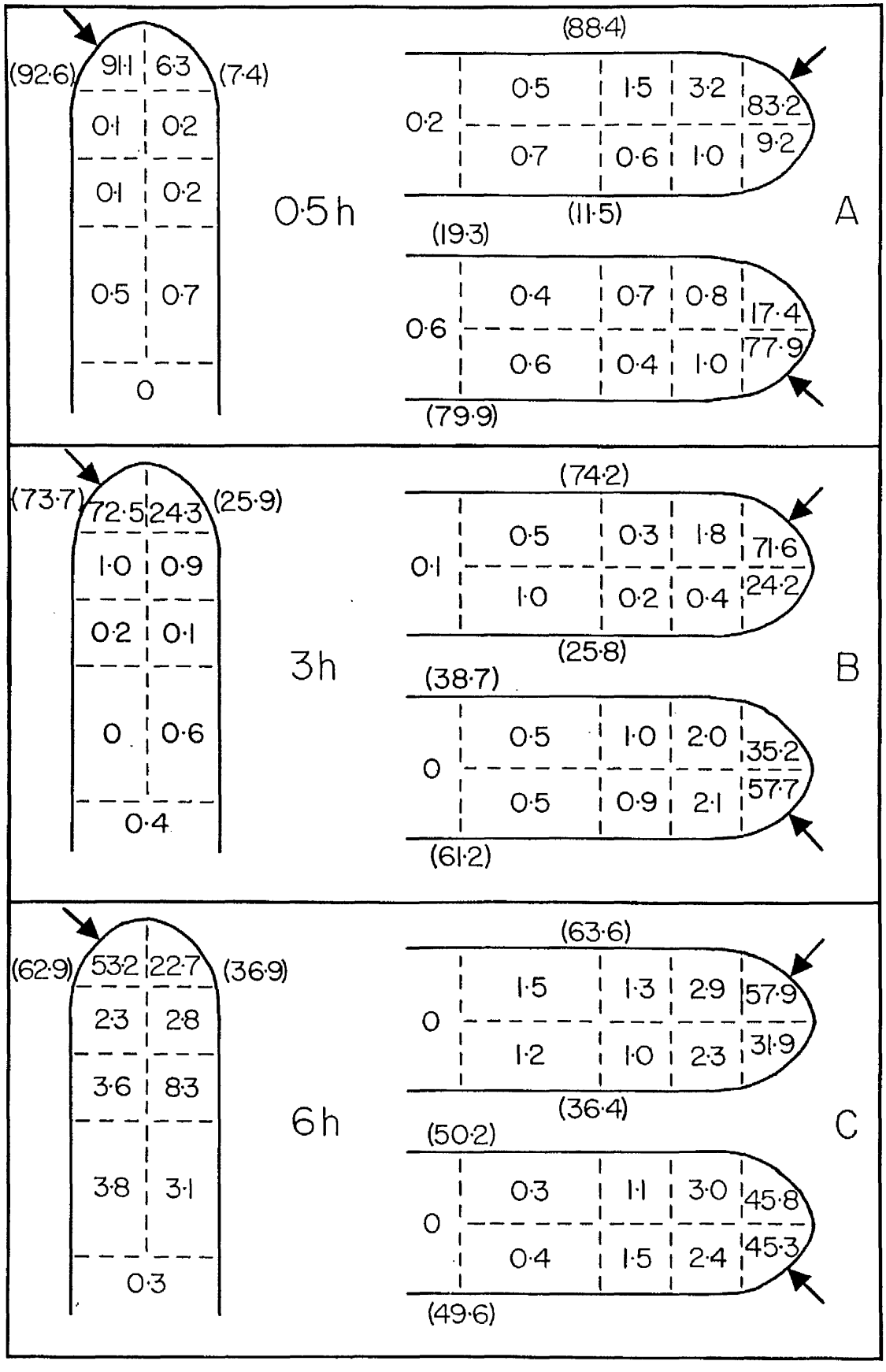
than the 3 mm region adjacent to the point of application and nearest to the basal end of the root. However the differential in the radioactive content of these two regions on either side of the point of application, decreased with time from 27% difference after 3 hours to a 13% differential after 12 hours.

On the basis of the above results it is apparent that there is some longitudinal movement of radioactivity in roots but that it exists in the basipetal as opposed to the acropetal direction ie. away from the root apex.

13. The Lateral Movement of Radioactivity from {^{14}C} GA$_3$ Applied Asymmetrically to Coleoptiles of Intact Zea Mays Seedlings during Geotropic Stimulation.

The distribution of radioactivity in both horizontal and vertical shoots of Zea mays seedlings after the asymmetric application of {^{14}C} GA$_3$ was followed as a function of time. The results which are shown in Figure 24 are the means of 3 independent experiments. In each orientation most of the radioactivity remained in the apical 5 mm of the coleoptile even after 6 hours; very little radioactivity moved into the more basal regions of the shoot. The distribution of radioactivity in the sub-apical region of the coleoptile was similar regardless of the orientation of the seedlings with respect to gravity ie. the distribution

Figure 24: Percent distribution of radioactivity in horizontal and vertical shoots of intact Zea mays seedlings. The tissue was bisected longitudinally and then transversely into three 5 mm portions and one 10 mm portion (dotted lines). The figures in brackets show the total radioactivity in each half of the shoot. The figure at the base of the shoot shows the total radioactivity in the rest of the shoot above the fruit. The arrows indicate the position of application of {^{14}C} GA_3. These data are the mean of two separate experiments.


(92·6)
91·1
6·3
(7·4)
0·1
0·2
0·1
0·2
0·5
0·7
0
0·5 h
(88·4)
0·2
0·5
1·5
3·2
83·2
0·7
0·6
1·0
9·2
(11·5)
A
(19·3)
0·6
0·4
0·7
0·8
17·4
0·6
0·4
1·0
77·9
(79·9)
(73·7)
72·5
24·3
(25·9)
1·0
0·9
0·2
0·1
0
0·6
0·4
3h
(74·2)
0·1
0·5
0·3
1·8
71·6
1·0
0·2
0·4
24·2
(25·8)
B
(38·7)
0
0·5
1·0
2·0
35·2
0·5
0·9
2·1
57·7
(61·2)
(62·9)
53·2
22·7
(36·9)
2·3
2·8
3·6
8·3
3·8
3·1
0·3
6h
(63·6)
0
1·5
1·3
2·9
57·9
1·2
1·0
2·3
31·9
(36·4)
C
(50·2)
0
0·3
1·1
3·0
45·8
0·4
1·5
2·4
45·3
(49·6)

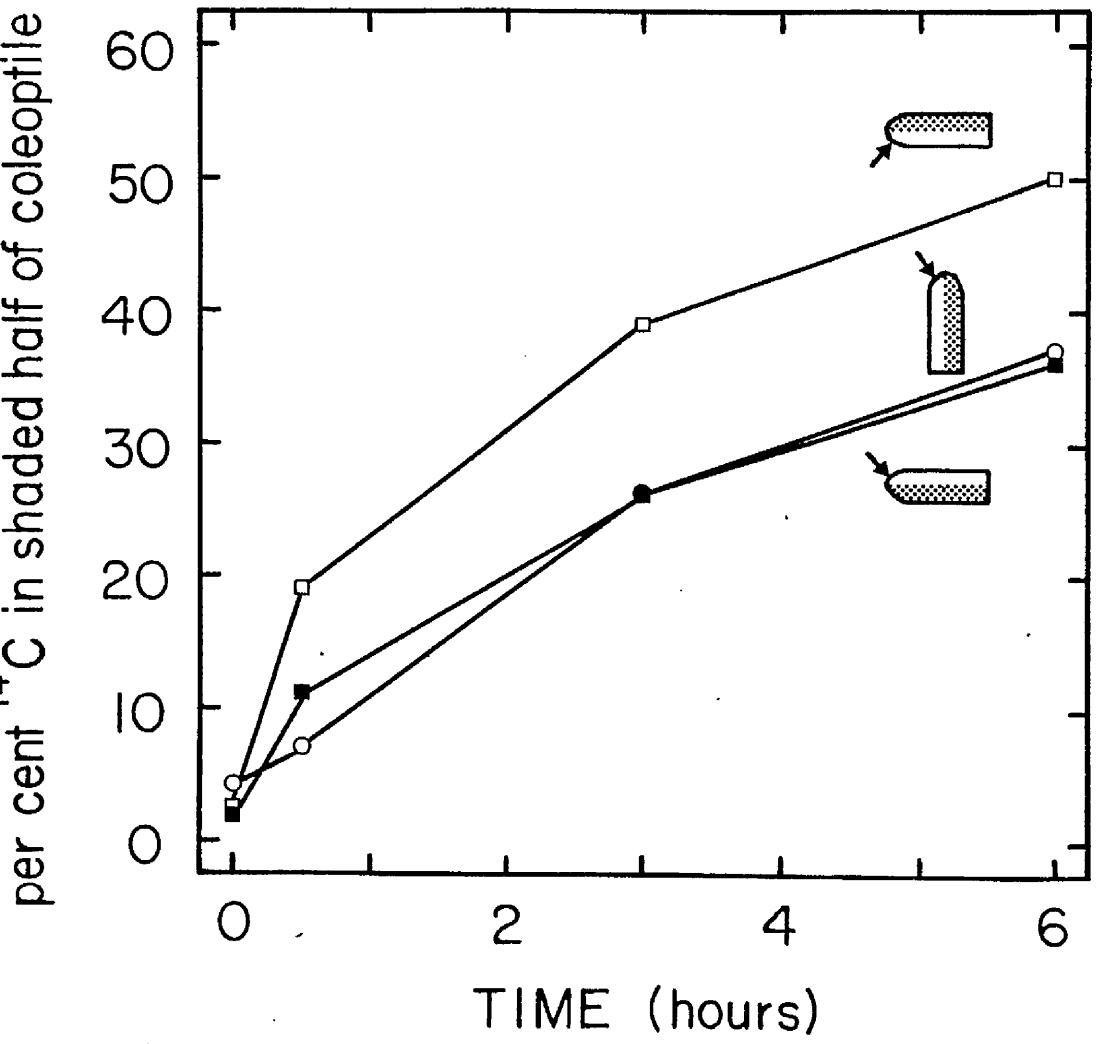
of radioactivity in the more basal regions of horizontal seedlings was indistinguishable from the distribution pattern in the corresponding zones of vertically orientated seedlings. The lateral distribution of radioactivity in the apical 5 mm of the coleoptile was, however, dependent upon orientation.

Figure 25 shows the lateral movement of radioactivity as a function of time. The proportion of radioactivity moving into the nondonated half of coleoptiles that were vertical or orientated horizontally with the point of application on the upper side, was closely similar and increased with time from 7.4% at 0.5 hour to 37% after 6 hours. In contrast the proportion of radioactivity in the nondonated half of the horizontal coleoptile was much greater when the point of application of {^{14}C} GA, was on the lower side, being 19.3% after 0.5 hour and 50.2% after 6 hours. After 0.5 hour there was therefore a net upward movement of 7.8%, which is significant at the 0.05 probability level, and this value increased with time to 12.9% after 3 hours and to 13.8% after 6 hours which are significant at the 0.01 probability level.

14. The Lateral Movement of Radioactivity from {^{14}C} GA, Applied Asymmetrically to Roots of Intact Zea Mays Seedlings during Geotropic Stimulation.

The distribution of radioactivity in horizontal and vertical primary roots of Zea mays seedlings 3 hours after

Figure 25: Lateral movement of radioactivity as a function of time. The proportion of the total radioactivity in the coleoptile in the half (shaded) opposite to the point of application of {^{14}C} GA_3 is shown for intact shoots of Zea mays seedlings for different orientations with respect to gravity. Inset diagrams show the point of application on the lower (□) or upper (■) side of a horizontal shoot or the side (○) of a vertical shoot.


per cent ^{14}C in shaded half of coleoptile
60
50
40
30
20
10
0
0
2
4
6
TIME (hours)

the asymmetric, point source application of {^{14}C} GA_3 is shown in Figure 26 in which the data are the means of 4 independent experiments. In each orientation more than 96% of the total radioactivity remained in the apical 5 mm of the root. There was very little longitudinal movement into the subapical regions of both horizontal and vertical roots. The proportion of the total radioactivity in the nondonated half of the root was approximately 37% when the root was kept vertical or placed horizontally with the point of application on the upper side. In contrast, 46% of the total radioactivity applied was recovered in the nondonated upper half of a horizontal root when the point of {^{14}C} GA_3 application was on the lower side. There is therefore a net upward movement of radioactivity of 9.3% after 3 hours, which is significant at the 0.001 probability level.

15. The Lateral Movement of Radioactivity from {^{3}H} GA_1 Applied Asymmetrically to Coleoptiles of Intact Zea Mays Seedlings during Geotropic Stimulation.

The distribution of radioactivity in horizontal and vertical coleoptiles of intact Zea mays seedlings 3 hours after the asymmetric application of {^{3}H} GA_1 is shown in Figure 27. The data are the means of 4 independent experiments.

As was the case for {^{14}C} GA_3, in both vertical and horizontal seedlings, most of the radioactivity remained in

Figure 26: Percent distribution of radioactivity after 3 h in horizontal and vertical roots of intact *Zea mays* seedlings. The root was bisected longitudinally and then divided transversely into three 5 mm portions and the remaining basal region (dotted lines). The figures in brackets show the total radioactivity in each half of the roots. The arrows indicate the position of application of {^{14}C} GA_3. These data are the mean of 4 separate experiments.

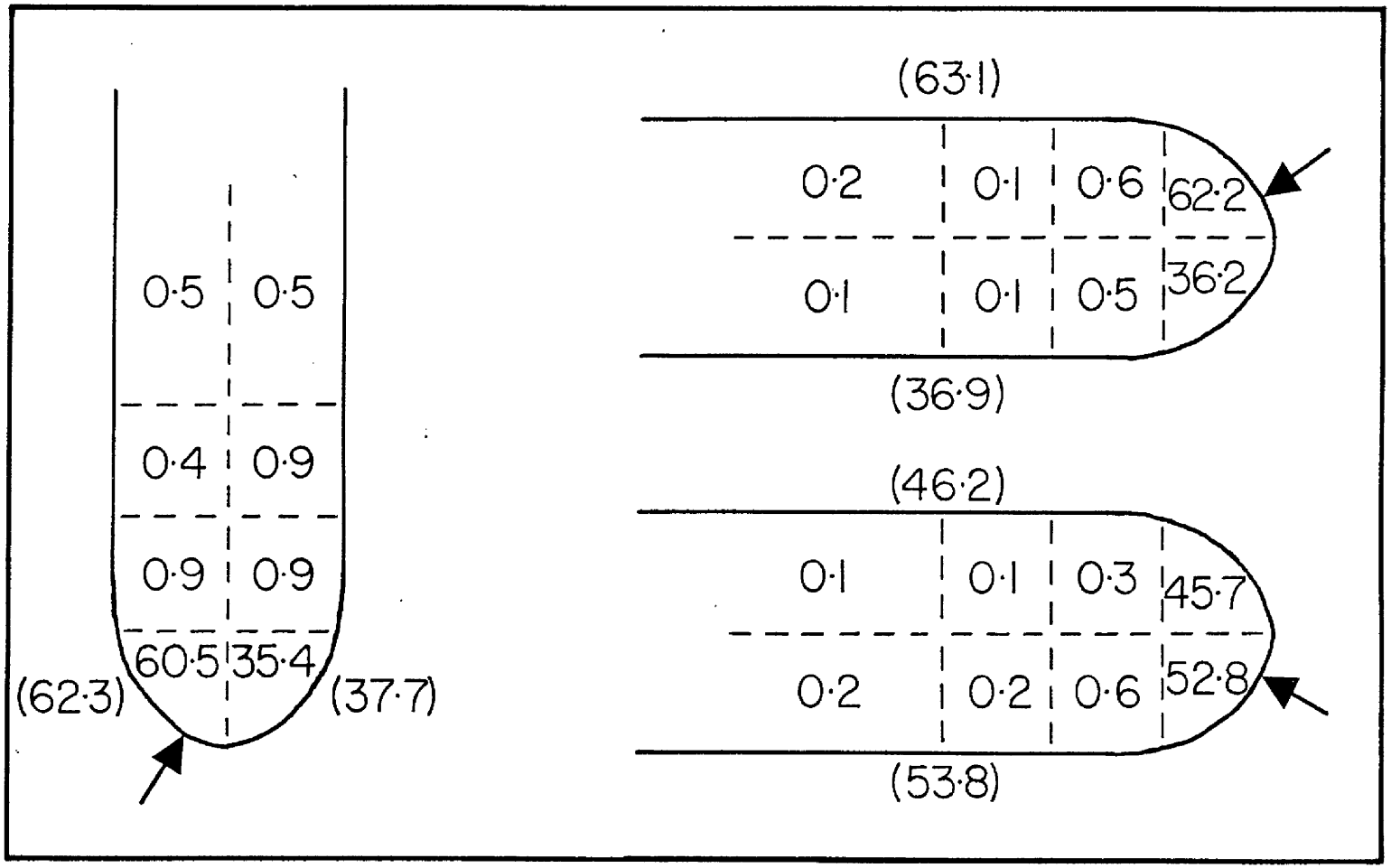
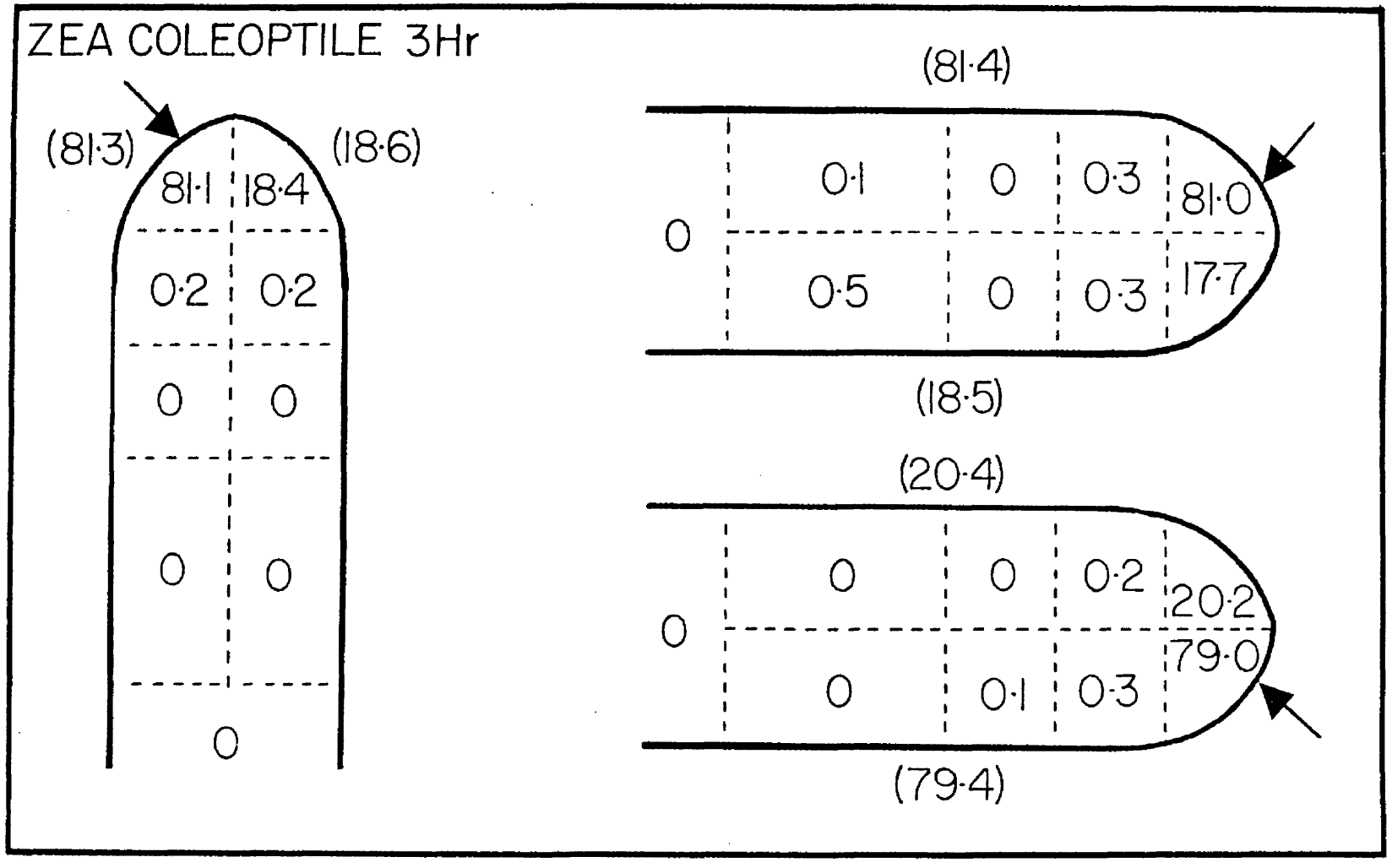

(63·1)
0·2
0·1
0·6
62·2
0·1
0·1
0·5
36·2
(36·9)
(46·2)
0·1
0·1
0·3
45·7
0·2
0·2
0·6
52·8
(53·8)
0·5
0·5
0·4
0·9
0·9
0·9
60·5
35·4
(62·3)
(37·7)

Figure 27: Percent distribution of radioactivity after 3 h in horizontal and vertical shoots of intact *Zea mays* seedlings. The tissue was bisected longitudinally and then transversely into three 5 mm portions and one 10 mm portion (dotted lines). The figures in brackets show the total radioactivity in each half of the shoot. The figure at the base of the shoot shows the total radioactivity in the rest of the shoot above the fruit. The arrows indicate the position of application of (^{3}H) GA_1. These data are the mean of four separate experiments.

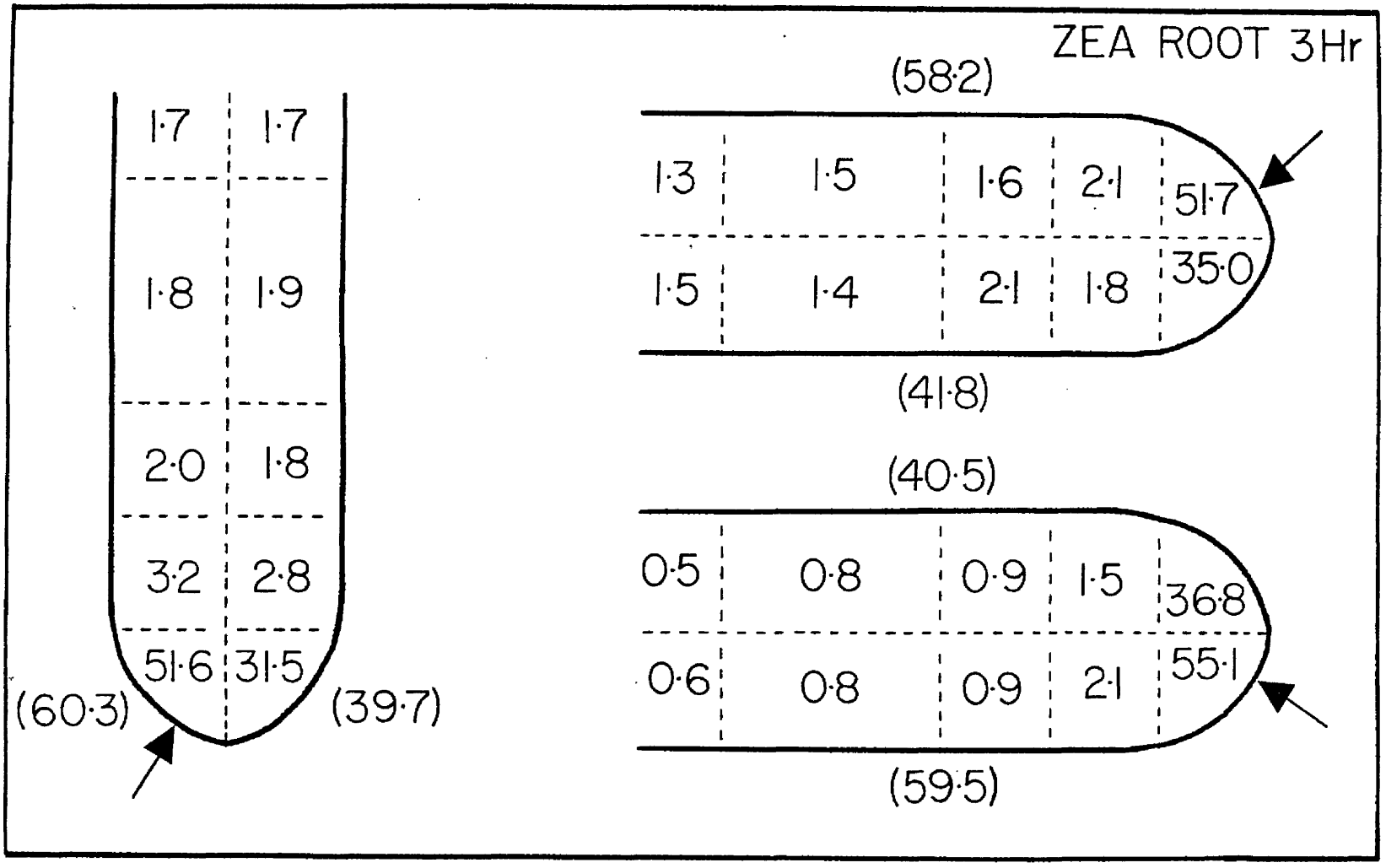

ZEA COLEOPTILE 3Hr
(81·3)
(18·6)
81·1
18·4
0·2
0·2
0
0
0
0
0
(81·4)
0
0·1
0
0·3
81·0
0·5
0
0·3
17·7
(18·5)
(20·4)
0
0
0·2
20·2
0
0
0·1
0·3
79·0
(79·4)

the apical 5 mm portion of the coleoptile. In fact only 1.2% or less of the total radioactivity applied was found in the more basal regions. However in contrast to the data for {^{14}C} GA_3, the lateral distribution of radioactivity within the tissues was independent of the geotropic orientation of the seedling. For both vertical and horizontal coleoptiles with either upper or lower donation, between 19 and 20% of the total applied radioactivity could be recovered from the side opposite to that of donation. No significant difference in the lateral redistribution of radioactivity could be detected between vertical and horizontal seedlings. Thus a gradient of radioactivity is not established between the upper and lower halves of horizontally orientated Zea coleoptiles. So in contrast to {^{14}C} GA_3 there appeared to be no evidence of an upward lateral movement of radioactivity from {^{3}H} GA_1 in coleoptiles of horizontal Zea seedlings after a transport period of 3 hours.

16. The Lateral Movement of Radioactivity from {^{3}H} GA_1 Applied Asymmetrically to Roots of Intact Zea Mays Seedlings during Geotropic Stimulation.

Figure 28 shows the distribution of radioactivity in both horizontal and vertical primary roots of Zea mays seedlings 3 hours after the asymmetric application of {^{3}H} GA_1. The data are the means of 4 independent experiments.

Figure 28: Percent distribution of radioactivity after 3 h in horizontal and vertical roots of intact Zea mays seedlings. The root was bisected longitudinally and then divided transversely into three 5 mm portions, one 10 mm portion and the remaining basal region (dotted lines). The figures in brackets show the total radioactivity in each half of the roots. The arrows indicate the position of application of {^{3}H} GA_1. These data are the mean of four separate experiments.


ZEA ROOT 3Hr
(58·2)
1·3
1·5
1·6
2·1
51·7
1·5
1·4
2·1
1·8
35·0
(41·8)
(40·5)
0·5
0·8
0·9
1·5
36·8
0·6
0·8
0·9
2·1
55·1
(59·5)
1·7
1·7
1·8
1·9
2·0
1·8
3·2
2·8
51·6
31·5
(60·3)
(39·7)

As was the case for coleoptiles, there appeared to be no lateral redistribution of radioactivity between the donated and nondonated halves of horizontal and vertical roots. About 40% of the total radioactivity applied, was found in the side opposite to that of donation, regardless of the root's orientation with respect to gravity. However, it is interesting to note that there appeared to be more longitudinal movement of radioactivity into the more basal regions of the root. Between 9 and 17% of the radioactivity applied was found in the sub-apical regions of the root. The extent to which the radioactivity moved longitudinally seemed to be dependent upon the orientation of the roots. For roots that were horizontal with the point of donation on the lower side, 8.9% of the total radioactivity was found in the more basal regions of the roots. In contrast, nearly twice as much (16.7%) radioactivity moved longitudinally into the sub-apical regions of vertically orientated roots. Some 13.4% of the applied radioactivity moved longitudinally into the more basal regions of horizontal roots that had been supplied with a pulse of {^{3}H} GA$_4$ on the upper side.

Metabolism and Chromatography

An essential question needed to be asked at this stage is concerned with the possible metabolic breakdown of gibberellic acid in the plant tissue and whether or not the radioactivity recovered from roots and coleoptiles which had been supplied with radioactive gibberellic acid, was

still associated with the gibberellic acid molecule. In an attempt to resolve this problem either {^{14}C} GA_3 or {^{3}H} GA_1 were applied asymmetrically to either roots or coleoptiles of intact Zea mays seedlings and the radioactivity which was subsequently recovered from the tissue was analysed by means of paper chromatography. The gibberellic acid was applied asymmetrically to a point 1 mm behind the apex of either roots or coleoptiles. Seedlings were orientated either vertically or horizontally with the point of application on either the upper or lower side. For experiments with {^{14}C} GA_3, transport was allowed to take place for 6 hours but with {^{3}H} GA_1 the transport time was only 3 hours. Coleoptiles were allowed to transport in the dark whereas the roots were maintained under white fluorescent light for the transport period. The extraction and chromatography procedures have already been described. In each case the experiment was performed on 4 separate occasions; two of the resulting extracts were chromatographed in acidic solvent systems and two in basic solvent systems. The results of the chromatographic analysis are shown in Figures 29 - 30.

17. The Extraction of Radioactivity from Shoots of Zea Mays Seedlings following the Application of {^{14}C} GA_3 to the Coleoptile.

The results obtained using an acidic solvent system

(ethyl methyl ketone : acetic acid :: 95 : 5) are shown in Figure 29. The major peak of radioactivity in chromatograms of extracts from all three geotropic orientations occurred between Rf 0.7 - 0.8 and co-chromatographed with the major peak of activity in the chromatograms of the stock solution of {^{14}C} GA_3 and unlabelled GA_3. Therefore the majority of the radioactivity extracted from the coleoptile tissue was indistinguishable from that of {^{14}C} GA_3.

Chromatographic analysis using a basic solvent system (isopropanol : ammonia : water :: 10 : 1 : 1) produced the results shown in Figure 30 which are essentially similar to those obtained using the acidic solvent system. Extracts from seedlings under all three geotropic orientations produced one major peak of activity between Rf 0.4 - 0.6 which corresponded with the major peak of activity from the stock solution of {^{14}C} GA_3 and unlabelled GA_3. The stock solution of {^{14}C} GA_3 contained an impurity at Rf 0.1 and this was also present as a very small peak at the same Rf value on chromatograms of each of the three plant extracts.

18. The Extraction of Radioactivity from Shoots of Zea Mays Seedlings Following the Application of {^{3}H} GA_1 to the Coleoptile.

Extracts from the three groups of seedlings under different orientations with respect to gravity were

Figure 29: Chromatographic analysis of ethyl acetate soluble extracts from vertical and horizontal Zea coleoptiles 6 h after application of {^{14}C} GA_3. Analyses were carried out on paper chromatograms developed in methyl ethyl ketone : acetic acid :: 95 : 5. Assayed by liquid scintillation spectrometry : horizontal bar indicates position of GA_3 : results confirmed in one further experiment.

