Movement and metabolism

of

ABA-2-^{14}C in plants

by

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E R R A T A

Contents, fourth page, line 4	For phtoperiod read photoperiod.
Abbreviations, line 1	For abscissic read abscisic.
line 6	Insert GC-MS gas chromatography-mass spectrometry.
line 12	Insert ppm parts per million.
Page 7, line 20	For is not believed read is believed not.
Page 10, line 28	For movement of estimated read movement was estimated.
Page 12, line 7	For in single type read in a single type.
Page 20, line 24	For Birch achenes, were read birch achenes were.
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Page 24, line 20	For P,lain read Plain.
Page 29, line 8	For $10^{o}C$ read $1^{o}C$.
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CONTENTS

Abbreviations

ABA	abscisic acid
cpm	counts per minute
dpm	disintegrations per minute
DNP	2,4-dinitrophenol
Fig.	figure
IAA	indoleacetic acid
LD	long days
M	molar
P	probability *** 0.001P ** 0.01 P * 0.05 P
RF or Rf	$\frac{\text{distance compound has moved from origin}}{\text{distance of solvent front from origin}}$
SD	short days
v/v	volume for volume
2,4-D	2,4-dichlorophenoxyacetic acid

SUMMARY

The uptake, movement and metabolism of ABA-2-^{14}C has been studied in coleoptile and root segments of Zea mays, petiole segments and whole plants of Phaseolus vulgaris and intact plants of Alnus glutinosa, Acer pseudoplatanus and Betula pubescens.

Using coleoptile segments ten millimetres in length, excised one millimetre from the apex, the following transport characteristics have been established: basal uptake of ^{14}C exceeds apical uptake; there is a slight, net basipetal polarity of accumulation of ^{14}C in tissue halves further from the donors; velocity of ^{14}C movement in either direction is less than 1.5 mmh^{-1}; movement of ^{14}C is independent of segment orientation, but dependent upon donor concentration; there is little or no export of ^{14}C into agar receivers; there is a metabolically-dependent step in the transport process; only a small proportion of the ^{14}C recovered from transporting tissue is chromatographically similar to ABA-2-^{14}C. Using segments of differing shapes, the more closely the segment resembles a uniform cylinder of tissue the more any differences between apical and basal uptake and acropetal and basipetal movement are abolished.

Using sub-apical root segments, there is a net acropetal polarity of ^{14}C accumulation in the tissue, rates of movement exceed those in coleoptiles, and lowering the temperature has less effect on root transport of ^{14}C than coleoptile transport.

Movement of ^{14}C in Phaseolus vulgaris petioles is non-polar at a rate of 5-7 mmh^{-1}. The movement of ^{14}C in whole bean plants is dependent upon the method and locus of application of ABA-2-^{14}C.

In general, ^{14}C distribution reflects movement from a source, through living tissues, to the more actively-growing regions of the plant. There are, however, differences between the ^{14}C distribution following ABA-2-^{14}C application and that following IAA-^{14}C or sucrose-^{14}C application. There is evidence of extensive metabolism of ABA-2-^{14}C in bean tissue. Root-applied ABA affects the plant-water relationships of P. vulgaris.

The movement and metabolism of ABA.2.^{14}C in A. glutinosa and B. pubescens are basically similar to those in bean plants. Continuous root-application of ABA to plants of A. glutinosa slightly reduces growth but does not induce the formation of a dormant state. Continuous leaf-application of ABA solution to plants of A. glutinosa and B. pubescens, maintained under long days, does not appear to inhibit growth or induce the formation of resting buds. The lack of response may be due to poor uptake, low mobility and/or extensive metabolism of the applied substance.

INTRODUCTION

The regulation of growth and development in higher plants is believed to involve the synthesis, movement and metabolism of certain discrete chemicals which exert profound effects at extremely low concentrations. These endogenous regulating substances have been termed plant hormones, or more generally, plant growth substances. Of special interest in developmental physiology is the hormonal correlation of growth activities in the plant. The elucidation of the characteristics of hormone transport forms an integral part of determining the nature of the growth regulating mechanism.

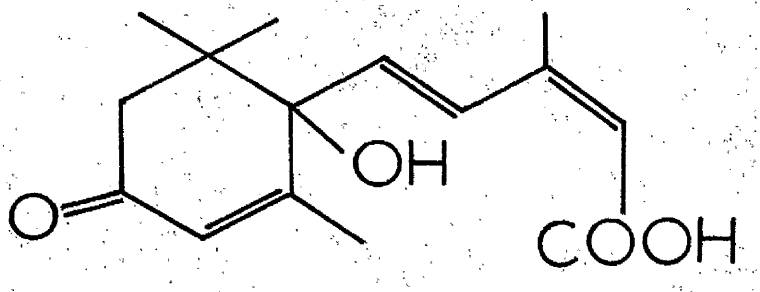
In 1922 Molisch suggested that inhibitory compounds may play a regulating role in growth. The concept was developed by later investigators, such as Hemberg, who suggested that inhibitors were involved in the dormancy mechanisms of potato tubers and buds of Fraxinus excelsior (Hemberg, 1949 a, b). The first inhibitory compound which is claimed to be a plant hormone is abscisic acid (ABA). This substance was discovered in two quite independent ways, one based on studies of bud dormancy, the other on the abscission of leaves.

Wareing and co-researchers, studying the role of inhibitors in the control of bud dormancy in woody species, extracted a single, highly active inhibitory substance from leaves of Acer pseudoplatanus (Robinson et al., 1963; Robinson and Wareing, 1964). This substance was termed 'dormin'. At almost the same time, in the course of a prolonged study of leaf abscission, a group of researchers under Addicott isolated a preparation from cotton fruits, termed abscisin II,

which promoted abscission (Ohkuma et al., 1963). Purification of abscisin II led to the isolation of a crystalline product which was identified as a sesqueterpenoid (Ohkuma et al., 1965). A comparison of the infra-red absorption spectra and other physical properties of dormin and abscisin II showed that they were identical (Cornforth et al., 1965a) . The compound is now named abscisic acid (Addicott et al., 1968).

Independently, the influence of fertilized lupin pods in causing the abscission of younger, unfertilized ones was traced to an abscission-promoting substance which also inhibited the elongation of coleoptile segments (Stevenick, 1958, 1959; Rothwell and Wain, 1964). This substance was later shown to be abscisic acid (Cornforth et al., 1966).

The structure of ABA was confirmed by synthesis by Cornforth et al., (1966) as follows:



The cis, trans (Z,E) and trans, trans (Z,Z) isomers show different physiological activities, the former being generally more active (Tamura and Nagao, 1970; Sandheimer and Walton, 1970).

The molecule contains an asymmetric carbon and therefore exhibits optical isomerism. The naturally occurring form is the (+) enantiomorph with an absolute stereochemistry of the 'S' configuration. The optical rotary dispersion of (+)-abscisic acid shows a very intense cotton effect in the ultraviolet region of the spectrum. Using this property the presence of ABA has been detected in partially purified extracts from a wide range of plant species (Cornforth, 1966; Milborrow, 1968). The widespread occurrence of ABA suggests it may have a number of different hormonal roles and consequently the effects of ABA have been studied on a number of physiological processes.

The general nature of the inhibition caused by ABA has been reviewed by Addicott and Lyon (1969). Most of the work was carried out on isolated pieces of plant tissues in which marked inhibitory activities were observed. The extent to which growth of an entire leafy shoot is retarded varies with many factors, including the method of application. Commonly a shortening of internodes may be the only effect, but a retardation of leaf expansion has also been observed (Lipe and Crane, 1966). More recently attention has been focused on the rapidity of ABA action on the growth of isolated tissue segments, especially the latent period between application of the substance and the beginning of the response. Warner and Leopold (1969) found a 5.1 min. latent period for inhibition of elongation of intact Alaska pea shoots by 10 μ M ABA. Zenk (1970) reported a latent period of about five minutes for 0.1 m M ABA inhibition of 0.1 μM IAA-promoted elongation in Avena coleoptiles. Rehm and Cline (1973), using an angular position sensing transducer to measure the elongation of Avena coleoptiles, also found a 4-5 min latent period, although

full growth inhibition was not established until about one hour after the application of ABA. The short term kinetics of the inhibition were not similar to those of any of the inhibitors of RNA and protein synthesis, tested in the same system. Information of this nature is very important in elucidating an hypothesis for the mode of action of abscisic acid in any given response. In many simple growth responses abscisic acid appears to antagonise the effects of other plant growth substances (Van Overbeek et al., 1967), an observation that supports the concept that plant growth is controlled by a hormonal balance.

The effect of ABA on the senescence of plant tissue is an area of controversy. In isolated leaf discs over short incubation periods, ABA accelerated the loss of chlorophyll in many species (Aspinall et al., 1967; El-Antably et al., 1967). Colquhoun and Hillman (1972), however, found that after 6 days incubation ABA apparently retarded chlorophyll decline compared to control discs. Senescent colour changes in leaves have been reported following foliar application of ABA (Cathey, 1968; Smith et al., 1969), but negative results have also been found (Colquhoun, pers. comm.).

The effect of ABA on abscission is a well-studied response, especially in cotton. Application of extracts containing ABA accelerated abscission of both intact fruit and defoliated pedicels (Addicott et al., 1964). Experiments with explants of cotton and other species (Smith et al., 1969; Dorffling, 1967) showed that applied ABA increased the rate of petiole abscission. In addition, application of ABA to the stem stump of the explant was almost as effective as application to the petiole stump, and application to a single petiole stump

was very effective in promoting abscission of the opposite petiole (Smith et al., 1969). This observation provides some evidence for the mobile nature of the abscission-promoting factor. When ABA was applied to intact leaves or foliage, abscission was promoted (Eagles and Wareing, 1964; Smith et al., 1969). Occasionally a single application sufficed, but usually several applications were required to induce leaf abscission. Application of ABA to young or actively growing plants were sometimes completely unsuccessful (Cathey, 1968) and the response in perennial species depended to some extent on the stage of development when application was made (Cooper et al., 1968). Addicott and Lyon suggest these variations in response may be due to variation in the levels of endogenous ABA with the environment, coupled with difficulties of poor absorption or rapid inactivation of the applied ABA. Clearly these are problems requiring further investigation.

There have been a number of reports of the inhibitory effects of ABA on the flowering of long day plants (e.g. Evans, 1966; El-Antably et al., 1967; Cathey, 1968). Wareing and Antably (1970) reported that extracts from the leaves of the long day plant Spinacia oleracea (spinach) contained more growth inhibitor, as measured by coleoptile bioassay, under short days than long days. Although there was no definite evidence that the inhibitory activity was due to ABA, the results showed that ABA may function in the regulation of flowering, being produced in the leaves and moving to the apex, inhibiting flowering in short days. Contrary to these results, however, Zeevart (1971) reported that levels of ABA in spinach extracts, as measured by ORD were actually higher in plants maintained under long days than short days. This suggests that the inhibitor levels as measured by bioassay may not give an accurate

estimation of the levels of endogenous ABA. Thus the involvement of ABA in the control of flowering in LDPs is questionable, although it is possible that inhibitors present in spinach, other than ABA, may participate in floral induction.

There have been many contradictory observations on the response of short day plants to the application of abscisic acid (see Addicott & Lyon, 1969). Plant age, variety, cultural conditions and photoperiod can all markedly influence flowering; coupled with variations in the time and method of ABA application, it would be difficult and probably unwise, to ascribe any role for ABA in floral induction until more is known about the fate of the applied substance in relation to these variables.

Apical dominance in the shoots of herbaceous plants is manifest by almost complete correlative inhibition of axillary bud outgrowth by the presence of an intact apex. The nature of the mechanism and the precise role of growth inhibitors are not fully understood, but the involvement of the latter is indicated by recent work. Axillary buds subject to correlative inhibition have been shown to contain higher concentrations of growth inhibitors than those released from apical dominance (Dorffling, 1966). Arney and Mitchell (1969) found that ABA applied to decapitated pea plants achieved inhibition of lateral bud outgrowth comparable to that produced by the intact apex. Hillman (1970), however, found that ABA alone promoted bud outgrowth slightly in Phaseolus vulgaris but with IAA and GA_3 gave inhibition almost comparable to the apex itself. Shein and Jackson (1971), expressing doubts about all the established hypotheses for apical dominance mechanisms, supported the view that the mechanism of apical dominance depended on an

interaction or balance between hormones. Much of the information on apical dominance has come from experiments using some degree of surgery on the plant or the exogenous application of growth substances or both. Cautious interpretation of the data from such experiments is necessary in view of the possible complications resulting from surgery and the lack of knowledge on the fate of the applied substance, or its correct site of application. Tucker and Mansfield (1971), fully aware of these difficulties, avoided both techniques in their study on Xanthium strumarium. They investigated the effects of light quality on apical dominance and the associated changes in endogenous levels of ABA and cytokinins. They found that apical dominance appeared to be influenced by changes in the balance of red and far-red wavelengths. Plants maintained in 16 hour photoperiods deficient in far-red light branched profusely, whereas the axillary buds of plants receiving sufficient far-red were inhibited. The levels of cytokinins and ABA were much higher in inhibited buds than in released buds, although there were considerable differences between plants of different ages. In their study ABA was measured by bioassay using the method of Tucker and Mansfield (1971), which is not believed to be subject to interference from other growth substances. They suggested that the control of lateral branching is achieved by changes in the balance of hormones, including cytokinins and ABA.

Perhaps the most interesting recent development in research on ABA is the most dramatic increase in the endogenous levels of ABA or ABA-like activity during the wilting of herbaceous plants (Wright and Hiron, 1969; Mizrahi et al., 1970; Zeevaart, 1971). It has also been shown that applied ABA suppresses stomatal opening (Imber and Tal, 1970; Jones and Mansfield, 1970; Mittelheuser and

Stevenick, 1969; Mizrahi et al., 1970) and increases the permeability of plant cells to water (Glinka and Renhold, 1971). These findings have led to the suggestion that ABA may function as an endogenous antitranspirent. Very recently, Mizrahi and Richmond (1973) have reported that abscisic acid content in tobacco leaves rose markedly when plants were deprived of nutrients by being transferred from a culture medium to distilled water. They suggested that the rise in leaf-ABA is not specific to water stress conditions, but may be involved with many conditions that impair the growth and development of plants; i.e. that ABA may be of considerable importance in stress physiology.

It has been considered that ABA, together with other hormones, may play an important part in the control of dormancy; not only bud dormancy of woody species, but also the dormancy of seeds and other plant organs such as rhizomes, corms, bulbs and tubers. As an inhibitor of seed germination, ABA is active in a great many species. It is particularly inhibitory to the germination of lettuce fruits (Aspinal et al., 1967; Wareing et al., 1968; McWha et al., 1973); seeds of grasses (Lipe and Crane, 1966) and several other species (Sankhla and Sankhla, 1968). It is not clear in most cases, however, whether a strict hormonal relationship exists in vivo. In many of the species whose seeds require stratification before they can germinate, the levels of ABA were lowered during stratification, and afterwards application of ABA to the seeds prevented germination (Lipe and Crane, 1966; Sondheimer and Galston, 1966). Similar correlations have been shown between the levels of ABA or inhibitor in tissues external to the embryo (e.g. seed coats, fruits) and the inhibition of germination (Black and Wareing, 1959; Lipe and Crane, 1966; Pieniazik and Rudnick, 1967; Irving, 1968;). In these cases a

hormonal relationship may well exist. In a number of cases (e.g. McWha et al., 1973), the effect of ABA was only temporary, suggesting rapid inactivation or metabolism of the applied compound (McWha and Hillman, 1973).

Wareing and Phillips (1970), described the similarities between seed and bud dormancy. Both can be broken by satisfying a chilling requirement, or by application of certain substances such as gibberellic acid. Both buds and seeds may show photoperiodic sensitivity in relation to dormancy induction. The close parallel between dormancy in buds and seeds is particularly clear in instances where the buds and seeds of a single species are compared, suggesting that the mechanism may be similar in both cases. Whether or not ABA plays a central role in this mechanism, both forms of dormancy provide further examples, like many of the responses discussed above, of a correlative process.

It is likely that all growth correlations are, in one way or another, affected by patterns of hormone distribution within the plant. The evidence discussed above suggests that ABA may be one of the hormones involved. But before ascribing a role to ABA in regulating correlative phenomena, it is essential to have a sound knowledge of the movement and metabolism of the substance in plants.

At the onset of this present investigation, knowledge of the movement of ABA was confined to: (a) detection of ABA or ABA-like activity in certain tissues likely to be involved with transport; (b) indirect evidence from the study of correlative phenomena; (c) three reports on the movement of applied ABA in isolated plant tissues. In the first category the occurrence of ABA in diffusates from fruits suggested that it may be readily transported (Addicott

et al., 1964). Furthermore ABA has been detected in both phloem and xylem sap (Bowen and Head, 1968; Lenton, Bowen and Saunders, 1968; Pieniazek and Rudinick, 1969; Davison and Young, 1973). In the second category, experiments such as that of Eagles and Wareing (1963), El-Antably et al., (1967) and Evans (1966), suggest that ABA is readily transported from the leaves to the stem apex. Similarly, abscission acceleration when ABA is applied to the petiole stumps of explants indicates a physiological response some distance from the site of application.

Dorffling and Bottger (1968) measured the transport of applied ABA in petiole and internode segments of Coleus. They applied synthetic and natural ABA extracted from tomato, to segments in a donor-tissue-receiver system. At intervals the receiver blocks were bioassayed for ABA activity. Thus these results must be considered in terms of the movement of ABA-like activity rather than ABA. In younger segments, transport was strongly basipetal; in older segments transport could take place in either direction. Rate of movement was estimated at between 24 - 36 mmh^{-1}. Using ABA-2-^{14}C synthesised by Cornforth et al., (1968), Milborrow (1969) found that basipetal transport in bean petioles was three times that of acropetal transport. He found that radioactivity did not move out into agar receiving blocks, but unfortunately he did not give any further details. Ingersoll and Smith (1970) studied the movement of abscisic acid in cotton explants. Unlabelled or ^{14}C-labelled ABA was applied to the petiole stumps and the movement of abscission promoting activity or radioactivity was followed through 5 mm petiole segments. The two techniques gave similar results. The rate of movement of estimated at between 20 to 30 mmh^{-1}, compared to 6 to 7 mmh^{-1} for IAA in Phaseolus, lens and Coleus tissues

(McCready and Jacobs, 1963; Pilet, 1965, 1968). Four hours after the application of ABA-2-^{14}C, 99% of the radioactivity extracted from the explant co-chromatographed with ABA. They suggested that the close agreement of the two techniques showed that the movement of ABA was an important factor in the ability to promote abscission in cotton. Thus these initial experiments showed that ABA or ABA-like activity was mobile in isolated plant segments at rates appreciably faster than IAA in similar tissues.. Polarity of movement was generally basipetal, but some difficulty was observed in the export of ABA into agar receiving blocks.

While Ingersoll and Smith found very little evidence of ABA breakdown in cotton, Milborrow estimated that under 50% of the radioactivity recovered from bean tissue after 12 hours was chromatographically similar to ABA. He separated three distinct, labelled products, presumably metabolites, from the bean tissue extracts. Similar products were found when ABA-2-^{14}C was incubated with tomato shoots or sycamore petiole sections, and the major products were subsequently identified as 6'hydroxymethyl ABA (Milborrow, 1969a) and abscisyl-β-D-glucopyranoside (Milborrow, 1970). The latter had been shown to occur naturally in the fruit of Lupinus luteus (Koshimizo et al., 1968) and in the pseudocarp of Rosa arvensis (Milborrow, 1970). The apparent lack of ABA breakdown in cotton may account for the rapid physiological response to the applied ABA.

Thus although Milborrow and subsequently Walton and Sondheimer (1972), have made extensive studies of the metabolism of ABA in certain plant tissues, there is clearly a need for more information on the uptake, movement and metabolism of ABA, in isolated plant sections and more importantly, in whole plants.

This lack of information, together with the availability of radioactively-labelled ABA for use as a tracer, led to the initiation of this present study.

There were essentially two ways to approach the investigation, given a limited supply of radioactively-labelled ABA and other resources: either to carry out a very detailed study of ABA transport in single type of plant tissue, elucidating as many characteristics as possible, or to make a more general survey of movement of ABA in a number of different plant species. Although results would probably dictate the ultimate course of the research programme, it was decided initially to compromise and study in reasonable detail the movement of ABA in three plant species: a monocotyledon, an herbaceous dicotyledon and a deciduous tree species.