V = coleoptiles orientated in vertical position

HU = coleoptiles orientated in horizontal position with {^{14}C} GA_3 application on upper side

HL = coleoptiles orientated in horizontal position with {^{14}C} GA_3 application on lower side

S = stock {^{14}C} GA_3 solution

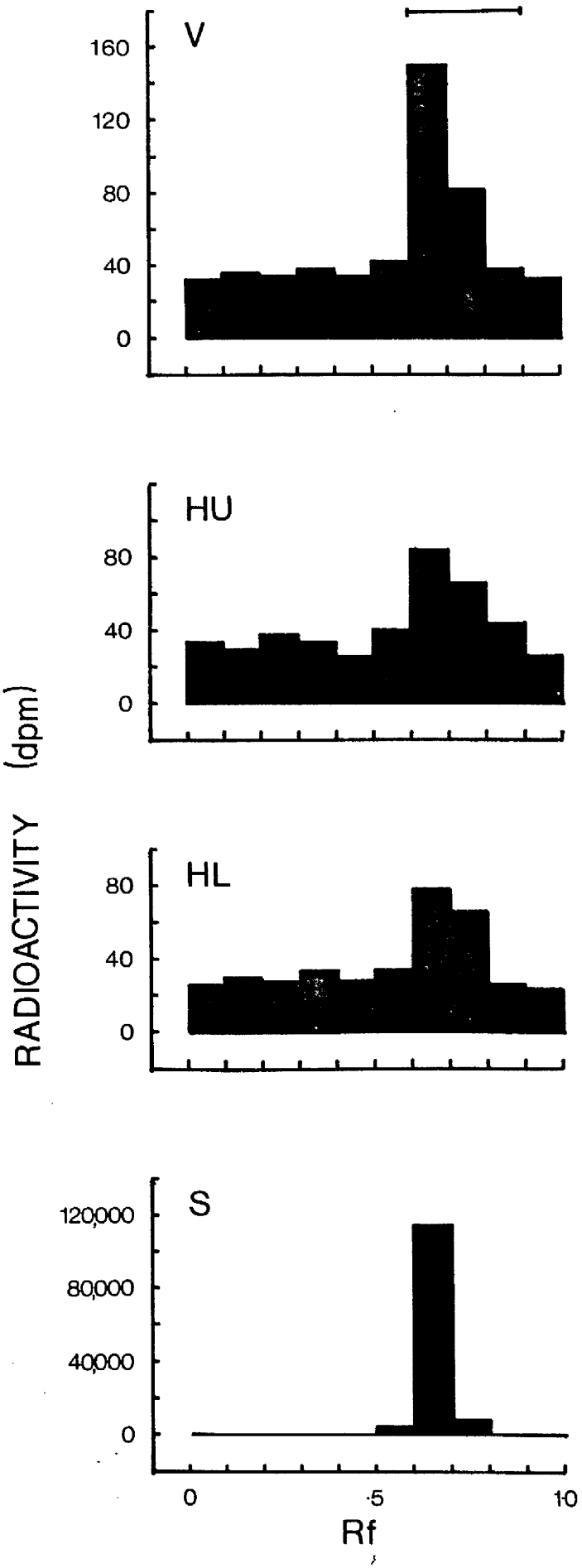

V
160
120
80
40
0
HU
80
40
0
HL
80
40
0
RADIOACTIVITY (dpm)
S
120,000
80,000
40,000
0
0
·5
1·0
Rf

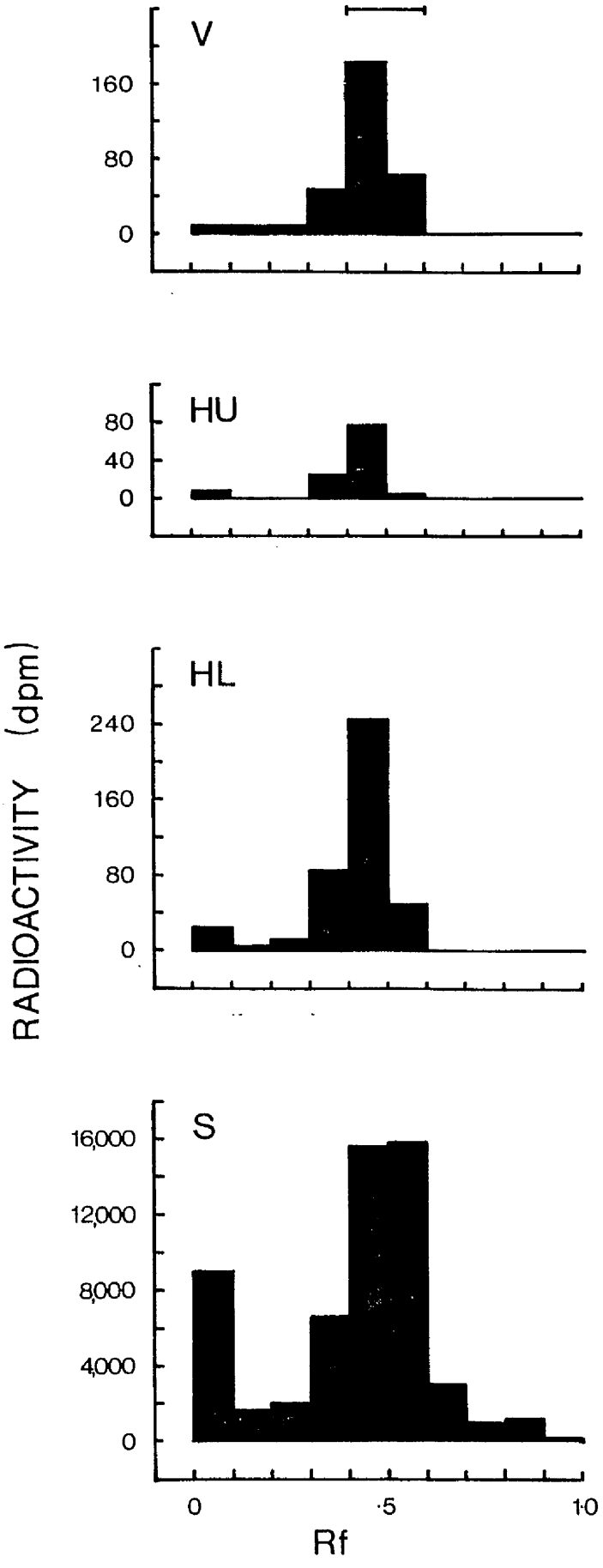
Figure 30: Chromatographic analysis of ethyl acetate soluble extracts from vertical and horizontal Zea coleoptiles 6 h after application of {^{14}C} GA_3. Analyses were carried out on paper chromatograms developed in isopropanol : ammonia : water :: 10 : 1 : 1. Assayed by liquid scintillation spectrometry ; horizontal bar indicates position of GA_3 : results confirmed in one further experiment.

V = coleoptiles orientated in vertical position.

HU = coleoptiles orientated in horizontal position with {^{14}C} GA_3 application on upper side.

HL = coleoptiles orientated in horizontal position with {^{14}C} GA_3 application on the lower side.

S = stock {^{14}C} GA_3 solution.


V
160
80
0
HU
80
40
0
HL
240
160
80
0
S
16,000
12,000
8,000
4,000
0
RADIOACTIVITY (dpm)
0
·5
1·0
Rf

chromatographed with the acidic solvent system and the results are shown in Figure 31. Each of the three chromatograms produced one major peak of activity between Rf 0.6 - 0.9 and this co-chromatographed with the major peak of activity produced in the chromatograms of the {^{3}H} GA_1 stock solution and unlabelled GA_1. The extract from seedlings that had been orientated horizontally with the point of application on the lower side also produced a minor peak of activity at Rf 1.0 and this co-chromatographed with a minor peak at the same Rf value which appeared as an impurity in the stock solution of {^{3}H} GA_1. The extracts from the other two batches of seedlings did not reveal this minor peak. The stock solution of {^{3}H} GA_1 also revealed another minor peak of activity at Rf 0.1 but this was not obtained on chromatograms of the tissue extracts.

Using the basic solvent system (Figure 32) all three extracts from the seedlings revealed a major band of activity between Rf 0.4 - 0.6 and this co-chromatographed with a corresponding band of activity in the chromatograms of stock solutions of {^{3}H} GA_1 and unlabelled GA_1. A minor peak of activity between Rf 0.8 - 1.0 was also produced by the plant extracts and although this was also observed on the {^{3}H} GA_1 stock solution chromatogram, it was much reduced. The stock solution of {^{3}H} GA_1 also produced a major peak of activity at Rf 0.1 which corresponded with a small peak at an identical Rf value on the chromatogram

Figure 31: Chromatographic analysis of ethyl acetate soluble extracts from vertical and horizontal Zea coleoptiles 3 h after application of {^{3}H} GA_1. Analyses were carried out on paper chromatograms developed in methyl ethyl ketone : acetic acid :: 95 : 5. Assayed by liquid scintillation spectrometry : horizontal bar indicates position of GA_1 : results confirmed in one further experiment.

V = coleoptiles orientated in vertical position

HU = coleoptiles orientated in horizontal position with {^{3}H} GA_1 application on upper side

HL = coleoptiles orientated in horizontal position with {^{3}H} GA_1 application on lower side

S = stock {^{3}H} GA_1 solution

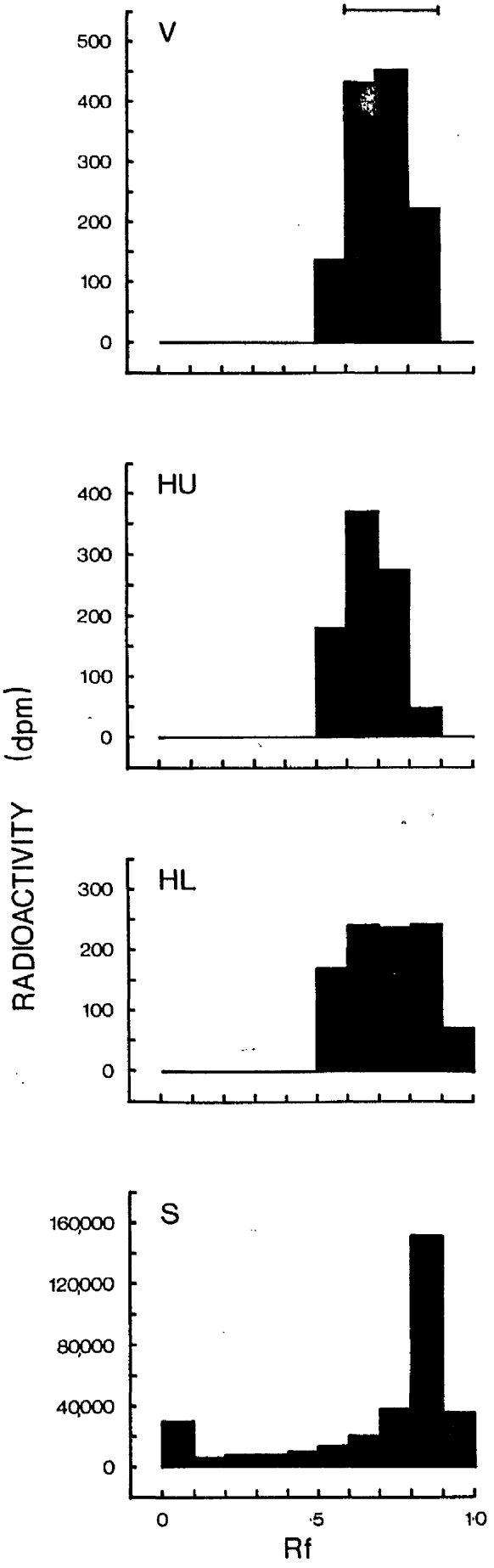

V
500
400
300
200
100
0
HU
400
300
200
100
0
HL
300
200
100
0
RADIOACTIVITY (dpm)
S
160,000
120,000
80,000
40,000
0
0
·5
1·0
Rf

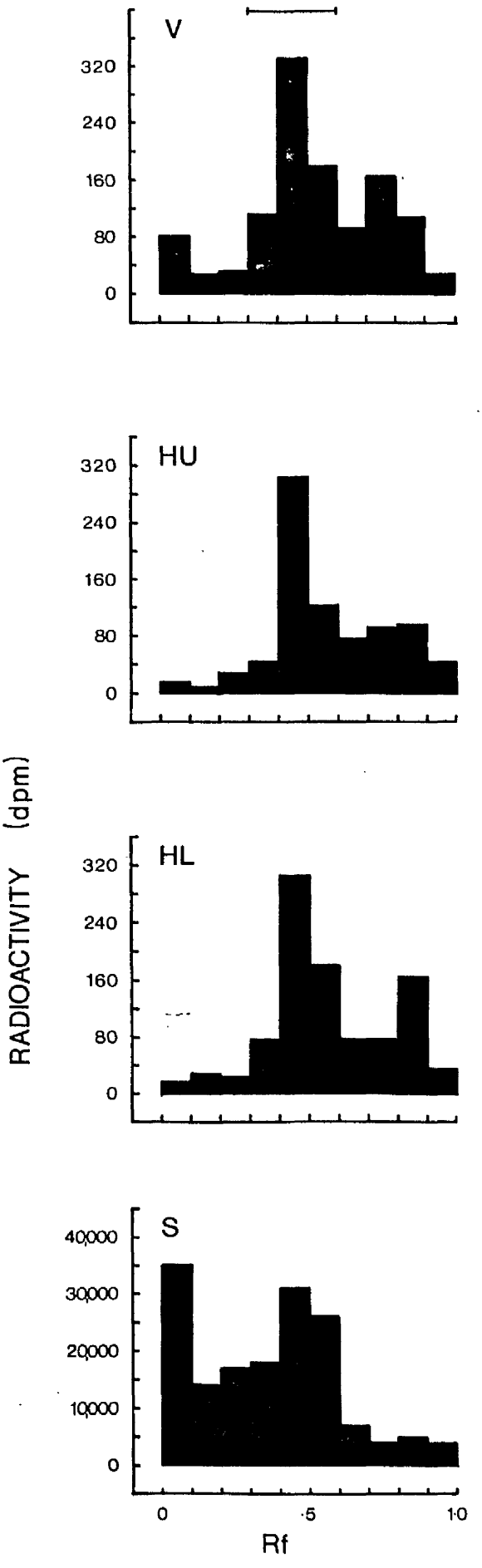
Figure 32: Chromatographic analysis of ethyl acetate soluble extracts from vertical and horizontal Zea coleoptiles 3 h after application of {^{3}H} GA_1.
Analyses were carried out on paper chromatograms developed in isopropanol : ammonia : water :: 10 : 1 : 1. Assayed by liquid scintillation spectrometry : horizontal bar indicates position of {^{3}H} GA_1 : results confirmed in one further experiment.

V = coleoptiles orientated in vertical position

HU = coleoptiles orientated in horizontal position with {^{3}H} GA_1 application on upper side

HL = coleoptiles orientated in horizontal position with {^{3}H} GA_1 application on lower side

S = stock {^{3}H} GA_1 solution


V
320
240
160
80
0
HU
320
240
160
80
0
HL
320
240
160
80
0
RADIOACTIVITY (dpm)
S
40,000
30,000
20,000
10,000
0
0
·5
1·0
Rf

of the extract from vertically orientated seedlings. A very small peak occurred at Rf 0.1 on the chromatograms of extracts from seedlings that were horizontally orientated.

19. The Extraction of Radioactivity from Roots of Zea Mays Seedlings Following the Application of {^{14}C} GA_3 to the Root Apex.

Using the acidic solvent system (Figure 33), extracts from all three batches of roots receiving geotropic stimulation, produced one major peak of activity between Rf 0.7 - 1.0 which also co-chromatographed with the major peak of activity from stock solutions of {^{14}C} GA_3 and unlabelled GA_3. The stock solution of {^{14}C} GA_3 also produced a minor peak of activity at the origin which was not present in chromatograms of tissue extracts. Similarly, with the basic solvent system (Figure 34), the majority of the radioactivity extracted from the root tissue was indistinguishable from the radioactivity present in the {^{14}C} GA_3 stock solution. All three extracts produced a band of activity between Rf 0.4 - 0.6 which corresponded exactly to the main zones of activity in the chromatograms of the stock solutions of labelled and unlabelled GA_3. The {^{14}C} GA_3 stock solution also revealed a minor peak of activity associated with the origin and this was also found to be present at an identical Rf value on the chromatograms of the extracts from the plant tissue.

Figure 33: Chromatographic analysis of ethyl acetate soluble extracts from vertical and horizontal Zea roots 6 h after application of {^{14}C} GA_3. Analyses were carried out on paper chromatograms developed in methyl ethyl ketone : acetic acid :: 95 : 5. Assayed by liquid scintillation spectrometry : horizontal bar indicates position of {^{14}C} GA_3 : results confirmed in one further experiment.

V = roots orientated in vertical position

HU = roots orientated in horizontal position with {^{14}C} GA_3 application on upper side

HL = roots orientated in horizontal position with {^{14}C} GA_3 application on lower side

S = stock {^{14}C} GA_3 solution

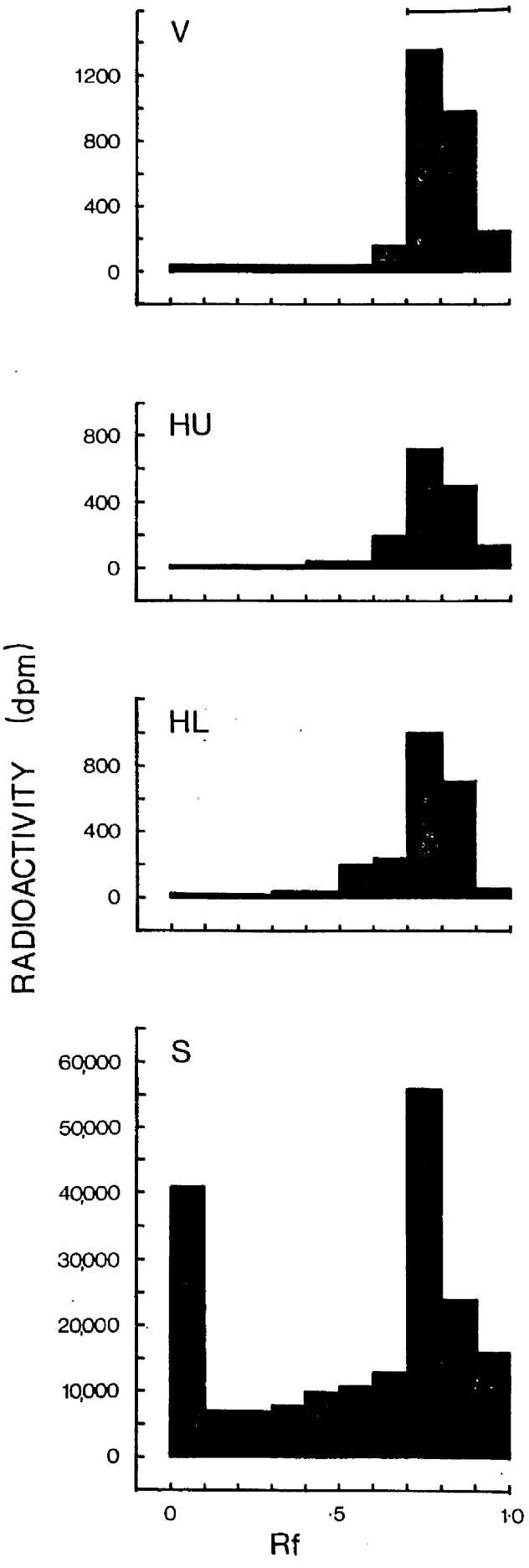

V
1200
800
400
0
HU
800
400
0
HL
800
400
0
RADIOACTIVITY (dpm)
S
60,000
50,000
40,000
30,000
20,000
10,000
0
0
·5
1·0
Rf

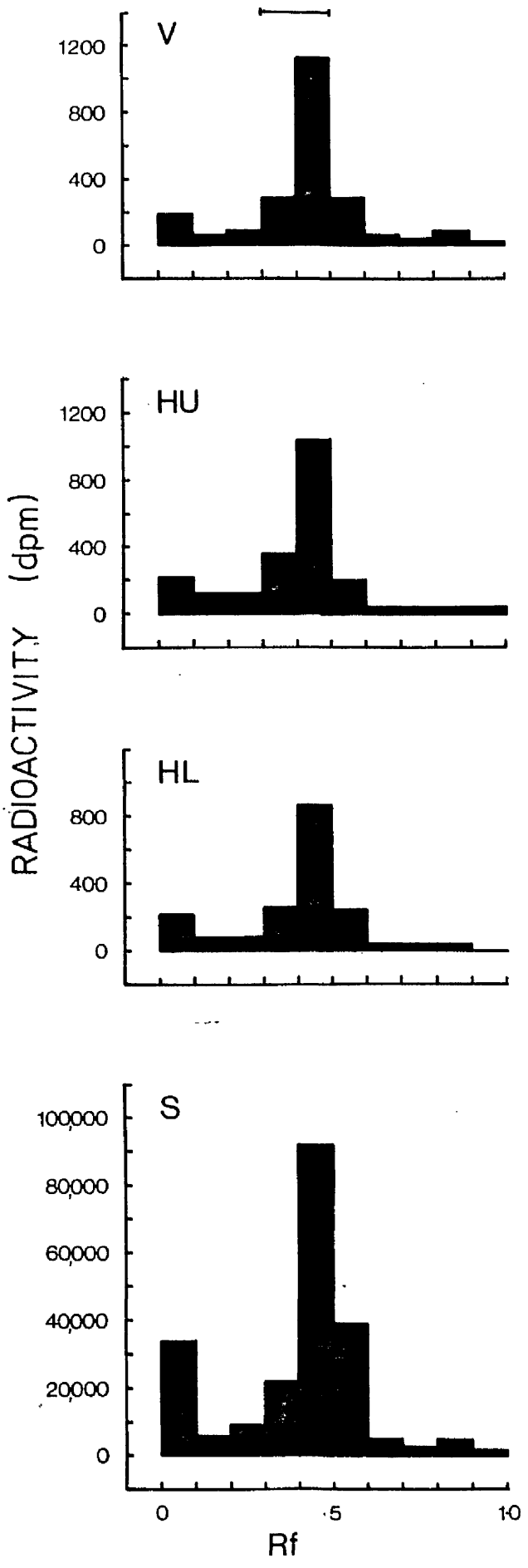
Figure 34: Chromatographic analysis of ethyl acetate soluble extracts from vertical and horizontal Zea roots 6 h after application of {^{14}C} GA_3. Analyses were carried out on paper chromatograms developed in isopropanol : ammonia : water :: 10 : 1 : 1. Assayed by liquid scintillation spectrometry : horizontal bar indicates position of GA_3 : results confirmed in one further experiment.

V = roots orientated in vertical position

HU = roots orientated in horizontal position with {^{14}C} GA_3 application on upper side

HL = roots orientated in horizontal position with {^{14}C} GA_3 application on lower side

S = stock {^{14}C} GA_3 solution


V
1200
800
400
0
HU
1200
800
400
0
HL
800
400
0
S
100,000
80,000
60,000
40,000
20,000
0
0
·5
1·0
Rf
RADIOACTIVITY (dpm)

20. The Extraction of Radioactivity from Roots of Zea Mays Seedlings following the Application of {^{3}H} GA_1 to the Root Apex.

All three extracts from the root tissue produced one major band of radioactivity when chromatographed with the acidic solvent system (Figure 35). Extracts from roots that were orientated either vertically or horizontally with the point of donation on the lower side produced a major peak of activity between Rf 0.7 - 0.9. However the extract from roots that were orientated horizontally with the point of donation on the upper side, produced a band of activity between Rf 0.8 - 1.0 and this co-chromatographed exactly with the major band of activity of the {^{3}H} GA_1 stock solution. In spite of this slight discrepancy, it seems reasonable to suppose that all four of the major peaks were associated with unchanged {^{3}H} GA_1. The unlabelled GA_1 produced a peak of activity at Rf 0.8 - 1.0. The chromatogram of the {^{3}H} GA_1 stock solution also revealed a minor peak of activity at Rf 0.1 but this was absent from the three chromatograms of the extracts from the plant tissues.

Similarly with the basic solvent system (Figure 36) one band of activity between Rf 0.4 - 0.6 was produced on all three chromatograms of extracts from the plant tissue which co-chromatographed with both unlabelled GA_1 and the major

Figure 35: Chromatographic analysis of ethyl acetate soluble extracts from vertical and horizontal Zea roots 3 h after application of {^{3}H} GA_1. Analyses were carried out on paper chromatograms developed in methyl ethyl ketone : acetic acid :: 95 : 5. Assayed by liquid scintillation spectrometry : horizontal bar indicates position of GA_1 : results confirmed in one further experiment.

V = roots orientated in vertical position

HU = roots orientated in horizontal position with {^{3}H} GA_1 application on upper side

HL = roots orientated in horizontal position with {^{3}H} GA_1 application on lower side

S = stock {^{3}H} GA_1 solution

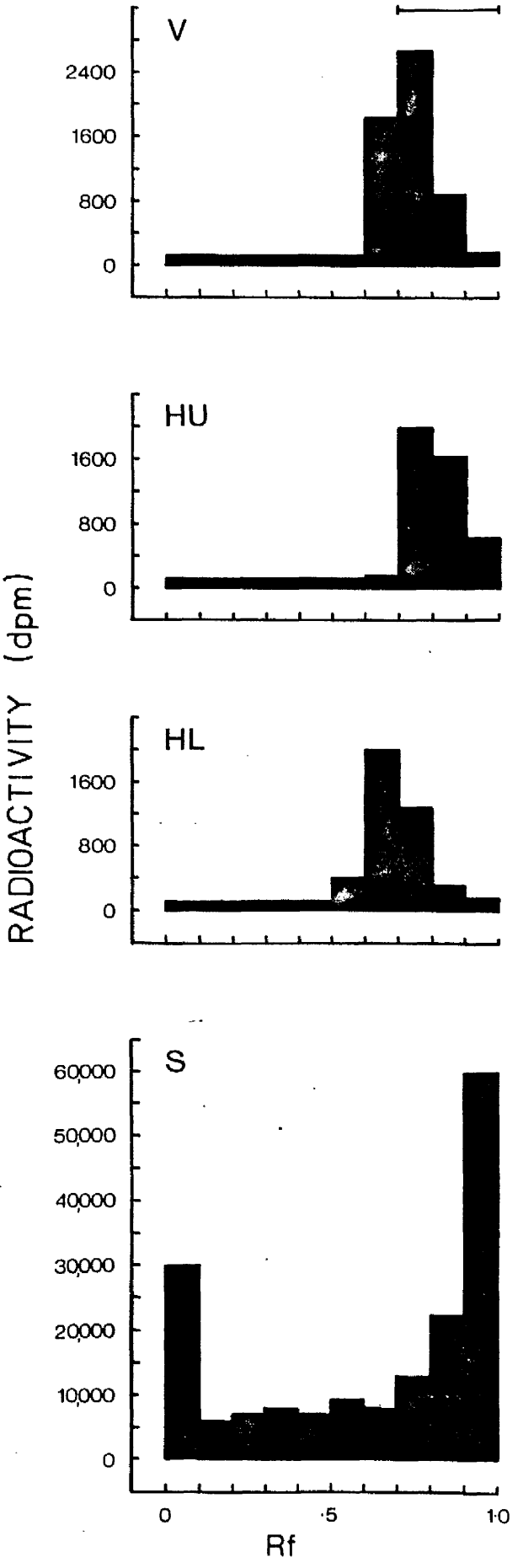

V
2400
1600
800
0
HU
1600
800
0
HL
1600
800
0
RADIOACTIVITY (dpm)
S
60,000
50,000
40,000
30,000
20,000
10,000
0
0
·5
1·0
Rf

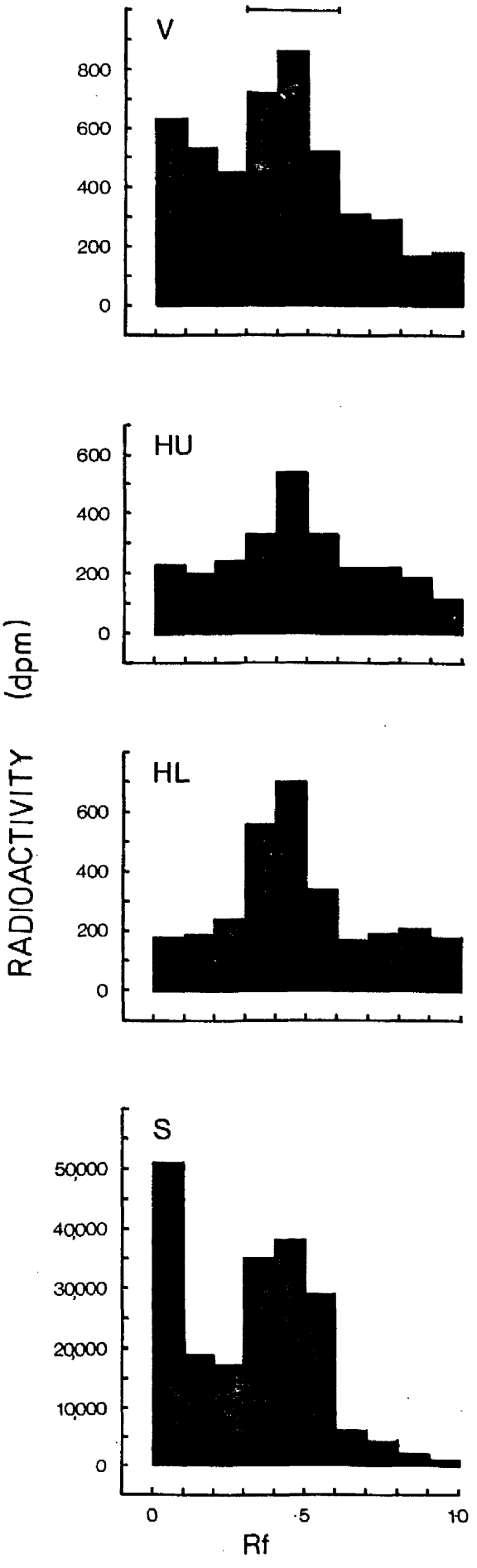
Figure 36: Chromatographic analysis of ethyl acetate soluble extracts from vertical and horizontal Zea roots 3 h after application of {^{3}H} GA_1. Analyses were carried out on paper chromatograms developed in isopropanol : ammonia : water :: 10 : 1 : 1. Assayed by liquid scintillation spectrometry : horizontal bar indicates position of GA_1 : results confirmed in one further experiment.

V = roots orientated in vertical position

HU = roots orientated in horizontal position with {^{3}H} GA_1 application on lower side

HL = roots orientated in horizontal position with {^{3}H} GA_1 application on lower side

S = stock {^{3}H} GA_1 solution


V
800
600
400
200
0
HU
600
400
200
0
HL
600
400
200
0
RADIOACTIVITY (dpm)
S
50,000
40,000
30,000
20,000
10,000
0
0
·5
1·0
Rf

peak of radioactivity from the {^{3}H} GA_1 stock solution. The chromatogram of the {^{3}H} GA_1 stock solution also revealed a second peak of activity at the origin. This was also produced on the chromatogram of the extract from vertical roots but was absent from those extracts of horizontally orientated roots.

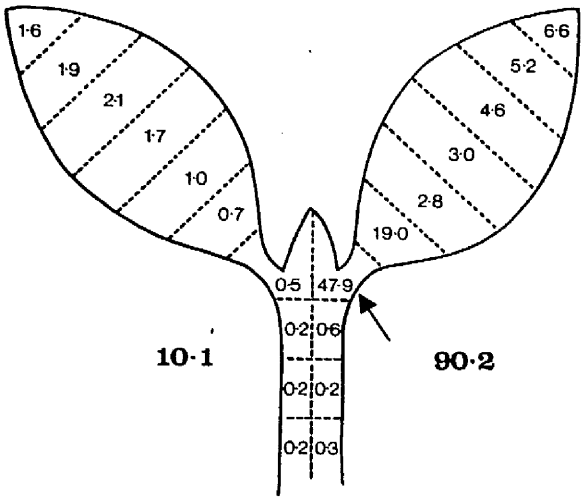
21. The Movement of Radioactivity from {^{3}H} GA_1 Applied Asymmetrically to Hypocotyls and Epicotyls of Intact Seedlings of Helianthus annuus.

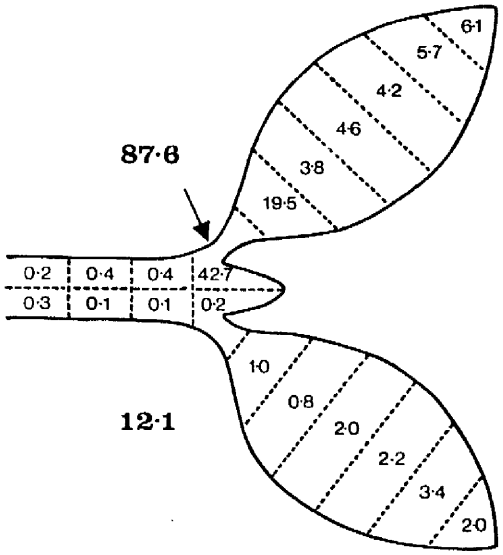
The longitudinal and lateral movement of radioactivity from {^{3}H} GA_1 has been investigated in both hypocotyls and epicotyls of geotropically stimulated, intact seedlings of Helianthus annuus. The {^{3}H} GA_1 was applied asymmetrically to either the hypocotyl, at a pre-determined point immediately below the cotyledonary node or to the epicotyl, at a point below the node of the first pair of true leaves in the first elongating internode (= epicotyl). After asymmetric application, the seedlings were orientated either vertically or horizontally with the point of application on either the upper or lower side. The seedlings were harvested and bisected for radioassay as described previously after either 3 h or 6 h. The results are shown in Figures 37 - 40 in which the data are the mean of at least 2 independent experiments.

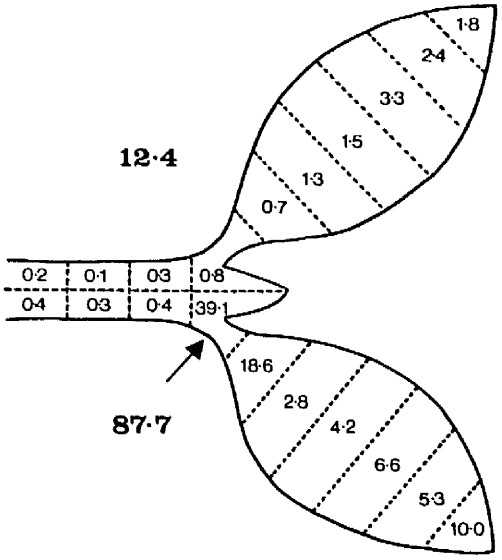
In horizontal hypocotyls (Figure 37) the proportion of the total applied radioactivity recovered from the side opposite to the point of donation after 3 h, was 12.1% when the donated side was uppermost and 12.4% when the donated side was lowermost. In vertically orientated seedlings, 10.1% was recovered from the side of the seedling opposite to that of donation. Therefore approximately 10 - 12% of the applied radioactivity was recovered from the non-donated side of the seedling regardless of the orientation of the seedlings with respect to gravity. Thus the lateral movement of radioactivity from {^{3}H} GA_1 was not enhanced by geotropic stimulation.

The pattern of distribution of radioactivity within the seedlings was similar for each orientation. There was virtually no basipetal movement of radioactivity. On the donated side of the seedlings, only 1% of the radioactivity was found below the point of application. Between 39 and 48% of the radioactivity remained in the region of application although there was a slight acropetal movement from the original application point up into the cotyledon. Approximately 19% of the applied radioactivity was found in the basal region of the cotyledon adjacent to the original point of application. However between 22 and 29% had moved upwards in an acropetal direction and was distributed throughout the cotyledon. On the nondonated side of the seedling between 0.7 and 1.4% of the radioactivity was present in the hypocotyl and between 9 and 11.4% was recovered from the cotyledon.

Figure 37: Percent distribution of radioactivity after 3 h in horizontal and vertical hypocotyls of intact Helianthus annuus seedlings. Cotyledons were removed by a cut immediately above the cotyledonary node and were then divided transversely into six equal sized portions. The hypocotyl was bisected longitudinally and then divided transversely into the cotyledonary node, two 10 mm portions and the remainder of the hypocotyl (dotted lines). The arrows indicate the position of application of {^{3}H} GA_1. These data are the mean of two separate experiments.


1·6
1·9
2·1
1·7
1·0
0·7
6·6
5·2
4·6
3·0
2·8
19·0
0·5
47·9
0·2
0·6
0·2
0·2
0·2
0·3
10·1
90·2

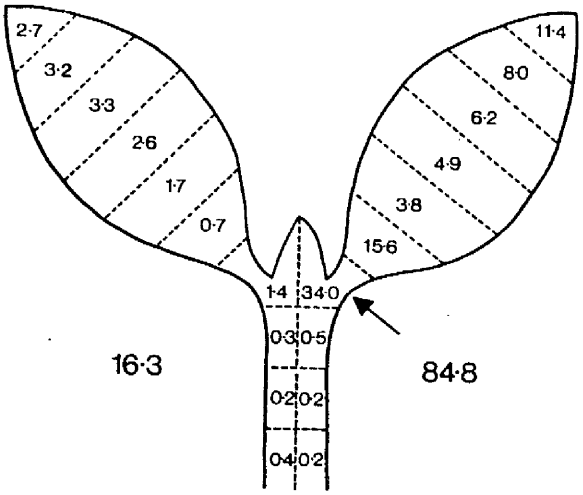

6·1
5·7
4·2
4·6
3·8
19·5
87·6
0·2
0·4
0·4
42·7
0·3
0·1
0·1
0·2
1·0
0·8
2·0
2·2
3·4
2·0
12·1

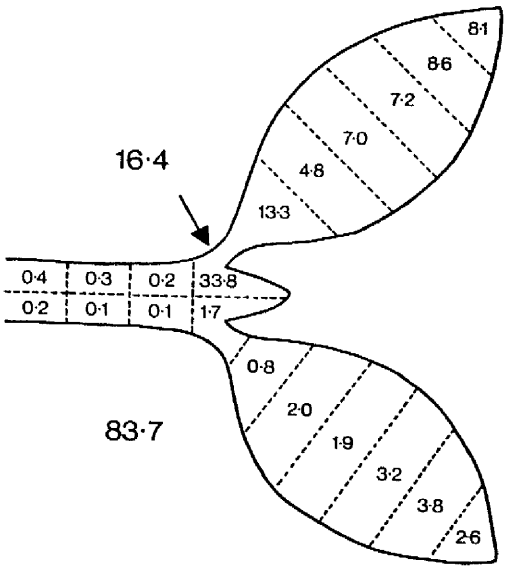

1·8
2·4
3·3
1·5
1·3
0·7
12·4
0·2
0·1
0·3
0·8
0·4
0·3
0·4
39·1
18·6
2·8
4·2
6·6
5·3
10·0
87·7

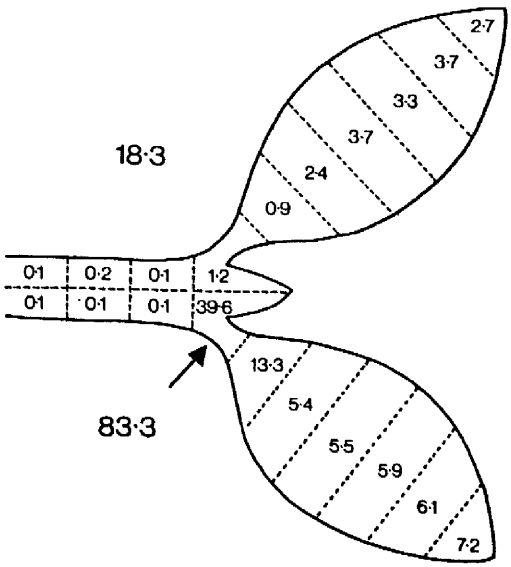
Similar results were obtained for hypocotyls after a 6 h transport period (Figure 38). Geotropic stimulation did not appear to enhance the lateral movement of radioactivity since between 16 and 18% of the total radioactivity applied was recovered from the nondonated side of the seedlings regardless of their orientation with respect to gravity. There was an obvious increase in the proportion of radioactivity that had moved laterally with the increase in time; i.e.10 - 12% after 3 h and 16 - 18% after 6 h. As was the case after 3 h, the distribution pattern within the seedlings was the same regardless of whether seedlings were orientated vertically or horizontally. Again there was still no basipetal movement even after the increase in the transport time. In fact on the donated side of the seedling, less radioactivity was found below the point of application after 6 h (0.3 - 0.9%) than after 3 h. Slightly less (34 - 40%) of the applied radioactivity was found where it was originally applied and approximately 13 - 16% was found in the basal region of the cotyledon adjacent to the point of application. There had therefore been an increase in the acropetal movement and 30 - 36% of the radioactivity was recovered from the middle and apical regions of the cotyledon. On the nondonated side 1.6 - 2.1% was found in the hypocotyl and between 14 and 17% was recovered from the cotyledon.

The results obtained with epicotyls were somewhat similar to those for hypocotyls in that no significant difference in the lateral distribution of radioactivity was

Figure 38: Percent distribution of radioactivity after 6 h in horizontal and vertical hypocotyls of intact Helianthus annuus seedlings. Cotyledons were removed by a cut immediately above the cotyledonary node and were then divided transversely into six equal sized portions. The hypocotyl was bisected longitudinally and then divided transversely into the cotyledonary node, two 10 mm portions and the remainder of the hypocotyl (dotted lines). The arrows indicate the position of application of {^{3}H} GA_1. These data are the mean of two separate experiments.

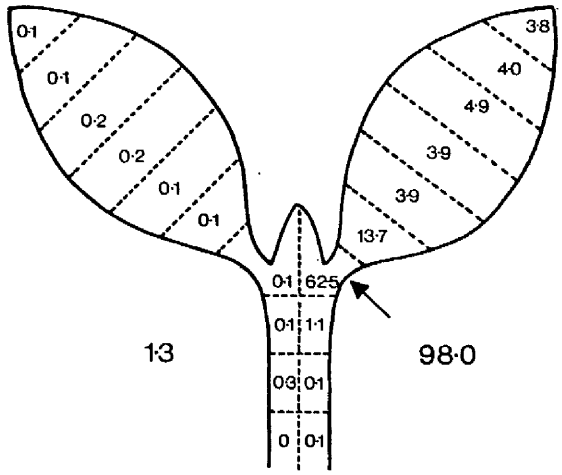

2·7
3·2
3·3
2·6
1·7
0·7
11·4
8·0
6·2
4·9
3·8
15·6
1·4
34·0
0·3
0·5
0·2
0·2
0·4
0·2
16·3
84·8

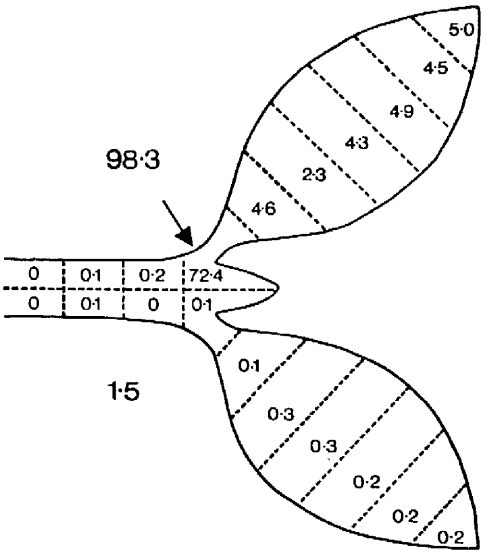

8·1
8·6
7·2
7·0
4·8
13·3
16·4
0·4
0·3
0·2
33·8
0·2
0·1
0·1
1·7
0·8
2·0
1·9
3·2
3·8
2·6
83·7


2·7
3·7
3·3
3·7
2·4
0·9
18·3
0·1
0·2
0·1
1·2
0·1
0·1
0·1
39·6
13·3
5·4
5·5
5·9
6·1
7·2
83·3

detected between vertically and horizontally orientated seedlings. In fact after 3 h, (Figure 39) there was very little lateral movement of radioactivity into the nondonated side at all. In vertical seedlings 1.4% was recovered from the nondonated side. The corresponding values for horizontal seedlings were 1.5% when the donated side was uppermost and 3.0% when the donated side was lowermost. On the donated side of seedlings there was virtually no basipetal movement i.e. only 1% of the applied radioactivity was found below the point of application. More than 58% of the radioactivity remained in the region of application although 26 - 37% was found fairly evenly distributed throughout the first true leaf. The small percentage of radioactivity recovered from the nondonated side of the seedling was distributed throughout the epicotyl and leaf tissue. After 6 h (Figure 40) there was an increase in the percentage of radioactivity which had moved laterally into the nondonated side of the seedling. Between 9 and 12% of the applied radioactivity was recovered from the side opposite to the donated side but there was no evidence of an enhanced lateral migration of radioactivity due to a prolonged geotropic stimulation. There was still no significant basipetal movement of radioactivity. In horizontal seedlings 55 - 60% of the applied radioactivity remained in the region of donation whereas in seedlings that were orientated vertically only 44% was recovered from the original region of application. In other words, there was more acropetal

Figure 39: Percent distribution of radioactivity after 3 h in horizontal and vertical epicotyls of intact Helianthus annuus seedlings. Leaves were removed by a cut immediately above the node and were then divided transversely into six equal sized portions. The epicotyl was bisected longitudinally and then divided transversely into the nodal region, two 10 mm portions and the remainder of the epicotyl (dotted lines). The arrows indicate the position of application of {^{3}H} GA_1. These data are the mean of two separate experiments.


0·1
0·1
0·2
0·2
0·1
0·1
3·8
4·0
4·9
3·9
3·9
13·7
0·1
6·25
0·1
1·1
0·3
0·1
0
0·1
1·3
98·0


5·0
4·5
4·9
4·3
2·3
4·6
98·3
0
0·1
0·2
72·4
0
0·1
0
0·1
0·1
0·3
0·3
0·2
0·2
0·2
1·5

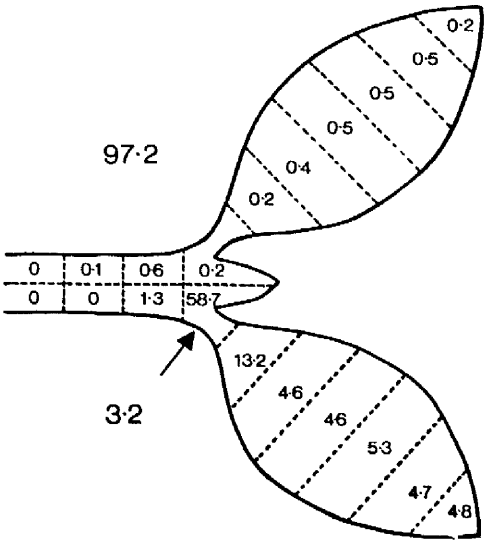
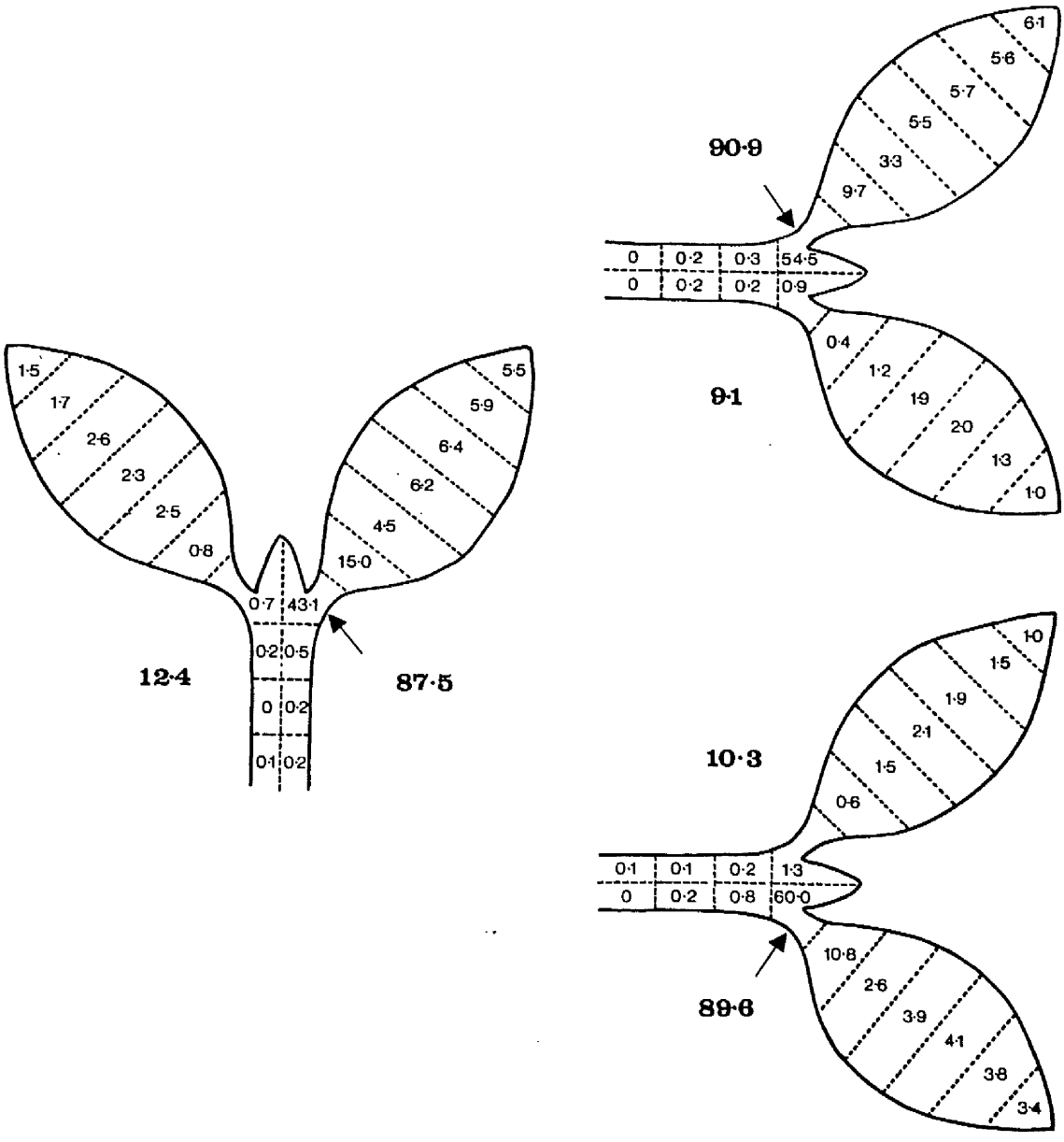

0·2
0·5
0·5
0·5
0·4
0·2
97·2
0
0·1
0·6
0·2
0
0
1·3
58·7
13·2
4·6
4·6
5·3
4·7
4·8
3·2

Figure 40: Percent distribution of radioactivity after 6 h in horizontal and vertical epicotyls of intact Helianthus annuus seedlings. Leaves were removed by a cut immediately above the node and were then divided transversely into six equal sized portions. The epicotyl was bisected longitudinally and then divided transversely into the nodal region, two 10 mm portions and the remainder of the epicotyl (dotted lines). The arrows indicate the position of application of [^{3}H] GA_1. These data are the mean of two separate experiments.


90·9
6·1
5·6
5·7
5·5
3·3
9·7
0
0·2
0·3
54·5
0
0·2
0·2
0·9
0·4
1·2
1·9
2·0
1·3
1·0
9·1
1·5
1·7
2·6
2·3
2·5
0·8
5·5
5·9
6·4
6·2
4·5
15·0
0·7
43·1
0·2
0·5
0
0·2
0·1
0·2
12·4
87·5
10·3
1·0
1·5
1·9
2·1
1·5
0·6
0·1
0·1
0·2
1·3
0
0·2
0·8
60·0
10·8
2·6
3·9
4·1
3·8
3·4
89·6

movement in vertical seedlings since 44% was present in the node and leaf above the point of application whereas in horizontal seedlings the percentage was less (29 - 36%). The radioactivity which was present in the nondonated side was distributed throughout the epicotyl and leaf tissue. There was no difference in the pattern of distribution with relation to the geotropic orientation of the seedlings.

Thus it is apparent from these results that there is no enhanced lateral movement of radioactivity in either hypocotyls or epicotyls as a result of geotropic stimulation.

B. ISOLATION OF ENDOGENOUS GIBBERELLINS.

The second part of this project has been concerned with attempts which have been made to identify and quantify the presence of endogenous gibberellin-like substances in and exported from, coleoptiles of Zea Mays and young shoot apices of Helianthus annuus. The results presented in the remainder of this section describe such experiments which were performed using a combination of extraction and 'agar diffusion' techniques.

1. Collection in Agar of Endogenous Gibberellin-like Substances from Coleoptiles of Zea Mays.

The 'agar diffusion' technique was used to collect gibberellin-like substances translocated out of isolated

coleoptiles apices. The agar was extracted with methanol, partitioned and the resulting fractions subjected to chromatography as described previously, before bioassay. Controls of plain agar, standard GA_3 in agar and standard GA_3 solutions were also tested.

(i) Standard GA_3

The procedure used to extract the agar was tested for efficiency with standard GA_3. A series of agar plates were made; each plate incorporating 10 ml of 20 g l^{-1} ion agar and 10 ml of the following standard solutions of GA_3:- 0.0001, 0.001, 0.01, 0.1, 1.0, 10.0 µg/ml. There was a control plate of plain agar. All plates were allowed to stand for 24 h in darkness at 25°C before being subjected to the extraction, partitioning and thin-layer chromatography procedure. Plates were developed in the basic solvent system and assayed with the lettuce hypocotyl bioassay (Figure 41). By reference to the GA_3 standards the approximate quantity of growth activity was estimated in µgs for each chromatogram and the total recovery from each plate was calculated by summing gibberellin activity from acidic and basic fractions (Table 1).

At the 0.0001 µg/ml concentration there was some growth activity at Rf 0.3 of the acidic ethyl acetate soluble fraction which promoted the length of the hypocotyl to 3.6 mm (1.6 mm more than the control) but since a similar value was also obtained at the same Rf in the acidic fraction of the plain agar extract, clearly the slight promotion of growth

Figure 41: Lettuce hypocotyl bioassay of acidic and basic plus neutral ethyl acetate soluble fractions from agar plates containing the following standard solutions of GA_3 :-

Chromatogram	GA_3 (µg/ml)
A	0
B	0.0001
C	0.001
D	0.01
E	0.1
F	1.0
G	10.0

Data are the mean length of lettuce hypocotyls for each Rf zone of chromatogram. Horizontal line (C) = control zone of chromatogram immediately behind the origin. Elongation in GA_3 control solutions are shown.

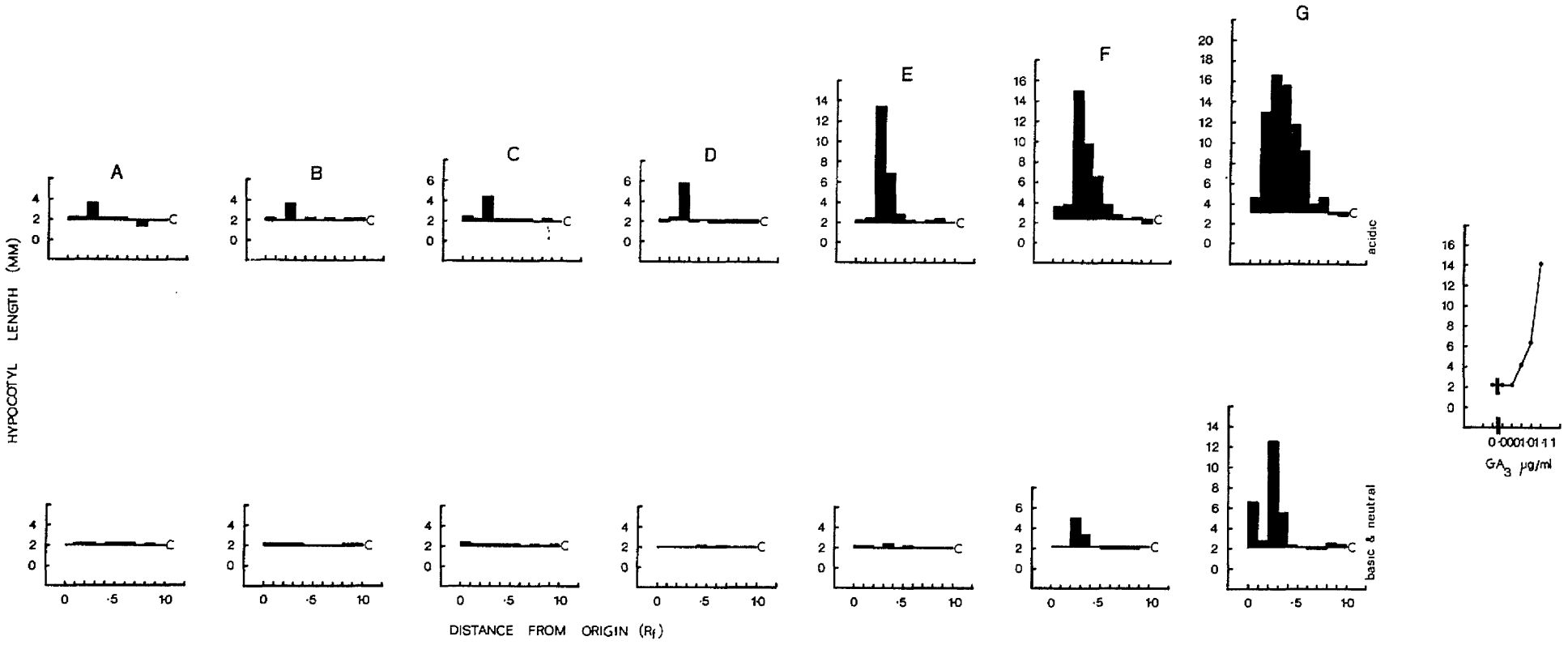

A
B
C
D
E
F
G
HYPOCOTYL LENGTH (MM)
DISTANCE FROM ORIGIN (Rf)
acidic
basic & neutral
0·0001 0·1 1 1
GA3 µg/ml

TABLE 1

Original conc. GA_3 / plate µg/ml	ACIDIC Rf zone	Hypocotyl Length mm	GA_3 equivalents µg/ml	Total recovery for each chromatogram µg	BASIC + NEUTRAL Rf zone	Hypocotyl Length mm	GA_3 equivalents µg/ml	Total recovery for each chromatogram µg	Total recovery for each GA_3 plate µg
0	0.3	3.6	0.014	0.014	No growth promoting activity				0.014
0.0001	0.3	3.6	0.014	0.014	No growth promoting activity				0.014
0.001	0.1	2.4	0.003	0.023	0.1	2.4	0.003	0.003	0.026
	0.3	4.4	0.02						
0.01	0.2	2.4	0.003	0.103	No growth promoting activity				0.103
	0.3	5.8	0.1						
0.1	0.2	2.4	0.003						
	0.3	13.4	1.8						
	0.4	6.8	0.16	1.972	0.4	2.4	0.003	0.003	1.975
	0.5	2.8	0.006						
	0.9	2.4	0.003						
1.0	0.1	3.6	0.014						
	0.2	3.8	0.016						
	0.3	16.0	2.0 *		0.3	5.0	0.04	0.052	3.071
	0.4	9.8	0.8		0.4	3.4	0.012		
	0.5	6.6	0.16	3.019					
	0.6	3.8	0.016						
	0.7	2.8	0.006						
	0.8	2.4	0.003						
	0.9	2.6	0.004						
10.0	0.1	4.6	0.04		0.1	6.6	0.16		
	0.2	13.0	1.6		0.2	2.8	0.006		
	0.3	16.6	2.0 *		0.3	12.6	1.4		
	0.4	15.6	2.0 *		0.4	5.6	0.08		
	0.5	11.8	1.2	7.314	0.5	2.4	0.003	1.656	8.97
	0.6	9.2	0.4						
	0.7	4.0	0.02						
	0.8	4.6	0.04						
	0.9	3.0	0.008		0.9	2.6	0.004		
	1.0	2.8	0.006		1.0	2.4	0.003		

was not due to the GA_3 but rather was as a result of the extraction procedure or the agar itself. The chromatogram of the extract from the 0.001 µg/ml concentration exhibited some growth activity at Rf 0.3 equivalent to 0.02 µg of GA_3. The hypocotyls were promoted to a length of 4.4 mm which was 2.4 mm more than the control. There was a slightly bigger increase in the growth promoting activity obtained at Rf 0.3 of the chromatogram of the acidic fraction of the 0.01 µg/ml concentration. Hypocotyls attained lengths of 5.8 mm which was equivalent to 0.1 µgs of GA_3. The acidic fraction from the 0.1 µg/ml extract produced a band of growth promoting activity at Rf 0.3 - 0.4; hypocotyls attained lengths of 13.4 mm and 6.8 mm, equivalent to 1.8 and 0.16 µgs of GA_3 respectively. Extracts from the lower concentrations i.e. 0.0001 - 0.1 µg/ml exhibited no significant growth promoting activity in the basic plus neutral ethyl acetate soluble fractions.

However at the concentration of 1.0 µg/ml there was growth activity exhibited in both the acidic and basic ethyl acetate soluble fractions. The acidic fractions produced a major band of growth promoting activity at Rf 0.3 - 0.5. It was difficult to calculate the approximate activity in µgs of GA_3 since at Rf 0.3, hypocotyls were promoted to a length of 16.0 mm, which was beyond the limits of the calibration curve for the range of standard GA_3 solutions. When constructing the standard curve, hypocotyl lengths of 14.2 and 14.0 mm were obtained at concentrations of 1.0 µg/ml and 10.0 µg/ml which tended to suggest that the hypocotyls

had reached their maximum growth; i.e. any further increase in GA_3 concentration would produce no further increase in length. This is difficult to reconcile with the hypocotyl lengths obtained from the extracts of the GA_3/agar plates, which were considerably greater than those elicited by 1 µg/ml in the calibration curve of the GA_3 standard solutions. At Rf 0.4 and 0.5, hypocotyls were 9.8 and 6.6 mm in length equivalent to 0.8 and 0.16 µg GA_3 respectively. The basic plus neutral fraction showed growth promoting activity at Rf 0.3 - 0.4 equivalent to 0.04 and 0.012 µg GA_3.

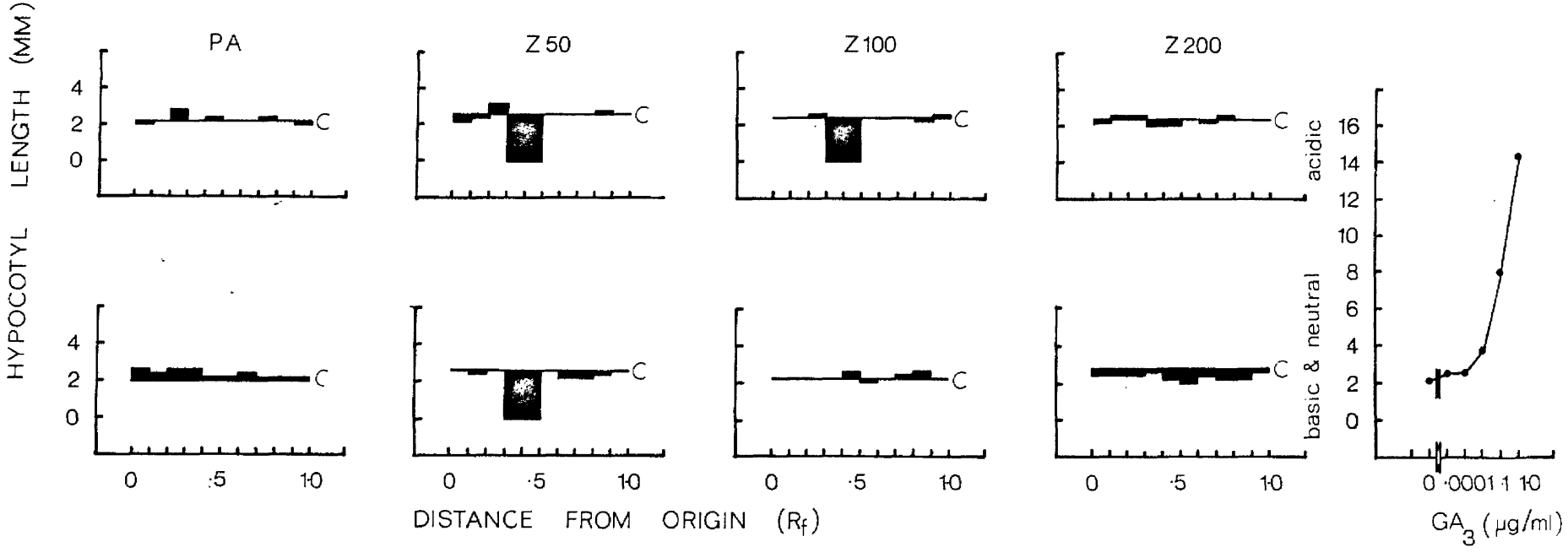
The acidic fraction from the 10.0 µg/ml extract produced a major band of activity between Rf 0.2 (1.6 µgs GA_3) - 0.6 (0.4 µgs GA_3) with two minor peaks at Rf 0.1 and 0.8. As was the case for the acidic fraction from the 1.0 µg/ml extract, it was difficult to calculate the GA_3 equivalent in µg from Rf 0.3 and 0.4 since the length of the hypocotyls, 16.6 and 15.6 mm respectively, were beyond the limit of the standard curve. The basic plus neutral fraction produced a minor peak of activity at Rf 0.1 (0.16 µg GA_3) and a major band of activity at Rf 0.3 (1.4 µgs) - 0.4 (0.08 µgs).

(ii) Coleoptile Apices.

Diffusion Experiment 1.

Preliminary experiments were conducted in order to determine the appropriate number of coleoptile apices to be diffused onto agar. Figure 42 shows the results of a bioassay of chromatograms of ethyl acetate soluble fractions from agar plates which had been in contact with batches of 50, 100 and 200 vertically orientated coleoptile apices for 24 hours. As a control, 20 ml of plain agar were treated

Figure 42: Lettuce hypocotyl bioassay of acidic and basic plus neutral ethyl acetate soluble fractions from agar plates which had been in contact with 50, 100 or 200 vertically orientated Zea coleoptile apices. A plain agar plate (PA) was extracted as a control. Data are the mean length of lettuce hypocotyls for each Rf zone of chromatogram. Horizontal line (C) = control zone of chromatogram immediately behind the origin. Elongation in GA_3 control solutions are shown.


HYPOCOTYL LENGTH (MM)
PA
Z 50
Z 100
Z 200
C
4
2
0
acidic
basic & neutral
16
14
12
10
8
6
4
2
0
0
·5
1·0
DISTANCE FROM ORIGIN (Rf)
0 ·0001 1 10
GA3 (µg/ml)

similarly. There was no significant growth activity detected in either the acidic or the basic plus neutral fractions from extracts of 50, 100 or 200 apices. Moreover, at Rf 0.4 - 0.5 there was strongly inhibitory activity in both fractions from the extract of 50 apices and in the acidic fraction from the extract of 100 apices. Surprisingly, this region of inhibitory activity was not observed in either fraction from the extract of 200 apices. Since it had been found in extracts from relatively small numbers of coleoptile apices, it is to be expected that an increase in the quantity of plant tissue extracted, would result in an increase in the amount of inhibitory activity present in that extract. This seemed not to be the case.

Diffusion Experiment 2.

Batches of 50, 100 and 200 coleoptile apices were left orientated in the vertical position on agar plates for 24 hours, alongside a control plate of plain agar. The methanolic extracts of each agar plate were partitioned and the resulting ethyl acetate soluble fractions chromatographed and bioassayed (Figure 43). There was no significant growth activity in any of the extracts.

Diffusion Experiment 3.

50, 100 and 200 coleoptile apices were placed in the vertical position on agar plates for 24 hours. A plain agar plate was treated similarly as a control. The results of a bioassay of the chromatograms of the ethyl acetate soluble fractions from the agar are shown in Figure 44. No significant amount of growth activity was detectable in any fraction.

Diffusion Experiment 4.

50, 100 and 200 coleoptile apices were placed horizontally on agar plates for 24 hours alongside a plain agar plate as a control. The results shown in Figure 45 were

Figure 43: Lettuce hypocotyl bioassay of acidic and basic plus neutral ethyl acetate soluble fractions from agar plates which had been in contact with 50, 100 or 200 vertically orientated Zea coleoptile apices. A plain agar plate (PA) was extracted as a control.
Data are the mean length of lettuce hypocotyls for each Rf zone of chromatogram. Horizontal line (C) = control zone of chromatogram immediately behind the origin.
Elongation in GA_3 control solutions are shown.