Section I of the results is concerned with studies on Zea mays. This species has been used in transport studies of other hormones for many years and this fact alone is sufficient justification for its selection as a basis for comparison. ABA transport has been studied primarily in coleoptile segments, also in root segments and whole seedlings. Emphasis has been placed on: (i) the basic characteristics of ABA movement in tissue segments as a function of time; (ii) the effects of certain environmental factors on these characteristics; (iii) the effect of segment age, shape and growth on these characteristics; (iv) metabolism of ABA-2-^{14}C in the tissue.

Section IIA considers the movement and metabolism of ABA-2-^{14}C in whole plants of P. vulgaris. In a number of responses the levels of leaf-ABA and the effects of the environment on these

levels have been considered to be of importance, so the export and distribution of ABA from a leaf source is of considerable interest. Consequently in this investigation emphasis has been placed on the export from leaves and subsequent distribution of applied ABA. This has been compared with the movement of other substances such as IAA and sucrose. In addition, the uptake and metabolism of applied ABA are of particular importance and these aspects have also been studied. Phaseolus vulgaris is a species which has been extensively used for physiological experiments, including transport studies. Relatively uniform plants can be produced quickly and easily and the large, flat, primary leaves are particularly suitable for the application of a substance. Also studies with this species may serve as useful preliminaries for Section III where plants are used which are more difficult to grow and take longer to reach a suitable experimental age. In Section IIB the effects of ABA on selected physiological processes, especially plant-water relationships, have been studied and related to ABA transport and metabolism.

The results of studies on three deciduous tree species are presented in Section III. This work was carried out with a view to examining the role of ABA in the induction of bud dormancy in these species.

Research on bud dormancy in woody plants began many years before the discovery of ABA and has been reviewed on a number of occasions (e.g. Wareing, 1956, 1965, 1969). In the annual cycle of growth of many woody species, resting buds are formed at some stage. In temperate species, such buds are formed typically in the late summer or autumn and the whole tree or shrub enters a dormant or resting phase. A resting bud normally contains a number of unexpanded

leaf primordia, which may be surrounded by either a number of modified stipules, as in Betula spp., or by modified leaves, as in Acer spp., which form the bud scales. Daylength is one environmental factor which has been shown to have a marked effect on the onset of dormancy (Wareing, 1956; Nitsch, 1957). Short days promote the formation of resting buds in the majority of woody species investigated. The effect of daylength is more marked in younger trees than in mature trees; in the latter other environmental factors may have a marked effect (Wareing, 1969). Consequently most of the work on bud dormancy induction has been carried out on tree seedlings, which are also easier to handle.

As in the photoperiodic control of flowering, the response at the shoot apex of some species such as Acer pseudoplatanus (sycamore) is controlled primarily by the length of day to which the leaves are exposed (Nitsch, 1963; Wareing, 1954). In Betula pubescens (birch) seedlings the shoot apex itself is sensitive to photoperiod, but renewed growth of the terminal bud, which occurs when the dormant seedlings are returned to long photoperiods, can be inhibited by exposing the mature leaves to short day conditions. These observations suggest that the formation of resting buds may be controlled by some influence, presumably hormonal in nature, which arises in the leaves under short days and is transmitted to the apex (Wareing and Saunders, 1971). It seems reasonable to suggest that cessation of growth is due to an inhibitory factor produced by the leaves under short days; in a wide context, however, the induction, dormant period and emergence from dormancy of buds is probably controlled by a balance between endogenous inhibitors and promoting substances, such as the gibberellins (Nitsch, 1957; Phillips and Wareing, 1959). Here we are

specifically concerned with the possible role of inhibitors in the induction of bud dormancy.

The validity of an 'inhibitor hypothesis' has been tested by two experimental approaches. Firstly, many attempts have been made to demonstrate a correlation between the photoperiodic imposition of dormancy, and the inhibitory activity or the inhibitor content of fractions derived from tissue extracts. Secondly, attempts have been made to induce the formation of resting buds by application of exogenous inhibitors or inhibitory fractions.

In the first case the levels of growth substances were measured in leaves and buds of mature sycamore trees growing under natural conditions and in young seedlings of the same species growing in controlled long day or short day environments (Phillips and Wareing, 1958, 1959; Robinson, 1962). Most inhibitory activity was found in leaves and apices of plants under short days. Studies by Dorffling (1963), however, on the same species did not show this rise in inhibitors under natural conditions. Using Betula pubescens both Kawasi and Nitsch (1958, 1961) and Eagles and Wareing (1963, 1964) were able to demonstrate increases in inhibitory activity under short days. Essentially similar results were obtained for the inhibitor content of leaves, phloem and xylem sap from Salix viminalis (Hoad, 1967; Bowen and Hoad, 1968; Bowen, 1969). Eliasson (1969), was working with Populus tremula, and found a rise in inhibitory activity in leaves in October from trees grown under natural conditions, but did not observe a short day induced increase in inhibitors from cuttings of Populus grown under controlled conditions. In all these cases activity was measured by bioassay using coleoptile segments, showing the activity of an inhibitory zone occurring at about Rf 0.6 on paper chromatograms developed in isopropanol: ammonia: water. This zone is the

β-inhibitor zone defined by Bennett-Clark and Kefford (1953). Several compounds which are inhibitory to plant growth have been found in this zone derived from various plants. Many of these compounds are weakly inhibitory, phenolic or aliphatic acids (Housley and Taylor, 1958; Lane and Bailey, 1964; Varga, 1957), but by far the most active constituent in many plant extracts appears to be abscisic acid (Milborrow, 1969). It is now known that the fractions bioassayed in the photoperiodic experiments referred to above, would all have contained ABA (Lenton, Perry and Saunders, 1971), but the tissues used to bioassay the extracts will respond not only to ABA but also to many other inhibitory or promoting compounds present in the inhibitor β zone. Therefore Lenton, Perry and Saunders (1972), utilising a newly developed technique based on quantitative gas chromatography which assays ABA specifically, measured the ABA content of birch, maple and sycamore plants grown under long and short photoperiods. No increase was observed in the ABA content when plants were transferred to dormancy inducing conditions. Thus, on the basis of these results, the observed rise in β-inhibitor activity in short days may not be due to an increase in the amount of ABA. This is supported by the results of Zeevart (1971 a, b) who demonstrated an increase in ABA content, as opposed to β inhibitor content, when spinach plants were transferred from SD to LD. Lenton et al., while realising that further work on this problem was required, concluded that if their results were substantiated, "the role of the compound (ABA) as a dormancy-inducing hormone (Eagles and Wareing, 1963) must inevitably be questioned." They emphasized the possible role of promotors in the control of bud dormancy.

The formation of resting buds in woody species by the application of exogenous β-inhibitor fractions or ABA has been reported on a number of occasions. Repeated leaf applications of a β-inhibitor fraction, extracted from birch, to actively growing birch plants maintained under a 14.5h photoperiod, completely arrested apical growth (Eagles, 1962; Eagles and Wareing, 1963). The compact structure of the arrested apices closely resembled the apices of plants rendered dormant by short days. Continuous application of ABA solution to actively growing plants of B. pubescens, A. pseudoplatanus and Ribes nigrum, maintained under 18 hour photoperiods, caused cessation of stem growth and formation of resting buds within 15 to 20 days (El-Antably et al., 1967). The ABA solution (25 p.p.m.) was applied to the youngest, fully-expanded leaf, the solution was renewed daily and when the treatment leaf showed signs of senescence, the solution was applied to the next youngest leaf. The apical region was also sprayed daily with ABA solution at the same concentration. Spraying the leaves alone was not effective for birch and sycamore plants. Successful induction of bud dormancy by application of ABA has been reported by other workers (Little and Eidt, 1968; Van der Veen, 1968).

Clearly the induction of bud dormancy by the application of ABA does not occur readily. The need for continuous application of fresh ABA solution (see Eagles, 1962) suggests one or more of the following possibilities: (i) very little of the applied ABA may have been taken up by the tissue; (ii) considerable breakdown of ABA may have occurred before it could be taken up; (iii) a large proportion of the ABA taken up may have been rapidly inactivated or metabolised (there is considerable evidence to support this view,

e.g. Addicott and Lyon, 1969; Milborrow, 1969); (iv) the ABA taken up may have been immobilised in the leaf of application; (v) only a small proportion of the mobile ABA may have moved towards the apical region; (vi) large amounts of ABA at the apex may have been required to induce bud dormancy. The availability of radioactively-labelled ABA has enabled these possibilities to be investigated.

It was decided to repeat and extend the studies of Eagles (1962) and El-Antably *et al.*, (1968) on *B. pubescens* and to examine the effects of ABA on another woody species, *Alnus glutinosa*. This species was chosen because a pure batch of seed was readily available and it had not been used before in this type of study. Section IIIA attempts to establish the basic characteristics of ABA movement and metabolism in these woody species, and Section IIIB relates these findings to the effects of applied ABA on the growth and dormancy induction in *B. pubescens* and *A. glutinosa*

MATERIALS AND METHODS

1. Plant Materials

The experiments in this thesis were carried out using coleoptile segments, root segments and whole seedlings of *Zea mays* L., c.v. Burpee Snowcross; petiole segments of *Phaseolus vulgaris* L., c.v. Canadian Wonder; whole plants of *Phaseolus vulgaris* L., c.v. Canadian Wonder, *Alnus glutinosa*, *Betula pubescens* and *Acer pseudoplatanus*. *Zea mays* fruits were obtained from W. Atlee Burpee Co., Philadelphia, Pa., U.S.A. Seeds of *P. vulgaris* were supplied by Hurst, Gurson, Cooper and Taber Ltd., Witham, Essex. *Alnus glutinosa* fruits were obtained from Prof. G. Bond, Dept. of Botany, University of Glasgow. Seeds of *B. pubescens* and *A. pseudoplatanus* were collected from single trees of each species; the precise taxonomic origins of these fruits were not ascertained.

For coleoptile experiments, the *Zea mays* fruits were soaked for twelve hours in running tap-water and planted 2.5 cm deep in moist vermiculite in plastic trays. These were placed in a cupboard in a growth room at $25 \pm 1^{\circ}C$ in the dark, and the coleoptiles used 5 - 6 days after the initial soaking.

For root segment experiments the seeds were soaked for 12 hours in running tap-water, set out on wet paper towels in covered plastic boxes and germinated in total darkness at $25 \pm 1^{\circ}C$. The roots were used when 2 - 3 days old.

In whole seedling experiments the *Zea mays* seeds were sown as for root segments, except that the seeds were first surface sterilised with 10% 'Chloros' solution for 30 seconds. The seedlings were used when 3 - 4 days old.

Plants of P. vulgaris were grown in 9 cm plastic pots containing John Innes No. 2. compost, in a heated greenhouse with supplementary lighting from Thorn 400W MBFR/U high pressure mercury vapour lamps. These lamps were switched on from 3 a.m. - 7 p.m. G.M.T., ensuring long-day conditions throughout the year. Although the temperature control in the greenhouse was not precise, it was sufficient to maintain satisfactory plant growth throughout the year. The plants were used when 2 - 3 weeks old.

Plants for water culture were raised in seed trays of moist vermiculite. When the primary leaves just started to expand, the seedlings were transferred to individual black perspex holders having a central hole for the stem. The holders rested on the lips of blackened pyrex glass, 250 ml conical flasks such that the roots were completely immersed in culture solution. The culture solution used was Long Ashton Nutrient solution (Table I). The solution was renewed every two weeks and under these conditions the plants grew well without forced aeration of the solution. Experiments were begun when the plants were 2 - 3 weeks old.

Seedlings of A. glutinosa, B. pubescens and A. pseudoplatanus were grown in seed trays of moist peralite placed in the long-day heated greenhouse. To improve germination, the Birch achenes, were first shaken with water for 3 days and then thoroughly rinsed. Plants to be grown in solid media were 'pricked' out at 3 weeks into 9 cm plastic pots containing J.I. No. 2. compost. The plants were watered twice a week with 'Vitafeed-S', (Vitax Ltd., Ormskirk), a commercial liquid manure.

Table I

Long Ashton Nutrient Solution.

Stock solution		concentration (g $1000cm^{-3}$)
1.	KNO_3	10.1
2.	$Ca(NO_3)_2 \cdot 4H_2O$	32.8
3.	$NaH_2PO_4 \cdot 2H_2O$	10.4
4.	$MgSO_4 \cdot 7H_2O$	18.4
5.	Ferric EDTA	0.245
6.	Mixture of: $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	3.5 (mg $1000cm^{-3}$)
	$MnSO_4 \cdot 4H_2O$	223
	$CuSO_4 \cdot 5H_2O$	24
	$ZnSO_4 \cdot 7H_2O$	29
	H_3PO_3	186

To make up 10 litres of composite stock solution take 200 cm^3 each of 1, 2, 3 and 4, 100 cm^3 of 6 and 5 cm^3 of 5 and make up to 10 litres with distilled water. Dilute x 5 for watering or culture solutions.

Plants of Alnus glutinosa were also grown hydroponically. The system adopted was very similar to that developed by Bond (1951) and Ferguson and Bond (1953). When about 3 weeks old, the plants were removed from the peralite, and the roots dipped into a brei of ground-up nodules taken from older, established plants. This brei served as a source of inoculum for the formation of nitrogen-fixing root nodules. The seedlings were then replanted in peralite moistened with ½ strength, nitrogen-free Crone's solution (Table II). When about 5 cm high the plants were mounted individually in the central holes of 5 x 5 x 0.7 cm black perspex holders, using split rubber rings and cotton wool wrapped around the base of the stem. The holders were placed on the top of blackened pyrex glass, 250 cm^3 conical flasks with the roots immersed in ¼ strength nitrogen-free Crone's solution. This was a self-replenishing nutrient solution which only required topping up with water periodically. The entire solution was renewed at monthly intervals and when grown in this manner the plants did not require forced aeration of the culture solution.

2. Radioactive Materials

Radioactive RS-Abscisic acid (ABA-2-^{14}C) used in these experiments had a specific activity of 45 $\mu Ci/mg$ and a limited supply was kindly donated by Hoffmann-La Roche Ltd., Basel, Switzerland. The solid was dissolved in a minimal volume of methanol and made up into a 10^{-3}M stock solution with distilled water. Chromatographic analysis of this solution showed one major radioactive peak which corresponded with other published data for ABA (Milborrow, 1968). Several minor radioactive spots were also present, but on a percentage basis these were in negligible amounts.

Table II

Crone's solution (Bond 1951)

Macronutrient	Concentration in final solution
KNO_3	0.75 g 1000 cm^{-3}
$Ca_3(PO_4)_2$	0.25
$CaSO_4 \cdot 2H_2O$	0.5
$Fe_3(PO_4)_2 \cdot 8H_2O$	0.25
$MgSO_4 \cdot 7H_2O$	0.5

Plus micronutrients (A-Z solution, Hoagland and Synder, 1933).

Amounts (in grams) in 18000 cm^3 of composite solution.

Add 1 cm^3 of this solution to 1000 cm^3 of macronutrients.

$Al_2(SO_4)_3$ 1.0; KI 0.5; KBr 0.5; TiO_2 1.0; $SnCl_2 \cdot 2H_2O$ 0.5;

LiCl 0.5; $MnCl_2 \cdot 4H_2O$ 7.0; H_3BO_3 11.0; $ZnSO_4 \cdot 7H_2O$ 1.0

$CuSO_4 \cdot 5H_2O$ 1.0; $NiSO_4 \cdot 6H_2O$ 1.0; $Co(NO_3)_2 \cdot 6H_2O$ 1.0.

IAA-1-^{14}C (specific activity 57 Ci/M), IAA-2-^{14}C (specific activity 52 Ci/M), and sucrose-^{14}C (600 Ci/M) were obtained from the Radiochemical Centre, Amersham, U.K. The IAA-1-^{14}C and IAA-2-^{14}C, which were at least 99% pure, were supplied in benzene solution in sealed vials. The benzene was dried down under a stream of nitrogen and the residue taken up in one drop of absolute ethanol and diluted with distilled water to give an aqueous stock solution. The solutions were stored in darkness at -15°C. The sucrose-^{14}C was supplied in aqueous solution, which was diluted as required.

3. Unlabelled growth regulating substances

RS-ABA was supplied by Hoffmann-La Roche, Basel, in powder form. This was dissolved in methanol and diluted with distilled water as required, keeping the concentration of methanol in the final solution below 500 p.p.m. Stock solutions were stored at 1°C in darkness. IAA was obtained from Sigma Chemicals, London, U.K., and stored at -15°C in darkness. Fresh solutions were prepared as required before an experiment, by dissolving in absolute ethanol and diluting with distilled water. In all growth experiments with ABA and IAA appropriate amounts of alcohol were added to control solutions to allow for any possible effects of the organic solvents. GA_3 was obtained from Sigma Chemicals, London, U.K., was dissolved in distilled water and stored at 1°C in the dark.

4. Growth Room Facilities

Except for two long term experiments using P. vulgaris, all experiments were carried out in controlled-temperature rooms, of which four types were used. Type 1 was a controlled-temperature dark-room at 25 ± 1°C, with dim green light in the spectral band

515-540 nm as the only source of light. Type 2 was a controlled-temperature light-room at 25 ± 1°C, illuminated by two 147 cm 65 Watt, white, Atlas fluorescent tubes, 82 cm above the working surface. Type 3 was a controlled-temperature growth room at 25 ± 2°C, illuminated by a bank of alternate daylight/warm white Atlas fluorescent tubes at 9 cm spacings, 76 cm above the working surface. The lights were automatically operated to provide 16 hour photoperiods. The side walls were faced with aluminium foil to improve light distribution. The experimental material was placed in a gravel bed which was watered daily. This arrangement gave some degree of humidity control. During the light period the relative humidity, as measured by a Thermohydrograph Type TK 155 (Gallenkamph & Co., Teeside), ranged between 55-65%. and in the dark period was relatively constant at 80%.

Type 4 was a cabinet with two separate, light-proof compartments which had common heating and cooling systems wired through matched thermostats, maintaining a high degree of temperature control to within ± 1°C of the required temperature. The lighting equipment was identical in both compartments, consisting of a bank of alternate daylight/warm white 147 cm, 65 Watt, Atlas fluorescent tubes. Two tubes in each bank were wired separately to time clocks to provide additional illumination if required. This arrangement enabled the cabinet to be used for photoperiodism studies. The lights were 150 cm above the growing area, and a 1.25 cm clear perspex sheet was fixed between the lights and the growing area. The complete interior walls of the cabinet were lined with Metalised Melinex. Unfortunately there was no humidity control in this cabinet.

5. Experimental procedures for studying the movement of ABA-2-^{14}C in plants

The majority of the experiments in this thesis are concerned with the study of the distribution of radioactivity in plants following the application of ABA-2-^{14}C. The methods adopted depended largely on the type of plant material studied.

Coleoptile segments All experiments were carried out in growth rooms 1 (25°C dark) or 2 (25°C light) unless otherwise stated. In experiments carried out in the dark the coleoptiles were excised and handled under green light in the spectral band 515-540 nm. The radiant flux density at the level of the plant material was 7.2×10^{-2} einsteins cm^{-2} sec^{-1}. Usually 10 mm coleoptile segments with the primary leaf removed, were excised 1 mm behind the apex of 5 day-old Zea mays seedlings using a razor blade cutter. Groups of 5 or 10 segments were held in the vertical position by means of a perspex holder. The ABA-2-^{14}C was supplied in an agar block to the cut ends of the segments. Molten 1.5% agar (Oxoid"Ion-agar" No. 2), containing the appropriate concentration of ABA-2-^{14}C was cast into brass moulds (25 x 20 x 1 mm) sandwiched between clean glass slides. The agar blocks thus formed were stored in petri-dishes lined with moist filter-paper until required. P,lain agar blocks were cast in a similar way and used as receiver blocks. For acropetal transport the basal end of the segment was placed in contact with the donor block. For basipetal transport the apical end was placed in contact with the donor block. At the end of the transport period, the receiver block was removed and placed in a scintillation vial. The five (or 10) segments were removed from the holder with forceps and placed on a razor blade cutter, which had 6 razor blades spaced 2 mm apart, with the donor

end of the segments up against one outside edge of the cutter. The 10 mm segments were cut into 5 x 2 mm sections. Each group of five replicate sections was placed in a scintillation vial. Additional tissue, formed as a result of growth of the segment during the transport period was placed in the vial containing the tissue sections furthermost from the donor. Two cm^3 of 95% ethanol were added to each vial and the lids screwed on. The tissue was extracted in the ethanol for at least 48 hours at $1^{o}C$ before being prepared for radio-assay.