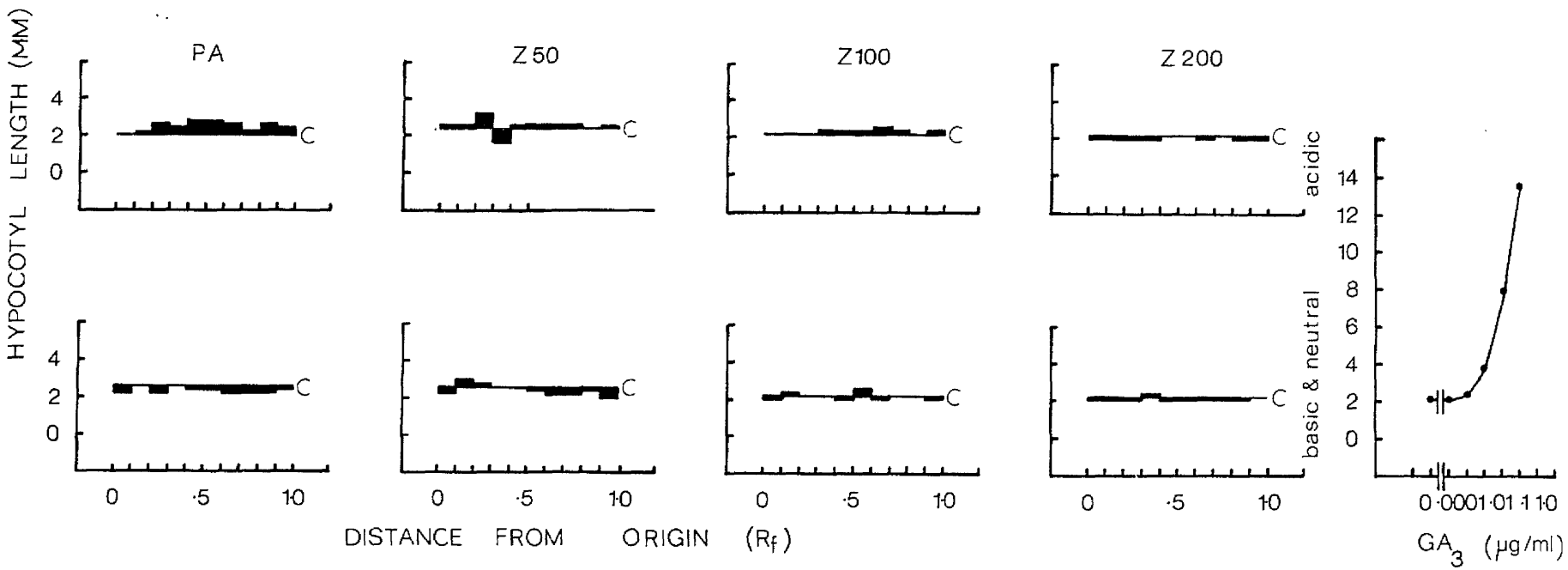

HYPOCOTYL LENGTH (MM)
PA
Z 50
Z100
Z 200
C
4
2
0
0
·5
1·0
DISTANCE FROM ORIGIN (Rf)
acidic
basic & neutral
14
12
10
8
6
4
2
0
0 ·0001 ·01 ·1 1·0
GA3 (µg/ml)

Figure 44: Lettuce hypocotyl bioassay of acidic and basic plus neutral ethyl acetate soluble fractions from agar plates which had been in contact with 50, 100 or 200 vertically orientated Zea coleoptile apices. A plain agar plate (PA) was extracted as a control. Data are the mean length of lettuce hypocotyls for each Rf zone of chromatogram. Horizontal line (C) = control zone of chromatogram immediately behind the origin. Elongation in GA_3 control solutions are shown.

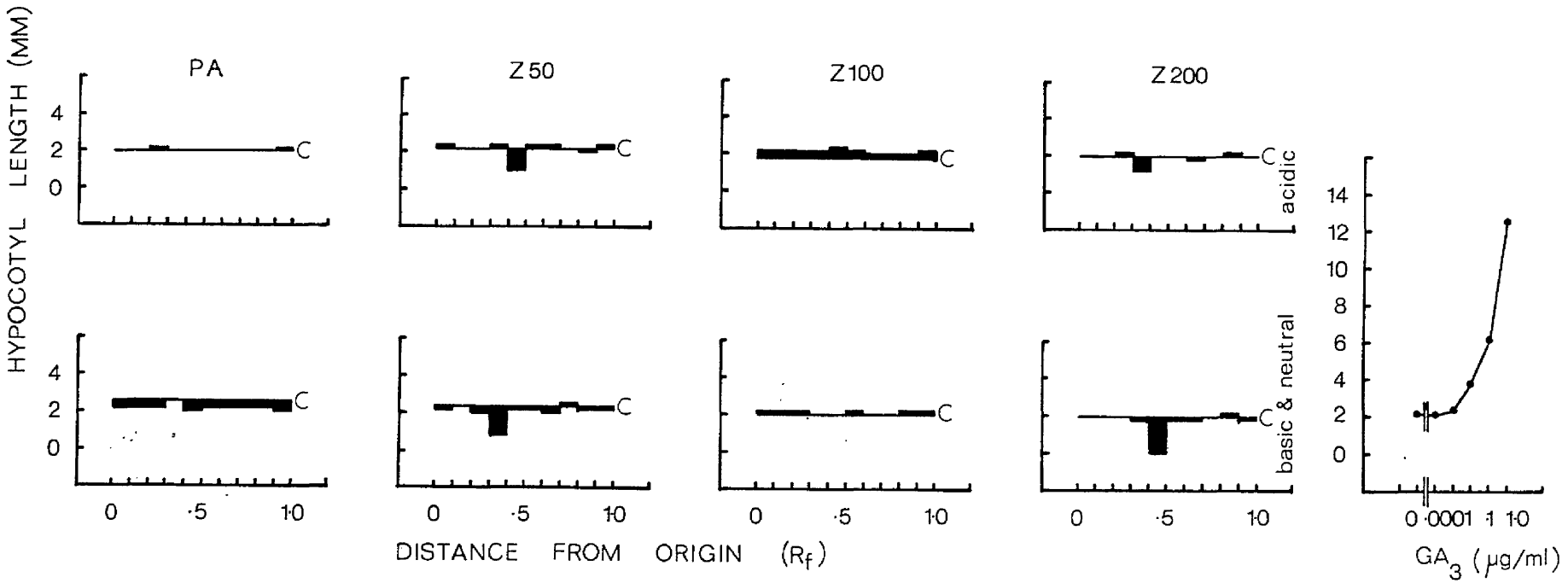
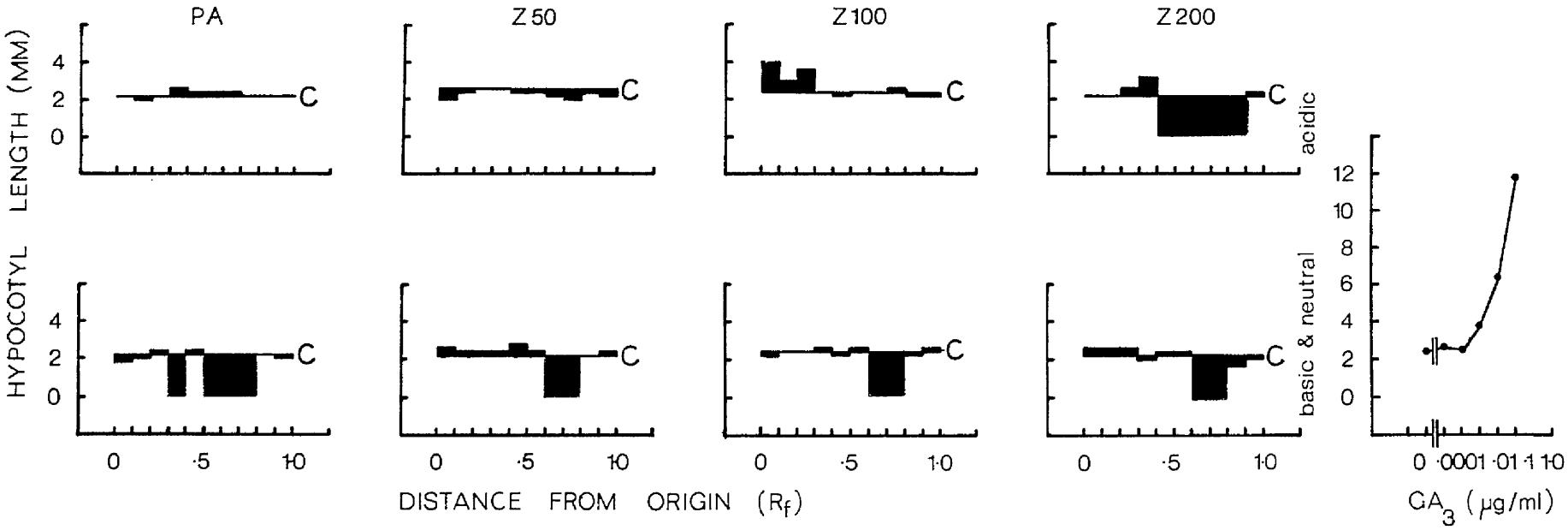

HYPOCOTYL LENGTH (MM)
PA
Z50
Z100
Z200
C
acidic
basic & neutral
4
2
0
0
·5
1·0
DISTANCE FROM ORIGIN (Rf)
14
12
10
8
6
4
2
0
0 ·0001 ·1 1·0
GA3 (µg/ml)

Figure 45: Lettuce hypocotyl bioassay of acidic and basic plus neutral ethyl acetate soluble fractions from agar plates which had been in contact with 50, 100 or 200 horizontally orientated Zea coleoptile apices. A plain agar plate (PA) was extracted as a control. Data are the mean length of lettuce hypocotyls for each Rf zone of chromatogram. Horizontal line (C) = control zone of chromatogram immediately behind the origin. Elongation in GA_3 control solutions are shown.


PA
Z50
Z100
Z200
HYPOCOTYL LENGTH (MM)
4
2
0
C
acidic
basic & neutral
12
10
8
6
4
2
0
0 ·5 1·0
DISTANCE FROM ORIGIN (Rf)
0 ·0001 ·01 ·1 1·0
GA3 (µg/ml)

obtained from bioassays of the agar extracts. There was no significant detectable growth activity in any of the extracts. Strongly inhibitory activity was present in the basic plus neutral ethyl acetate soluble fractions of all extracts; Rf 0.4 - 0.8 plain agar, Rf 0.7 - 0.8 coleoptiles and in the acidic fractions from plain agar (Rf 0.8) and 200 coleoptile apices (Rf 0.5 - 0.9).

Diffusion Experiment 5.

Batches of 50, 100 and 200 coleoptile apices were left on agar plates for 24 hours in the horizontal position alongside a control plate of plain agar. A bioassay (Figure 46) of chromatograms of both fractions from each of the four agar extracts revealed no significant growth activity. Considerable inhibitory activity was present in all the basic plus neutral ethyl acetate soluble fractions from all four extracts and in the acidic fractions of extracts from plain agar and 100 coleoptile apices.

Diffusion Experiment 6.

430 coleoptile apices were orientated horizontally and placed such on agar plates for 24 hours alongside a control plate of plain agar. Bioassay results (Figure 47) of both acidic and basic plus neutral ethyl acetate soluble fractions from both the agar extracts revealed no growth promoting activity to be present. Inhibitory activity was revealed at Rf 0.6 - 0.7 in the basic plus neutral fraction from the coleoptile agar extract.

Figure 46: Lettuce hypocotyl bioassay of acidic and basic plus neutral ethyl acetate soluble fractions from agar plates which had been in contact with 50, 100 or 200 horizontally orientated Zea coleoptile apices. A plain agar plate (PA) was extracted as a control. Data are the mean length of lettuce hypocotyls for each Rf zone of chromatogram. Horizontal line (C) = control zone of chromatogram immediately behind the origin. Elongation in GA_3 control solutions are shown.

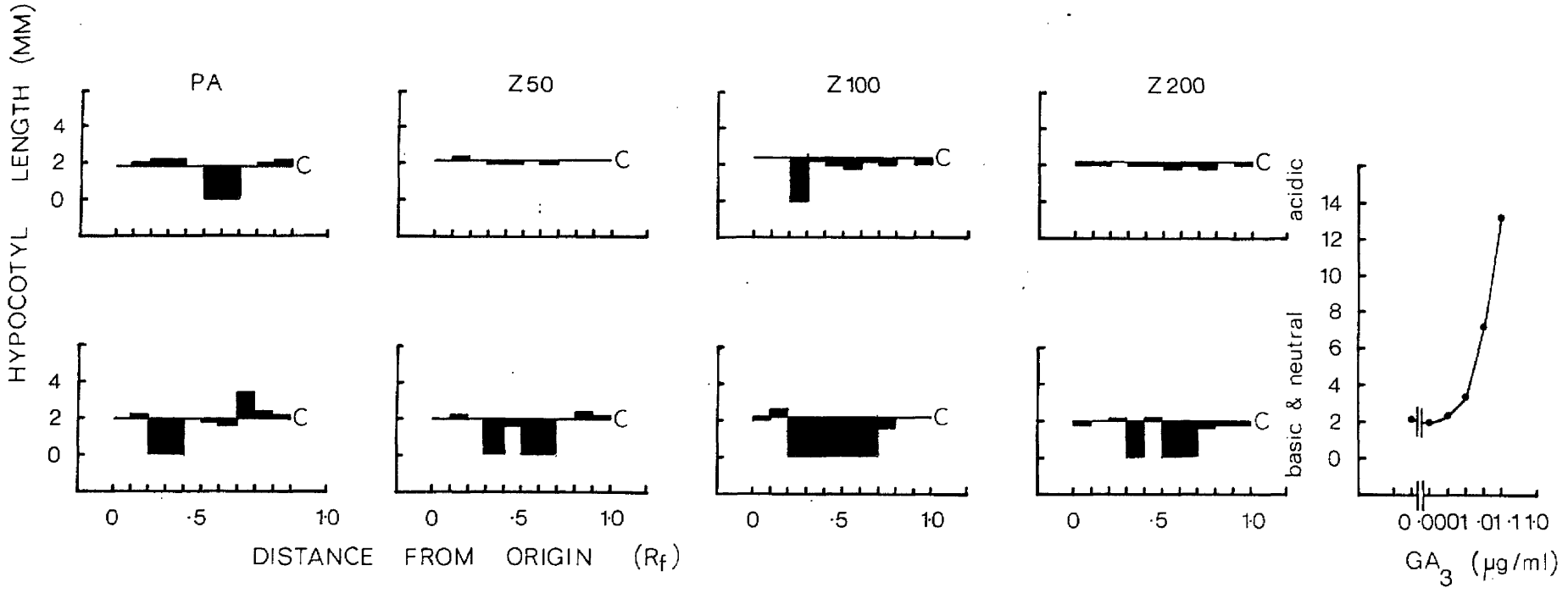
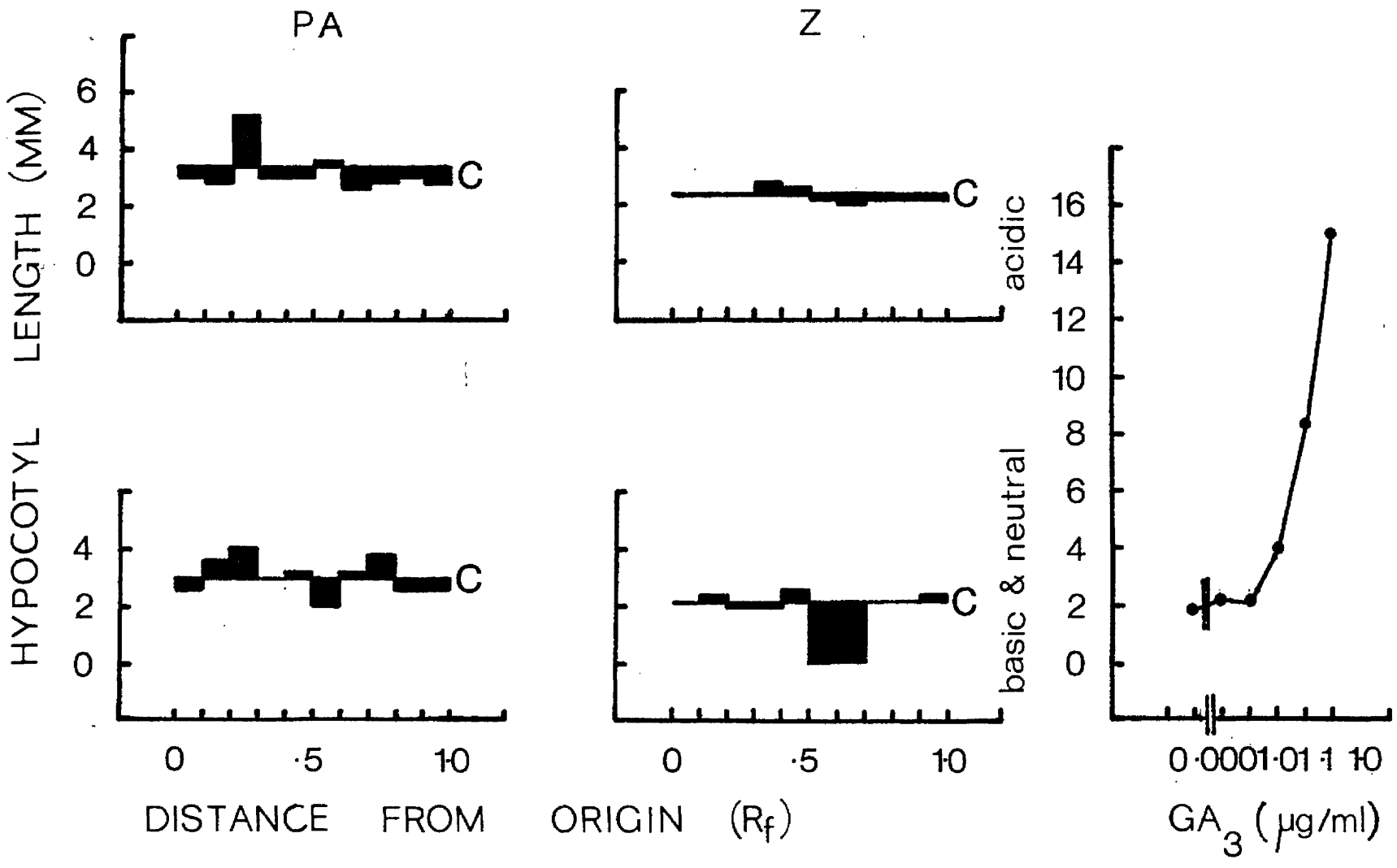

HYPOCOTYL LENGTH (MM)
PA
Z50
Z100
Z200
C
4
2
0
0
·5
1·0
DISTANCE FROM ORIGIN (Rf)
acidic
basic & neutral
14
12
10
8
6
4
2
0
0
·0001
·01
·1
1·0
GA3 (µg/ml)

Figure 47: Lettuce hypocotyl bioassay of acidic and basic plus neutral ethyl acetate soluble fractions from agar plates which had been in contact with 430 horizontally orientated Zea coleoptile apices. A plain agar plate (PA) was extracted as a control. Data are the mean length of lettuce hypocotyls for each Rf zone of chromatogram. Horizontal line (C) = control zone of chromatogram immediately behind the origin. Elongation in GA_3 control solutions are shown.


PA
Z
HYPOCOTYL LENGTH (MM)
6
4
2
0
C
C
acidic
basic & neutral
16
14
12
10
8
6
4
2
0
4
2
0
C
C
0
·5
1·0
0
·5
1·0
DISTANCE FROM ORIGIN (Rf)
0·0001
·01
·1
1·0
GA3 (µg/ml)

Diffusion Experiment 7. - adapted procedure

Approximately 500 coleoptile apices were placed horizontally on agar plates for 24 hours alongside a plain agar control plate. The methanolic extracts of the agar plates were divided into two. One half of each extract was partitioned to produce acidic and basic plus neutral ethyl acetate soluble fractions and both of these were subjected to a dilution procedure which produced fractions diluted to a concentration $\frac{1}{10}$ and $\frac{1}{50}$ of the original fraction. The resulting diluted fractions were chromatographed and bioassayed (Figure 48). There was no detectable growth activity in any of the dilutions from either of the two fractions from both agar extracts. However since there were no regions of growth inhibition on the chromatograms, the technique of diluting the fractions appeared to have been successful in their elimination.

The remaining halves of the methanolic extracts were also partitioned to produce acidic and basic plus neutral ethyl acetate soluble fractions and each of these was subjected to purification on a charcoal/celite column and the resulting eluates chromatographed and bioassayed (Figure 49). There was no significant growth activity in either fraction from the coleoptile agar extract or the acidic fraction from the extract of plain agar. However growth activity equivalent to 0.16 µg GA_3 was observed at Rf 0.3 in the basic plus neutral fraction of the extract of plain agar.

Figure 48: Lettuce hypocotyl bioassay of acidic and basic plus neutral ethyl acetate soluble fractions from agar plates which had been in contact with 500 horizontally orientated Zea coleoptile apices. Fractions were purified by dilution to produce concentrations $\frac{1}{10}$ and $\frac{1}{50}$ of the original:-

	Histogram		Concentration
Plain Agar	A	=	original
	B	=	$\frac{1}{10}$
	C	=	$\frac{1}{50}$
Zea	D	=	original
	B	=	$\frac{1}{10}$
	F	=	$\frac{1}{50}$

A plain agar plate (PA) was extracted as a control. Data are the mean length of lettuce hypocotyls for each Rf zone of chromatogram. Horizontal line (C) = control zone of chromatogram immediately behind the origin. Elongation in GA_3 control solutions are shown.

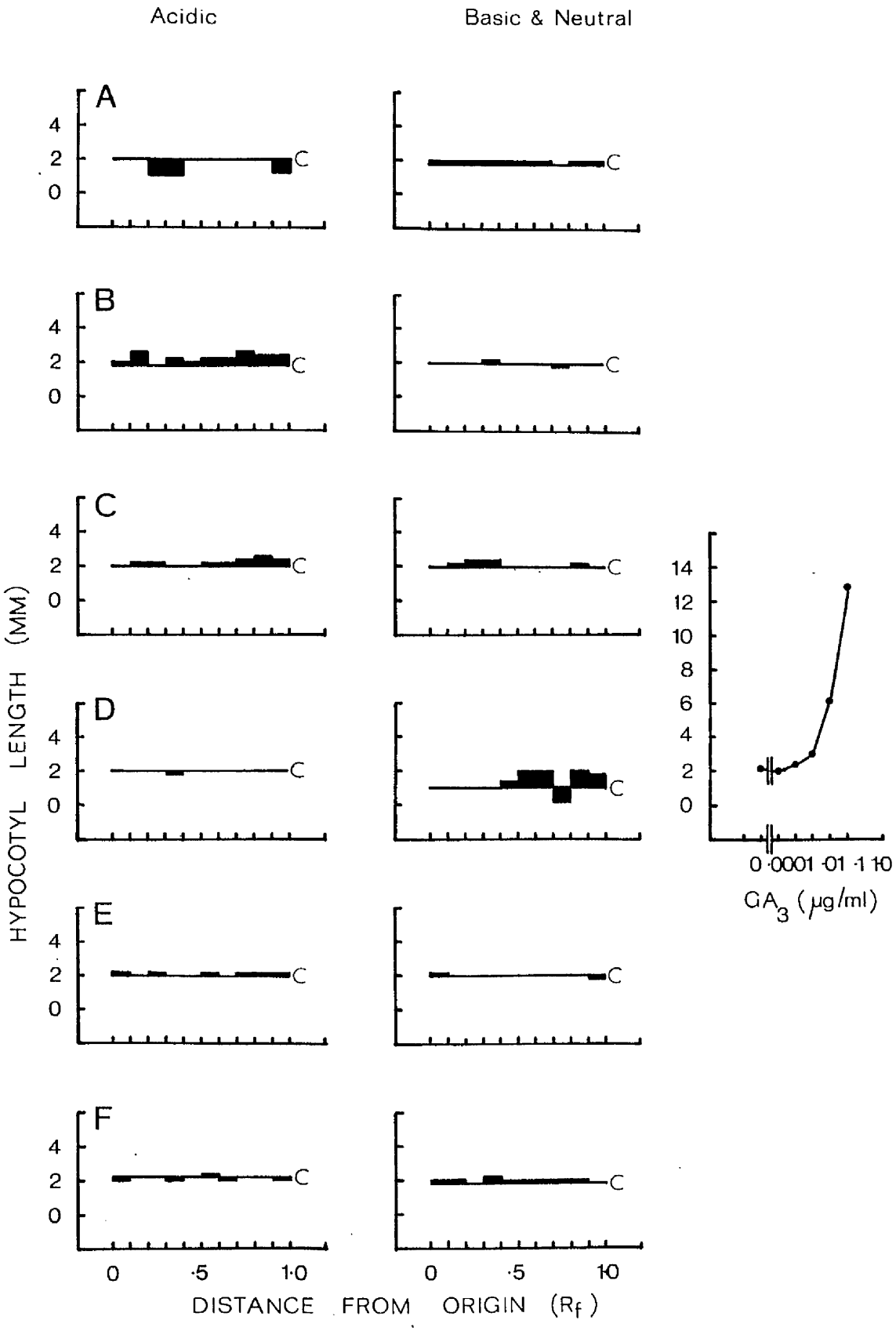
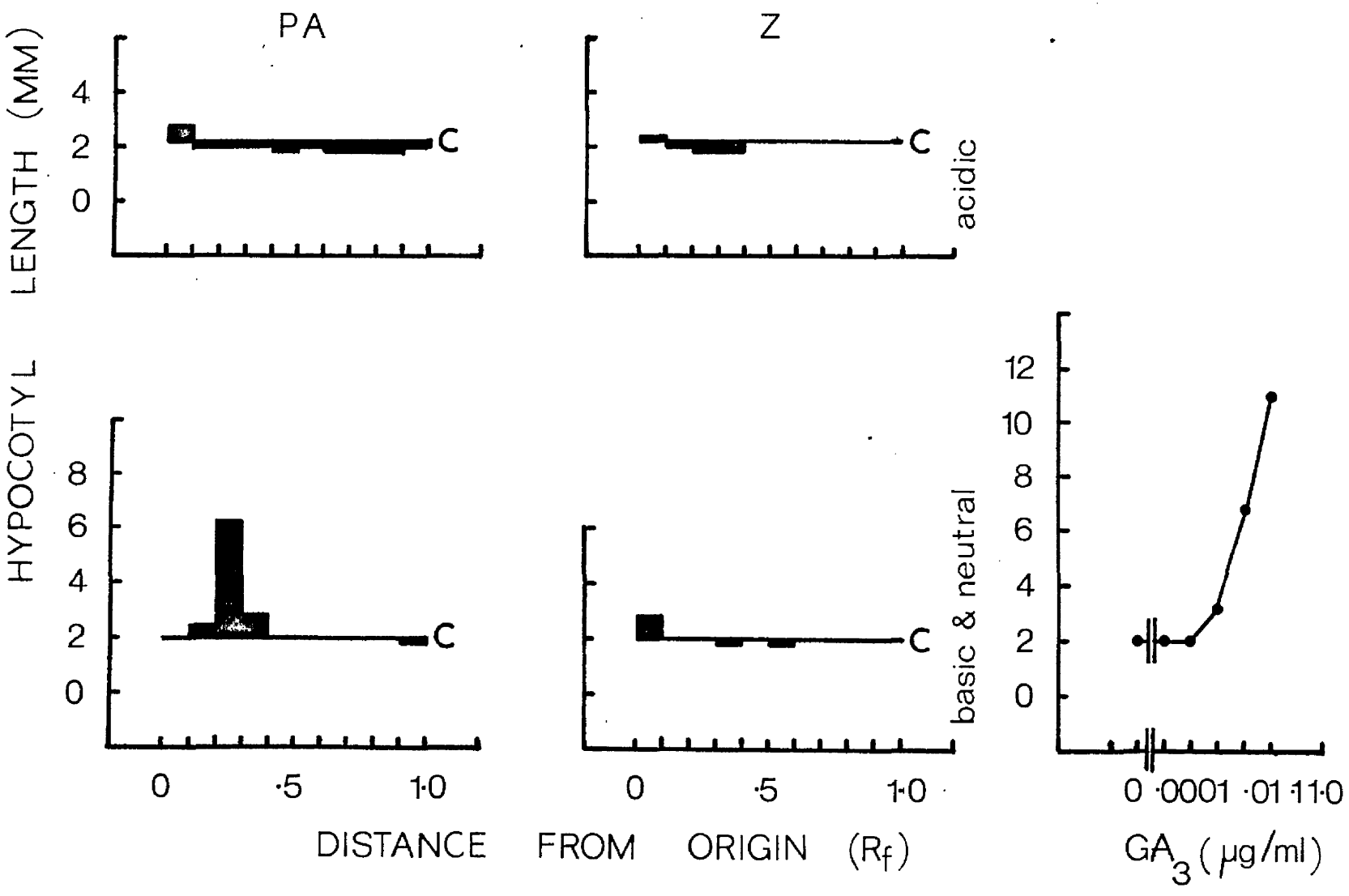

Acidic
Basic & Neutral
A
B
C
D
E
F
4
2
0
C
HYPOCOTYL LENGTH (MM)
0
·5
1·0
DISTANCE FROM ORIGIN (Rf)
14
12
10
8
6
4
2
0
0 ·0001 ·01 ·1 10
GA3 (µg/ml)

Figure 49: Lettuce hypocotyl bioassay of acidic and basic plus neutral ethyl acetate soluble fractions, following purification on a charcoal/celite column, from agar plates which had been in contact with 500 horizontally orientated Zea coleoptile apices. A plain agar plate (PA) was extracted as a control. Data are the mean length of lettuce hypocotyls for each Rf zone of chromatogram. Horizontal line (C) = control zone of chromatogram immediately behind the origin. Elongation in GA_3 control solutions are shown.


PA
Z
HYPOCOTYL LENGTH (MM)
4
2
0
C
acidic
8
6
4
2
0
basic & neutral
12
10
8
6
4
2
0
0 ·5 1·0
DISTANCE FROM ORIGIN (Rf)
0 ·0001 ·01 1·0
GA3 (µg/ml)

2. Extraction of Endogenous Gibberellin-like Substances from Coleoptiles of Zea Mays.

Coleoptiles were excised from batches of 4 day old dark grown Zea seedlings, deleaved and extracted wih methanol. The extracts from five different batches of seedlings were subjected to purification, chromatography and bioassay as described previously. The extraction procedure was tested for efficiency with standard GA, and for possible artifacts with methanol only.

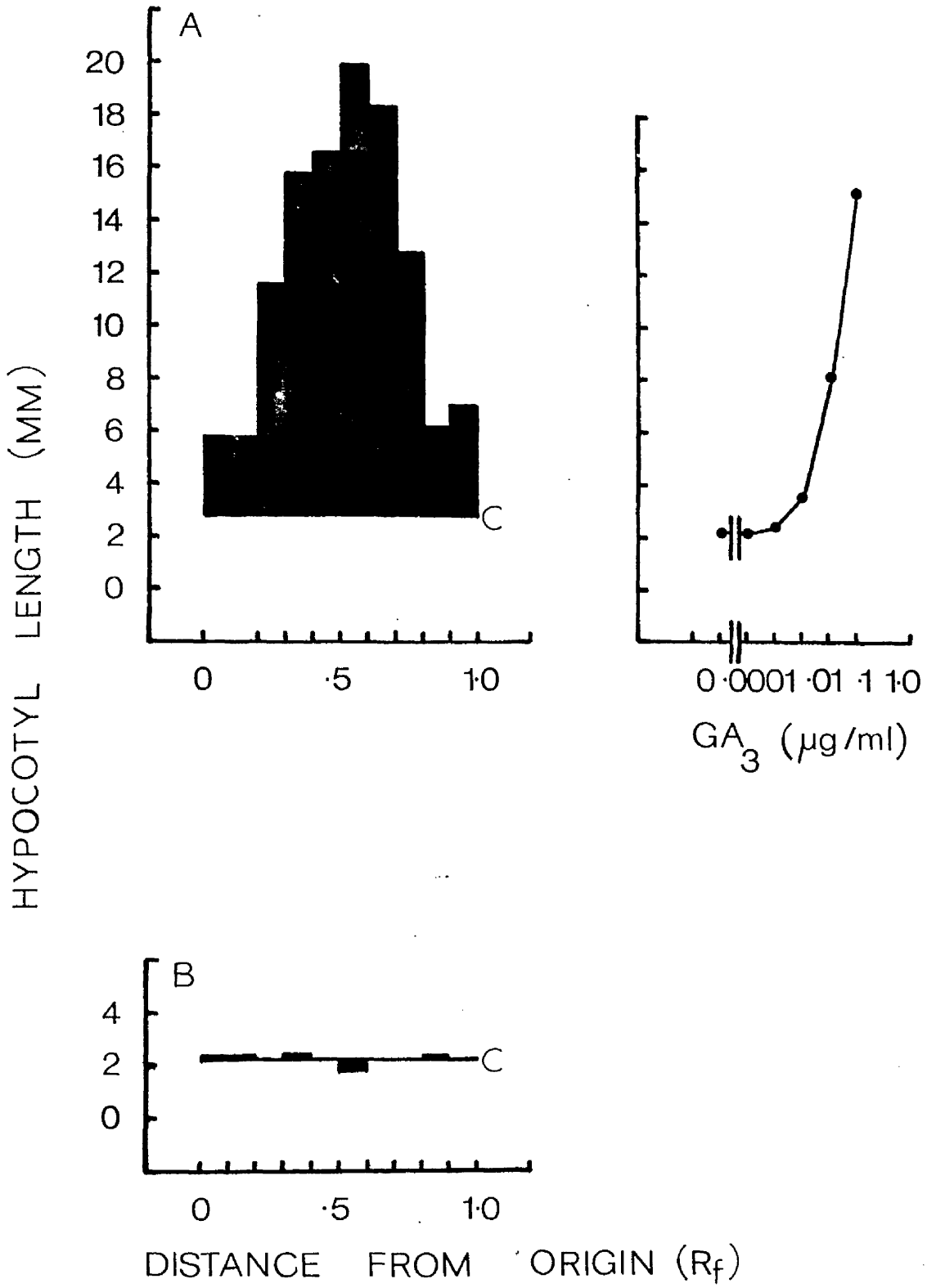
(i) Standard GA,

Approximately 14 g of coleoptiles with the primary leaves removed, together with 100 µg GA, were placed in methanol in darkness and subjected to the extraction, partitioning and chromatography procedure described previously, before assay with the lettuce hypocotyl bioassay (Figure 50). Growth promoting activity equivalent to 0.08 µgs or more was observed at each Rf value. There was a major bond of activity between Rf 0.3 and 0.8 and at Rf 0.6 there was gibberellin-like activity equivalent to at least 2.0 µgs.

(ii) Solvent blank

In order to ascertain that the procedures used in the extraction would not themselves give rise to growth promoting activity in the bioassay, 50 ml of methanol was added to 50 ml of distilled water and left in darkness before being subjected to the purification and chromatography procedures.

Figure 50: Lettuce hypocotyl bioassay of extract of A: c. 100µg standard GA_3 and B: solvent blank. Data are the mean length of lettuce hypocotyls for each Rf zone of chromatogram. Horizontal line (C) = control zone of chromatogram immediately behind the origin. Elongation in GA_3 control solutions are shown.


A
20
18
16
14
12
10
8
6
4
2
0
C
0
·5
1·0
0·0001 ·01 ·1 1·0
GA3 (µg/ml)
HYPOCOTYL LENGTH (MM)
B
4
2
0
C
0
·5
1·0
DISTANCE FROM ORIGIN (Rf)

The results of a lettuce hypocotyl bioassay (Figure 50) showed no growth promoting activity to be present.

(iii) Coleoptiles.

Extraction Experiment 1.

The methanolic extract from approximately 600 coleoptiles weighing 43 g, was partitioned, purified on a polyvinylpyrollidone column and strip loaded onto a glass backed silica gel plate which was developed in an acidic solvent system (chloroform : ethyl acetate : formic acid :: 50 : 50 : 1) before assay with the lettuce hypocotyl bioassay (Figure 51). There was a small peak of growth promoting activity at Rf 1.0 equivalent to 0.2 µg GA_3. There was a region of inhibitory activity at Rf 0.1 - 0.4.

Extraction Experiment 2.

Figure 52 shows a lettuce hypocotyl bioassay of the methanolic extract from approximately 550 coleoptiles weighing 40 g, which had been partitioned and subsequently chromatographed in the acidic solvent system. There was no indication of any gibberellin-like activity; indeed there was inhibition of hypocotyl growth at Rf 0.1 - 0.2.

Extraction Experiment 3.

Approximately 560 coleoptiles weighing 41 g were extracted in methanol, partitioned and chromatographed in the basic solvent system (isopropanol : ammonia : water :: 10 : 1 : 1). The results of a lettuce hypocotyl bioassay are shown in Figure 53. Growth promoting activity

Figure 51: Lettuce hypocotyl bioassay of the methanolic extract from c. 600 Zea coleoptiles. Data are the mean length of lettuce hypocotyls for each Rf zone of chromatogram. Horizontal line (C) = control zone of chromatogram immediately behind the origin. Elongation in GA_3 control solutions are shown.

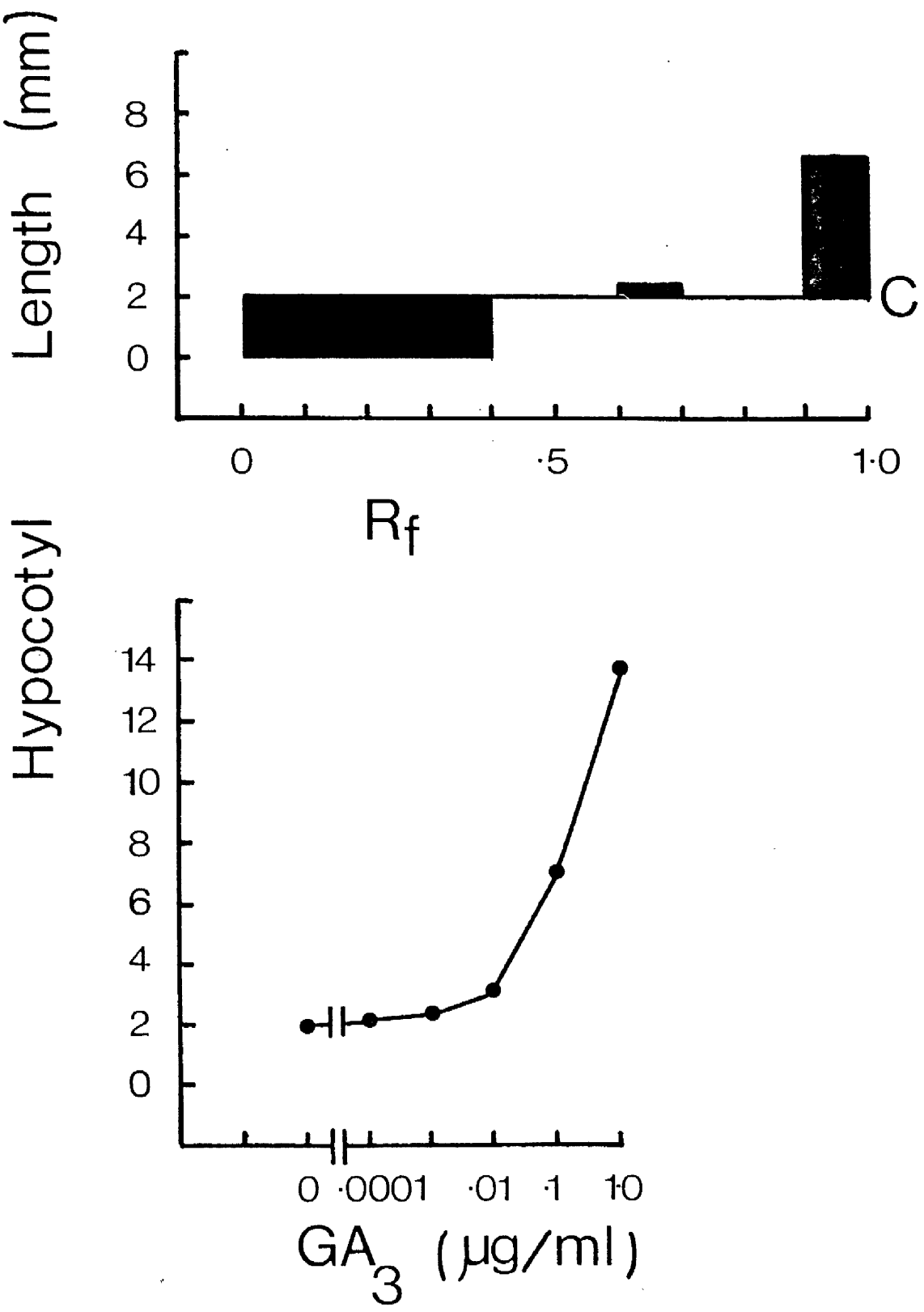

Length (mm)
8
6
4
2
0
C
0
·5
1·0
R_f
Hypocotyl
14
12
10
8
6
4
2
0
0 ·0001 ·01 ·1 1·0
GA_3 (µg/ml)

Figure 52: Lettuce hypocotyl bioassay of the methanolic extract from c. 550 Zea coleoptiles. Data are the mean length of lettuce hypocotyls for each Rf zone of chromatogram. Horizontal line (C) = control zone immediately behind the origin. Elongation in GA_3 control solutions are shown.

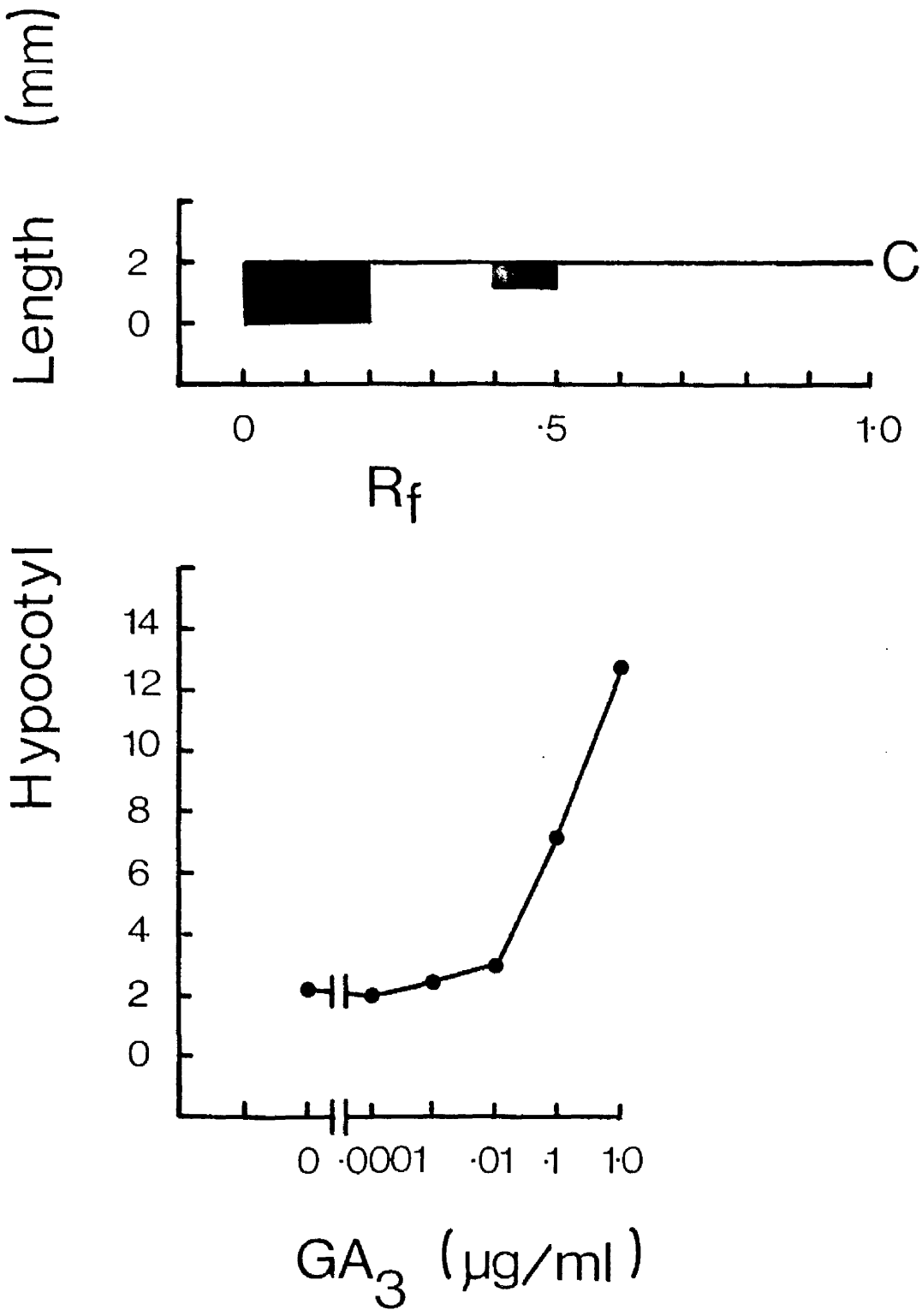
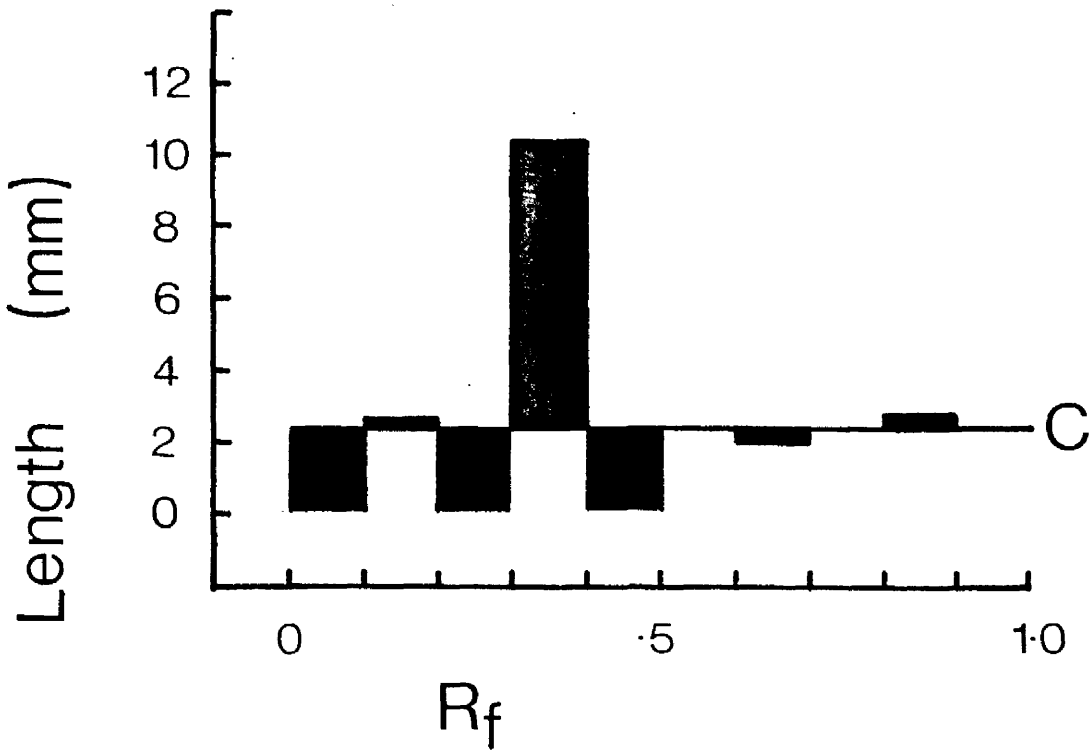
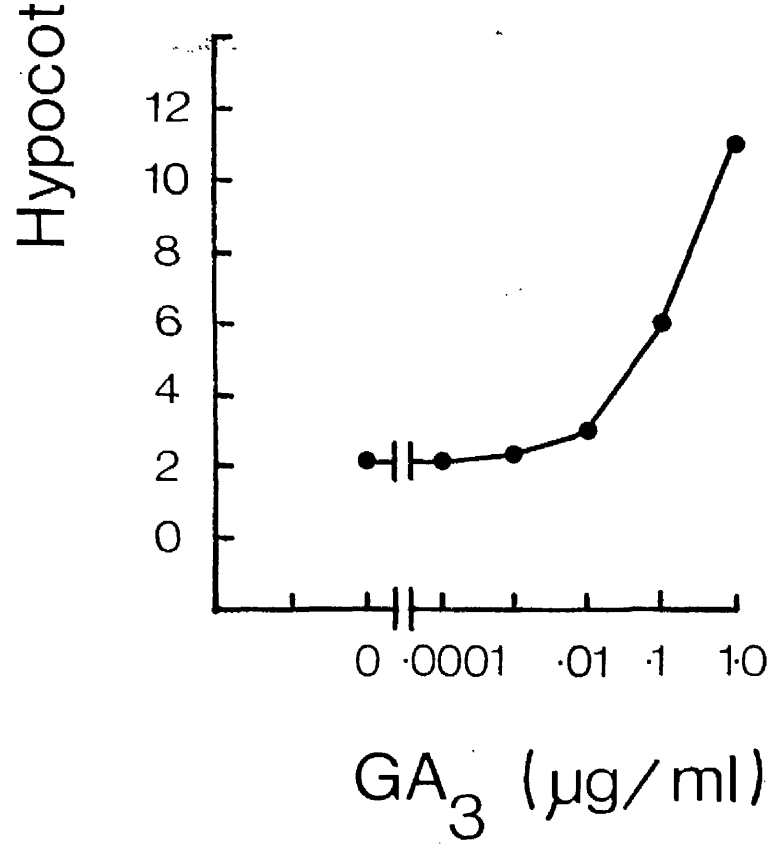

Length (mm)
2
0
C
0
·5
1·0
R_f
Hypocotyl
14
12
10
8
6
4
2
0
0 ·0001 ·01 ·1 1·0
GA_3 (µg/ml)

Figure 53: Lettuce hypocotyl bioassay of the methanolic extract from c. 560 Zea coleoptiles. Data are the mean length of lettuce hypocotyls for each Rf zone of chromatogram. Horizontal line (C) = control zone of chromatogram immediately behind the origin. Elongation in GA_3 control solutions are shown.


Hypocotyl Length (mm)
12
10
8
6
4
2
0
C
0
·5
1·0
R_f


12
10
8
6
4
2
0
0 ·0001 ·01 ·1 1·0
GA_3 (µg/ml)

equivalent to 2.0 µg GA_3 was present at Rf 0.4. Inhibitory activity was observed at Rf 0.1, 0.3, 0.5.

Extraction Experiment 4.

The methanolic extract from approximately 550 5 d old coleoptiles weighing 40 g was purified by partitioning and chromatographed in the basic solvent system. There was no growth activity in a lettuce hypocotyl bioassay (Figure 54). The elongation of the hypocotyls was inhibited at Rf 0.5 - 0.7.

Extraction Experiment 5.

The methanolic extract from 620 5 d old coleoptiles weighing 45 g was purified by partitioning and chromatography in the basic solvent system before assay with the dwarf rice bioassay (Figure 55). There was no indication of any growth promoting activity.

3. Collection in Agar of Endogenous Gibberellin-like Substances from Shoot Apices of Helianthus annuus.

A technique for agar diffusion similar to that used for Zea coleoptile apices was employed to collect gibberellin-like substances translocated basipetally out of isolated shoot apices of Helianthus seedlings. The agar was extracted with methanol and purified by a partitioning and chromatography procedure which was described previously, before bioassay. Extracts of plain agar, standard GA_3 in agar and standard GA_3 solutions were also tested.

Figure 54: Lettuce hypocotyl bioassay of the methanolic extract from c. 550 Zea coleoptiles. Data are the mean length of lettuce hypocotyls for each Rf zone of chromatogram. Horizontal line (C) = control zone of chromatogram immediately behind the origin. Elongation in GA_3 control solutions are shown.

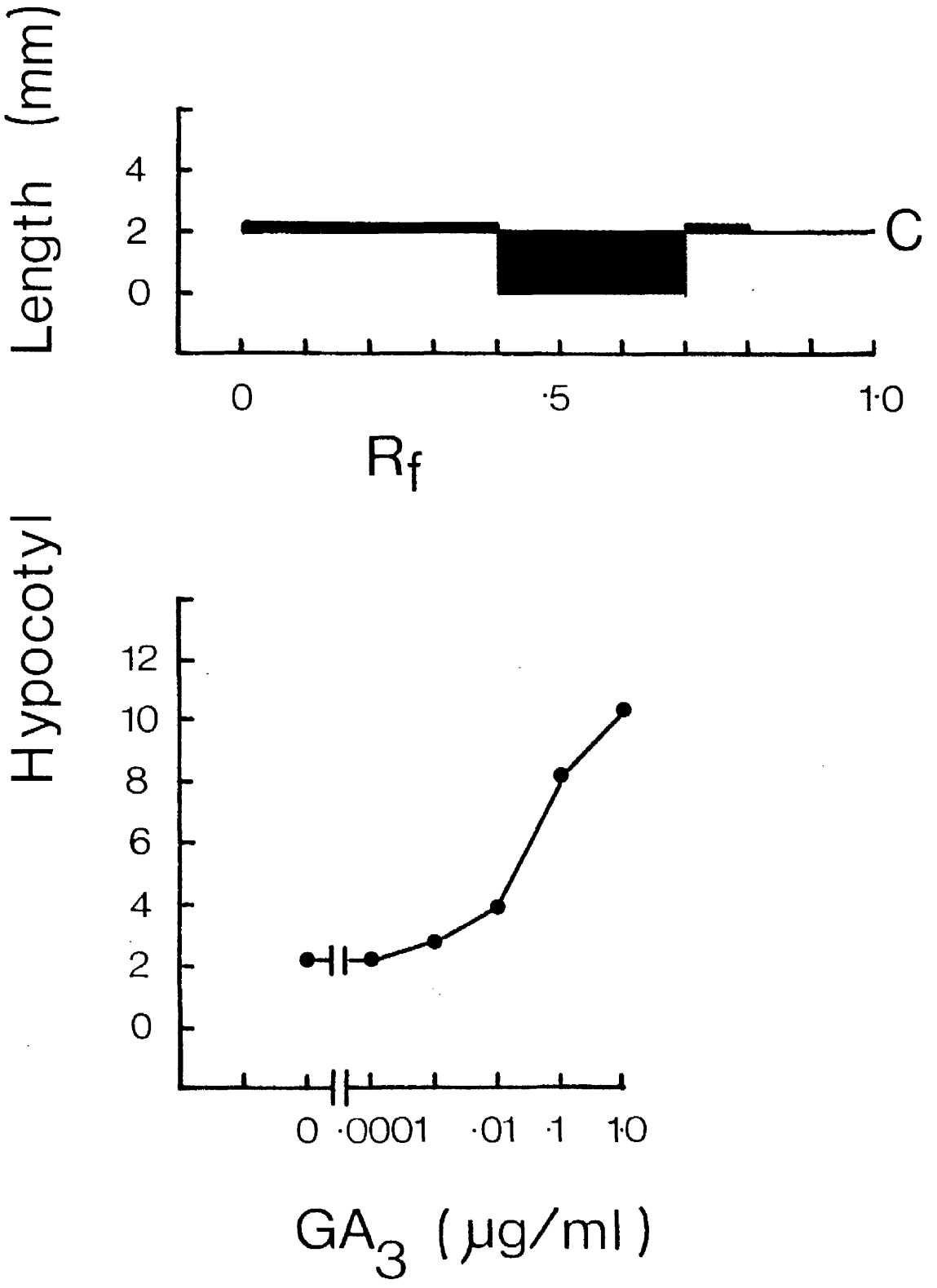
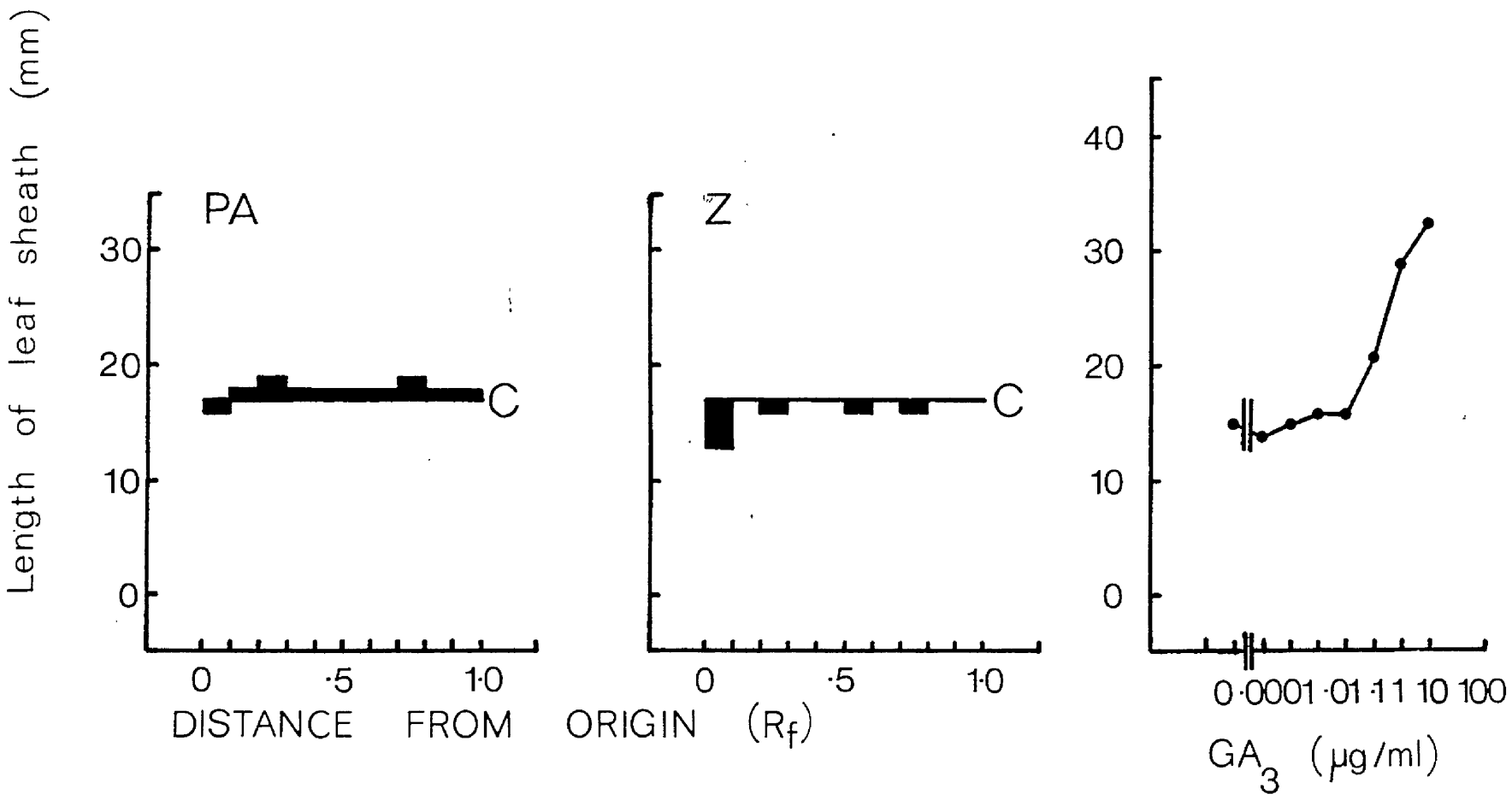

Length (mm)
4
2
0
C
0
·5
1·0
R_f
Hypocotyl
12
10
8
6
4
2
0
0 ·0001 ·01 ·1 1·0
GA_3 (µg/ml)

Figure 55: Dwarf rice bioassay of the methanolic extract from c. 620 Zea coleoptiles. Data are the mean length of the leaf sheath for each Rf zone of chromatogram. Horizontal line (C) = control zone of chromatogram immediately behind the origin. Elongation in GA_3 control solutions are shown.


Length of leaf sheath (mm)
PA
Z
C
C
30
20
10
0
0
·5
1·0
DISTANCE FROM ORIGIN (Rf)
40
30
20
10
0
0·0001 ·01 ·1 1 10 100
GA3 (µg/ml)

(i) Standard GA_3

In order to test the efficiency of the extraction procedure, a series of agar plates were made; each plate incorporating 50 ml of 20 gl^{-1} ion agar and 50 ml of the following concentrations of stock solutions of GA_3: 0.0001, 0.001, 0.01, 0.1, 1.0, 10.0 µg/ml. There was a control plate incorporating ion agar and 50 ml of distilled water. The plates were allowed to stand for 24 h at 25°C under white fluorescent light before methanolic extraction and purification by partitioning and chromatography. The resulting acidic ethyl acetate fractions from each plate were assayed with the lettuce hypocotyl bioassay (Figure 56). As for the coleoptile diffusion experiments, the approximate quantity of growth promoting activity in µgs was estimated for each chromatogram, by comparison with standard GA_3 solutions and the total recovery from each chromatogram was calculated (Table 2).

At a concentration of 0.0001 µg/ml there was a region of growth promoting activity between Rf 0.5 - 0.6 which was equivalent to 0.12 µg but since this peak was also observed at the same Rf value of the chromatogram of the plain agar extract, it seemed likely that this activity was caused by an artifact of the extraction procedure or else was due to the agar itself. This phenomenon also occurred in the extraction trial for the Zea, when growth promoting activity was observed at Rf 0.3 on chromatograms of the acidic fractions from extracts of both plain agar control and GA_3 at a concentration of 0.0001 µg/ml. Again this suggested the possibility that the activity could be due either to an artifact of the extraction procedure or to the agar itself. However, if this was so, then chromatograms of all the extracts would show some slight growth promoting activity, whether or not they had been in contact with coleoptile tips or sunflower apices and this is clearly not evident.

Figure 56: Lettuce hypocotyl bioassay of acidic ethyl acetate soluble fractions from agar plates containing the following standard solutions of GA_3:-

Chromatogram	GA_3 (µg/ml)
A	0
C	0.0001
E	0.001
G	0.01
B	0.1
D	1.0
F	10.0

Data are the mean length of lettuce hypocotyls for each Rf zone of chromatogram. Horizontal line (C) = control zone of chromatogram immediately behind the origin. Elongation in GA_3 control solutions are shown.

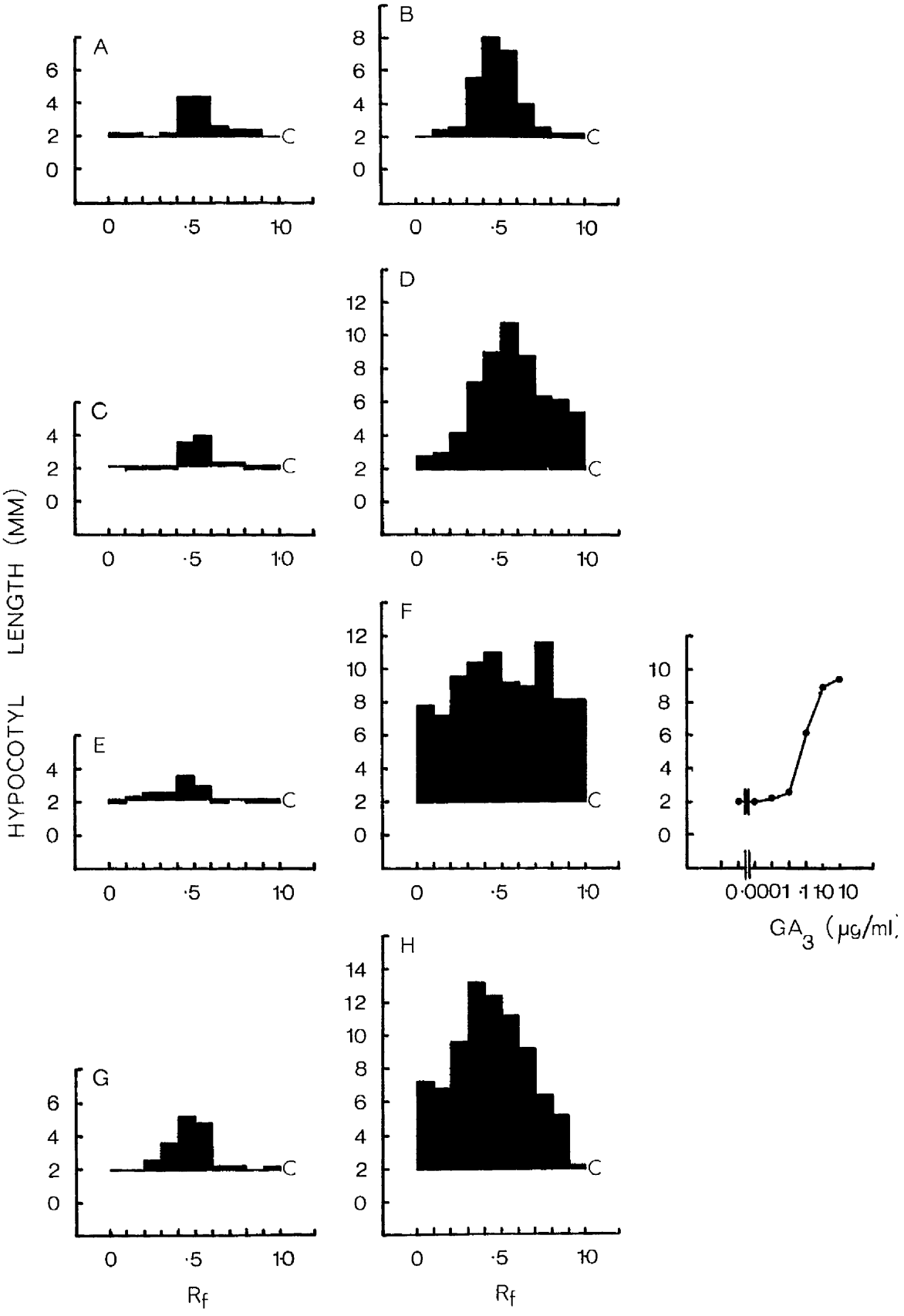

A
B
C
D
E
F
G
H
C
0
·5
1·0
HYPOCOTYL LENGTH (MM)
Rf
0·0001 ·1 1·0 10
GA3 (µg/ml)

TABLE 2

Original conc. GA_3/ plate µg/ml	Rf zone	Hypocotyl lengths mm	GA_3 equivalents µg	Total recovery for each chromatogram µg
0	0.5	4.4	0.18	
	0.6	4.4	0.18	
	0.7	2.6	0.03	0.426
	0.8	2.4	0.018	
	0.9	2.4	0.018	
0.0001	0.5	3.6	0.12	
	0.6	4.0	0.12	0.276
	0.7	2.4	0.018	
	0.8	2.4	0.018	
0.001	0.2	2.4	0.018	
	0.3	2.6	0.013	
	0.4	2.6	0.03	0.258
	0.5	3.6	0.12	
	0.6	3.0	0.06	
0.01	0.3	2.6	0.03	
	0.4	3.6	0.12	0.57
	0.5	5.2	0.24	
	0.6	4.8	0.18	
0.1	0.2	2.4	0.018	
	0.3	2.6	0.03	
	0.4	5.5	0.24	
	0.5	8.0	1.8	3.138
	0.6	7.1	0.9	
	0.7	4.0	0.12	
	0.8	2.6	0.03	
1.0	0.1	2.8	0.045	
	0.2	3.0	0.06	
	0.3	4.2	0.135	
	0.4	7.2	1.2	
	0.5	9.0	3.0	38.13
	0.6	10.8*	30.0*	
	0.7	8.8	2.7	
	0.8	6.4	0.45	
	0.9	6.2	0.3	
	1.0	5.4	0.24	
10.0	0.1	7.7	1.5	
	0.2	7.1	0.9	
	0.3	9.6*	30.0*	
	0.4	10.4*	30.0*	
	0.5	11.0*	30.0*	135.6
	0.6	9.2	6.0	
	0.7	8.9	2.7	
	0.8	11.6*	30.0*	
	0.9	8.2	2.4	
	1.0	8.1	2.1	

* beyond limit of standard GA_3 curve

At the 0.001 µg/ml concentration there was a slight growth promoting activity at Rf 0.5 - 0.6 equivalent to 0.12 and 0.06 µgs GA_3 respectively. Growth activity equivalent to 0.12, 0.24, 0.18 µg GA_3 was observed at Rf 0.4, 0.5, 0.6, at the concentration of 0.01 µg/ml. The extract from the 0.1 µg/ml plate, produced a peak of activity between Rf 0.4 - 0.7, equivalent to 0.24, 1.8, 0.9, 0.12 µgs GA_3. There were several peaks of growth promoting activity in the extract from the 1.0 µg/ml plate but the major region of activity was a band from Rf 0.4 - 0.7 which was equivalent to 1.2, 3.0 and 2.7 µgs. It was impossible to estimate the GA_3 equivalents at Rf 0.6, since at this Rf, the hypocotyls were promoted to a length of 10.8 mm and this was beyond the limits of the calibration curve. The extract from the 10.0 µg/ml plate produced growth promoting activity at each Rf value which was equivalent to 0.9 µgs of GA_3 or more. There were two major peaks of activity at Rf 0.5 and 0.8 but again these were beyond the limits of the standard curve used for estimating GA_3 equivalents.