In experiments studying the effects of temperature on ABA movement, a growth room set at the appropriate temperature was used. All plant material and apparatus was allowed to equilibrate at the chosen temperature for at least one hour prior to experimentation. To achieve anoxic conditions the method of Wilkins and Martin (1967) was adopted. Essentially this involved mounting the segments in a perspex holder inside an airtight perspex cylinder which could be sealed and subsequently evacuated. After 6 evacuations and flushing of the cylinder with oxygen-free nitrogen gas the donor block was lowered using a threaded perspex plunger onto the cut ends of the segments. In this way the central cavity of the coleoptile segment could be evacuated and filled with nitrogen prior to donation. During the transport period nitrogen gas was passed slowly through the experimental chamber, having first been humidified through a water bubbler. At the same time a control system was run, using compressed air instead of oxygen-free nitrogen.

Root Segments The procedures used for root segments of Zea mays were very similar to those used for coleoptiles. Ten segments of

3 day-old roots were excised 1 mm behind the apex and mounted, in groups of 5 or 10, in perspex holders with one cut surface in contact with a receiver block and the other in contact with a donor block. The holder was placed in a petri-dish, inside a plastic box lined with moist paper towels. The experiments were carried out in growth rooms Type 1 and 2. At the end of the transport period the segments were cut up and extracted in exactly the same way as the coleoptile segments. The one deviation from coleoptile procedures was in experiments on the effects of anoxic conditions. Since roots do not have a large internal lumen they were mounted in holders in the normal way and then placed in a vacuum desiccator. This was alternately evacuated and flushed with oxygen-free nitrogen six times, and on the final flushing a slight negative pressure was left inside the desiccator to improve the seal between base and lid. A mercury barometer was inserted in the vacuum/pressure line to monitor the flushing process. Again a control system was run using compressed air instead of oxygen-free nitrogen.

Whole seedlings of Zea mays In the first series of experiments a simple petri-dish system was used to supply aqueous ABA-2-^{14}C solution at various concentrations to the root tips of 3 day-old seedlings. Eight cm^3 of ABA-2-^{14}C was pipetted in a 5 cm petri dish. Five 6 mm holes were drilled in the lid through which the primary root was passed so that the grain rested on the lid while the first 2 mm of the root tip dipped into the solution. In the second series the ABA-2-^{14}C was applied in 3 x 3 mm agar donor blocks to the shoot or root apex. One mm of the apex was excised prior to application.

After application the seedlings were placed in translucent plastic boxes lined with moist paper towels in the dark room (Type 1) or the light room (Type 2). At the end of the transport period each seedling was divided up into coleoptile, mesocotyl, fruit and primary root, each part weighed and then cut up and placed in a separate scinillation vial. Two cm^3 of 95% ethanol were added to each vial and the lid screwed on. The tissue was extracted for at least 48 hours at $1^{o}C$ before being prepared for radio-assay. The donor block or an aliquot of the ABA-2-^{14}C solution, taken before and after the experiment, was also prepared for counting.

Petiole segments of P. vulgaris. The experiments were carried out in growth room Type 2 ($25^{o}C$ light). Segments, 10 mm in length, were cut from the primary leaf petioles or the first trifoliate leaf petiole of 3 week-old P. vulgaris plants. The segments were always taken from the middle portion of the petiole and were cut from the plant at the same time of day in each experiment. Segments in groups of 5 or 10 were mounted in perspex holders, similar to those used for coleoptiles, in the vertical position with either all the distal cut ends in contact with the donor block (basipetal movement) or all the proximal ends in comtact with the donor block (acropetal movement). Agar receiver blocks were placed on the opposite cut ends. At the end of the transport period the segments were cut up into four equal portions and each portion placed into a scintillation vial containing 2 cm^3 of 95% ethanol. The donor and receiver blocks were treated likewise. The material was extracted for at least 48 hours at $1^{o}C$ before being prepared for counting.

Whole plants of P. vulgaris, A. pseudoplatanus, A. glutinosa, B. pubescens. The experimental procedures used for the study of ABA-2-^{14}C movement in these species were very similar. Any differences, including the exact locus of application, are noted in the results section. The procedures described here are similar to those already published (Hocking, et al., 1972).

Nearly all the experiments with whole plants were carried out in growth rooms Type 3 and 4. The plants were brought in from the greenhouse at least 3 hours, and usually 1 day, prior to experimentation. The ABA-2-^{14}C was usually applied in a small agar block (4 x 5 x 1 mm) to the leaf surface. The blocks were made by cutting up the large blocks, described above, into 8 equal pieces. To facilitate uptake, the leaf surface was first lightly abraded with carborundum powder and the leaf thoroughly rinsed and dried. The agar block was covered with a small square of clear polythene to reduce the rate at which the block dried up. The applications were always made at the same time of day, relative to the photoperiod.

In a few experiments with P. vulgaris and A. glutinosa an aqueous solution of ABA-2-^{14}C was injected into the distal end of the leaf petiole using a Hamilton syringe. In experiments using hydroponically-cultured plants the ABA-2-^{14}C was added to the culture solution using a glass pipette. In these experiments aliquots of the culture solution were taken at the beginning and end of the transport period.

Experiments with P. vulgaris using IAA-1-^{14}C, IAA-2-^{14}C and sucrose ^{14}C were carried out in exactly the same way as that described for the leaf application of ABA-2-^{14}C.

At the end of the transport period the plants were removed from the pot (or culture flask) and the roots washed free of soil (or solution). The entire plant was then divided into small portions and each portion was placed in a scintillation vial containing 2 cm^3 of 95% ethanol. The donor block was carefully removed from the leaf surface and extracted separately, as was the area of tissue immediately beneath the donor block. The material was extracted for at least 48 hours at 10°C before being prepared for radio-assay.

In a number of experiments with P. vulgaris the stem of the plant was steam-girdled prior to application. On the day before application the plants were brought in from the greenhouse and tied to supporting stakes. Steam from a steam-can was passed through a Pasteur pipette and directed onto a small cylinder of stem either below or above the primary leaf node. About 30 seconds of this treatment was sufficient to produce an effective steam-girdle. The remainder of the plant was protected from the steam with paper tissue. The plants were then placed in the appropriate growth room to equilibrate for 24 hours before the tracer was applied. The positions of plants in the growth room were randomised.

6. Detection of Radioactivity in extracted material

The radioactivity in extracted plant material, agar blocks and aqueous solutions was determined using liquid scintillation spectrometry.

The material which had been extracted in 95% ethanol for at least 48 hours at 1°C was first reduced to dryness in vacuo.

Ten cm^3 of a scintillation mixture comprising 2, 5-diphenyl oxozole (PPO) dissolved in toluene ($^4g\ l^{-1}$) were added to each vial. The samples were assayed in one of four counting systems:-

(i) a Packard Tri-carb with Automatic Activity Analyser

(ii) a Packard Tri-carb

(iii) a Tracerlab Corumatic 2024 BSL four channel counter

(iv) a Beckman LS200

All samples of variable quench were counted on the Packard Tri-carb with Automatic Activity Analyser. This machine was calibrated according to the manufacturer's instruction manual 2096 (Packard Instrument Co. Inc., 2200 Warrenville Rd., Powners Grove, Ill. 60515. U.S.A.) to correct automatically for background and quenching, printing out data in disintegrations per minute (dpm). A quench correction curve was plotted for each of the other machines, and all counts were corrected for background and quench, and expressed as dpm.

7. Experimental procedure for studying the metabolism of ABA-2-^{14}C in plants

These experiments involved primarily the chromatographic analysis of extracts of plant tissues which had been treated with ABA-2-^{14}C under a number of different environmental conditions. The metabolism of ABA-2-^{14}C was studied in coleoptile and root segments of Zea mays and whole plants of P. vulgaris and A. glutinosa. The extraction and purification procedures used prior to chromatography varied according to the type of tissue used.

Coleoptile and root segments. Ten mm sub-apical segments of coleoptiles or roots were set up as described above for acropetal and basipetal transport, both in the light and darkness at 25°C. ABA-2-^{14}C was supplied in agar donor blocks at a concentration of 10^{-5}M. At the end of the transport period a millimetre section of tissue adjacent to the donor was removed, and the remainder of the tissue was placed in a vial and extracted with 95% ethanol for 2 days at 1°C in the dark. During subsequent procedures care was taken to avoid exposure of the extracts to direct sunlight. The extracts were then reduced to dryness on a rotary evaporator and re-dissolved in 0.1 cm^3 of re-distilled absolute ethanol. As the extracts had been obtained from very small amounts of unmacerated tissue (usually 10 segments) no purification was required before chromatography. The extracts were analysed using both descending paper (Whatman's No. 1.) and ascending thin-layer (300 μ silica gel) chromatography. A number of different solvent systems were used to develop the chromatograms. For paper chromatograms the solvents used were:- n-butanol: n-propanol: ammonia: water :: 2:6:1:2 v/v; n-butanol: acetic acid: water :: 5:1:2:2 v/v; Isopropanol: ammonia: water :: 8:1:1 v/v. For thin layer chromatograms only 2:6:1:2 was used. After development and drying the chromatograms were assayed for radioactivity by two methods; liquid scintillation counting and autoradiography.

For scintillation counting the chromatograms were divided into 10 (complete Rf) or 20 (half Rf) zones. Each zone was either cut up, or in the case of TLC, scraped off, and placed in a scintillation vial. Ten cm^3 of the PPO/toluene scintillation

mixture was added and the samples counted on one of the counting systems described above. Counts were corrected for background but not quench, and expressed as cpm.

For autoradiography the dried chromatograms were placed in contact with Ilford 'Red Seal' X-ray film in total darkness for 1 month. At the end of this period the film was developed in Ilford 'Phenisol' X-ray developer and 'Kodafix' fixer, and finally dried.

P. vulgaris and A. glutinosa. ABA-2-^{14}C was applied either in an agar block to a leaf surface, or in aqueous solution to the culture solution of hydroponically cultured plants, as described above. The exact transport periods and environmental conditions used are given in the results section. The amount and type of tissue extracted varied in different experiments. In a large experiment with P. vulgaris 40 plants were used and divided up into donor leaves, apices, upper stems, lower stems, roots and nodules. With A. glutinosa the donor leaf, apex and stem were usually extracted separately. Extensive purification was necessary prior to chromatography. The procedure was standardised and followed in part the method of Lenton et al., (1971).

At the end of the transport period the tissue was extracted with 80% re-distilled methanol (10 ml/g tissue) at 1°C overnight. The material was then homogenised in a Waring blender and re-extracted twice with 80% methanol, 12 hours for each extraction. The combined extracts were reduced to an aqueous solution on a rotary evaporator at 35°C avoiding exposure to

direct sunlight. The solution was then frozen, thawed and centrifuged at 16,000 g for 1.5 h to remove suspended material. The pH of the supernatant was adjusted to 3.5 with 2N HCl and extracted three times with equal volumes of re-distilled diethyl ether. With some extracts the ether was removed at this stage and the material re-dissolved in methanol for chromatography. The aqueous phase was also monitored for possible losses of radioactivity. On at least one occasion the aqueous phase was dried, re-dissolved in methanol and chromatographed. For other extracts the purification was taken further by alternately extracting the ether phase four times with $\frac{1}{4}$ volume of 5% soldium bicarbonate solution and $\frac{1}{4}$ volume of water. After re-adjusting the pH to 3.5 the combined aqueous extracts were extracted 3 times with equal volumes of ether and the final ether extracts dried over annhydrous sodium sulphate before removing the ether on a rotary evaporator. The dried extracts were re-dissolved in re-distilled methanol for chromatographic analysis. Whatman's No. 3. chromatography paper was used, with the following solvent systems:

(i) n-butanol: n-propanol: ammonia: water :: 2:6:1:2 v/v;

(ii) n-butanol: acetic acid: water :: 5:1:2:2 v/v;

(iii) Isopropanol: ammonia: water :: 8:1:1 v/v.

After development and drying the chromatograms were assayed using liquid scintillation counting or autoradiography.

The metabolism of IAA-1-^{14}C and IAA-2-^{14}C in whole plants of P. vulgaris was studied in the same way as that described for ABA-2-^{14}C.

8. Statistical analysis

The statistical analyses used in this thesis were calculated as follows:

(i) Standard error of a mean:

$$S.E. = \pm \frac{\text{Standard Deviation}}{\sqrt{n}}$$

where n = number of observations

$$\text{standard deviation} = \sqrt{\frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n-1}}$$

(ii) Student's t-test:

$$t = \frac{\text{Difference of means}}{\text{Standard error of difference}}$$

$$= \frac{\bar{X}_A - \bar{X}_B}{\sqrt{(S.E._A)^2 + (S.E._B)^2}}$$

where A and B are the two populations.

The t - value obtained was compared with the values in the Student's t - Distribution table in Fisher and Yates' 'Statistical Tables for Biological, Agricultural and Medical Research', Oliver Boyd Ltd., Edinburgh, using $n_A + n_B - 2$ degrees of freedom, at probability levels not higher than 0.05P. Percentage data were first transformed using Angular Transformation Tables.

RESULTS Section I Studies on Zea Mays

1A Movement and metabolism of ^{14}C in coleoptile segments of Zea mays

(i) Uptake and movement of ^{14}C in coleoptile segments.

Since 1928, when Went devised the donor-segment-receiver system, the question of polarity has dominated the study of growth regulator movement in isolated segments of plant tissue. Thus, as a first step in this investigation the uptake and movement of ABA-2-^{14}C in Zea coleoptile segments were studied in acropetal and basipetal directions as a function of time.

The segments were set up as described in the Methods section, in growth room 2 ($25^{o}C$. light), and 10^{-6}M ABA-2-^{14}C in an agar block applied to the apical or basal ends of the segments. After 24 hours there was no detectable accumulation of radioactivity in the apical or basal receiving blocks. In the tissue, however, there was a significantly greater uptake (to 0.001P) of ^{14}C from basal donor blocks after 8 hours than from apical blocks. (Fig.1.) After 24 hours 80% more radioactivity had accumulated in the acropetally transporting segments than in the basipetally transporting segments. This difference may be explained by the fact that a considerably greater area of tissue was in contact with the donor block at the basal end than the apical end of the segment, an aspect considered in greater detail in a later section.

Because there was no accumulation of radioactivity in the receiving blocks it was not possible to assay them for ABA transport polarity and velocity. Consequently, the movement and distribution of radioactivity through the coleoptile tissue was studied in detail. This approach has the advantage of eliminating possible complications arising from the movement of ^{14}C from the tissue into the agar

receiving blocks or vice versa.

The coleoptiles were set up as before, and at the end of a transport period the 10mm segments were cut up into five 2mm sections which were radioassayed in groups of five replicates. In this way the movement of ^{14}C through the segment was followed at 2mm intervals. These sections were numbered 1 to 5, starting at the donor block end. Over longer time periods the extra tissue remaining as a result of growth of the segment was grouped with the fifth section, which became the 'receiver' of the tissue transport system. The results are shown in Figure 2. The radioactivity in each section is expressed as a percentage of the total radioactivity in the segment. The use of percentage values allows data from separate experiments to be compared, as there were large variations in uptake between experiments.

In the first tissue section a greater uptake of radioactivity from the basal donor block was apparent, and a greater percentage dpm remained in section 1 of acropetal segments throughout the time-course, compared to basipetal segments. In section 2 this difference was not apparent but in remaining sections the difference appeared to have been reversed and by the fifth section a clear basipetal polarity of movement of ^{14}C was established. This polarity occurred despite the fact that the acropetal segments had a much greater loading of radioactivity in section 1. Figure 3 shows the summation of the % dpm in sections 3, 4 and 5 as a function of time. After 12 hours there was a significant difference (to 0.01P) between the accumulation of radioactivity in sections 3 - 5 of basipetally transporting segments and the accumulation in acropetally transporting segments; i.e. there was a significant basipetal polarity of ^{14}C movement on the basis of percentage distribution following application of 10^{-6}M ABA-2-^{14}C. The statistical significance of this difference, however, declined with time.

FIGURE 1

The basal, —●— , and apical, —○— , uptake of ^{14}C into coleoptile segments as a function of time. The points, with vertical bars representing twice the S.E. of the mean, are mean values of four experiments, each experiment using five 10mm coleoptile segments of Zea mays supplied with donors containing 10^{-6}M ABA-2-^{14}C at 25°C in the light.

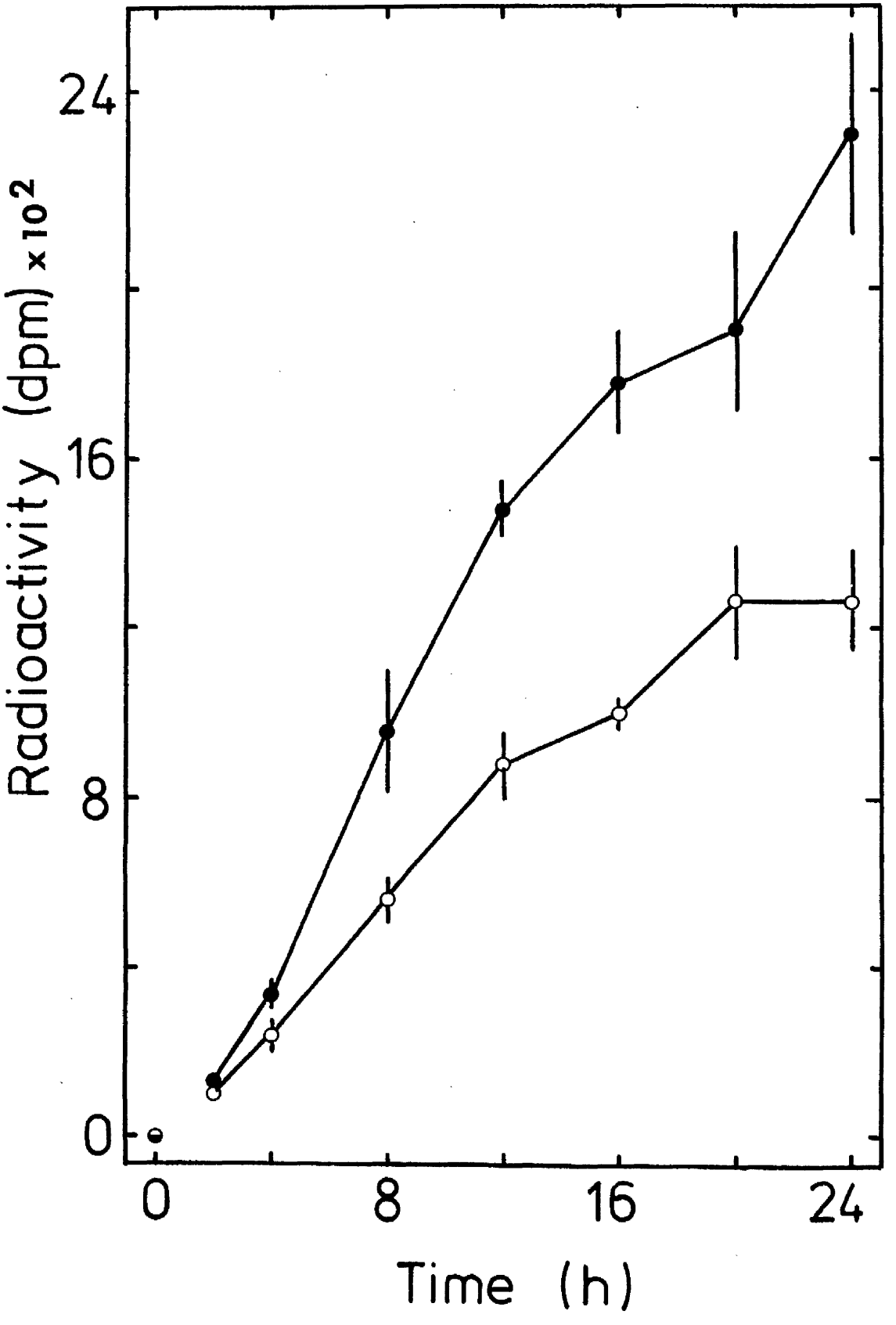

24
16
8
0
Radioactivity (dpm) x 10²
0
8
16
24
Time (h)

FIGURE 2

The accumulation of ^{14}C, as a percentage of the total uptake per segment, in sections away from the donor blocks of acropetally, —●— , and basipetally, —○— , transporting segments of Zea mays. Experimental details are as in Fig. 1.