(ii) Helianthus Shoot Apices

Diffusion Experiment 1.

550 apices were excised from 18 d old plants and placed vertically on agar plates for 24 h alongside control plates of plain agar. The results of a lettuce hypocotyl bioassay

of ethyl acetate soluble fractions from both extracts are shown in Figure 57. The chromatogram of the shoot apices showed a region of growth promoting activity between Rf 0.4 - 0.6 which was equivalent to atotal of 1.83 ugs GA_3.

Diffusion Experiment 2.

500 apices from 16 d old plants were placed vertically on agar plates alongside control plates of plain agar. A lettuce hypocotyl bioassay of both extracts (Figure 58) showed that there was no growth promoting activity in either extract.

Diffusion Experiment 3.

503 apices excised from 18 d old plants were left in the upright position on agar plates alongside control plates of plain agar for 24 h. The results of a lettuce hypocotyl bioassay (Figure 59) revealed no growth promoting activity.

Diffusion Experiment 4.

800 apices from 20 d old plants were placed vertically on agar plates alongside control plates of plain agar for 24 h. A peak of growth promoting activity equivalent to 0.24 ug GA_3 was present at Rf 0.5 of the chromatogram from the shoot apices (Figure 60). The plain agar control chromatogram exhibited some growth promoting activity at Rf 1.0.

Figure 57: Lettuce hypocotyl bioassay of the acidic ethyl acetate soluble fraction from agar plates which had been in contact with 550 Helianthus shoot apices. A plain agar plate (PA) was extracted as a control. Data are the mean length of lettuce hypocotyls for each RF zone of chromatogram. Horizontal line (C) = control zone of chromatogram immediately behind the origin. Elongation in GA_3 control solutions are shown.

Approximate estimated quantity of growth activity present on chromatogram:-

RF zone	GA_3 equivalents (µg)
0.4	0.18
0.5	1.2
0.6	0.45

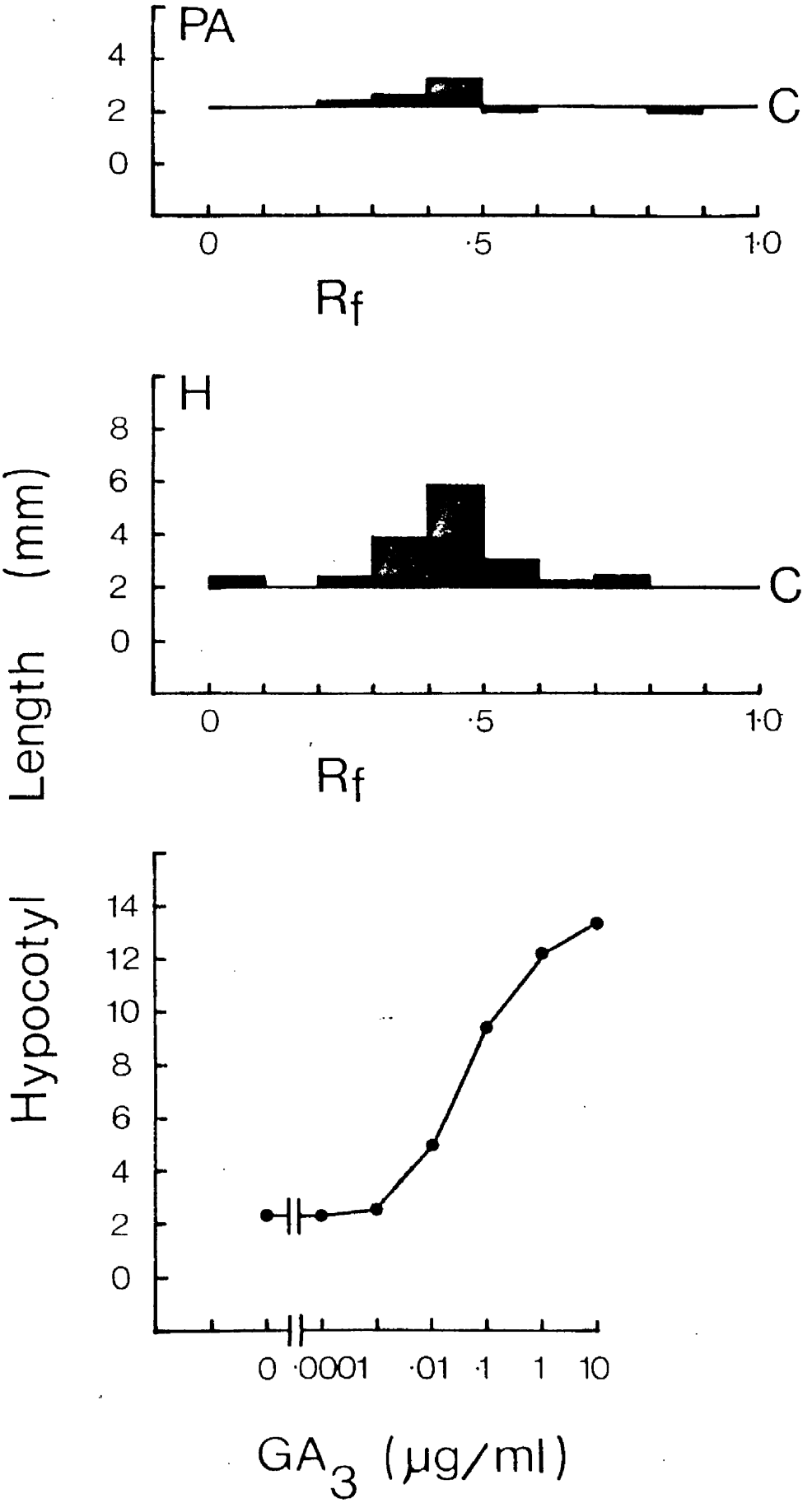

PA
4
2
0
C
0
·5
1·0
Rf
H
8
6
4
2
0
C
0
·5
1·0
Rf
Length (mm)
Hypocotyl
14
12
10
8
6
4
2
0
0
·0001
·01
·1
1
10
GA3 (µg/ml)

Figure 58: Lettuce hypocotyl bioassay of the acidic ethyl acetate soluble fraction from agar plates which had been in contact with 500 Helianthus shoot apices. A plain agar plate (PA) was extracted as a control. Data are the mean length of lettuce hypocotyls for each Rf zone of chromatogram. Horizontal line (C) = control zone of chromatogram immediately behind the origin. Elongation in GA_3 control solutions are shown.

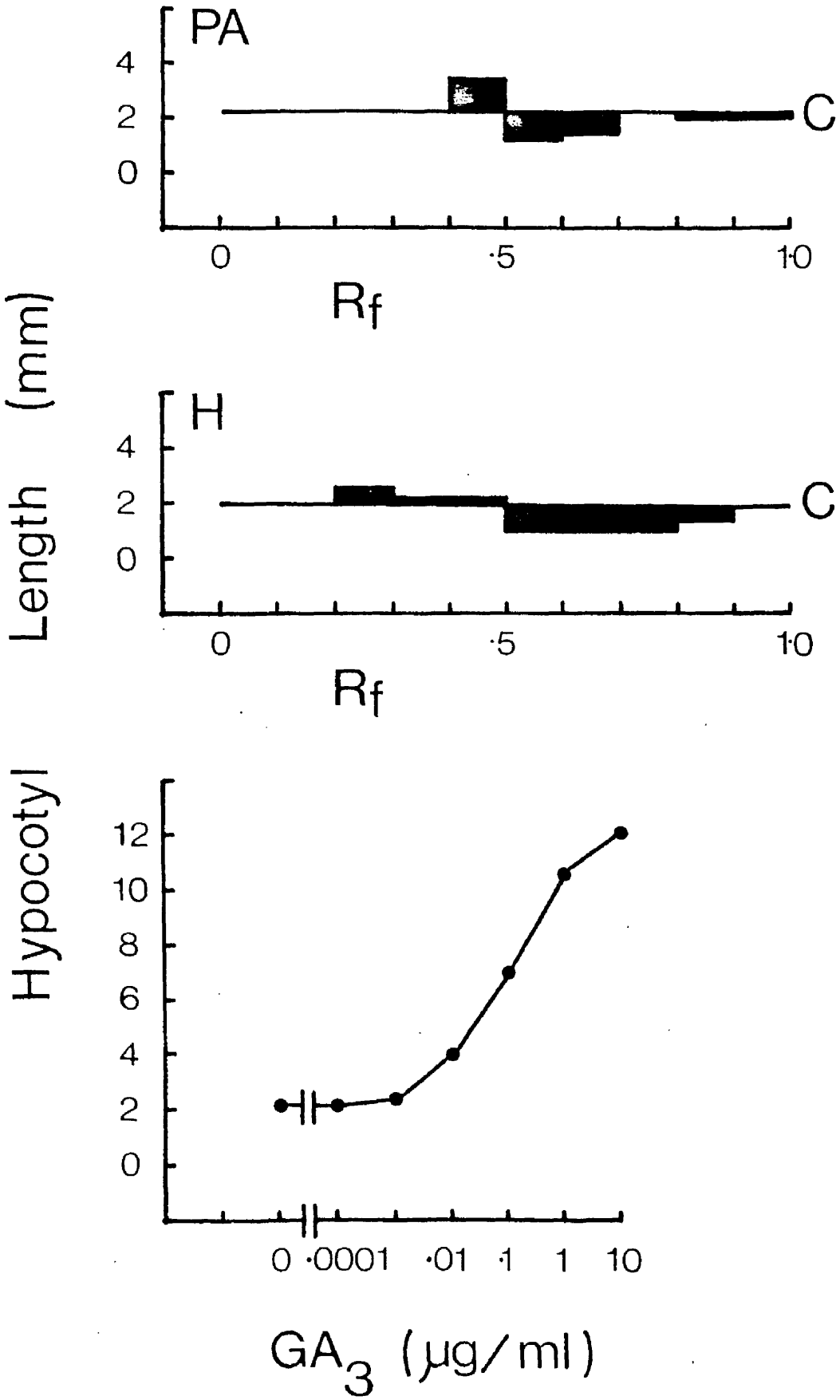

PA
4
2
0
C
0
·5
1·0
Rf
H
4
2
0
C
0
·5
1·0
Rf
Length (mm)
Hypocotyl
12
10
8
6
4
2
0
0 ·0001 ·01 ·1 1 10
GA3 (µg/ml)

Figure 59: Lettuce hypocotyl bioassay of the acidic ethyl acetate soluble fraction from agar plates which had been in contact with 503 Helianthus shoot apices. A plain agar plate (PA) was extracted as a control. Data are the mean length of lettuce hypocotyls for each Rf zone of chromatogram. Horizontal line (C) = control zone of chromatogram immediately behind the origin. Elongation in GA_3 control solutions are shown.

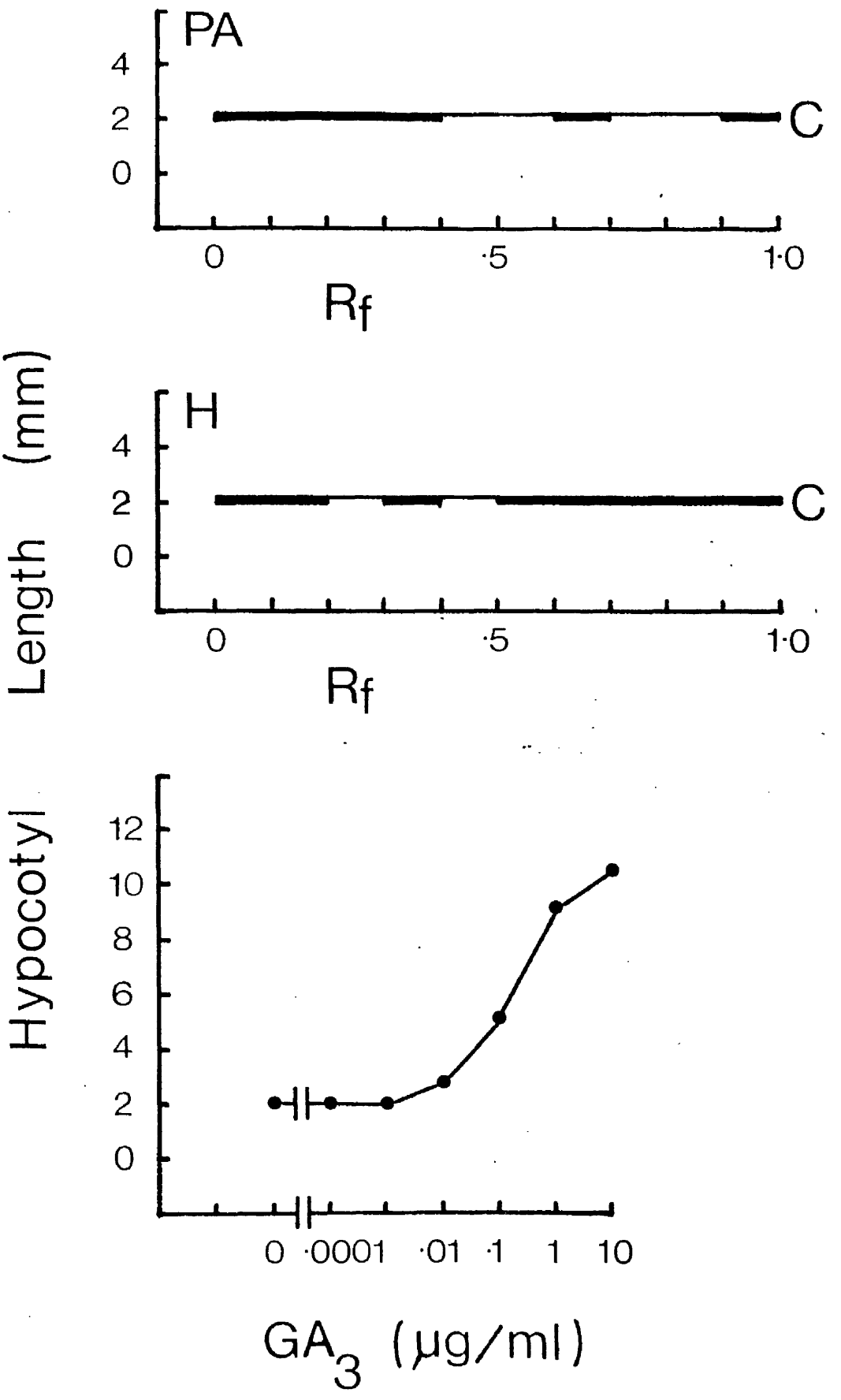
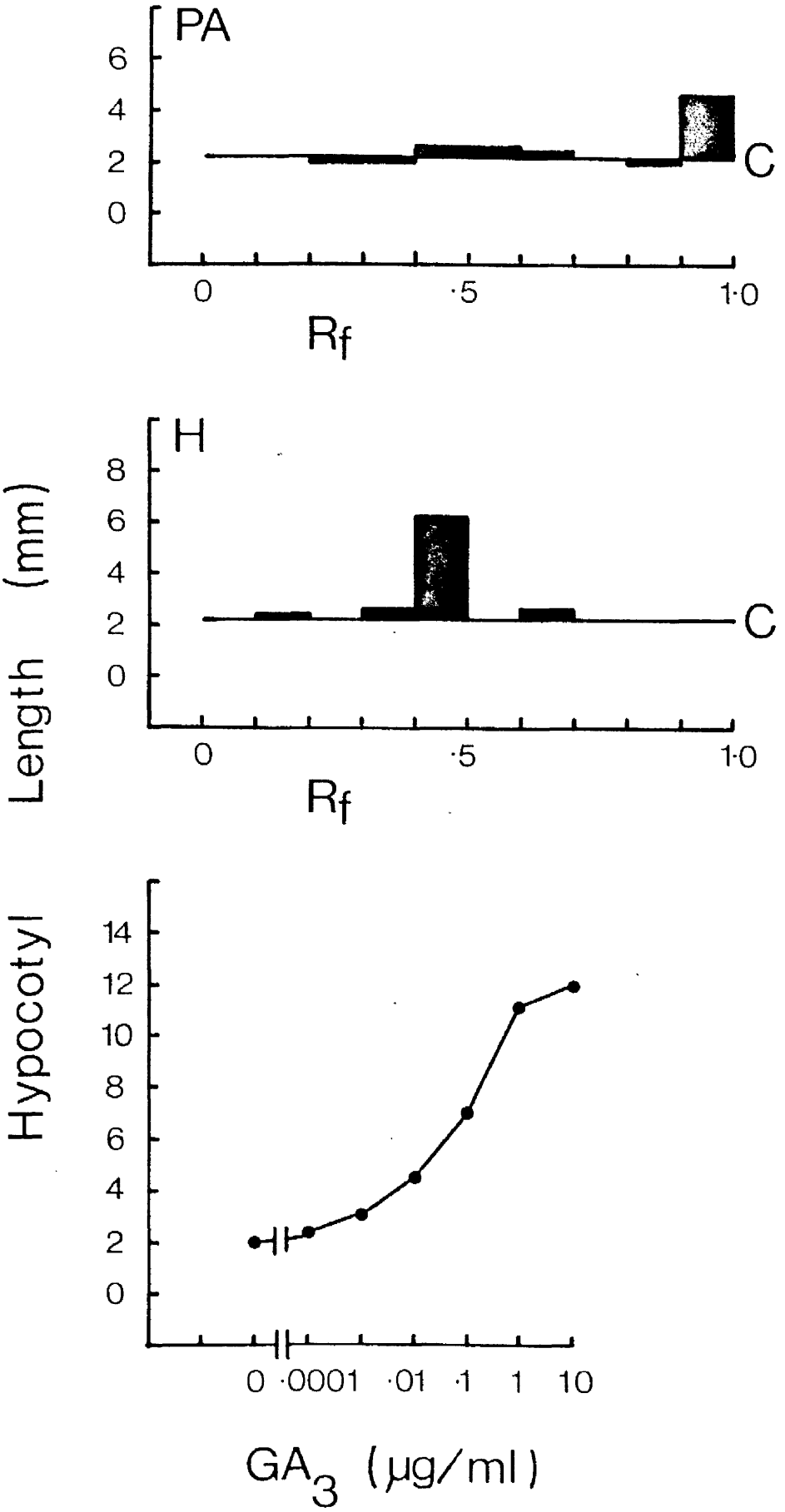

PA
4
2
0
C
0
·5
1·0
Rf
H
4
2
0
C
0
·5
1·0
Rf
Hypocotyl Length (mm)
12
10
8
6
4
2
0
0 ·0001 ·01 ·1 1 10
GA3 (µg/ml)

Figure 60: Lettuce hypocotyl bioassay of the acidic ethyl acetate soluble fraction from agar plates which had been in contact with 800 Helianthus shoot apices. A plain agar plate (PA) was extracted as a control. Data are the mean length of lettuce hypocotyls for each Rf zone of chromatogram. Horizontal line (C) = control zone of chromatogram immediately behind the origin. Elongation in GA_3 control solutions are shown.

Approximate estimated quantity of growth activity present on chromatogram:-

Rf zone	GA_3 equivalents (µg)
0.5	0.24


PA
6
4
2
0
C
0
·5
1·0
Rf
H
8
6
4
2
0
C
0
·5
1·0
Rf
Hypocotyl Length (mm)
14
12
10
8
6
4
2
0
0 ·0001 ·01 ·1 1 10
GA3 (µg/ml)

Diffusion Experiment 5.

515 apices excised from 14 d old plants were positioned vertically on agar plates alongside control plates of plain agar. Figure 61 shows the results of a lettuce hypocotyl bioassay of the acidic ethyl acetate soluble fractions from both extracts. There was no indication of any growth promoting activity.

Diffusion Experiment 6.

600 apices from 19 d old plants were placed vertically on agar plates alongside controls of plain agar for 24 h. The chromatogram of the shoot apices showed a slight growth promoting activity at Rf 0.5 - 0.6 in a lettuce hypocotyl bioassay (Figure 62) which was equivalent to 0.02 and 0.021 µgs GA_3.

Diffusion Experiment 7.

500 apices were excised from 18 d old plants and orientated vertically on agar plates alongside control plates of plain agar for 24 h. Bioassay results (Figure 63) indicated no growth promoting activity to be present in either extract.

Diffusion Experiment 8.

538 apices excised from 18 d old plants were placed on agar plates in an upright position alongside control plates of plain agar for 24 h. Figure 64 shows the result of a lettuce hypocotyl bioassay of both extracts. The chromatogram of the extract from shoot apices exhibited some growth promoting activity at Rf 0.4 - 0.6, equivalent to 0.06 µg GA_3 at Rf 0.5.

Figure 61: Lettuce hypocotyl bioassay of the acidic ethyl acetate soluble fraction from agar plates which had been in contact with 515 Helianthus shoot apices. A plain agar plate (PA) was extracted as a control. Data are the mean length of lettuce hypocotyls for each Rf zone of chromatogram. Horizontal line (C) = control zone of chromatogram immediately behind the origin. Elongation in GA_3 control solutions are shown.

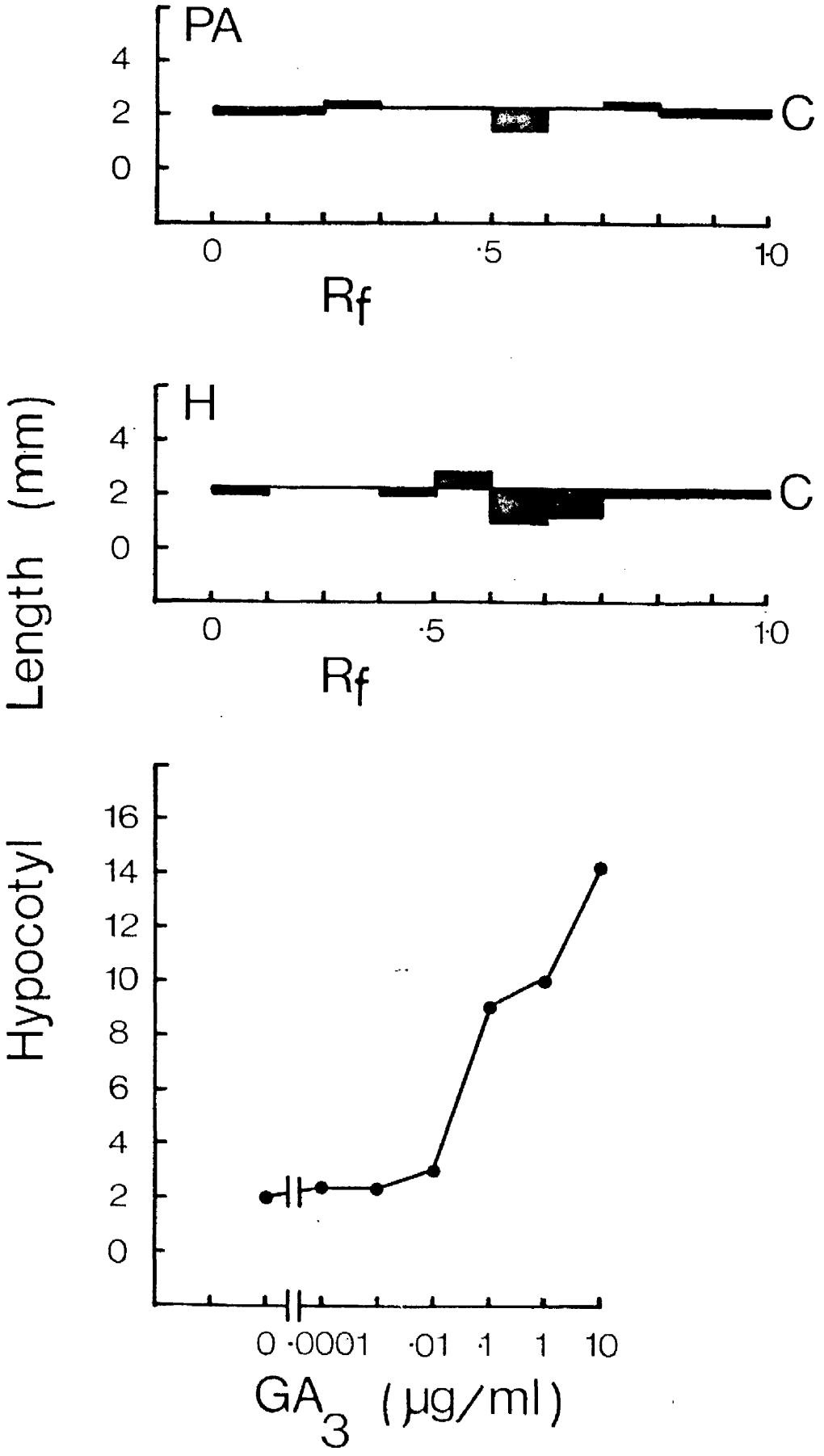

PA
4
2
0
C
0
·5
1·0
Rf
H
4
2
0
C
0
·5
1·0
Rf
Length (mm)
Hypocotyl
16
14
12
10
8
6
4
2
0
0
·0001
·01
·1
1
10
GA3 (µg/ml)

Figure 62: Lettuce hypocotyl bioassay of the acidic ethyl acetate soluble fraction from agar plates which had been in contact with 600 Helianthus shoot apices. A plain agar plate (PA) was extracted as a control. Data are the mean length of lettuce hypocotyls for each Rf zone of chromatogram. Horizontal line (C) = control zone of chromatogram immediately behind the origin. Elongation in GA_3 control solutions are shown.

Approximate estimated quantity of growth activity present on chromatogram:-

Rf zone	GA_3 equivalents (µg)
0.5	0.06
0.6	0.21

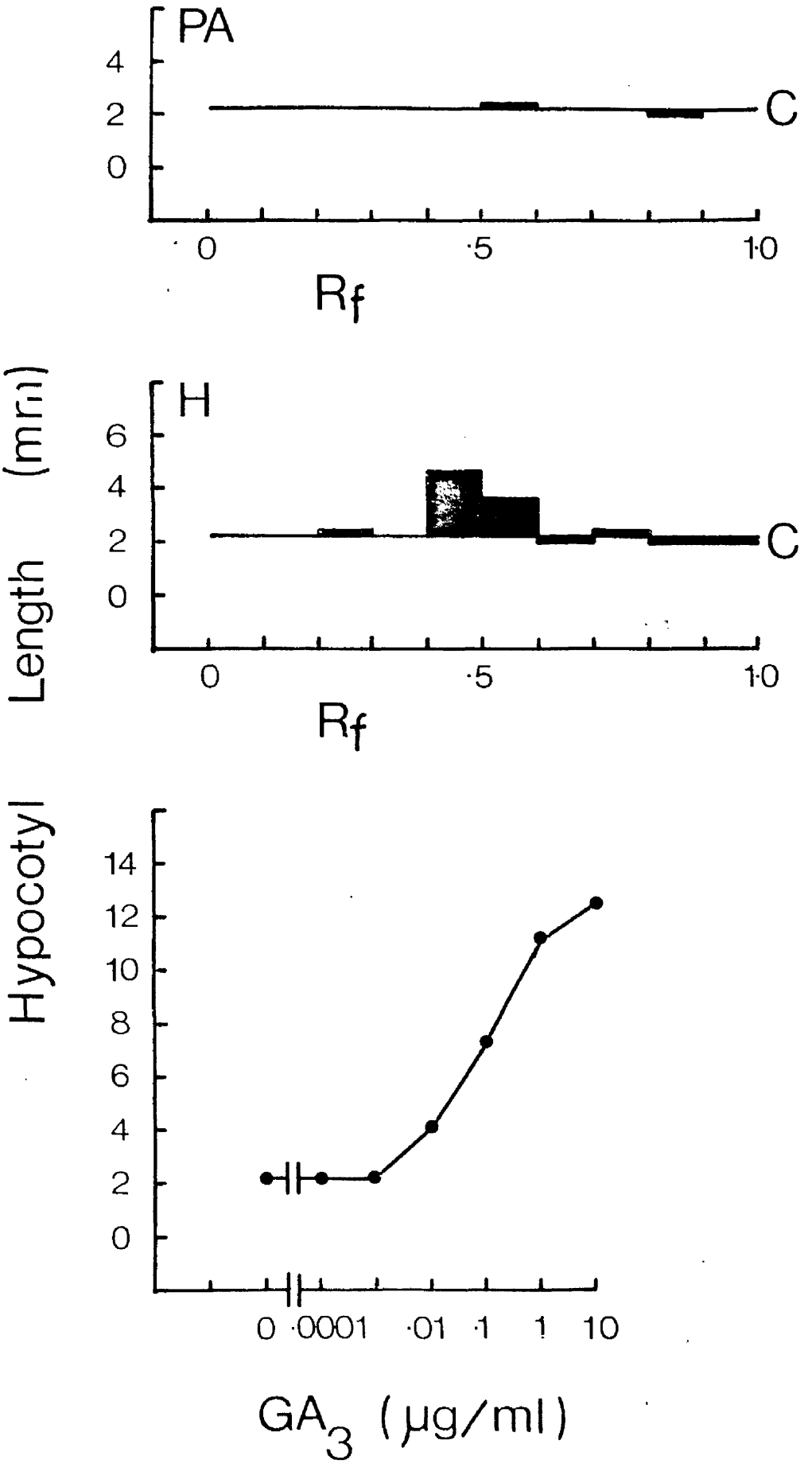

PA
4
2
0
C
0
·5
1·0
Rf
H
6
4
2
0
C
0
·5
1·0
Rf
Hypocotyl Length (mm)
14
12
10
8
6
4
2
0
0
·0001
·01
·1
1
10
GA3 (µg/ml)

Figure 63: Lettuce hypocotyl bioassay of the acidic ethyl acetate soluble fraction from agar plates which had been in contact with 500 Helianthus shoot apices. A plain agar plate (PA) was extracted as a control. Data are the mean length of lettuce hypocotyls for each Rf zone of chromatogram. Horizontal bar (C) = control zone of chromatogram immediately behind the origin. Elongation in GA_3 control solutions are shown.

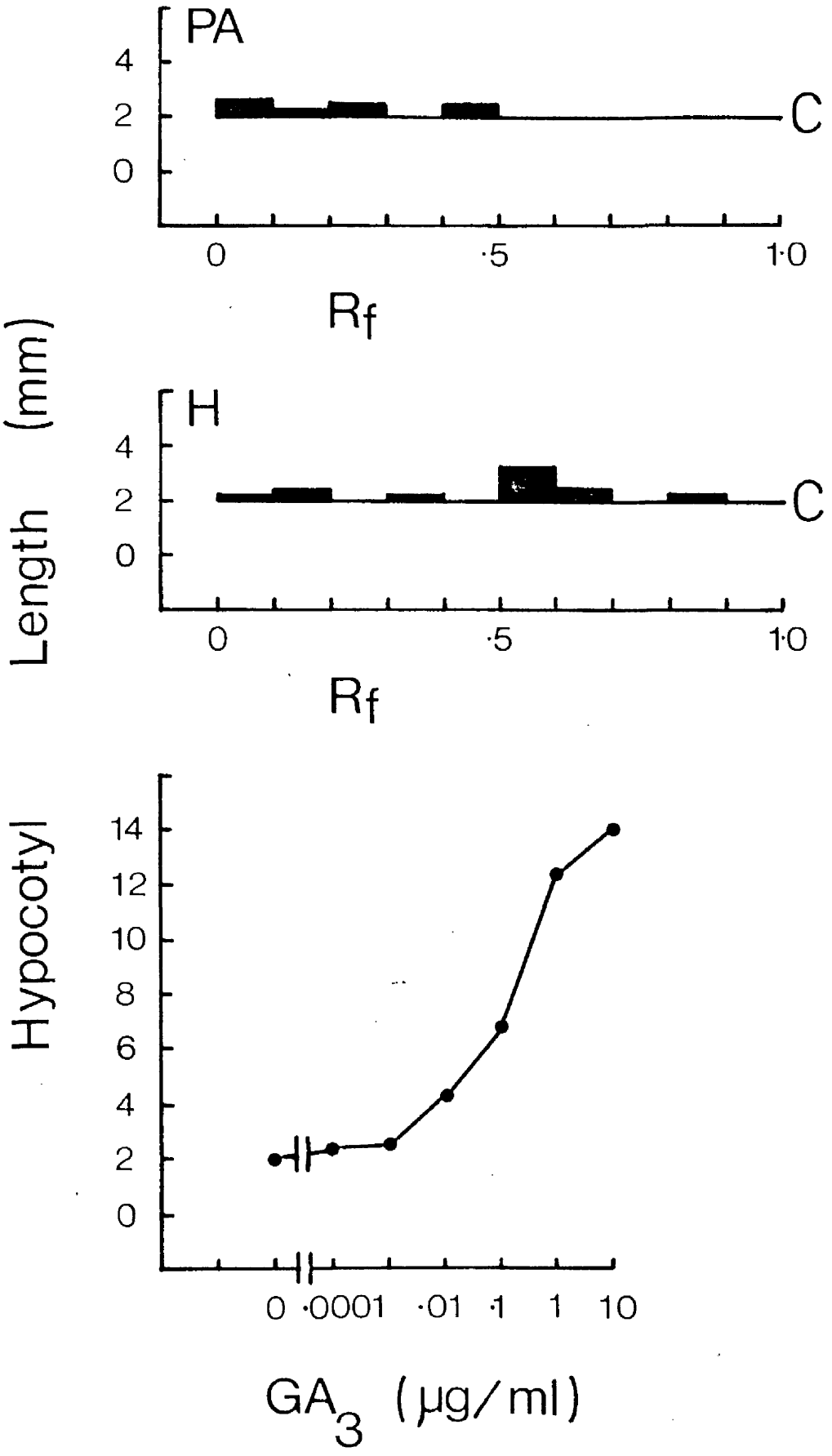
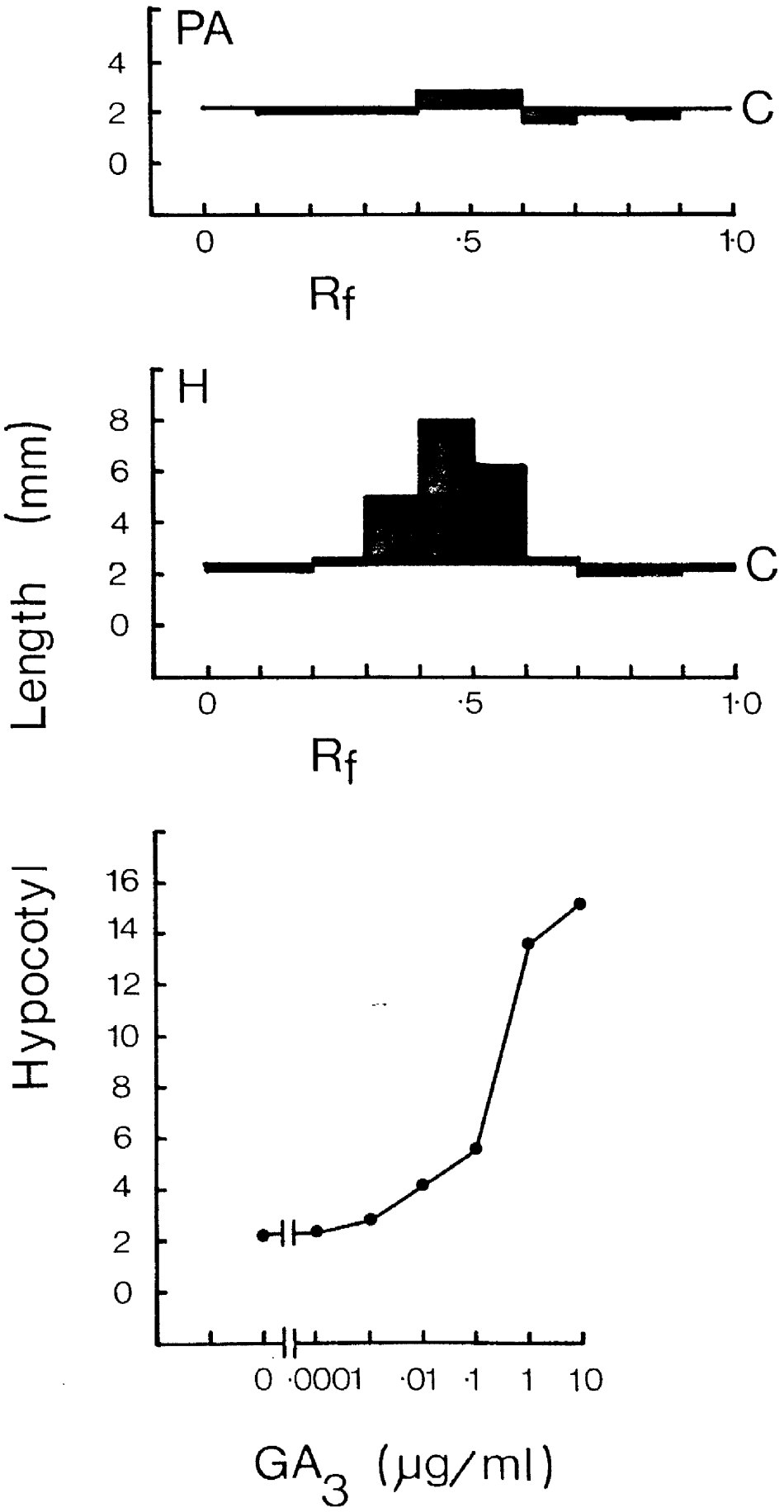

PA
4
2
0
C
0
·5
1·0
Rf
H
4
2
0
C
0
·5
1·0
Rf
Length (mm)
Hypocotyl
14
12
10
8
6
4
2
0
0 ·0001 ·01 ·1 1 10
GA3 (µg/ml)

Figure 64: Lettuce hypocotyl bioassay of the acidic ethyl acetate soluble fraction from agar plates which had been in contact with 538 Helianthus shoot apices. A plain agar plate (PA) was extracted as a control. Data are the mean length of lettuce hypocotyls for each Rf zone of chromatogram. Horizontal line (C) = control zone of chromatogram immediately behind the origin. Elongation in GA_3 control solutions are shown.

Approximate estimated quantity of growth activity present on chromatogram:-

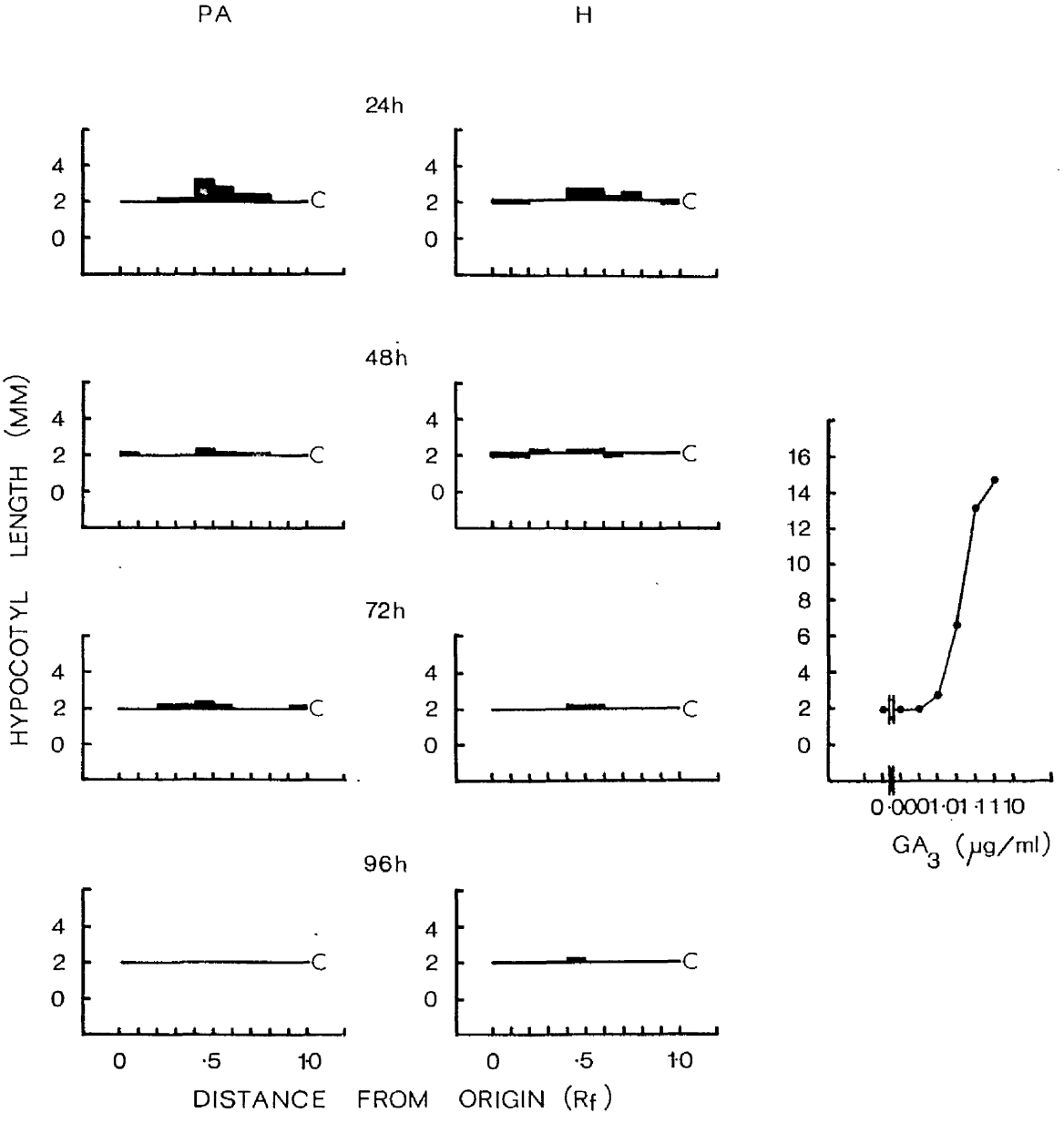
Rf zone	GA_3 equivalents (µg)
0.4	0.012
0.5	0.06
0.6	0.006


PA
4
2
0
C
0
·5
1·0
Rf
H
8
6
4
2
0
C
0
·5
1·0
Rf
Length (mm)
Hypocotyl
16
14
12
10
8
6
4
2
0
0 ·0001 ·01 ·1 1 10
GA3 (µg/ml)

Diffusion Experiment 9.

Batches of approximately 60 shoot apices from an identical batch of 16 d old plants were placed vertically on agar plates and allowed to diffuse for time periods of 24, 48, 72 and 96 h in the light at 25°C. Control plates of plain agar were placed alongside for each time point. The results of a lettuce hypocotyl bioassay are shown in Figure 65. There was no indication of any growth promoting activity.

Figure 65: Lettuce hypocotyl bioassay of the acidic ethyl acetate-soluble fraction from agar plates which had been in contact with c. 60 Helianthus shoot apices for time periods of 24, 48, 72 and 96 h. Control plates of plain agar (PA) were extracted at each time point. Data are the mean length of lettuce hypocotyls for each Rf zone of chromatogram. Horizontal line (C) = control zone of chromatogram immediately behind the origin. Elongation in GA_3 control solutions are shown.


PA
H
24h
48h
72h
96h
C
4
2
0
HYPOCOTYL LENGTH (MM)
0
·5
1·0
DISTANCE FROM ORIGIN (Rf)
16
14
12
10
8
6
0·0001
0·01
0·1
1
10
GA3 (µg/ml)

DISCUSSION

The role of gibberellin transport in the geotropic response of roots and shoots has been examined in Zea mays and Helianthus annuus seedlings. These have both proved popular test plants for hormone transport studies; particularly Zea mays seedlings which are easy to grow and produce large populations relatively quickly. Moreover cereal coleoptiles have been used extensively as an experimental material to investigate the relationship between hormone transport and tropisms. The investigation began with an appraisal of the characteristics of gibberellin transport in roots and shoots of Zea mays seedlings. Initial experiments were performed using {^{14}C}IAA and the classical agar block technique (WENT, 1928) in order to develop techniques of hormone transport studies and to confirm the existing knowledge concerning the nature of the IAA transport mechanism in Zea coleoptile segments.

Data presented in Figures 3 and 4 showed that when Zea coleoptile segments are supplied continuosly with {^{14}C}IAA, applied in agar blocks to either their apical or basal cut ends, more radioactivity moved into basal receiver blocks than into apical receiver blocks throughout the duration of the twelve hour time course. These data confirm earlier findings that IAA moves with a basipetal polarity under these conditions and establishes that the polarity exists

even after a twelve hour transport period. This basipetal polarity is a distinctive characteristic of auxin transport in sections of shoot (WENT, 1928). Confirmation that more auxin is transported basipetally than acropetally has been repeatedly established in a variety of tissues and species (VAN DER WEIJ, 1932; 1934; WENT and WHITE, 1939; JACOBS, 1954; 1961; 1967; LEOPOLD, 1961; 1963; 1964; GOLDSMITH and THIMANN, 1962; MCCREADY and JACOBS, 1963a; 1963b; KALDEWEY, 1965; PILET, 1965a).

Under anaerobic conditions (Figures 5 and 6) the basipetal movement of IAA into receiver blocks at the end of a 10 mm coleoptile segment is much reduced during a time course of 12 hours. With apical donor blocks, smaller, but still significant, amounts of radioactivity reached basal receiver blocks throughout the time course whereas the amounts of radioactivity which reached apical receiver blocks never exceeded 10 dpm. This suggests that the basipetal movement of IAA in Zea coleoptile segments under anaerobic conditions is not totally abolished and is still greater than acropetal movement. As already discussed in the Results section, this suggestion gains support from the uptake and distribution of radioactivity in the segment tissue.

The finding that the longitudinal basipetal transport of IAA is reduced in segments which have been deprived of oxygen, is in partial agreement with previously published data. WILKINS and MARTIN (1967) using 5 mm Zea coleoptile segments and only a 2 hour transport period, found that

anaerobic conditions reduced the uptake of radioactivity from an apical donor by 74% and the proportion of the total radioactivity found in the receiver block by 45%. With a basal donor, anaerobic conditions reduced the total uptake by 58% but no radioactivity reached apical receiver blocks under either aerobic or anaerobic conditions. In nitrogen, uptake from both apical and basal donor blocks was similar. Since radioactivity was found to be present in basal receiver blocks and absent in apical receiver blocks under nitrogen, it is apparent that even under anaerobic conditions, the transport of IAA is basipetally polarised, although only at a fraction of the rate established in air. In contrast to the present data however, WILKINS and MARTIN (1967) reported that with 10 mm Zea coleoptile segments, the basipetal polarity was completely inhibited under anaerobic conditions and there was no radioactivity present in receiver blocks at the basal end of the segments.

WILKINS and WHYTE (1968) reported that the polar flux observed by them under anaerobic conditions is more marked after a 3 hour transport period than after the 2 hour transport period used by WILKINS and MARTIN (1967). Data from the present experiments revealed the polarity to be even more marked after 4 hours, and by 12 hours under nitrogen, more radioactivity was present in receiver blocks than in the coleoptile tissue. Possibly after 12 hours in an atmosphere of nitrogen, the uptake process had ceased and any radioactivity present within the coleoptile tissue had moved into receiver blocks.

Confirmation of WILKINS and MARTIN'S (1967) findings came from GOLDSMITH (1967a) who supplied a pulse of {^{14}C} IAA to the apical end of 20 mm segments of Zea coleoptiles under both aerobic and anaerobic conditions and found that the rate of movement of the radioactive pulse was between 12 - 15 mm/hour in air but was reduced to only 1 - 2 mm/hour in nitrogen. Under aerobic conditions, 70% of the total radioactivity within the segment had moved 10 mm or more down the segment from the initial peak whereas under anaerobic conditions, less than 10% of the total radioactivity had moved as far in the same time period. Movement of the radioactive pulse was therefore sharply reduced by anaerobic conditions but not totally abolished.

In contrast, NAQVI, DEDOLPH and GORDON (1965) reported that the amount of radioactivity present in receiver blocks at the basal end of 10 mm Zea coleoptile segments was reduced by 50% during a 2 hour transport period under nitrogen. The authors maintained that the rate of basipetal transport was unaffected and since they reported that the amount of auxin transport in either air or nitrogen was proportional to uptake, suggested that anaerobic conditions affected the overall transport from donor to receiver by directly inhibiting the uptake.

GOLDSMITH (1967a) however argued against this since she demonstrated a direct inhibition of transport distinct from uptake, and showed that the proportion of radioactivity

which moved at least 10 mm beyond the initial position of the peak was significantly reduced in nitrogen.

Both WILKINS and MARTIN (1967) and GOLDSMITH (1967a) attributed the discrepancy between their results and those of NAQVI et al. (1965) to the use of conditions by the latter authors that were not sufficiently anaerobic to reduce basipetal transport to the level observed by WILKINS and MARTIN (1967) and GOLDSMITH (1967a). NAQVI et al. (1965) did not employ an evacuation procedure to make certain that no residual oxygen remained within the coleoptile cavity and tissues.

Similarly HERTEL and LEOPOLD (1963b) also claimed a smaller reduction in basipetal transport of IAA through Zea coleoptile segments under anaerobic conditions than that reported by WILKINS and MARTIN (1967) and GOLDSMITH (1967a) and this discrepancy was probably due again to lack of an evacuation procedure (WILKINS and MARTIN, 1967).

Experimental evidence has shown therefore that some movement of IAA persists in Zea coleoptile segments under nitrogen. Two possibilities have been suggested to explain this; either sufficient oxygen remains within the coleoptile tissue to support the transport at a reduced level or alternatively anaerobic metabolism, that is, glycolysis, is supplying the energy for the much reduced transport (GOLDSMITH, 1967a; WILKINS and WHYTE, 1968). The first possibility seems unlikely in view of the elaborate evacuation procedure employed and it is unlikely that

sufficient oxygen could remain within the tissue to support the reduced rate for up to 12 hours as shown by these present experiments. In addition, movement did not decline under anaerobic conditions, which would be likely if the oxygen supply was gradually being depleted, but rather it continued steadily but at a much reduced rate. It seems much more likely to be maintained by energy derived from glycolysis. Also since growth of the coleoptiles was totally inhibited under nitrogen, it is indicative that oxygen is not available. GOLDSMITH (1967a) suggested that the ratio of ATP production by glycolysis to that produced by aerobic metabolism, is correlated with the rate of anaerobic transport to that of aerobic. Therefore it seemed sagacious to ascertain the effect of a metabolic inhibitor such as sodium fluoride, which is known to block stages in the Embden-Meyerhof pathway, on this sustained polar flux.

From the data presented in Figures 7 and 8, pretreating the segments with sodium fluoride prior to exposure to nitrogen, did not result in complete abolition but rather diminished further, the already reduced polar flux found to exist under anaerobic conditions. Since treatment with sodium fluoride had very little effect upon the acropetal movement of IAA in both air and nitrogen, and no effect upon the basipetal polarity in air during an 8 hour transport period, it is suggestive that the basipetal polar flux

observed in aerobic conditions is independent of energy derived from the glycolytic pathway. Therefore when normal aerobic metabolism can occur, the Embden-Meyerhof pathway is not essential for energy production. However glycolysis does provide the energy necessary to maintain the polar flux under anaerobic conditions.

Although it was found that treatment with sodium fluoride did reduce further the already reduced polar flux which was found to exist under nitrogen, some discrepancy exists when this result is compared with those of previous authors. WILKINS and WHYTE (1968) reported that in the presence of two metabolic inhibitors, sodium fluoride and iodoacetic acid, the polar flux which existed under anaerobic conditions was totally abolished and reduced to that of acropetal movement which indicated a dependence upon aerobic metabolism. One possible explanation for this discrepancy is an incomplete evacuation and therefore oxygen was not totally excluded from the system. Thus the coleoptiles were not sufficiently anaerobic to reduce transport to the level observed by WILKINS and WHYTE (1968). However, this possibility does seem unlikely in view of the elaborate precuations taken. Another reason for the inconsistency might be that the coleoptile tissue required soaking in the sodium fluoride solution for a longer time period. WILKINS and WHYTE (1968) also employed a 2 h soaking period but their transport times were only 3 h, considerably less than the 8 h used in the present investigation.

WILKINS and WHYTE (1968) found too that acropetal movement was unaffected by sodium fluoride under both aerobic and anaerobic conditions and that in air, the inhibitor had no effect upon uptake from an apical donor or basipetal transport. They reported that with sodium fluoride, uptake from an apical donor under nitrogen was decreased by 30 - 45% which suggested that 55 - 70% of the total uptake of IAA was due to diffusion. The present results indicate however that uptake from an apical donor was not affected

by the inhibitor treatment under both aerobic and anaerobic conditions. However, the amount of the total radioactive uptake that was found to be present in the receiver blocks, was increasingly reduced throughout the time course from 59% after 2 hours, to 73% after 8 hours. Sodium fluoride would appear to have more of an inhibitory effect upon the exit of radioactivity from the coleoptile tissue into the receiver block than upon the uptake of radioactivity from a donor block by the coleoptile tissue. It would be interesting to investigate whether the reduction of movement of radioactivity into the receiver blocks would continue to increase if the duration of the time course was extended.

Extensive experimentation with cereal coleoptiles has led to an understanding of the relationship that exists between auxin transport and tropisms in these organs. It was suggested as early as 1937 (WENT and THIMANN) that geotropic stimulation led to a lateral redistribution of endogenous auxin in the stimulated coleoptile and it has now been unequivocally demonstrated that this is so (GOLDSMITH and WILKINS, 1964).

Figure 9 shows that when Zea coleoptile segments were supplied with asymmetric donor blocks containing {^{14}C} IAA, between 2 and 3 times as much of the total radioactivity taken up by the segments, moved laterally in horizontal segments with the donor blocks on the upper apical cut surface, as in vertical segments and in horizontal segments with donor blocks on the lower cut

surface. Since the total amount of radioactivity recovered from the segments is similar for all geotropic orientations, the difference in distribution cannot be due to differences in uptake from the donor blocks. Therefore radioactivity recovered from the non-donated half of the segment, opposite to the asymmetric donor block, must be as a result of lateral transport within the tissue.

These results support those of GOLDSMITH and WILKINS (1964) who reported that a lateral polar flux of IAA occurred towards the lower half of geotropically stimulated Zea coleoptile segments. The present data are closely similar to those of GOLDSMITH and WILKINS (1964) who found that approximately 10% of the radioactivity in vertical segments was recovered in the half opposite the asymmetric donor block whereas in horizontal segments, about 25% was found opposite an upper source but only 4% opposite a lower source. This represents a net downward lateral transport of 21%. The corresponding values from the present experiments are that 13% of the total radioactivity was recovered from the non-donated half of vertical segments, 31% from horizontal segments with upper donors and 11.9% from horizontal segments with lower donors. The magnitude of the net downward lateral movement of radioactivity was therefore 19.5%.

Similarly, both HERTEL (1962) and GILLESPIE and THIMANN (1963) divided Zea coleoptile segments longitudinally and applied agar donor blocks containing {^{14}C} IAA to the apical

cut surfaces. Radioactivity was assayed in receiver blocks which were placed against the longitudinal cut surfaces of the divided segments. GILLESPIE and THIMANN (1963) found that 3 times as much radioactivity moved into lateral receivers when movement was from the upper half of a horizontal segment than from half of a vertical segment. HERTEL (1962) found 2.5 times as much radioactivity moved laterally downward as upwards (GOLDSMITH and WILKINS, 1964). The present experiments also revealed that 2.5 times as much radioadctivity was recovered from the lower half of horizontal segments than from the upper. Therefore geotropic stimulation results in a lateral movement of IAA across vertical coleoptile segments which is less than moves into the lower half of a horizontal segment but more than moves towards the upper side of horizontal segments. Normally, the amount of radioactivity recovered from the non-donated side of horizontally orientated segments with lower donors would be less than from the non-donated side of vertical segments and Figure 10 illustrates this for both segments one and two. In contrast, the data in Figure 9 show no difference in the amounts of radioactivity from {^{14}C} IAA recovered from the non-donated sides of vertical segments and horizontal segments supplied with lower donors.

GOLDSMITH and WILKINS (1964) suggested that radioactivity could be transported laterally anywhere along the length of a 15 mm coleoptile segment, after which it could again be transported longitudinally. It follows as a consequence of this that the asymmetric distribution of radioactivity varies with the length of the coleoptile segment. As early as 1930, DOLK suggested that the asymmetrical distribution of auxin in receiver blocks, increased with the length of the coleoptile tips. Figure 10 showed that lateral transport of radioactive IAA occurred to a similar extent in 10 mm segments cut 1 mm and 11 mm

behind the apex. This would seem therefore to extend the suggestion of GOLDSMITH and WILKINS (1964) to include a greater length of segment. The uptake and distr ibution pattern of radioactivity in the coleoptile segments (Figure 10) were closely similar for the two types of tissue and they both showed a net downward lateral movement of radioactivity of approximately 30%.

The results of this experiment do not agree entirely with the findings of DE LA FUENTE and LEOPOLD (1968) who developed a technique which was a refinement of the one used by GOLDSMITH and WILKINS (1964). In their experiments unilateral application was accomplished by making a vertical cut through the diameter of the coleoptile segment and removing half of the tissue cylinder for a distance of 5 mm from the donor block. Radioactive IAA was thus supplied unilaterally to one half of a 2.5 cm or 3.0 cm long segment. Further horizontal incisions were interposed at 5 mm intervals on the non-donated side which restricted the longitudinal movement of auxin and permitted lateral IAA transport to be assessed in each isolated 5 mm section of the whole segment. The results of their experiments suggested that the lateral movement of IAA is markedly superior in the region nearest the extreme apical tip and declines in the successively lower regions. The lateral transport showed a striking responsiveness to gravity in the more apical sectors of the segment. The uppermost sector showed 25% lateral movement when gravity opposed the lateral movement, 50% when coleoptile segments were

vertical and 67% lateral movement when gravity is pulling in the direction of lateral movement. Gravity responsiveness was found to decline with distance down the coleoptile segment, increasing lateral movement at the more basal end of the segment only from 16% to 30%.

The greater facility for lateral movement in the younger, more apical tissue of Zea coleoptile segments, as reported by DE LA FUENTE and LEOPOLD (1968), is in agreement with the conclusion of BRIGGS (1963) who showed that the lateral distribution of auxin was relatively insensitive to the insertion of a razor blade to various depths from the basal ends of the coleoptile tips. The declining gradient of basipetal transport down a stem or coleoptile (JACOBS, 1950; LEOPOLD and LAM, 1962) is suggestive of the declining facility of lateral transport. Similarly SHAW et al. (1973) reported that in intact Zea coleoptile apices, the lateral movement of {^{3}H} IAA after 2 hours, was 3 to 4 times greater after application 1 mm behind the apex than in the more basal tissues, 5 mm behind the apex. A significant net downward movement of radioactivity was detected in the extreme apex of the Zea coleoptile but not in the more basal regions. This result agrees with both GOLDSMITH and WILKINS (1964) and DE LA FUENTE and LEOPOLD (1968), that lateral transport could occur along the entire length of 15 mm long sub-apical segments (SHAW et al. 1973). HERTEL et al. (1969) and FILNER et al. (1970)

have also reported that lateral transport of radioactivity from IAA is greater in segments excised from the apical region of the coleoptile than in segments excised from the more basal regions. Since the zones of elongation are in the more apical regions of the coleoptile as opposed to the more basal, it is generally acclaimed that lateral transport should occur to a greater extent in these apical regions, since the increased concentration of auxin which results from the lateral redistribution would lead to an increased growth rate.

Since the transport of gibberellin may be involved in the regulation of geotropic curvature in roots and shoots, it was clearly of relevance to establish the features of the transport system for molecules of this configuration in plant tissues.

There is clearly very little longitudinal movement of radioactivity from {^{14}C} GA_3 in Zea coleoptile segments. No polarity in the longitudinal movement of radioactivity from {^{14}C} GA_3 could be detected on the basis of the distribution of radioactivity within the tissue and no radioactivity above background was recovered from agar receiver blocks placed at either the apical or basal cut ends of the segments. This finding is in close agreement with previous reports based on experiments with sub-apical coleoptile segments and agar donor blocks.

HERTEL et al. (1969) found little longitudinal movement of {^{3}H} GA$_7$ in 2 mm segments of Zea coleoptiles after 1.5 hours. Virtually no radioactivity was found in either apical or basal receiver blocks. Neither was there any indication of a polarity based on the distribution of radioactivity within the plant tissue.

Similarly WILKINS and NASH (1974) found no evidence for any polar movement of radioactivity from {^{3}H} GA$_3$ in Zea coleoptile segments. 63% of the total radioactivity applied was found to be confined to the apical 5 mm portion of a 15 mm sub-apical Zea coleoptile segment after 8 hours and scarcely any significant amounts of radioactivity were found in either the apical or basal receiver blocks even after transport periods of 24 hours.

Likewise, PHILLIPS (pers. comm., 1975) reported that HARTUNG was unable to detect any movement of radioactive GA through coleoptile segments into agar receiver blocks.

Although his experiments were performed on pea epicotyl tissue rather than Zea mays coleoptile segments, CLOR (1967) found that GA did not exhibit the polar transport in pea stem segments observed for IAA. Since no receiver blocks were used in these experiments, conclusions were based only on the distribution of GA within the tissue.

The finding of an essentially non-polar pattern of gibberellin movement in Zea coleoptile segments is substantiated by the recent experiments of PHILLIPS and HARTUNG (1974) using segments of Phaseolus coccineus internodes.

Although the movement of {^{3}H} GA_1 was found to occur freely through internode segments ranging from 6 - 8 cm in length, only small amounts of radioactivity appeared in agar receiver blocks, particularly when transport was measured in young internode segments that were elongating. Little or no metabolism of {^{3}H} GA_1 seemed to take place during a 16 hour transport period. With 6 mm segments there was no indication of any longitudinal polarity in either young or mature segments. Using 8 cm long segments, an acropetal polarity occurred in young but not mature segments. However it seemed most likely that this was not a true acropetal polarity but rather that it was influenced by the position of the growing regions. Therefore if {^{3}H} GA_1 was being transported away from the point of application, towards the regions of growth where it was withdrawn from the transport system, then the rate at which {^{3}H} GA_1 was transferred from the point of application into the elongating zones would increase and this would produce an apparent acropetal polarity.

It seems apparent that the relatively small quantities of radioactivity which emerge into agar receiver blocks are a typical feature of the longitudinal transport of exogenous gibberellin (KATO, 1958; HERTEL et al., 1969; SCOTT and MOST, 1972; WILKINS and NASH, 1974). Thus transport of exogenous gibberellin differs from the usual behaviour of exogenously applied auxin in similar situations. Since

exogenously applied gibberellins do not appear to be transported into agar receiver blocks as easily as endogenous ones (RAILTON and PHILLIPS, 1973), it is possible that at least in Zea mays coleoptiles, exogenous and endogenous gibberellins are transported in separate channels. It may well be that although GA_1 and GA_3 do not appear to be transported into agar receiver blocks, some other endogenous gibberellins may be. JONES (1973) observed that some endogenous gibberellins diffuse into agar more readily than others.

In the present experiments, the logarithmic profile (Figure 13) was observed regardless of the length of the transport time or whether the segments were supplied with apical or basal donor blocks. Radioactivity was seen consistently to decrease along the length of the coleoptile segment with increasing distance from the donor. Similarly PHILLIPS and HARTUNG (1974) reported a logarithmic profile for acropetal and basipetal transport in longer older segments of Phaseolus coccineus internodes but in younger segments such a profile only existed for basipetal transport. A more complicated longitudinal polarity was found to exist for acropetal transport.

Although the present experiments indicated that the uptake of radioactivity by coleoptile segments was consistently greater from a basal donor than from an apical one, this can probably be attributed to differences in the surface area at either end of the coleoptile segment rather than in the

uptake process itself being greater at one end than at the other.

The inhibition of aerobic metabolism by using an atmosphere of nitrogen, has been used previously in transport experiments with IAA to establish that the basipetal polarity is an energy requiring process and is largely dependent upon aerobic metabolism (WILKINS and MARTIN, 1967). Even though there seemed to be no longitudinal polarity of {^{14}C} GA$_3$ transport, it was thought that the utilisation of anaerobic conditions would establish whether or not the uptake mechanism of {^{14}C} GA$_3$ from the donor blocks was dependent upon metabolic energy. Under nitrogen, the profile of the distribution of radioactivity within the coleoptile tissue which emerged, was essentially the same as was observed in an aerobic atmosphere, suggesting that the movement of radioactivity was as a result of passive diffusion along the segment rather than an active process. However, uptake was shown to be largely dependent upon aerobic metabolism since an atmosphere of nitrogen reduced it by a factor of between 75% and 87%. Seemingly the mechanism whereby gibberellin is taken up into the plant tissue is an energy requiring process but thereafter any movement along the coleoptile segment occurs by diffusion and subsequent to uptake, the gibberellin is not actively transported. Under nitrogen there was still no significant movement of radioactivity from the tissue into the receiver blocks.