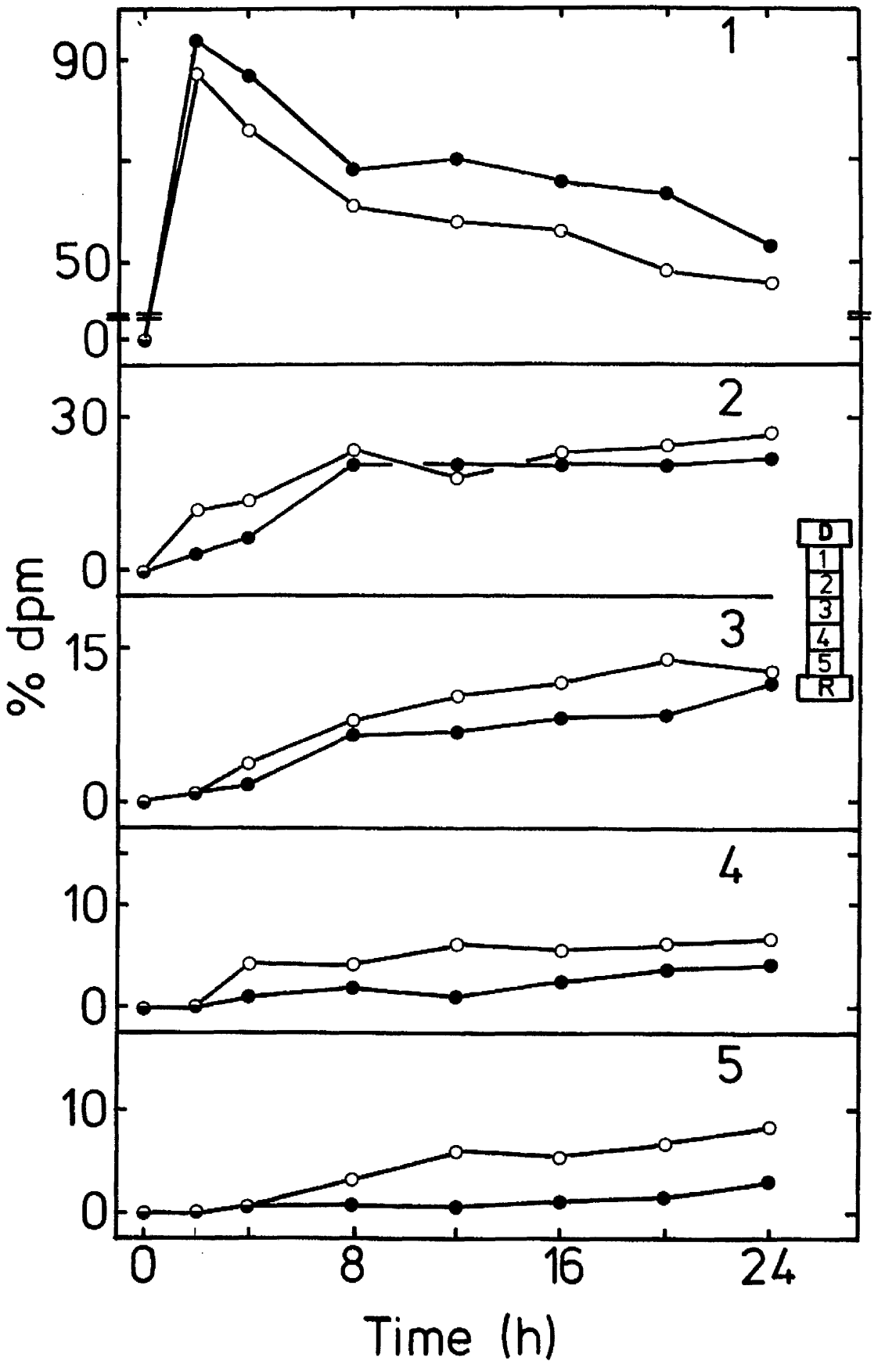
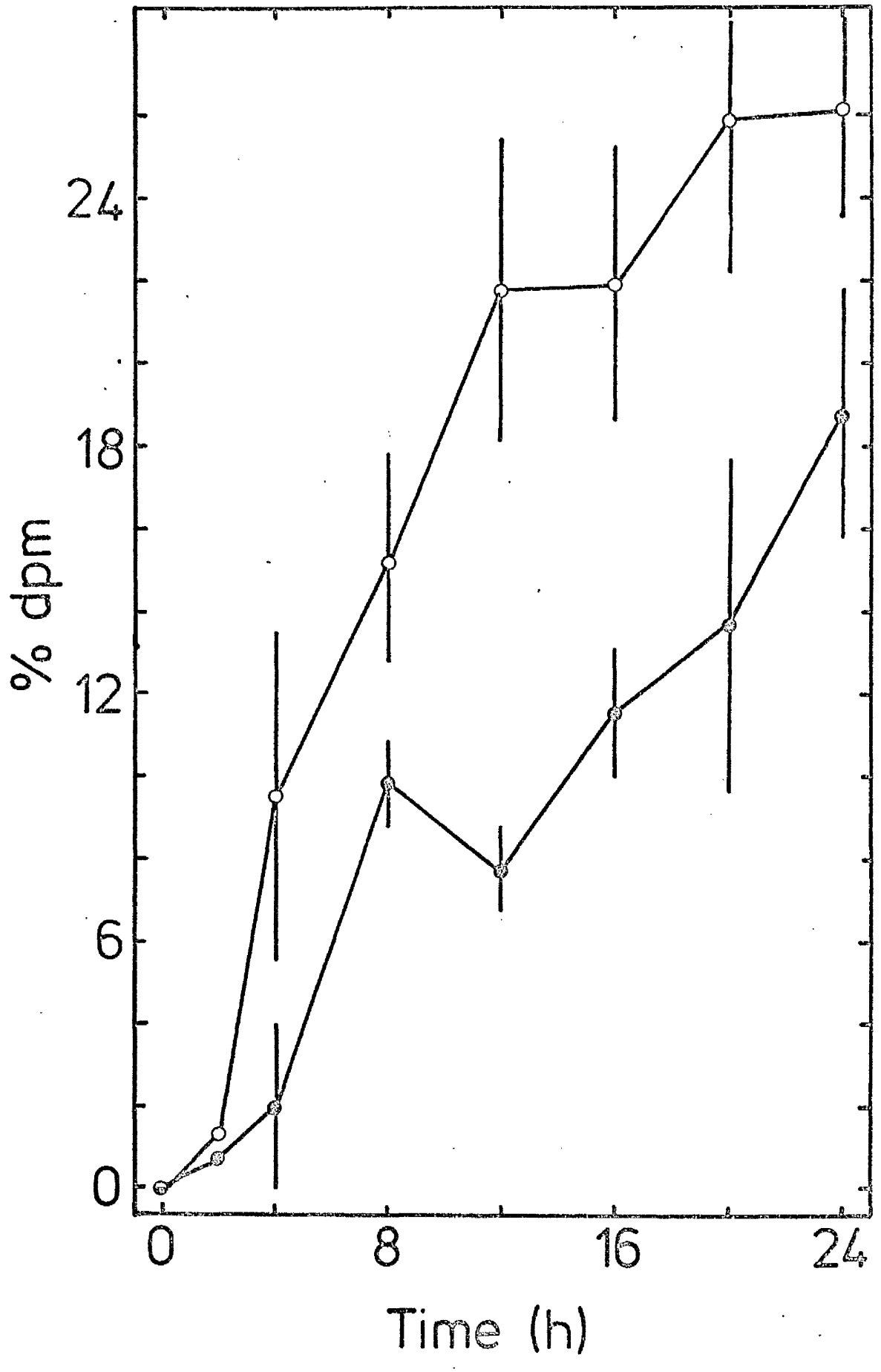

90
50
0
1
30
0
2
15
0
3
10
0
4
10
0
5
% dpm
D
1
2
3
4
5
R
0
8
16
24
Time (h)

FIGURE 3

The accumulation of ^{14}C, as a percentage of the total uptake per segment in the 3rd., 4th. and 5th. sections of acropetally, —●— , and basipetally, —○— , transporting segments of Zea mays. Experimental details are as in Fig. 1.


24
18
12
6
0
% dpm
0
8
16
24
Time (h)

(ii) Velocity of ^{14}C movement in coleoptile segments

In the past, three methods have been used for the measurement of growth regulator velocities in isolated tissue segments. Van der Weij (1932) devised the intercept method for auxin transport in coleoptile segments on the basis that the amount of auxin in a basal receiver increased nearly linearly with time. Goldsmith (1967) developed an alternative method using pulse-labelling of corn coleoptiles with ^{14}C-IAA, and Newman (1963) designed a third, electrical, method. The last two methods were devised specifically for auxin movement, but it was considered possible to adapt the intercept method of Van der Weij for the study of abscisic acid movement in corn coleoptiles.

Since the agar receiver block data were unattainable within practical time periods, the analysis of transport velocities was carried out using the fifth section of tissue as the 'receiver' of the system. The coleoptiles were set up as described above and the accumulation of radioactivity in section 5 was plotted as a function of time. (Fig. 4.) An approximately linear relationship was obtained for both acropetal and basipetal movement. Using van der Weij's method the curve obtained was extrapolated by eye back to the time axis, the intercept giving the time taken for radioactivity to appear in the receiving 5th section. For basipetal transport the intercept was between 6 and 8 hours, indicating a net rate of basipetal movement of ^{14}C through 8 mm of tissue of 1.0-1.3mm h^{-1}. The equivalent rata for acropetal transport was 0.5-0.8mm h^{-1}. The slope of the velocity plot gives the flux, i.e. the amount of radioactivity moved in unit time. The two plots for basipetal and acropetal movement were vitually parallel, showing that the fluxes were similar at between

5 and 7 dpm $^{-1}$. From these results, which were the pooled values of four separate experiments, it could be inferred that the basipetal polarity observed after 24 hours was due to a faster rate of basipetal movement compared to acropetal movement, rather than a greater flux. The capacities of the basipetal and acropetal systems for the movement of ^{14}C from ABA-2-^{14}C would appear to be similar. Whether or not the difference in rates is sufficient to indicate that different processes are involved in acropetal and basipetal movement remains to be determined.

In this respect, it is of interest to plot the distribution of radioactivity in the tissue after a certain time period as a function of distance moved from the donor block. In Figure 5, the data from Figure 2 are shown with % dpm plotted against distance moved from the donor block. Small quantitative differences of uptake and rate of movement can be discerned, but the overal shapes of the distribution profiles are similar.

FIGURE 4

The accumulation of ^{14}C into the fifth section of acropetally, —●—, and basipetally, —○—, transporting segments of Zea mays coleoptiles, as a function of time. The plotted values are means of four experiments, each using five coleoptile segments supplied with 10^{-6} M ABA-2-^{14}C at 25°C in the light. Lines plotted by eye.

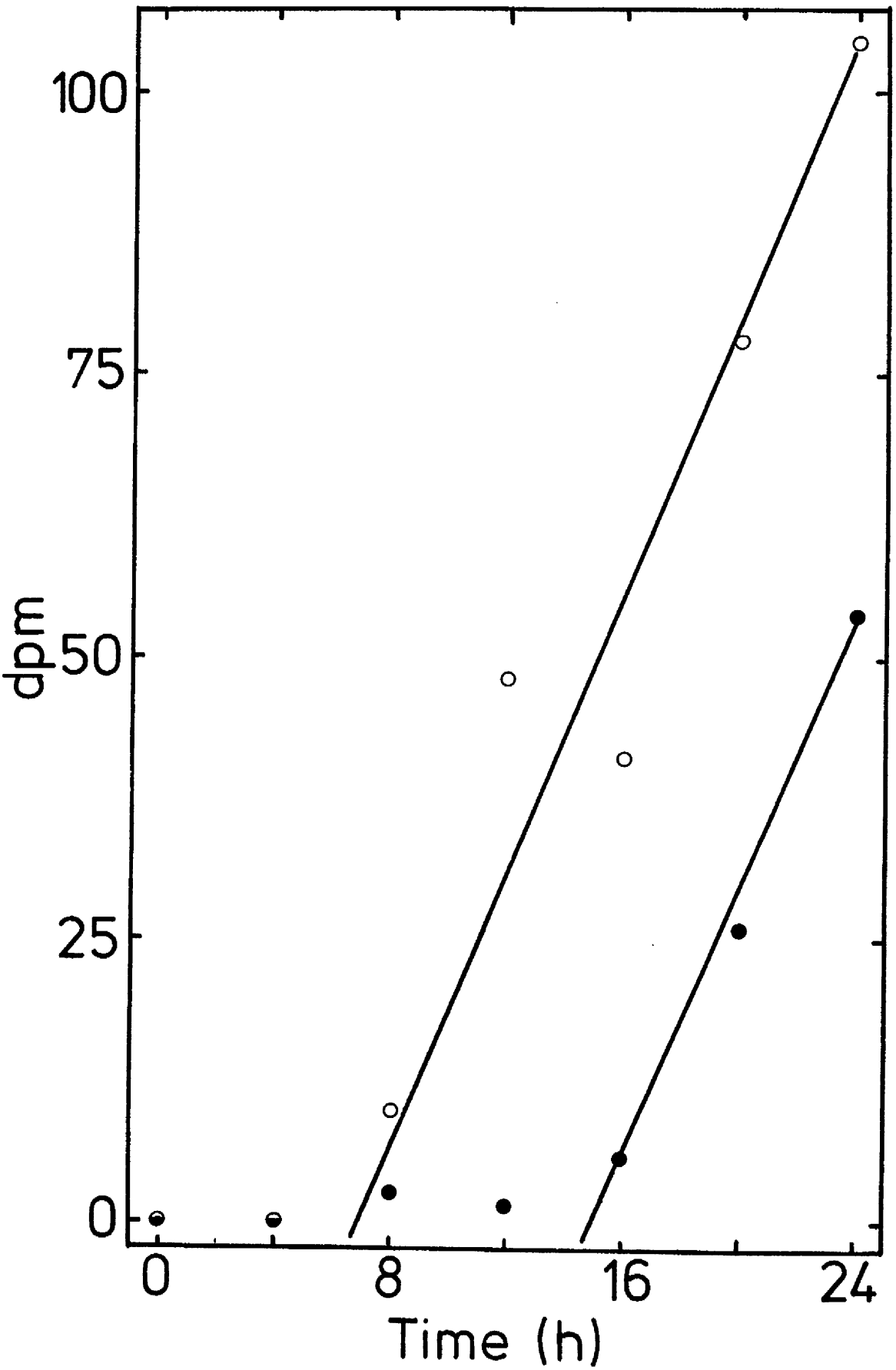
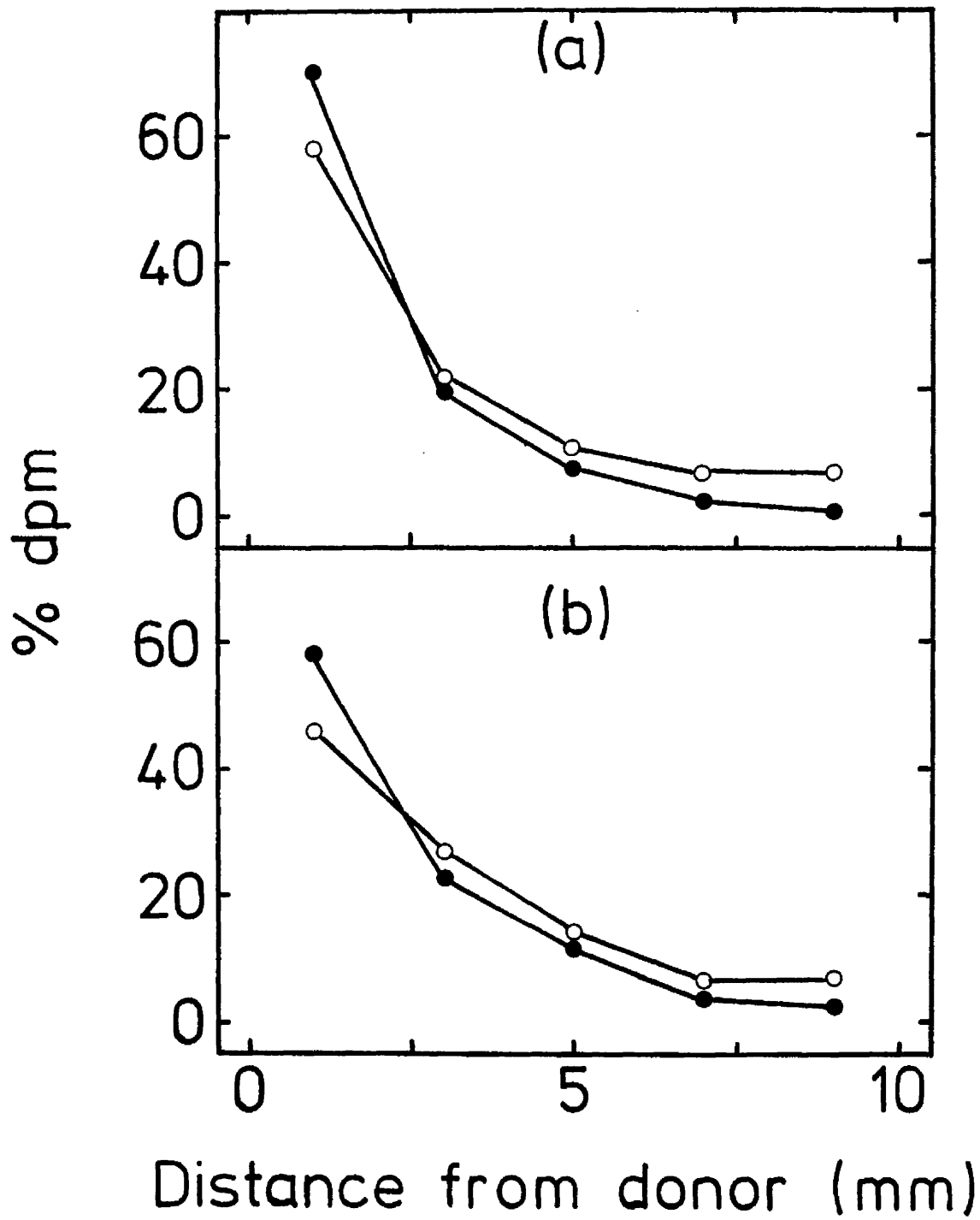

100
75
50
25
0
dpm
0
8
16
24
Time (h)

FIGURE 5

The distribution of radioactivity in acropetally, —●—, and basipetally, —○—, transporting segments, as a function of distance from the donor. Experimental details as in Fig. 4.


(a)
(b)
60
40
20
0
% dpm
0
5
10
Distance from donor (mm)

(iii) The effect of inversion on the uptake and movement of ^{14}C in coleoptile segments.

When investigating the polarity of movement of a substance in plant tissue, the orientation of the tissue and consequent effects of gravity must be taken into account. Indeed, a criterion for polar transport through an organ is that it should occur irrespective of the spatial orientation of that organ; i.e. basipetal movement is from the morphological apex to the morphological base, irrespective of orientation.

Previous experiments have all been carried out with the donor block at the bottom of the segment, and thus for acropetal transport the segments were upright and for basipetal transport the segments were inverted. This system has two major advantages. Firstly it simplifies the setting up procedure. Secondly, it ensures that the coleoptiles are always in firm contact with the donor blocks, held down by their own weight and the weight of the receiver blocks. However, over longer time periods, the inverted segments develop considerable curvatures which complicate the cutting-up procedure. Moreover, gravity may influence the movement of the substance under investigation. The alternative system of keeping the segments upright and re-orientating the donor blocks has two main disadvantages. Firstly, the contact with the donor block may be different for acropetal and basipetal transport. Secondly, if growth of the segments is uneven, the donor block may be lifted clear of the slower growing segments during basipetal transport. Because of these problems, it is necessary to study the movement of the substance using both systems, to ensure that any differences observed when using one system are not due to artifacts arising from that system.

The four possible orientations of segments and donor/receiver blocks are shown in Figure 6.

Segments, 10mm in length, were used and set up in the four orientations shown in Figure 6, applying 5μM ABA-2-^{14}C in the donor blocks. Transport was considered over 24 hours in the dark, at 25^{o}C. Figure 7 shows the effect of inversion on uptake of ^{14}C into the segments. Basal uptake was always significantly (to 0.001P) greater than apical uptake of ^{14}C, but there was no significant effect of inversion on uptake.

Figure 8 shows the effect of inversion on the distribution of ^{14}C down the coleoptile. Again there was no significant effect of inversion. Furthermore, inversion did not alter the basipetal polarity of movement of ^{14}C into sections 3 - 5 of the coleoptile segments. (Fig. 9.) The effect of inversion on the straight growth of 10mm coleoptile segments during the transport period was also determined. At the end of the experiment the segments were shadowgraphed at a magnification of x5. Table 1 shows the results from three separate experiments. Although the growth of the segments varied between experiments, there was no significant (to 0.05P) effect of inversion on growth in any one experiment.

These results show that the differences observed in acropetal and basipetal movement of ^{14}C in segments supplied with ABA-2-^{14}C were independent of orientation of the segments.

Table 1. The effect of inversion on the straight growth over 24 hours at 25°C in the light of *Zea mays* coleoptile segments. Mean, final, magnified lengths (cm) standard errors are given for three experiments, twenty replicates in each experiment.

Acropetal			Basipetal		
Upright	Inverted	't' valve	Upright	Inverted	't' valve
6.95 ± 0.04	7.01 ± 0.07	0.74 n.s.	7.20 ± 0.04	7.08 ± 0.08	0.84 n.s.
6.52 ± 0.08	6.53 ± 0.05	0.54 n.s.	6.52 ± 0.04	6.67 ± 0.07	0.98 n.s.
6.56 ± 0.04	6.59 ± 0.04	0.63 n.s.	6.58 ± 0.05	6.60 ± 0.06	0.90 n.s.

FIGURE 6

The four possible orientations of 10mm coleoptile segments with donor and receiver blocks.

A = morphological apex

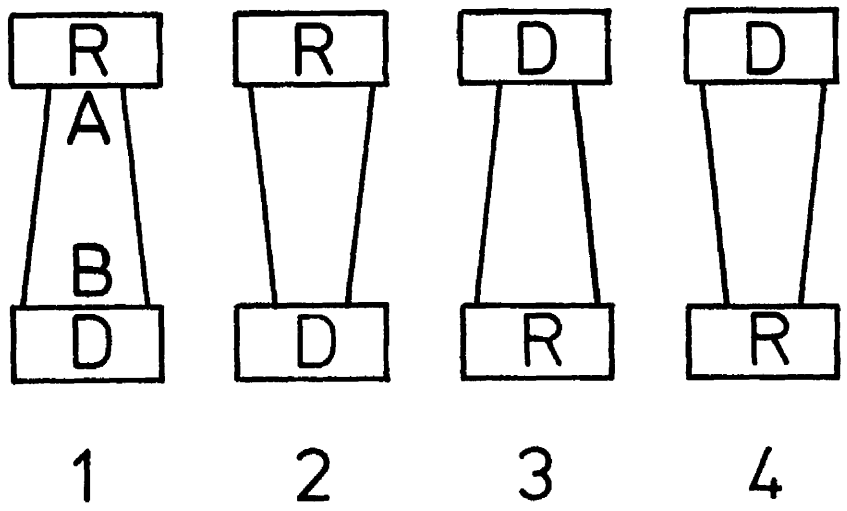
B = morphological base

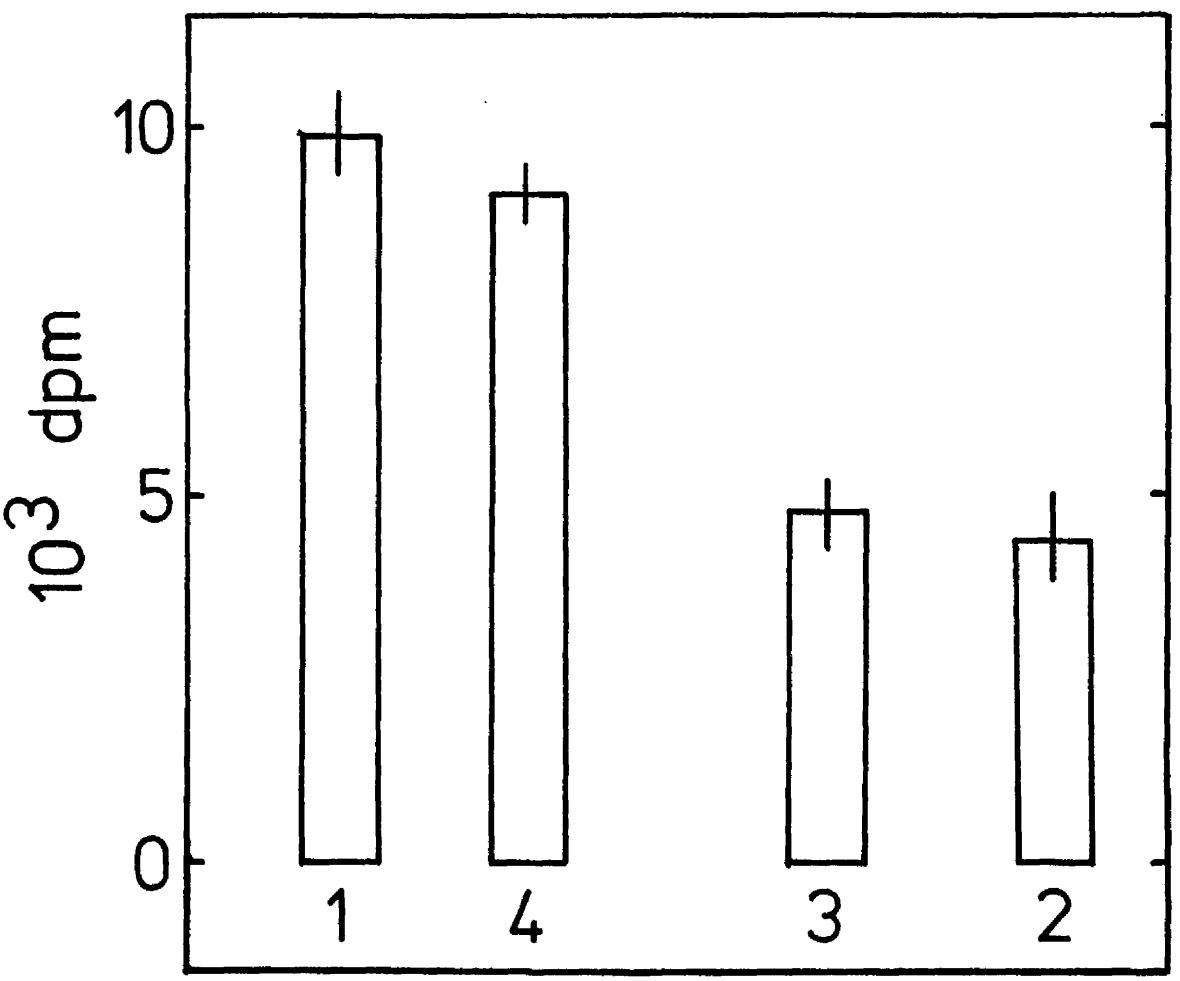
D = donor block

R = receiver block

FIGURE 7

The effect of inversion on the total uptake of ^{14}C into 10mm Zea coleoptile segments after 24 hours in the light at 25°C, supplied with blocks containing 5 μM ABA-2-^{14}C. The values given are the means (vertical bar represents twice S.E.) of 60 replicates from three experiments.


R
A
B
D
R
D
D
R
D
R
1
2
3
4


10
5
0
10^3 dpm
1
4
3
2

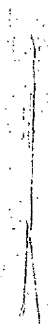
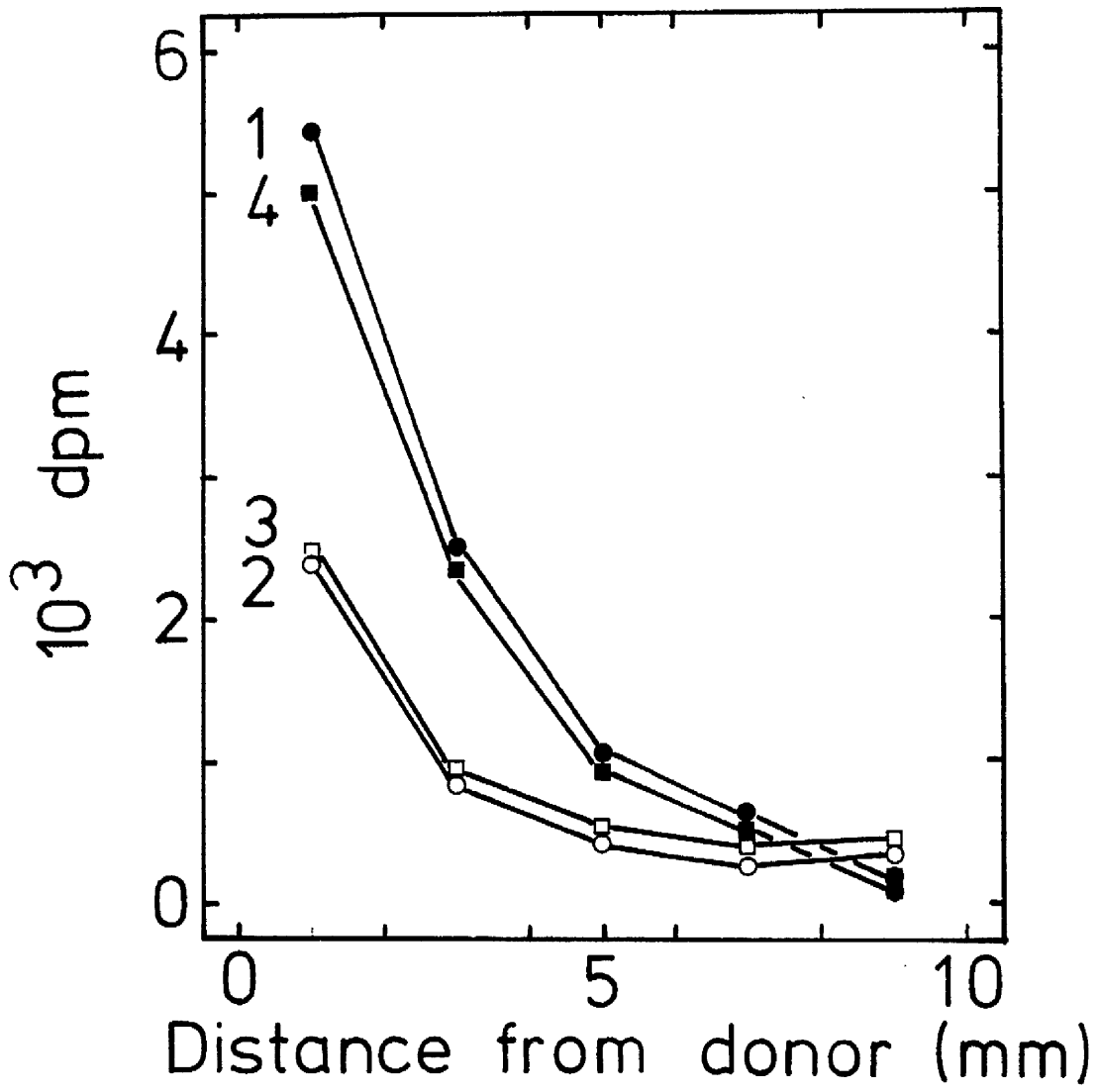


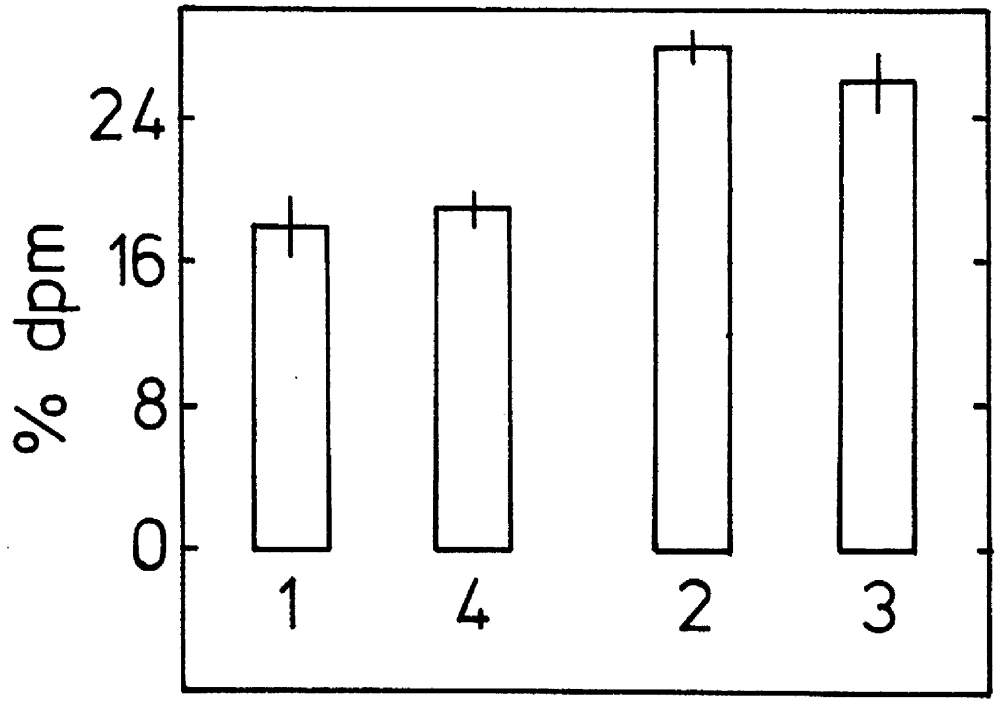
FIGURE 8

The effect of inversion on the distribution of ^{14}C in the coleoptile segment as a function of distance from the donor block. Experimental details as in Figure 7.

FIGURE 9

Effect of inversion on the accumulation of ^{14}C, expressed as a percentage of the total uptake, in the 3rd., 4th. and 5th. sections of Zea mays coleoptile segments after 24 hours. Experimental details as in Figure 7.


6
4
2
0
10^3 dpm
1
4
3
2
0
5
10
Distance from donor (mm)


24
16
8
0
% dpm
1
4
2
3

(iv) The effect of light on the movement of ^{14}C in coleoptile segments.

The preceding experiments were carried out in the light using coleoptiles which, at the beginning of the experiment, had been grown for five days in total darkness. Etiolated coleoptiles undergo a number of morphological and physiological changes when placed in the light for the first time. Also light may affect the breakdown of ABA-2-^{14}C. Hence it is possible that light may influence the pattern of ^{14}C movement in coleoptile segments. It was important, therefore, to determine whether there were any observable differences in ^{14}C movement in the dark, compared to movement in the light, following application of ABA-2-^{14}C.

The experiments were carried out in growth room 1 (dark 25°C) and growth room 2 (light 25°C). In the dark room the coleoptiles were excised and handled under dim green light, and during the period of ABA transport they were maintained in complete darkness. The total uptake of ^{14}C into the tissue as a function of time was considered first. (Fig. 10.) The pattern of uptake was similar in the light and dark. Basal uptake was always greater than apical uptake and the difference between the two was similar in the light and dark. There were some quantitative differences in uptake in light and darkness but these were probably due more to variation in uptake between experiments rather than to any significant effect of light. Throughout this investigation uptake in different experiments was variable, although within experiments there was only a small variation in uptake between replicates. The true cause of this variation was not discovered, but possibly reflected slight variations in the physiological condition of different batches of coleoptiles.

The distribution of ^{14}C in tissue segments was also examined. Figure 11 shows the effect of light on the accumulation of ^{14}C in sections 3, 4 and 5 of the coleoptile segments as a function of time. When considered in this way there was a marked basipetal polarity of ^{14}C movement, regardless of exposure to light which was established by 12 hours and maintained for the remainder of the 24 hour period. One difference between light and dark treatments was that the polarity was significant (to 0.01P) after only 2 hours in the dark, whereas in the light the polarity was not clearly established until after 12 hours. This initial polarity in the dark decreased after 4 hours, and then increased again, indicating that there was a rapid surge of radioactivity (on a percentage basis) down the segment in the basipetal direction in the dark immediately after the application of the ABA-2-^{14}C. It was not clear whether this was caused by a high initial rate of movement, or a high initial flux. After two hours the pattern of distribution stabilised and followed quite closely the pattern in the light. Possibly these small quantitative differences may be due to an effect of light on the immobilisation or breakdown of ABA-2-^{14}C in the tissue. The effect of light on the metabolism of ABA-2-^{14}C in tissue is discussed elsewhere. It would seem therefore, that light does not markedly alter the basic characteristics of ^{14}C movement in coleoptile segments supplied with ABA-2-^{14}C.

FIGURE 10

The total uptake of ^{14}C into 10mm coleoptile segments of Zea mays in light or darkness at 25°C as a function of time. The segments were supplied with donor blocks containing 1 M ABA-2-^{14}C. The plotted values are means of two experiments, five replicates in each.

—●— Basal uptake (acropetal movement) in the light

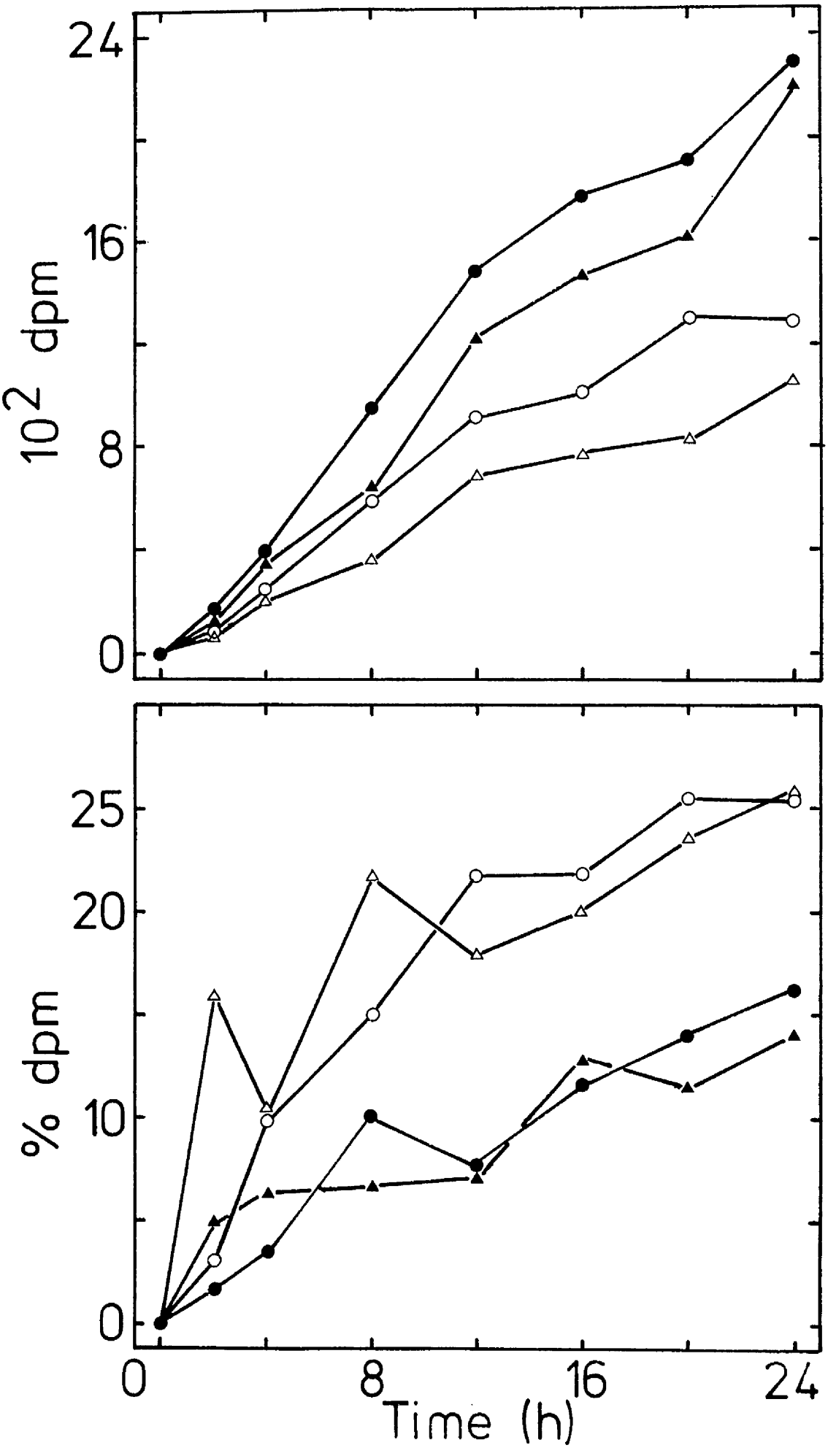
—○— Apical uptake (basipetal movement) in the light

—▲— Basal uptake (acropetal movement) in the dark

—△— Apical uptake (basipetal movement) in the dark.