Although RAILTON and PHILLIPS (1973) reported that endogenous gibberellins were capable of movement into agar receiver blocks and GA_3 applied exogenously to coleoptile segments did not confirm this finding, RAILTON and PHILLIPS (1973) were using intact coleoptile apices in their experiments rather than the sub-apical segments used both in the present experiments and also by previous authors (HERTEL et al., 1969; WILKINS and NASH, 1974). Comparing results obtained with sub-apical segments to those obtained with intact coleoptile apices does not seem justifiable. Accordingly, a series of experiments were designed whereby a pulse of {^{14}C} GA_3 was applied as a liquid to detached but intact Zea coleoptile apices. These experiments substantiated the earlier findings obtained using sub-apical segments and agar donor blocks; that no longitudinal polarity of gibberellin transport existed in coleoptiles and when the pulse was applied to the apex, there was no movement of radioactivity into basal receiver blocks. It would appear that the gibberellin-like activity detected in agar reciver blocks by RAILTON and PHILLIPS (1973) is clearly not GA_3 since in these present experiments, the application of exogenous GA_3 either to sub-apical coleoptile segments or to intact apices, did not result in any transport of radioactivity into receiver blocks.

Of course other endogenous gibberellins apart from the GA_3 used both in these experiments and by WILKINS and NASH (1974), may be diffusing from the coleoptiles into agar

receiver blocks. Alternatively the bioassay used by RAILTON and PHILLIPS (1973) may not be as specific as was originally envisaged and other substances in the receiver blocks may be active in promoting the extension of the lettuce hypocotyls. In addition an intact apex may be an essential requirement for the operation of a longitudinal transport system of gibberellin and for export of gibberellin into receiver blocks to occur (WILKINS and NASH, 1974).

When a pulse of {^{14}C} GA$_3$ was applied to the apices of intact primary roots of Zea, the results obtained indicated a very limited capacity for longitudinal transport. The profiles obtained for the distribution of radioactivity along the root tissue with increasing length of transport period, showed that even after 12 hours, most of the radioactivity was still confined to the region of application. Some radioactivity was found up to 12 mm from the original point of application but this amounted to only 2% of the total amount applied. Geotropic stimulation did not alter the longitudinal transport pattern in anyway since the results obtained for horizontally orientated roots were virtually the same as for vertical roots. Similarly, application of a pulse of {^{14}C} GA$_3$ to a pre-determined point 15 mm behind the root apex resulted in virtually no longitudinal movement of radioactivity further than the 3 mm region on either side of the point of application. Seemingly there was no detectable acropetal polarity.

There is very little published work concerning gibberellin transport in roots, especially roots of intact seedlings. JACOBS and PRUETT (1973) concluded that both GA_3 and {^{14}C} GA_3 moved with a basipetal polarity in 4.8 mm long segments cut from the young regions of Zea mays roots. Only gibberellin present in the agar receiver blocks was assayed and no studies were made of the distribution of gibberellin within the tissues of the root segments.

More recently HARTUNG and PHILLIPS (1974) reported that the movement of both {^{3}H} GA_1 and {^{14}C} GA_3 through apical elongating root segments from Phaseolus coccineus seedlings, was basipetally polarised on the basis of the radioactivity recovered from the receiver blocks. No difference was found in the amount of radioactivity in the root tissue after either basipetal or acropetal transport. With older non-elongating segments, higher levels of radioactivity were found in receiver blocks. Similarly no difference was found in tissue radioactivity following basipetal and acropetal transport. A comparison of gibberellin transport in both stelar and cortical tissues revealed that the basipetal polarity was restricted to the stele and was absent from the cortical tissues.

IAA has been shown to be transported in root segments with an acropetal polarity (WILKINS and SCOTT, 1968 a and b; SCOTT and WILKINS, 1968; 1969; WILKINS and CANE, 1970; CANE and WILKINS, 1970; WILKINS, CANE and MCCORQUODALE, 1972 a and b). Use of a micro-application technique has

confirmed that this acropetal polarity exists in intact Zea roots (SHAW and WILKINS, 1974) and substantiated the finding of BOWEN et al. (1972), who used soluble compound micro-autoradiography and girdling techniques, that the transport mechanism occurs only in the stele. It is apparent therefore, that in Zea roots, IAA and gibberellin are transported longitudinally in opposite directions. However, since it has now been shown unequivocally that IAA is synthesised in the apices of coleoptiles (GREENWOOD et al., 1972), is basipetally transported away from the shoot apex and acropetally transported along the roots and since there is evidence of the sites of gibberellin synthesis and/or conversion being the root tip tissues (BUTCHER, 1963; JONES and PHILLIPS, 1966; KENDE and SITTON, 1967; SITTON, RICHMOND and VAADIA, 1967; SKENE, 1967; CARR and REID, 1968; CROZIER and REID, 1972; FRYDMAN and WAREING, 1973 a and b), both IAA and gibberellin are being longitudinally transported away from where they were synthesised.

As mentioned previously for gibberellin transport in coleoptiles, it is difficult to compare results obtained with excised root segments and agar donor blocks where polarity is based on radioactivity in agar receiver blocks, (JACOBS and PRUETT, 1973; HARTUNG and PHILLIPS, 1974) with the present experiments using roots of intact seedlings, a pulse of radioactivity and where polarity is based on the distribution of radioactivity within the root tissue. Nevertheless, it does seem that some longitudinal movement of gibberellin is apparent in the basipetal direction. Of

course it is possible that the profile of radioactivity along the root, observed in the present experiments, may be as a result of diffusion and not an active process. Inhibiting aerobic metabolism would enable this to be investigated.

Asymmetric application of a pulse of {^{14}C} GA_3 to both horizontally and vertically orientated coleoptiles and roots of intact Zea mays seedlings showed that there was clearly very little longitudinal movement of radioactivity within the tissue and therefore confirmed the findings of previous experiments and published reports (HERTEL et al., 1969; WILKINS and NASH, 1974) which used sub-apical coleoptile segments and agar donor blocks incorporating the radioactive gibberellic acid.

No downward lateral movement of radioactivity was detected and this has supported the experiments of WILKINS and NASH (1974) who were unable to detect any downward lateral movement of {^{3}H} GA_3 in horizontally orientated sub-apical Zea coleoptile segments which had been supplied with asymmetric donor blocks. Thus the present experiments have substantiated for intact seedlings what was previously known for sub-apical coleoptile segments.

However the present experiments have revealed that a marked net upward lateral movement of radioactivity from {^{14}C} GA_3 occurred from the lower to the upper half of both horizontal coleoptiles and roots. WILKINS and NASH (1974) did not observe this upward lateral movement of radioactivity

since they compared the movement and distribution of radioactivity from {^{3}H} GA_3 in vertical coleoptiles only with that in coleoptiles which had been orientated horizontally with agar donor blocks in contact with the upper half of the apical end. They did not examine the distribution of radioactivity in horizontal coleoptiles with agar donor blocks in contact with the lower half of the apical end.

The observations of a significant upward lateral movement of radioactivity from {^{14}C} GA_3 in intact coleoptiles provides further discrepancy in comparison with the report of RAILTON and PHILLIPS (1973). They claimed that a gradient of gibberellin-like activity arises in favour of the lower of two receiver blocks at the basal cut end of a horizontally orientated detached Zea coleoptile apex. A plausible explanation of their results may be the differential synthesis and/or release of other endogenous gibberellins present in the upper and lower halves of the horizontal apex which may be concealing or reversing the effect of an existing upward lateral movement of GA_3. It is possible that GA_3 may exist as a naturally occurring gibberellin in Zea mays coleoptiles. The endogenous gibberellins present in Zea mays coleoptiles have not yet been identified although GA_3 has been found to be present in Zea mays seeds (JONES, 1964).

It has already been discussed that in roots, GA_3 and IAA seem to be transported longitudinally in opposite directions and a similar situation seems to exist in

geotropically orientated coleoptiles. The existence of an upward lateral transport of radioactivity from {^{14}C} GA_3 in horizontal coleoptiles in which an active downward lateral transport of {^{3}H} IAA is known to be taking place (SHAW et al., 1973) demonstrates the operation of two opposed polar transport systems in the same organ, each with very different molecular specificity.

The geotropic response of a coleoptile, that is; the curvature which arises as a result of the different rates of elongation of the upper and lower halves of a horizontal coleoptile, is usually attributed to the result of a downward lateral transport of IAA from the upper to the lower halves of the coleoptile, such that a high concentration of auxin is established in the lower half. Since IAA is known to stimulate the elongation of a coleoptile, the rate of elongation of the lower half is greater than the upper which results in upward curvature. However, if this upward movement of GA_3 does occur, it may well be that it is as a result of the downward IAA transport. Alternatively, gibberellin could be inhibiting extension growth on the upper half of the coleoptile or at least contributing towards reducing the growth rate of the upper side which would also result in upward curvature. As yet gibberellins have not been shown to inhibit the elongation growth of coleoptiles. A factor in favour of this possible hypothesis is that the reports of gibberellin acting as a growth

promoter of coleoptiles are for the large part confusing and the gibberellin is usually applied in the presence of an IAA source. HAYASHI and MURAKAMI (1953b) reported that gibberellin had no effect on the extension of wheat coleoptile sections, either alone or in the presence of IAA. BRIAN et al., (1955), on the other hand, reported a quite definite response but again less than that from IAA and with a characteristic flat-topped log-dose response curve. HAYASHI and MURAKAMI (1953b) reported a very slight increase in extension of etiolated oat coleoptile sections which was not detectable in the presence of IAA but NITSCH and NITSCH (1956) using more refined techniques, found that GA_3 increased the extension of etiolated oat coleoptile and oat first-internode sections, though it was far less active than IAA. Their data also suggest a flat-topped log-dose response curve. If the RAILTON and PHILLIPS (1973) hypothesis is to be believed, the gibberellin-like activity which they claim undergoes downward lateral movement, must be shown to be capable of promoting the growth of coleoptiles. As yet, gibberellin promotion of coleoptile extension growth has only been demonstrated with exogenously applied gibberellins, usually in the presence of exogenous auxin as well. It would be interesting to collect endogenous gibberellin-like substances from Zea coleoptile apices, in agar receiver blocks and to reapply these agar blocks to coleoptile segments to see if they induce any curvature. Obviously plain agar blocks could be used for controls. This has not yet been attempted.

The net upward lateral transport of radioactivity from {^{14}C} GA_3 which has also been observed to occur in horizontally orientated primary roots of intact Zea mays seedlings, is in close accord with the recent paper of EL. ANTABLY and LARSEN (1974a) who reported that extracts from the upper halves of horizontally orientated primary roots of Vicia

faba exhibited between 1.8 and 3.7 times as much gibberellin-like activity as extracts from the lower halves after only 30 minutes geotropic stimulation. The gibberellin-like activity was measured by the lettuce hypocotyl bioassay. GA_3 was found to be present naturally in these roots and to be able to promote the extension growth of root cells. If GA_3 does play a role in geotropism, then this lateral gradient of extractable gibberellin-like activity could be responsible for the downward geotropic curvature of roots, by stimulating the elongation of the upper half of the root.

If the transport system in Vicia faba roots is closely similar to that in Zea mays, that is; if (^{14}C) GA_3 is found to be laterally transported upwards, then this upward movement may be wholly or partially responsible for the establishment of the lateral gradient of gibberellin-like activity observed by EL. ANTABLY and LARSEN (1974a). A more recent paper by EL. ANTABLY and LARSEN (1974b) confirmed the finding in the earlier paper of a lateral gradient of gibberellin-like activity, using the more precise and sensitive technique of gas-liquid chromatography rather than bioassay. In addition, application of GA_3 to Vicia faba and Lepidium sativum roots showed that low concentrations of this compound do stimulate root cell elongation within a few hours and possibly even within 1 hour. The stimulation of root elongation by gibberellins has also been demonstrated in other species (RICHARDSON, 1958; BUTCHER and STREET, 1960; ABDALLA and EL. ANTABLY, 1970; EL HINNAWY, 1973).

It does therefore seem plausible that gibberellins play a contributing role in the development of the positive geotropic curvature in roots.

As in shoots, roots seem to have two oppositely polarised lateral transport systems with different molecular specificities, the one carrying gibberellin upwards and the other inhibitory substances downwards (GIBBONS and WILKINS, 1970; SHAW and WILKINS, 1973).

Similar experiments applying a pulse of {^{3}H} GA_1 to horizontal roots and coleoptiles revealed little longitudinal transport in either, which was a similar result to the one obtained with {^{14}C} GA_3. Surprisingly however, in contrast to {^{14}C} GA_3, no upward lateral movement of radioactivity occurred in horizontal coleoptiles and roots. There was no lateral redistribution of {^{3}H} GA_1 upon geotropic stimulation. Since there have been no previous published reports of any lateral movement of {^{3}H} GA_1 it seems unlikely therefore that GA_1 plays any significant role in the establishment of geotropic curvature in these organs. If this is so, then the lateral transport system for gibberellins must be extremely specific if it can distinguish between one double bond.

A qualitative analysis of the radioactivity present within the tissues of coleoptiles and roots, either 3 hours or 6 hours after the asymmetric application of a pulse of either {^{14}C} GA_3 or {^{3}H} GA_1, showed that virtually almost

all the {^{14}C} and {^{3}H} were still associated with the GA_3 and GA_1 molecules. Obviously both {^{14}C} GA_3 and {^{3}H} GA_1 are either not metabolised or alternatively metabolised extremely slowly in these tissues. Geotropic stimulation seemed to have no effect on the metabolism. Few data have been published on the metabolism of gibberellins in Zea mays seedlings. HERTEL et al. (1969) in their transport experiments with coleoptile segments, did not establish whether the radioactivity was still confined to the {^{3}H} GA_1 molecule. Similarly WILKINS and NASH (1974) did not analyse the radioactivity present within the coleoptile segments and receiver blocks to see if the {^{3}H} GA_3 molecules had been metabolised. The only published results from experiments investigating the movement of {^{14}C} GA_3 in Zea mays roots neglected to investigate the possible metabolism of {^{14}C} GA_3 (JACOBS and PRUETT, 1973). PHILLIPS and HARTUNG (1974) in their paper on {^{3}H} GA_1 transport in internode segments of Phaseolus coccineus reported that {^{3}H} GA_1 was recovered unmetabolised as assayed by TLC using a solvent system of ethyl acetate: chloroform: acetic acid :: 50 : 10 : 1. Similarly {^{3}H} GA_1 injected into cotyledons and roots of intact Phaseolus coccineus seedlings (HARTUNG and PHILLIPS, 1974), and segments of Helianthus annuus internodes (PHILLIPS and HARTUNG, 1976) was not metabolised as measured by the same solvent system.

Since it is obviously erroneous to assume that what has been established for coleoptiles of etiolated seedlings, can be extended to include intact green plants, the next series of experiments were designed using Helianthus annuus seedlings. Asymmetric application of a pulse of {^{3}H} GA_1 to both hypocotyls and epicotyls of geotropically stimulated intact seedlings of Helianthus annuus revealed virtually no basipetal longitudinal transport. Most of the radioactivity remained where it was applied although there was a slight acropetal movement. Surprisingly there was no lateral redistribution of radioactivity as a result of geotropic orientation. This is disconcerting when viewed against the results of PHILLIPS (1972a) who investigated the diffusible gibberellins that were collected from Helianthus apical buds in relation to geotropic stimulation. Almost 10 times as much gibberellin-like activity was obtained from the lower as from the upper tissues of horizontal shoot tips whereas approximately equal quantities were obtained from the two halves of vertical buds. PHILLIPS (1972a) suggested that this lateral gradient in the distribution of gibberellin-like activity may arise as a result of lateral transport of gibberellins and also enhanced gibberellin synthesis in buds orientated horizontally.

Of course the same arguments are applicable here as they were for the RAILTON and PHILLIPS (1973) hypothesis for Zea coleoptile apices. GA_1 may not be a naturally occurring

gibberellin of Helianthus annuus which would possibly explain why it did not undergo any lateral redistribution when applied to intact seedlings. Alternatively endogenous gibberellins other than GA_1 may be undergoing differential synthesis or downward transport in the horizontal detached shoot apices. Also the endogenous gibberellins which are collected in agar receiver blocks may not be a true reflection of those present in the transport system but rather may be gibberellins which are produced as a "wounding effect" (RAPPAPORTH and SACHS, 1967) by the injured cells at the cut surfaces in contact with the agar receiver blocks. This difference in behaviour between endogenous and exogenously applied gibberellins suggests that their transport processes are different. On the other hand, JONES (1973) reported that some endogenous gibberellins of higher plants do diffuse into agar more readily than others. Therefore it is possible that the transport behaviour of endogenous and exogenous gibberellins is essentially similar (PHILLIPS and HARTUNG, 1974). In addition it seems hardly reasonable to compare results obtained from isolated excised apical buds with those from intact seedlings complete with an extensive root system.

Since previous work has shown that the young leaves of the apical buds of Helianthus annuus seedlings are the sites of gibberellin synthesis and that gibberellins are concerned with the regulation of cell extension, (JONES and PHILLIPS, 1966; 1967) it is difficult to comprehend why no mechanism

exists for the basipetal longitudinal transport of gibberellins from these sites of synthesis to the regions of growth. Recent results obtained by HARTUNG and PHILLIPS (1974) compare favourably with the minimal longitudinal transport in Helianthus seedlings observed in these present experiments. They observed that neither {^{3}H} GA_1 or {^{14}C} GA_3 moved out of the growing leaves of the apical buds of Helianthus seedlings. Unfortunately they give no further details of these experiments. By way of a contrast to Helianthus seedlings, they reported that {^{3}H} GA_1 which was injected into the cotyledons of Phaseolus coccineus seedlings moved with an acropetal polarity into the roots.

However, the finding in the present study, that the lateral movement of radioactive gibberellic acid in intact Helianthus seedlings is unaffected by gravity, is confirmed by the very recent publication of PHILLIPS and HARTUNG (1976) on the effects of gravity upon transport of exogenous {^{3}H} GA_1 and {^{14}C} IAA in isolated segments of Helianthus internodes. They claimed that the distribution of radioactivity within the tissues of 2.5 or 3.0 cm internode segments was unaffected by geotropic stimulation, that is; displacing segments from the vertical to the horizontal position. This was true whether agar donor blocks were applied symmetrically or asymmetrically across the apical cut surfaces of the segments and gravity did not influence either the downward or upward lateral movement of gibberellin

or auxin. When vertical Helianthus stem segments were incubated for 6^{h} with either symmetrically or asymmetrically applied agar donor blocks containing GA_1 or GA_3, no growth curvature developed, although elongation growth was enhanced. In contrast, horizontally orientated stem segments exhibited marked negative curvature when GA_1 was applied either symmetrically or asymmetrically to the apical cut surfaces. Horizontally orientated segments exhibited only slight negative geotropic curvature with plain agar donors and no curvature at all when no agar blocks were applied. The elongation growth induced by gibberellic acid in vertical and horizontal segments was equal. Although the need to be cautious, when comparing results obtained with segments to those obtained with intact plants, cannot be over-emphasised, it does seem that the results obtained in the present study agree with those of PHILLIPS and HARTUNG (1976).

An essential requirement for the demonstration of the involvement of a substance in a physiological process is that the substance must be present in the organism (JACOBS, 1959). It seemed prudent therefore to establish that gibberellins were naturally occurring in higher plants since their possible role in geotropism was under investigation. Since the first reports on the presence of gibberellin-like substances in higher plants (RADLEY, 1956; WEST and PHINNEY, 1956) most investigations of the naturally occurring endogenous gibberellins have been conducted using solvent

extraction techniques. Although agar diffusion techniques have been used extensively in, and form the basis of, research on endogenous auxins, it was not until 1964 that attempts were made to obtain gibberellins from the plant tissue by the agar diffusion method. JONES and PHILLIPS (1964)showed that gibberellins readily diffused from apical buds of Helianthus annuus and that the technique could be used for the quantitative determination of the gibberellin content of plant organs. Further evidence of the usefulness of agar diffusion for obtaining gibberellins from plant organs was shown by JONES and PHILLIPS (1966). The technique of agar diffusion offers several distinct advantages over extraction methods. An estimation of endogenous gibberellins by extraction indicates the gibberellin content of the extracted organ at a single point in time. Agar diffusion, however, enables an estimation of hormone production over a specific time period (JONES and PHILLIPS, 1966; 1967). The advantages of agar diffusion for a quantitative estimation of endogenous gibberellin content have been shown by BAILISS and WILSON, 1967; STODDART and LANG, 1967 (JONES, 1968a).

SCOTT and JACOBS (1964) in a critical examination of the techniques available for the isolation of auxin from plant tissues, concluded that the most suitable method was that called 'agar diffusion' in which isolated plant organs

were placed with a cut surface against agar which is subsequently assayed for growth activity (WENT, 1928). SCOTT and JACOBS (1964) indicated the incorrectness of the term 'diffusion' for this technique and therefore it has been called 'collection in agar' in the present investigation. The use of this technique results in an indication of the transportable growth substances within the plant tissue and these may be of a greater physiological significance than those extractable substances.

Accordingly, the method of RAILTON and PHILLIPS (1973) was employed in the present study. Authentic standard GA_3 was incorporated into some plain agar plates at a range of concentrations and passed through the similar procedures used for plants. The maximum recovery was equivalent to 8.97 µgs of GA_3 and indicated a 8.97% recovery compared with the 100 µgs of GA_3 which was the maximum amount incorporated into the plates. However the amount of standard GA_3 applied was above the maximum response level of the lettuce hypocotyl bioassay and therefore the recovery for this method would be expected to be greater. The maximum sensitivity for the lettuce hypocotyl bioassay is reported to be 10 µg/ml (BAILISS and HILL, 1971). A more accurate result would have been produced if extracts had been subjected to a dilution procedure, prior to bioassay, in order to bring the amount of gibberellin activity being detected to within the range of the standard curve. The determination of GA_3 equivalents by reference to a standard curve has a very limited accuracy. A standard curve plots growth against logarithmic dose and obviously only very large differences will be detected. The lettuce hypocotyl bioassay is very inadequate for quantifying gibberellins and in future studies this aspect needs to be viewed extremely critically. A more accurate result would be obtained by incorporating {^{3}H} GA_1 into a series of agar plates and following its fate through the extraction procedure with scintillation counting. Alternatively a fluorescence technique might prove useful in detecting areas of gibberellin activity on the chromatograms.

Results from the 'agar collection' experiments were systematically negative; that is, no growth promoting activity in the lettuce hypocotyl bioassay was detected on any occasion. It is very difficult to explain these results

in light of those obtained by RAILTON and PHILLIPS (1973). They employed a closely identical technique and reported that gibberellin-like activity equivalent to as much as 0.4 µg of GA_3, was the total yield from 100 vertically orientated coleoptile apices after 20 hours diffusion. By geotropically stimulating the apices, that is, placing them horizontally, the yield was increased 5 fold to 2.1 µgs which is equivalent to 0.02 µgs of GA_3 per coleoptile.

Failure in the present study to obtain any gibberellin-like activity from fairly small numbers of coleoptile apices in both vertical and horizontal orientations, prompted an investigation into large numbers of apices. A consequential result therefore was to use batches of 500 apices but this too failed to produce any growth promotion in the lettuce hypocotyl bioassay. Personal communication with RAILTON also failed to produce insight into the reason why his results could not be repeated. One possibility was thought to be too low a light intensity for the growth of the lettuce seedlings throughout the 3 days of the bioassay but since it is the magnitude of the response but not the sensitivity, that is affected by the light source (TINKLIN, 1968) and since GA_3 control solutions produced a standard response on every occasion, this was clearly not a contributing factor in the continual failure of the 'agar collection' experiments.

A prominent feature in every experiment was the inhibition of growth that occurred at Rf 0.3 - 0.5 which was the expected Rf value for any possible gibberellin-like activity. Since this inhibitory effect could be negating any gibberellin effect that might be present, experimental procedures were adapted in an attempt to remove possible inhibitory substances (RAILTON pers. comm., 1974). The lettuce hypocotyl bioassay is known to be very sensitive to endogenous inhibitors (BAILISS and HILL, 1971). RAILTON and PHILLIPS (1975) also obtained these regions of inhibition in their experiments. Two adaptations to the original technique: purification by a dilution procedure and purification by using a charcoal/celite column, were introduced on extracts of agar which had been in contact with 500 horizontally orientated apices but this too proved unsatisfactory in revealing any gibberellin-like growth promoting activity, although the problem of growth inhibition was alleviated.

Since the 'collection in agar' experiments had failed to demonstrate the presence of any gibberellin-like substances in Zea coleoptile apices and since RAILTON (pers. comm., 1974) was insistent of their presence at high concentrations in Zea coleoptile tips; alternative experimental techniques were explored. Moreover GA_3 has been shown to be present in the seeds of Zea mays by the demonstration of growth promoting activity in the following bioassays:- dwarf pea, cucumber hypocotyl, lettuce hypocotyl and barley endosperm

(JONES, 1964). GA_3 has also been found to exist in seed extracts of Hordeum vulgare (JONES, MACMILLAN and RADLEY, 1963), Festuca pratensis and Phaseolus multiflorus. Gibberellins are formed in considerable quantities during seed development (CLELAND, 1969) and the largest amounts are being produced when the nucellus and/or endosperm degenerate (CORCORAN and PHINNEY, 1962; JACKSON and COOMBE, 1966; LUCKWILL, WEAVER and MACMILLAN, 1969; CHACKO, SINGH and KACHRU, 1970). As seed development proceeds towards dormancy, free gibberellins are converted to bound forms as glycosides of gibberellic acid (BARENDSE et al., 1968; BARENDSE, 1971; SEMBDNER et al., 1972). In a number of dicotyledonous species, free gibberellins have been shown to be released from these bound forms after the germination of the seeds (SHELDRAKE, 1973). Therefore it is possible that GA_3 may exist in Zea seedlings since it has been found in the seeds.

JONES and LANG (1968) and JONES (1968a) have demonstrated clearly with apical buds of pea, that there were discrepancies in both the quality and quantity, between extractable and 'diffusible' gibberellins. Both GA_1 and GA_3-like gibberellins were appraised by extraction but only the GA_1-like form was obtained by 'diffusion'.

Accordingly the present study was continued using an extraction procedure devised by CROZIER (pers. comm., 1975). Methanol was used, being a good extractant for plant material.

Extracts were partially purified by running through a polyvinylpyrollidone column and further purification was achieved by TLC. Chromatograms were developed in either acidic or basic solvent systems prior to bioassay with the lettuce hypocotyl or dwarf rice bioassay. (MURAKAMI, 1968).

By passing standard GA_3 through the procedures used for extracts of plant tissue, a figure of at least 10% recovery was obtained. Although there was considerable variation between experiments, evidence was obtained in only 1 out of 5 extraction experiments, of a substance that was active in promoting the growth of lettuce hypocotyls. The position of this activity on the chromatogram co-chromatographed with the expected position of GA_3. Unfortunately a marker spot of GA_3 was not run on the same chromatogram as the plant extract in this experiment, for fear of contamination.

The quantity of activity detected, equivalent to approximately 2.0 µg GA_3, compares favourably to that obtained by RAILTON and PHILLIPS (1973) from coleoptile apices by agar diffusion. They reported a figure of 0.004 µgs of GA equivalents per coleoptile apex (0.4 µgs from 100 apices). In the present study, activity equivalent to approximately 2.0 µg was obtained from 560 coleoptiles and this represents 0.0036 µgs per coleoptile.

PVP is known to be highly effective in the purification of gibberellin-like substances in plant extracts, presumably by the selective removal of phenolic compounds and possibly other organic acids. GLENN et al (1972) used a PVP column, 1.9 cm x 30 cm, eluted with 0.1M pH 8.0 phosphate buffer to determine the elution pattern of a broad spectrum of GAs. They reported that their seven sample GAs (GA_1, GA_3, GA_4, GA_5, GA_7, GA_8, GA_9) were recovered in the 60 - 160 ml range. Comparing the volume of the column used in the present investigation (61.39 cm^3) to that used by GLENN et al (85.07 cm^3), it is possible to calculate that most GAs would be recovered in the 43 - 115 ml fractions. Since only the first 60 ml were collected, it is highly probable that much of the gibberellin-like activity was still retained on the column. As a preliminary, the elution pattern of {^3H} GA_3 should have been determined prior to the column being used for plant extracts.

Again, as in the 'agar collection' experiments, inhibition of the elongation growth of hypocotyls was observed, even though extracts had been partially purified by apolyvinylpyrollidonecolumn prior to chromatography. The concept that this inhibitory effect may be masking any growth promoting activity that could be present, led to an alternative bioassay being used. The rice microdrop assay whereby test seedlings are treated with only 1 µl of the test solution, is sensitive to GA_3 at a range of 0.05 - 100 mg/ml whereas the lettuce hypocotyl bioassay is only sensitive at a range of 0.01 - 10 mg/ml. Nonetheless no growth promoting activity was detected.

In view of the large discrepancy that was found to exist for Zea mays coleoptiles, using both 'collection in agar' and extraction methods for the identification of gibberellin-like substances, compared with published work (RAILTON and PHILLIPS, 1973), attempts were made to collect gibberellin-like substances from detached shoot apices of Helianthus annuus. It is firmly established that gibberellins are present in this tissue (PHILLIPS, 1972a) and thus provided an opportunity to establish whether it was the technique of the operator or the Zea coleoptile tissue that was causing the discrepancy (PHILLIPS pers. comm., 1975). Of course as mentioned previously, it may well be that gibberellin-like substances are not present in Zea mays coleoptile tissue or possibly that they may be present but

in such small quantities that the technique is not sufficiently sensitive enough to detect them. The method used was as described by PHILLIPS (1972a). In a similar manner to the methods used for Zea coleoptiles, the efficiency of the extraction procedure was measured by incorporating a range of concentrations of standard GA_3 solutions into agar plates. Unfortunately the efficiency was not calcuable since it was beyond the limits of the response curve obtained from standard GA_3 solutions. Variable results were observed in these experiments but gibberellin-like activity as measured by the lettuce hypocotyl bioassay was observed in 4 out of 8 experiments using Helianthus. The maximum response observed was obtained from 550 Helianthus shoot apices and was equivalent to 1.83 µgs GA_3. This represents 0.0033 µgs per Helianthus shoot apex. PHILLIPS (1972a) reported values of 0.170 nanograms from 70 upright buds. If this is converted to comparable units, it represents 0.000 0024 µgs per bud.

A standard collection period of 24 hours was used in all experiments of this type. However the question did arise that this might be insufficient time for collection and this prompted a study to be made of 'diffusion' over a time course ranging from 24 hours to 96 hours. The lettuce hypocotyl bioassay failed to detect any growth activity at all. JONES and PHILLIPS (1964) have shown moreover that yields of gibberellin-like activity declined if the period allowed for diffusion is too long. They observed that 24 hours is the ideal maximum.

The transport experiments in the first part of this study revealed that no significant amounts of radioactivity from {^{14}C} GA$_3$ were exported from Zea coleoptile segments into agar receiver blocks. Since no gibberellin-like activity could be detected in detached Zea coleoptile apices using agar collection and extraction techniques but was found in Helianthus, it seems improbable that gibberellins are present in Zea coleoptiles.

Obviously in this investigation only very crude 'collection in agar' and extraction techniques have been employed. Bioassays are not specific and in any case do not provide unequivocal evidence of a substance being present in a tissue. Similarly TLC and PC can really only be regarded as means for separation and not identification of given substances. Until quite recently techniques used for characterisation and quantification of the endogenous gibberellins in plant tissues had been based almost entirely on bioassays and chromatography of extracts. At their best, these methods provide only circumstantial evidence of identification even when authentic samples of gibberellins are available. The use of mass spectrometry in combination with various chromatographic techniques (GCMS) has recently been shown to be invaluable in providing conclusive and unequivocable evidence for the identification of the gibberellins present in plant tissue. Such a technique requires at least 100 - 200 ngs of gibberellin to be present

in the extract, is expensive and not widely available. In this present study no attempt was made to employ the technique of GCMS to detect gibberellin-like activity but obviously if time had permitted this would have been an indubitable line for further investigation.

In conclusion, the transport of exogenously applied GA_1 and GA_3 has been examined in both Zea mays and Helianthus annuus seedlings in relation to geotropism. The resultsof the lateral transport experiments involving roots and coleoptiles of intact Zea seedlings, suggest the possible involvement of GA_3 in the geotropic response of these organs but whether the observed upward lateral movement of GA_3 is the cause or result of geotropic curvature is unknown and will require further investigation. The experiments involving the transport of GA_1 in both Zea and Helianthus seedlings indicate that GA_1 has a negative role in geotropic curvature. Since the structural difference between GA_3 and GA_1 is only double bond, the present experiments lend evidence to the existence of an extremely specialised mechanism for gibberellin transport in plant tissue. The wide range of radioactively-labelled gibberellins which are becoming increasingly available, particularly since the onset of this investigation, should assist in the resolution of this mechanism.

Virtually nothing is known as to how the mechanism of gibberellin transport occurs; which tissues are involved and whether or not it is an active process. The micro-

pipette technique might be usefully employed to elucidate this since it enables gibberellic acid to be applied to a very specific region of tissue.

The physiological significance of the upward movement of GA_3 is obscure and it is not known whether the role of gibberellin is that of growth promoter or inhibitor. Use of a displacement transducer could clarify this.

The studies on the endogenous gibberellins were not very conclusive in providing evidence for the existence of gibberellin in Zea mays seedlings. However only the crudest techniques were used. Ideally, as mentioned previously, if both plant tissue and manual labour were plentiful, GCMS could be employed to determine unequivocally, whether or not gibberellins were a constituent of Zea mays seedlings and furthermore, whether there was an enhanced synthesis or lateral redistribution upon geotropic stimulation. Clearly much more experimentation is essential before the role of gibberellins in geotropism is understood.

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