FIGURE 11

The accumulation of ^{14}C, expressed as a percentage of the total uptake in dpm, into sections 3, 4 and 5 of Zea mays coleoptile segments in light or darkness at 25°C as a function of time. Experimental details and symbols as in Figure 10.


24
16
8
0
10^2 dpm
25
20
10
0
% dpm
0
8
16
24
Time (h)

(v) The effect of donor concentration of ABA-2-^{14}C on the movement of ^{14}C in coleoptile segments.

Any detailed study of the movement of a growth substance in plant tissue must include some consideration of whether the movement has a molecular specificity and is metabolically dependent. These two factors are interrelated since it is unlikely that any degree of specificity of movement within the tissue could be achieved without metabolic activity, and the converse is also probably true. Evidence for the existence of a specific, metabolically dependent mechanism can be drawn from such experiments as the effects of donor concentration, temperature, anoxic conditions and metabolic inhibitions, on the movement of the substance in the tissue. The existence of polar movement does, of course, suggest some degree of specificity within the tissue.

In respect of the effects of donor concentration it is important to discover if the movement is independent of donor concentration, and if the system is saturated at higher concentrations. Evidence of a saturation response might suggest a system analagous to an enzymic system, possibly supporting the concept of a specific transport site.

In this study the coleoptiles were set up as before, but using a range of concentrations of ABA-2-^{14}C in the donor blocks. Uptake and movement were determined over a 24 hour period in the light at 25^{o}C. (Fig. 12.) The relationship between donor concentration and uptake of radioactivity was virtually linear over the range of concentrations used for both basipetal and acropetal movement, showing that uptake is proportional to, and possibly dependent on, donor concentration. The movement of ^{14}C into section 5 of the segments

was also proportional to donor concentration for both basipetal and acropetal movement. (Fig. 13.) The basipetal polarity was maintained over the range of 10^{-4} to 10^{-6}M ABA-2-^{14}C. This is shown clearly in Figure 14, where the movement of ^{14}C into sections 3, 4 and 5 is plotted on a percentage basis as a function of donor concentration. The difference between basipetal and acropetal distribution of ^{14}C in these sections remained relatively constant throughout. Thus donor concentration over the range 10^{-6}M to 10^{-4}M ABA-2-^{14}C did not affect the basic characteristics of ^{14}C movement in coleoptile segments. Uptake and movement of ^{14}C into the receiving section of the segment were proportional to donor concentration in both basipetal and acropetal directions. There was no evidence of a saturation response over a hundredfold increase in donor concentration and more important no evidence that basipetal movement was affected in a different way to acropetal movement by increasing donor concentrations.

FIGURE 12

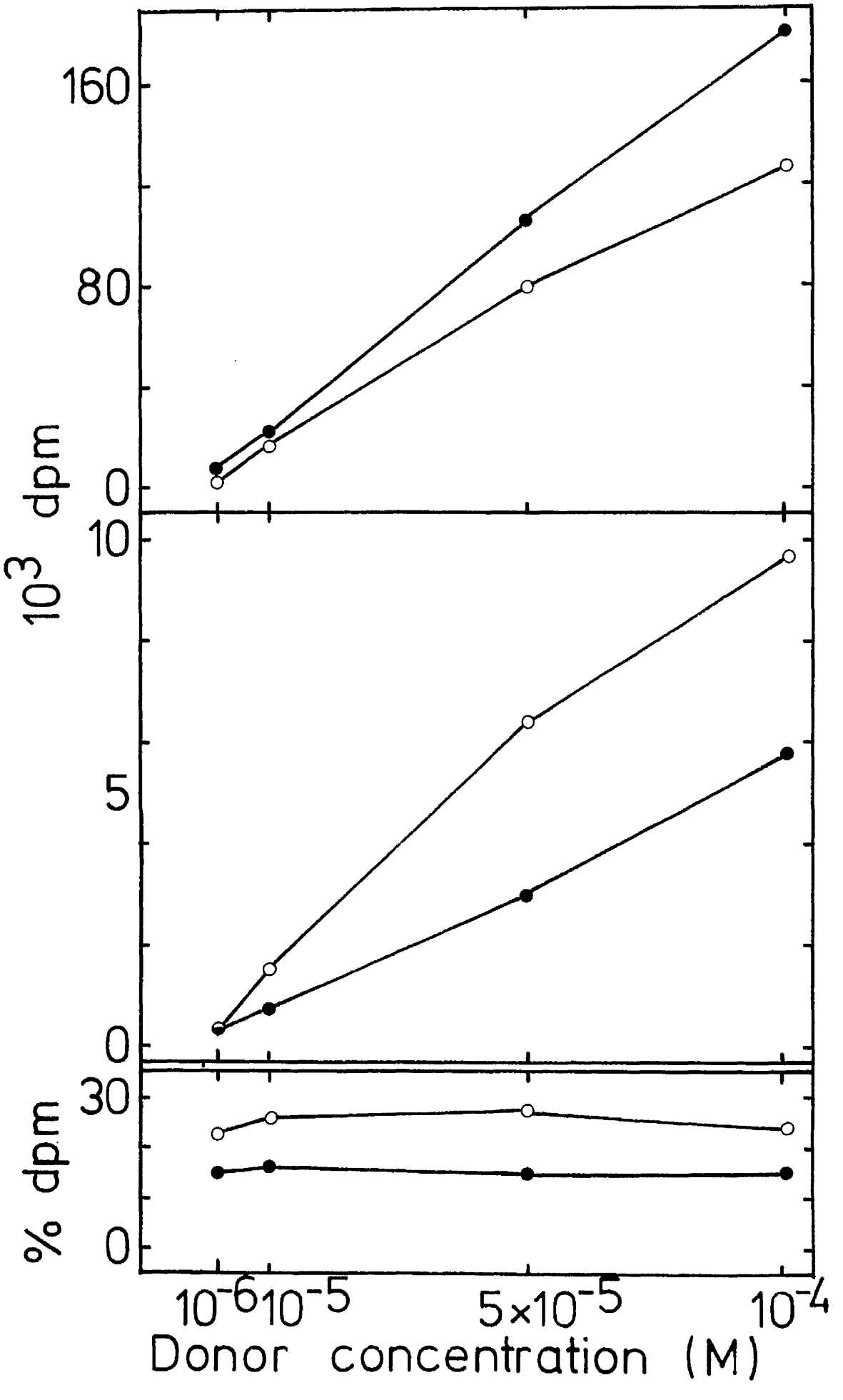
The basal, —●—, and —○—, apical uptake of ^{14}C into 10mm *Zea mays* coleoptile segments after 24 hours in the light at 25°C, as a function of donor concentration of ABA-2-^{14}C. The plotted values are means of fifteen replicates.

FIGURE 13

The accumulation of ^{14}C into the fifth section of basipetally, —○—, and acropetally, —●—, transporting segments after 24 hours in the light at 25°C, as a function of donor concentration. The plotted values are means of fifteen replicates. Details as in Figure 12.

FIGURE 14

The accumulation of ^{14}C, expressed as a percentage of the total uptake per segment, into sections 3, 4 and 5 of basipetally, —○—, and acropetally, —●—, transporting segments as a function of donor concentration of ABA-2-^{14}C. Experimental details as in Figure 12.


160
80
0
10^3 dpm
10
5
0
30
% dpm
0
10^{-6} 10^{-5} 5×10^{-5} 10^{-4}
Donor concentration (M)

(vi) The effect of temperature on the uptake and movement of ^{14}C in coleoptile segments.

In the study of the relationship between movement of a substance and the metabolic activity of the tissue, the effects of temperature on the movement of ^{14}C in coleoptile segments supplied with 10^{-6}M ABA-2-^{14}C must be considered.

Coleoptile segments, ten millimetres in length, were set up for acroptetal and basipetal transport over 24 hours at three temperatures:- 0,* 10 and 25°C. Lowering the temperature markedly reduced both basal and apical uptake. (Fig. 15.) At 0°C basal uptake was reduced by 90% compared to the uptake at 25°C, and apical uptake was reduced by 86%, making the uptake similar in both directions. However, the amounts of radioactivity involved at this temperature were very small, and any slight differences in basal and apical uptake may not have been detected. At 10°C uptake was again markedly reduced, but generally basal uptake was still greater than apical uptake although the difference between the two was lost after 24 hours. The significance of this observation is difficult to assess. It is tempting to suggest that basal uptake was more temperature dependent than apical uptake and hence had a greater metabolically dependent component. However a number of processes, both metabolic and physical, would be affected by temperature changes and the data did not lend itself to detailed analysis of temperature coefficients. One complicating factor may be the effect of temperature on the expansion of the cross-sectional area of tissue in contact with the donor block. Although no measurements were taken of cross-sectional areas during the experiment, longitudinal growth was certainly reduced at 10°C, and completely inhibited at 0°C, so it is possible that any

* the temperature was actually just above zero, such that the agar blocks did not freeze.

increase in cross-sectional area was similarly affected.

The effects of temperature on the movement of ^{14}C through the coleoptile segments were also considered. The experiments were carried out as described previously by analysis of the accumulation of ^{14}C in 2mm sections of the segments, with time. Figure 16 shows the effect of temperature on the accumulation of radioactivity in the 3rd. 4th and 5th sections of 10mm coleoptile segements as a function of time. At $0^{o}C$ less than 3% of the radioactivity had reached the 3rd section after 24 hours in the basipetal direction, but no radioactivity was detected in the acropetal direction. Thus, clearly at $0^{o}C$ the movement of ^{14}C in both directions in the tissue was very much reduced.

At $25^{o}C$ and $10^{o}C$ there was a greater percentage accumulation of radioactivity in the basipetally transporting segments than the acropetally transporting segments. Thus at $10^{o}C$ the basipetal polarity of ^{14}C movement was maintained. The percentage dpm in the 3rd - 5th sections in the basipetal direction, however, was only 9% at $10^{o}C$, compared with 24% at $25^{o}C$. Similarly, the acropetal accumulation was reduced from 12% to 3% after 24 hours at $10^{o}C$. The time taken for ^{14}C to appear in the 3rd section at $10^{o}C$ was increased, suggesting that the rate of movement of ^{14}C had been reduced. This was investigated further by plotting the accumulation of ^{14}C into the receiving end of the tissue against time. At $10^{o}C$ this was only possible with basipetal transport, using the 3rd section away from the donor as the receiver, since by 24 hours the accumulation in the 5th section was too low for meaningful analysis. This meant, in effect, considering basipetal tramsport over a 4mm section (i.e. through sections 1 and 2) at $10^{o}C$, shown in Figure 17 Plot I. This was compared with basipetal

transport at 25°C through an 8mm segment, using the 5th section as receiver (Plot II); and basipetal transport at 25°C through a 4mm segment using the 3rd section and beyond as a receiver (Plot III). The intercept on the 'X' axis of Plot II was 6 to 7 hours, giving a rate of movement, for an 8mm segment at 25°C, of 1.2 - 1.3mm $hour^{-1}$. From Plot III the calculated rate of movement was between 1.1 - 1.2mm $hour^{-1}$ for a 4mm segment at 25°C. Thus the rate of movement of ^{14}C in the basipetal direction at 25°C was relatively independent of segment length with a value of 1.1 - 1.3mm $hour^{-1}$. This agreed closely with a rate of movement obtained in an earlier calculation (Fig. 4.). At 10°C the intercept on the 'X' axis was about 9 hours, giving a calculated rate of movement of 0.4 - 0.5mm $hour^{-1}$ through a 4mm segment in the basipetal direction. Thus a 15°C change in temperature reduced the rate of basipetal movement of ^{14}C by over 60%.

Comparing the slopes of Plots I and III it can be seen that the flux of basipetal movement through a 4mm segment was also reduced at 10°C compared to the flux at 25°C. At 10°C the calculated basipetal flux was 3 - 4 dpm $hour^{-1}$, whereas at 25°C it was 15 - 20 dpm $hour^{-1}$.

These results suggest that both the rate and the flux of basipetal movement of ^{14}C in Zea mays coleoptile segments supplied with 10^{-6}M ABA-2-^{14}C were partly dependent on temperature. Much longer time periods would be necessary before meaningful analysis could be applied to acropetal transport at those lower temperatures.

FIGURE 15

The effect of temperature on the total apical and basal uptake of ^{14}C into 10mm Zea mays coleoptile segments supplied with donor blocks containing 10^{-6}M ABA-2-^{14}C in the light. The plotted values are means of two experiments, Five replicates in each experiment.

—●— Basal uptake (acropetal movement) at 25°C.

—○— Apical uptake (basipetal movement) at 25°C.

—■— Basal uptake (acropetal movement) at 10°C

—□— Apical uptake (basipetal movement) at 10°C.

—▲— Basal uptake (acropetal movement) at 0°C.

—△— Apical uptake (basipetal movement) at 0°C.

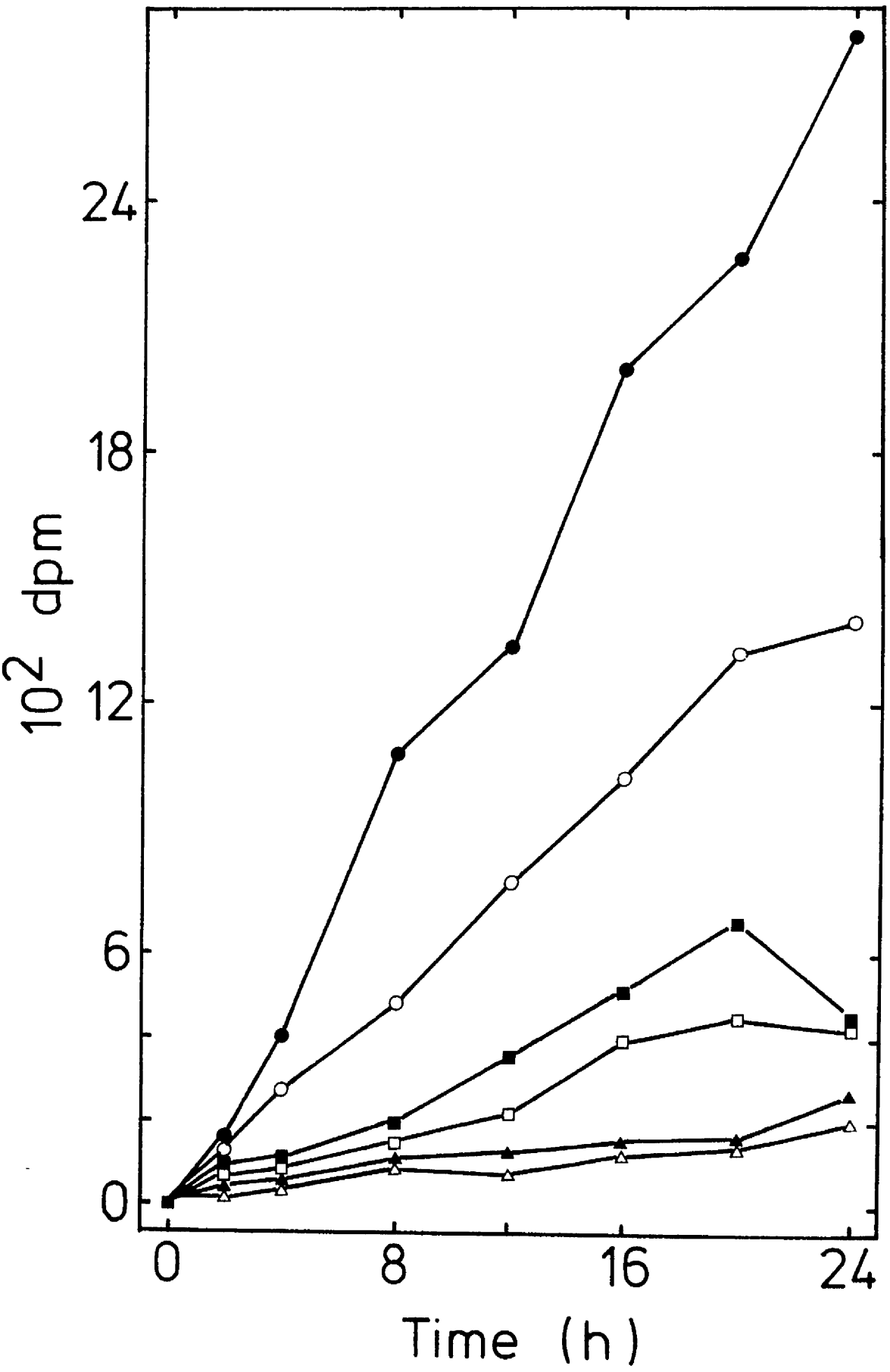

24
18
12
6
0
10^2 dpm
0
8
16
24
Time (h)

FIGURE 16

The effect of temperature on the accumulation of ^{14}C, expressed as a percentage of the total uptake per segment, in the 3rd, 4th and 5th sections of acropetally and basipetally transporting segments. Experimental details and symbols are as in Figure 15.

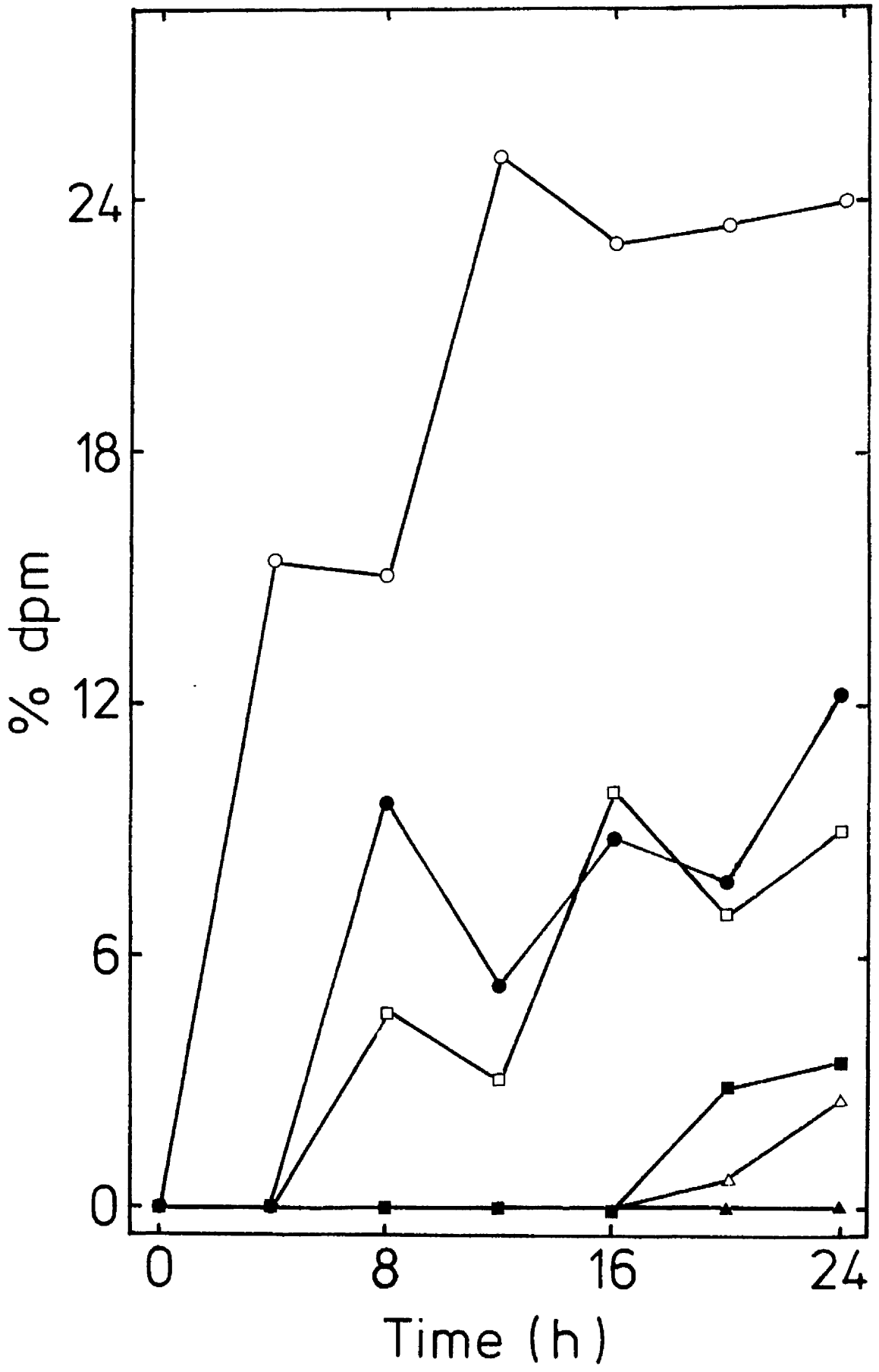

24
18
% dpm
12
6
0
0
8
16
24
Time (h)

FIGURE 17

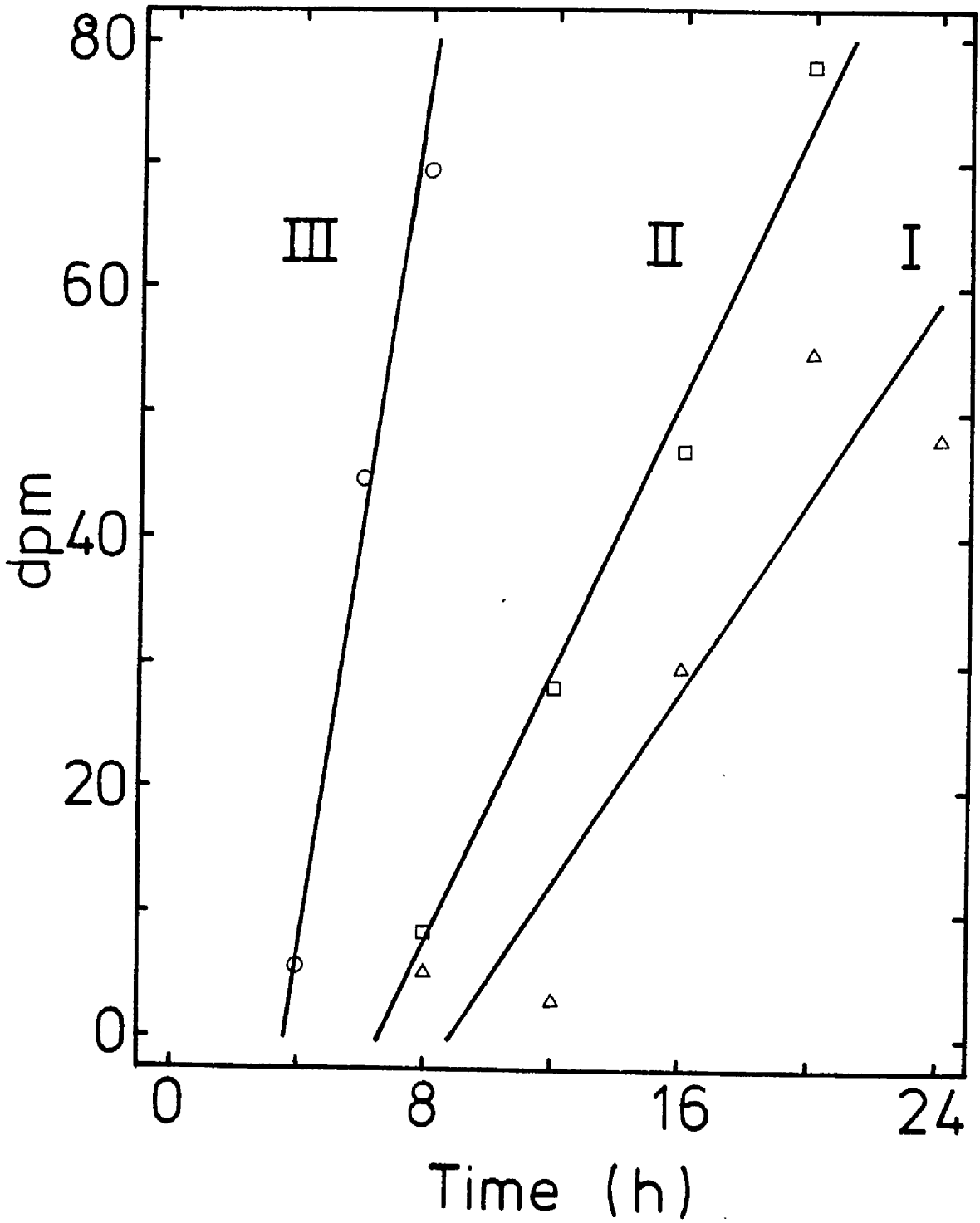
The effect of temperature on the accumulation of ^{14}C into 'tissue receivers of Zea mays coleoptile segments supplied with donor blocks containing ABA-2-^{14}C. Experimental details as in Figure 15.

I —△— Basipetal transport at 10°C through a 4mm segment i.e. accumulation of ^{14}C into the 3rd section.

II —□— Basipetal transport at 25°C through a 8mm segment i.e. accumulation of ^{14}C into the 5th section.

III —○— Basipetal transport at 25°C through a 4mm segment i.e. accumulation into the 3rd section.

The lines were plotted by eye.


80
60
40
20
0
dpm
0
8
16
24
Time (h)
III
II
I

(vii) Effect of anoxic conditions on the uptake and movement of ^{14}C in coleoptile segments.

Previously it was found that temperature was a factor affecting the uptake and movement of ^{14}C in coleoptile segments. Lowering the temperature brought about a decrease in uptake and movement of ^{14}C. It was suggested in consequence that the uptake and movement of ^{14}C in coleoptile segments supplied with ABA-2-^{14}C was to some extent dependent on metabolic activity. Another way to examine the dependence of a transport system on active metabolism is to carry out transport experiments under anoxic conditions. By excluding oxygen from the system the activity of aerobic metabolism should decrease. These conditions can be simply achieved by surrounding the tissue with oxygen-free nitrogen. With coleoptiles it is essential that the central cylinder is also depleted of oxygen. This was achieved by using an experimental chamber in which the donor block was applied to the coleoptiles after the central cavities were filled with nitrogen. Normally this was done by repeatedly evacuating the chamber and refilling with nitrogen but in the experiments evacuation was not used, and instead the chamber was purged with a vigorous stream of nitrogen for 1 hour prior to application of the donor block. During the experiment a small flow of nitrogen was maintained through the chamber. As a control a second chamber was set up using compressed air instead of nitrogen.

This experimental system was satisfactory for the nitrogen treatments, but was unsuitable for the air controls. It was originally designed for short time course experiments with IAA, but with ABA-2-^{14}C where longer transport periods were required, the growth of the coleoptiles in air was considerable and this was

hindered by the rigid application of the donor block. As a consequence the segments grew through the donor block and were distorted against the top of the chamber. This distortion did not occur under nitrogen as very little growth occurred, even over 24 hours, indicating that aerobic metabolism was effectively inhibited. To overcome this problem the air treatments were carried out in conventional chambers as described in the Methods section, and thus they should not be regarded as true controls. The results must be considered with this in mind. Although it would have been possible to completely resolve this problem by designing a more sophisticated chamber, this was not attempted.

The effect of anoxic conditions on the total uptake of ^{14}C over 24 hours is shown in Figure 18. Basal uptake was reduced by 64% under nitrogen and apical uptake by 51%. Thus the difference between basal and apical uptake was less marked under nitrogen. This result was similar to that obtained with low temperatures and suggested that a greater proportion of basal uptake was dependent on aerobic metabolism compared to apical uptake.

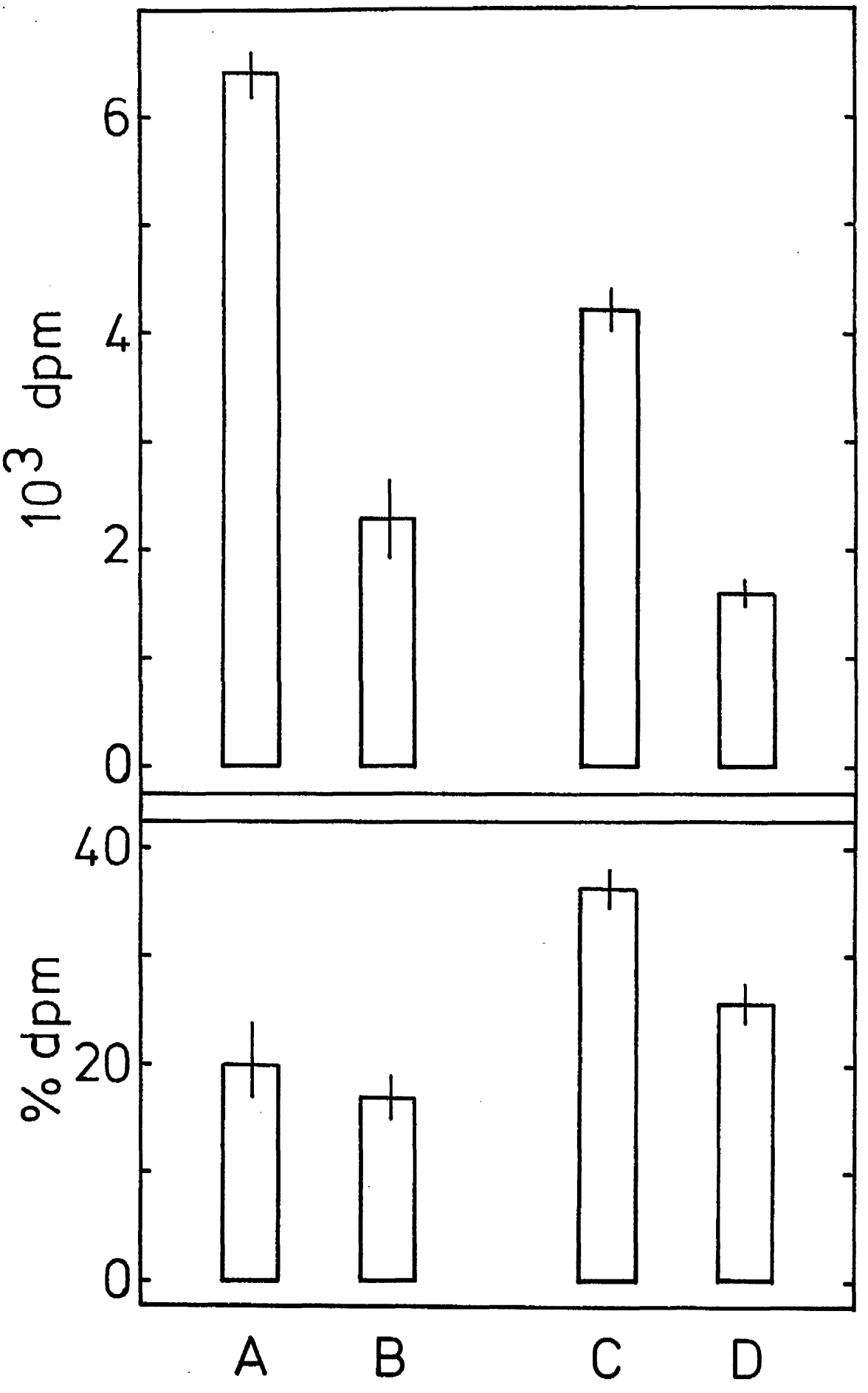
The effect of anoxic conditions on the polarity of ^{14}C movement was also considered by examining the accumulation of ^{14}C into sections 3 - 5 of the coleoptile segments after 24 hours under nitrogen and in air (Fig. 19.) While anoxic conditions reduced the % dpm in these sections, the polarity of movement was not destroyed, more radioactivity accumulating in the basipetally transporting sections.

FIGURE 18

The basal uptake of ^{14}C in air (A) and under nitrogen (B), and the apical uptake in air (C) and under nitrogen (D) by 10mm Zea mays coleoptile segments after 24 hours in the light at 25°C, supplied with donor blocks containing 5 μM ABA-2-^{14}C. The values given are means from two experiments, ten replicates in each experiment. The vertical bars represent twice the standard errors of the means.

FIGURE 19

The accumulation of ^{14}C, expressed as a percentage of the totals uptake per segment, by sections 3 - 5 of 10mm Zea mays coleoptile segments, in air (A - acropetal, C - basipetal) and under nitrogen (B - acropetal, D - basipetal). Experimental details as in Figure 18.


6
4
2
0
10^3 dpm
40
20
0
% dpm
A
B
C
D

(viii) The effect of tissue age on the uptake and movement of ^{14}C in coleoptile segments.

In most transport studies on Zea mays, the coleoptiles were used when 5 days old. Between 5 and 6 days old in constant darkness they continued to grow actively, but by 6 to 7 days old the expanding primary leaf had often penetrated the coleoptile tip, at which stage the coleoptile ceased to grow and senesced. Thus between 6 and 7 days the coleoptile was probably ageing and a number of metabolic processes might have been in decline, coinciding with a decrease in the rate of extension growth. It was decided to investigate the effect of coleoptile age on the transport of ^{14}C in segments supplied with ABA-2-^{14}C.

Segments, 10mm long, excised from 6 day-old coleoptiles were used for this experiment. These coleoptiles which had been pierced by the primary leaf were not used. The uptake of ^{14}C by segments supplied with donor blocks containing 10^{-6}M ABA-2-^{14}C was considered as a function of time (Fig. 20.) There was a tendency for basal uptake to exceed apical uptake.

Analysis of the accumulation of ^{14}C in 2mm tissue sections of the segments was carried out to study the polarity of ^{14}C movement in the tissue. Figure 21 shows the percentage radioactivity accumulated by sections 3 - 5 of acropetally and basipetally transporting segments. The results, show no clearly defined polarity of ^{14}C movement in 6 day-old coleoptile segments over 24 hours. Thus the age of coleoptiles used in transport experiments seems to be critical. As the primary observable difference between 5 day-old and 6 day-old coleoptiles in a decline in the rate of extension growth, it is possible that the apparent polar movement of ^{14}C is dependent upon, or related to, the continued growth of

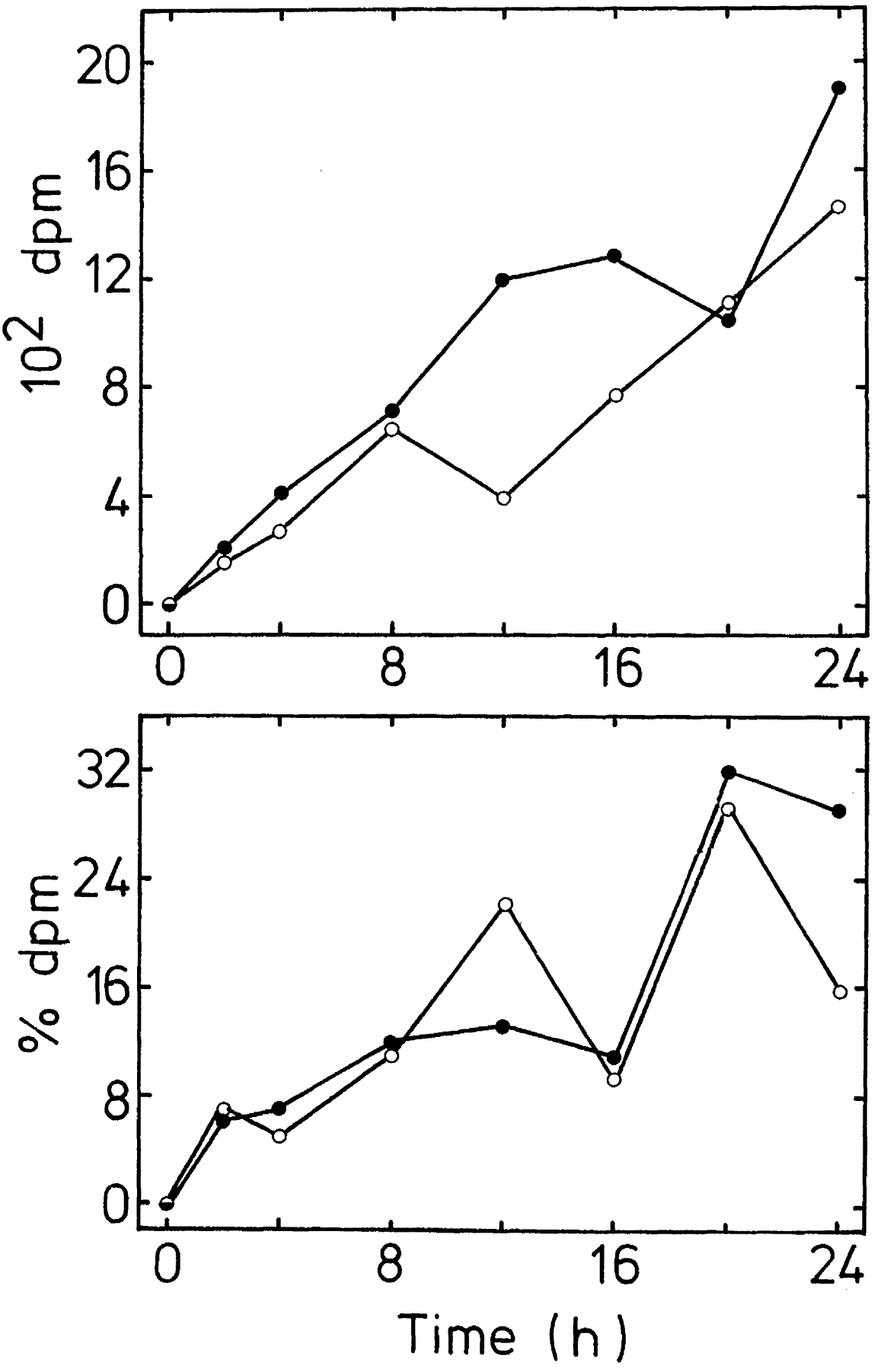
the segment. A detailed consideration of the inter-relationship between growth and movement of ^{14}C in coleoptile segments supplied with ABA-2-^{14}C is given in the next two sub-sections.

FIGURE 20

The basal ——●—— and apical ——○—— uptake of ^{14}C by 6 day-old coleoptile segments as a function of time. ABA-2-^{14}C was supplied in donor blocks at a concentration of 10^{-6} M and the experiment was carried out at 25°C in the light. The plotted values are means from two experiments, ten replicates in each experiment.

FIGURE 21

The accumulation of ^{14}C, expressed as a percentage of the total uptake per segment, in sections 3 - 5 of acropetally ——●—— and basipetally ——○—— transporting segments of 6 day-old Zea mays coleoptiles. Experimental details as in Figure 19.


20
16
12
8
4
0
10^2 dpm
0
8
16
24
32
24
16
8
0
% dpm
0
8
16
24
Time (h)

(ix) The effect of ABA-2-^{14}C on the straight growth of coleoptile segments.

In previous sections it has been suggested that growth of the segment may be a factor affecting the pattern of ^{14}C movement. Since ABA is known to have growth regulating activity it was important to examine the effect on segment growth of supplying ABA-2-^{14}C in agar blocks, to their basal and apical ends.

Segments, 10mm in length, were excised from 5 day-old coleoptiles and supplied with three different concentrations of ABA-2-^{14}C in agar donor blocks. At the end of a given transport period at 25^{o}C in darkness the segments were measured using a shadow-graphing technique. After measurement the segments were then assayed individually on a liquid scintillation spectrometer to give uptake data as dpm segment^{-1}. Figure 22 shows the acropetal and basipetal uptake of ^{14}C into the tissue from three concentrations of ABA-2-^{14}C in the donor blocks, as a function of time. The uptake over 24 hours increased linearly with time and was related to concentration. There was also the typical pattern of greater uptake from the basal end. Figure 23 shows the growth data for the same sets of coleoptiles, plotting increase in length against time, at three donor concentrations and with a plain agar donor block as a control. The growth rate of the control was linear over 24 hours. Increasing concentrations of ABA-2-^{14}C in the donor blocks inhibited growth over 24 hours. The highest concentrations in the donors caused the greatest significant (to 0.05P) inhibition of straight growth compared to the control. The differences between concentrations were not significant, perhaps attributable to insufficient replication.

There were no significant differences in growth between acropetally and basipetally transporting segments supplied with the same concentration of ABA-2-^{14}C.

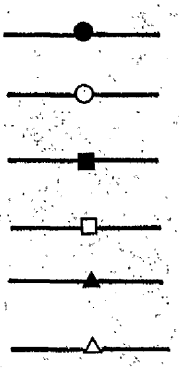
These results show that ABA-2-^{14}C supplied to Zea mays coleoptile segments in agar donor blocks inhibited straight growth at 25°C in the dark, over 24 hours. This inhibition increased with increasing concentrations of ABA-2-^{14}C in the donor blocks, which corresponded with increasing uptake of ^{14}C into the tissue.

The growth data given above were obtained from experiments carried out in darkness. The effect of ABA-2-^{14}C on growth in the light was also considered. The experimental procedure was carried out as above, with one set of coleoptiles maintained at 25°C in the light and one set in darkness at 25°C, over a single time period of 24 hours, but with a range of concentrations of ABA-2-^{14}C (Fig. 24.) The straight growth was expressed as the % change in length of the segment compared to the control after 24 hours at 25°C in light and dark. In the light, low concentrations of ABA-2-^{14}C in the donor caused slight, significant (0.05P) promotion of straight growth in basipetally transporting segments compared to the control. At higher concentrations there were no significant differences between treated segments and the controls. At no concentration were there any significant differences in growth between acropetal and basipetal segments. In the dark at 5×10^{-6}M and 5×10^{-4}M there were no significant differences between treated segments and controls after 24 hours, although there was a observable tendency for growth to be inhibited. 10^{-4}M ABA-2-^{14}C in the donor blocks caused a significant (to 0.05P) inhibition of growth compared to the control after 24 hours.

These results suggest that the growth regulating activity of ABA-2-^{14}C when applied to coleoptile segments in donor blocks was affected by light. However light alone can have an effect on the growth of dark-grown coleoptiles, so a simple interpretation of the results is not possible. Further experimentation is necessary to fully resolve the problems of the relationships between straight growth, light and ABA-2-^{14}C in transport mechanisms.

FIGURE 22

The total uptake of ^{14}C into 10mm *Zea mays* coleoptile segments supplied with three different concentrations of ABA-2-^{14}C in the donors, as a function of time, at 25°C in darkness. The values given are the means of twenty replicates with the vertical bars representing twice the standard error. Radioactivity expressed as dpm segment^{-1}.



10^{-4}M ABA-2-^{14}C Basal uptake.

10^{-4}M ABA-2-^{14}C apical uptake.

5×10^{-5}M ABA-2-^{14}C basal uptake.

5×10^{-5}M ABA-2-^{14}C apical uptake.

5×10^{-6}M ABA-2-^{14}C basal uptake.

5×10^{-6}M ABA-2-^{14}C apical uptake

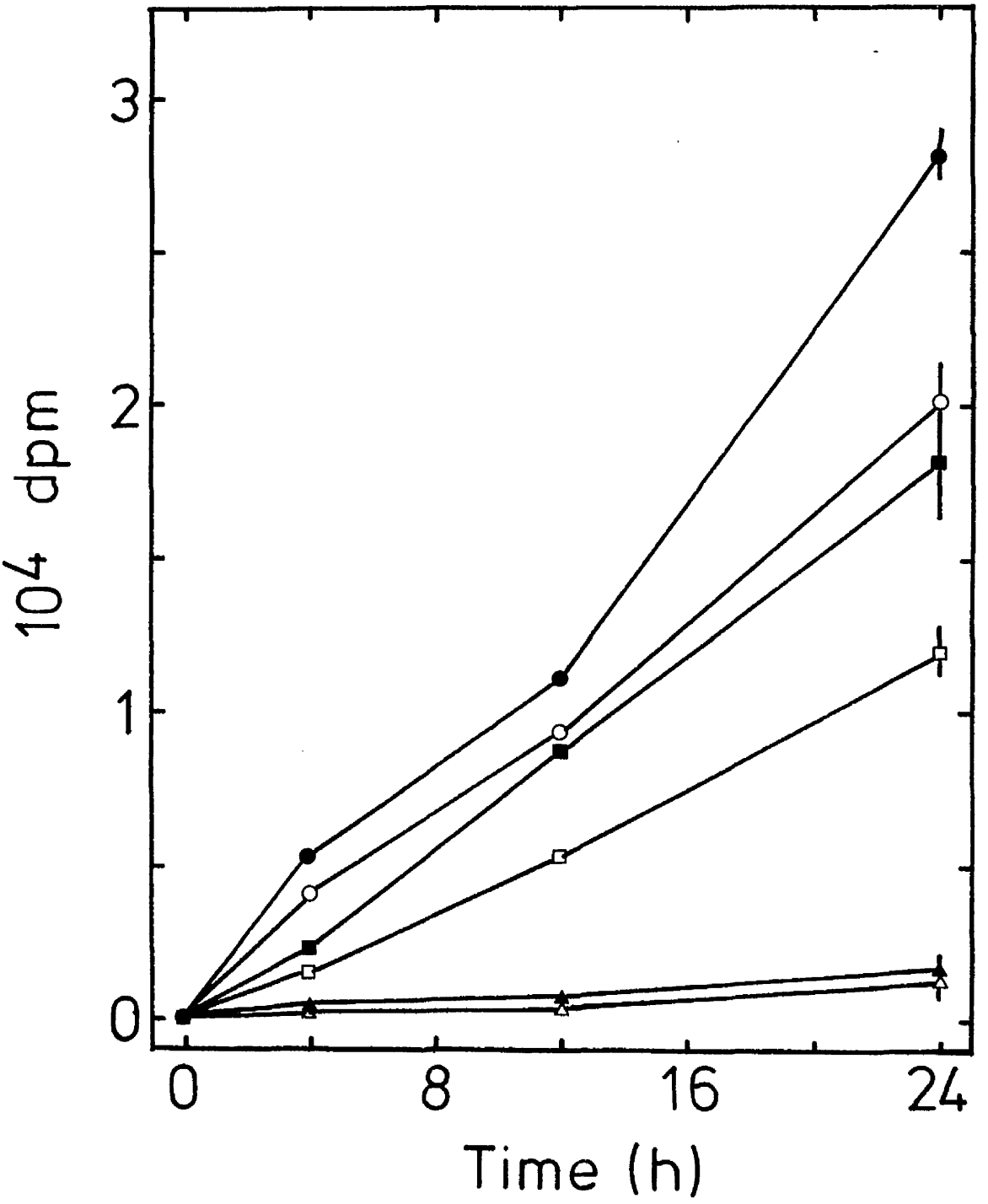

3
2
1
0
10⁴ dpm
0
8
16
24
Time (h)

FIGURE 23 (a) & (b)

The straight growth of 10mm Zea mays coleoptile segments supplied with three different concentrations of ABA-2-^{14}C in the donors, as a function of time. Experimental details and symbols as in Figure 22, in addition:- ——×—— plain agar control

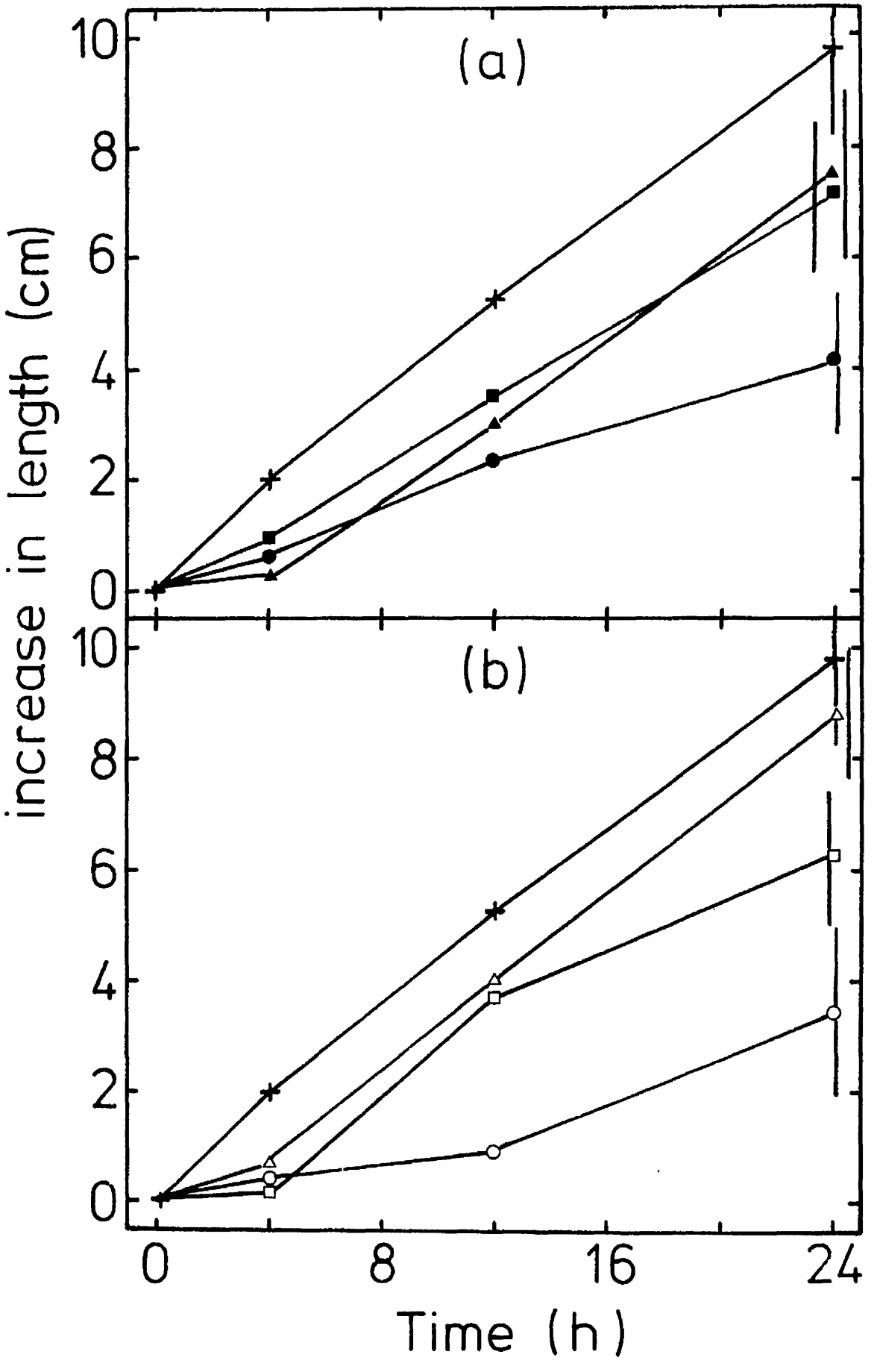

(a)
(b)
increase in length (cm)
Time (h)
0
2
4
6
8
10
16
24

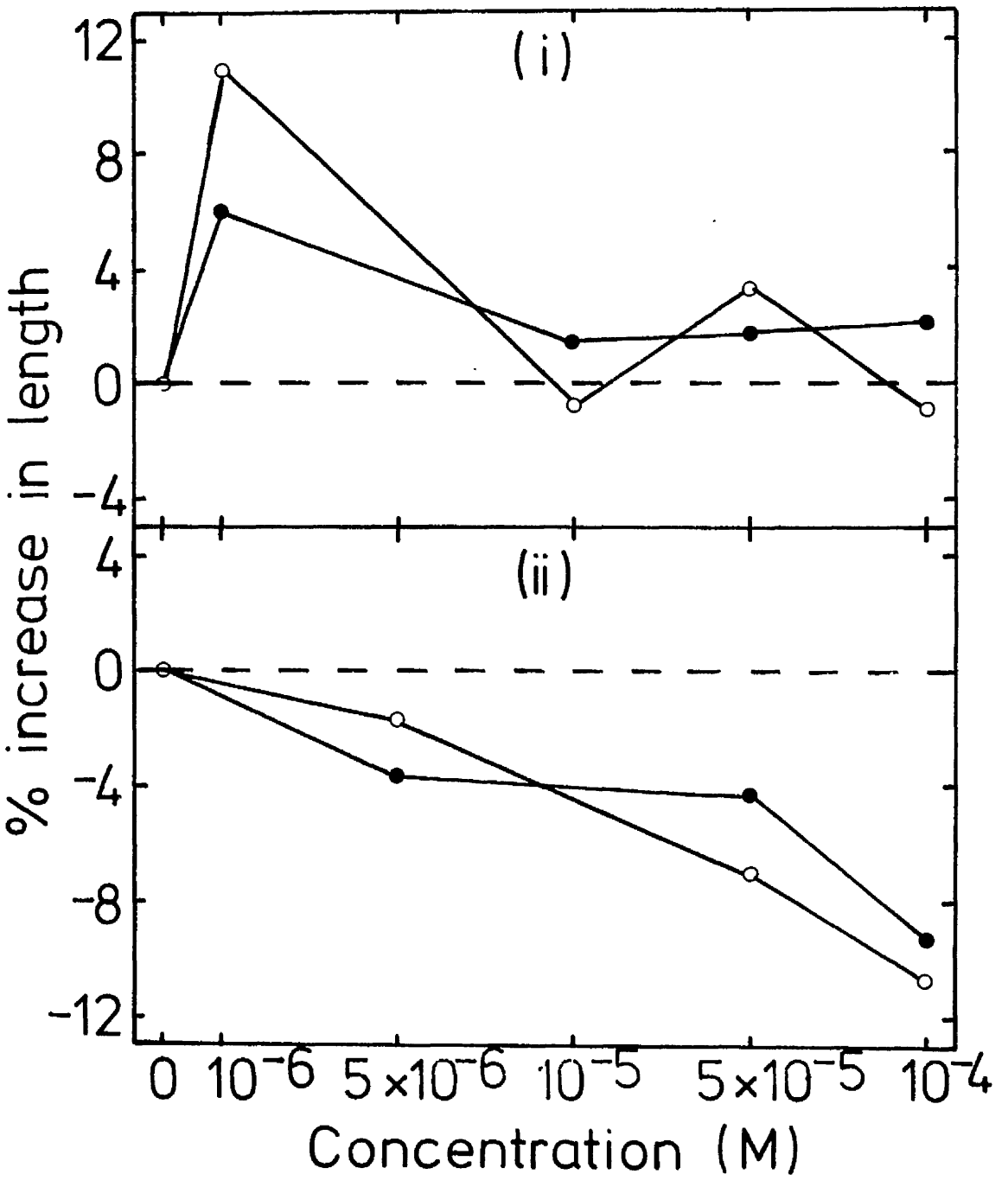
FIGURE 24

The straight growth of Zea mays coleoptile segments originally 10mm long, expressed as the percentage inhibition/promotion compared to the control after 24 hours, as a function of donor concentration of ABA-2-^{14}C.

(i) Basipetally —○— and acropetally —●— transporting segments in the light at 25°C.

(ii) Basipetally —○— and acropetally —●— transporting segments in the dark at 25°C.

The plotted values are means of twenty replicates.


(i)
(ii)
12
8
4
0
-4
-8
-12
% increase in length
0
10⁻⁶
5×10⁻⁶
10⁻⁵
5×10⁻⁵
10⁻⁴
Concentration (M)

(x) An analysis of uptake, polarity and growth in coleoptile segments

In an earlier section it was suggested that the observed difference between basal (acropetal) and apical (basipetal) uptake of ^{14}C from donor blocks containing ABA-2-^{14}C may simply be a function of the cross-sectional area of the tissue in contact with the block. Because the coleoptile tapers towards the tip, there is a difference between the cross-sectional areas of the apical and basal ends of a 10mm segment excised 1mm from the apex. The difference in area decreases with segments excised further from the apex. This relationship can also be expressed in terms of the fresh weight differences between thin, 2mm sections cut from the apical and basal ends of 10mm segments excised 0, 1, and 6mm from the apex (Table 2).

Table 2. Fresh weight (g) of apical and basal 2mm sections of 10mm segments excised 0, 1, 6mm from the apex of Zea mays coleoptiles. (Means of five replicates).

Distance from apex (mm)	Apical section	Basal section
0	0.0104 ± 0.0020	0.0203 ± 0.0010
1	0.0124 ± 0.0010	0.0297 ± 0.0025
6	0.0259 ± 0.0015	0.0315 ± 0.0020

To investigate any relationship between segment shape and transport characteristics, a transport experiment was carried out using 10mm coleoptile segments excised (i) with intact apices, (ii) 1mm and, (iii) 6mm from the apex. The segments were supplied with 5μM ABA-2-^{14}C for 24 hours, and the radioactivity was analysed in 2mm tissue sections, weighed prior to radioassay. The total basal uptake expressed

in dpm, did not change markedly in the three types of segment used, but the apical uptake increased in segments excised further from the apex, such that the ratio of basal uptake : apical uptake decreased from 48.7 in apical segments to 1.26 in segments excised 6mm from the apex. (Table 3). The very low apical uptake by segments with intact apices may be misleading because the intact cuticle, may have been a considerable barrier to uptake of radioactivity from the donor block. However, the results from the two types of sub-apical segments suggest that the differences in uptake observed may be largely dependent upon the shape of the coleoptile segment. When the uptake was expressed as dpm g^{-1} fresh weight of section 1 (Table 3, lines 3,4,) the ratio of basal uptake : apical uptake by each type of segment decreased; the ratios of the two sub-apical segments approached unity. Thus, by correcting for the weight of tissue immediately adjacent to the donor block, the difference between apical and basal uptake of ^{14}C from donor blocks containing ABA-2-^{14}C was virtually eliminated.

Table 3 also shows the ratio of basipetal to acropetal accumulation of ^{14}C in sections 3, 4 and 5 of the three types of segment used (line 5). This ratio, which represents the polarity of ^{14}C movement in the coleoptile segment, was lowest and nearest to unity (i.e. no polarity) in the segments excised 6mm from the apex. Thus the observed polarity of ^{14}C movement in coleoptile segments supplied with ABA-2-^{14}C may also be a function of segment shape. The more closely the segment resembles a uniform cylinder of tissue the more the polarity is abolished. When expressed on a fresh weight basis, the ratios of basipetal to acropetal accumulation of ^{14}C in sections 3, 4 and 5 were less than one, for all segment types. Thus although the total amount of radioactivity accumulated may be greater in the basipetal direction, the concentration of radioactivity in the tissue on a fresh weight basis

Table 3. An analysis of the uptake and accumulation of ^{14}C in three different types of coleoptile segment supplied with donor blocks containing ABA-2-^{14}C for 24 hours in the light at 25°C. The polarity ratios are given, unless otherwise stated, by

$$\frac{\text{Basipetal accumulation of } ^{14}C \text{ sections } 3 - 5}{\text{Acropetal accumulation of } ^{14}C \quad " \quad " \quad "}$$

Values given are means of ten replicates. Segments were originally 10mm long.

	(i) Segments excised intact apices		(ii) Segments excised 1mm from apex		(iii) Segments excised 6mm from apex	
	Basal	Apical	Basal	Apical	Basal	Apical
Uptake (dpm)	11744	241	12699	5290	12442	9802
Uptake Ratio $\frac{\text{Basal}}{\text{Apical}}$	48.70		2.40		1.26	
Uptake ($dpmg^{-1}$ section 1)	578522	23173	430474	426612	397507	377000
Uptake Ratio $(\frac{\text{Basal}}{\text{Apical}})$	24.96		1.01		1.05	
Polarity ratio	1.398		1.392		1.108	
Polarity ratio (Fresh weight basis)	0.680		0.669		0.866	
Polarity ratio (Section 5 only)	2.638		2.574		1.373	
Polarity ratio (section 5 fresh weight basis)	0.398		0.844		0.95	
Final segment length (x5)	6.66(cm)	6.93	7.05	7.01	7.52	7.45

after 24 hours, was actually lower.

The polarity of accumulation of ^{14}C in segment 5 only (i.e. movement through 8mm of tissue) showed a similar pattern, except that the ratios were slightly higher.

The last line of Table 3 shows the final length of the segments after 24 hours, (magnified x 5) which were originally 10mm in length. The segments excised 6mm from the apex were significantly (to 0.001P) longer than the segments with intact apices. This result raises the problem that certain zones down the coleoptile elongate at faster rates than other zones. If these zones of faster growth occur in one part of a transport segment then growth will not be uniform over the entire length of the segment and this may influence the movement of ^{14}C more in one direction than the other. This effect would be greater over longer transport periods, such as twenty-four hours.

The growth of the transporting tissue was considered in more detail by measuring the growth of isolated zones of tissue over 24 hours in water, methand control solution, (methanol was used to dissolve the ABA) and increasing concentrations of ABA solution. The zones, excised at different distances from the apex were: A 1 - 11mm; B 1 - 6mm; C 6 - 11mm; D 11 - 16mm. All showed the same pattern of decreasing growth over 24 hours with increasing ABA concentration. (Fig. 25.) The inhibition by 10^{-4}M ABA solution was significant (to 0.001P) for all zones. On a percentage increase basis, zone C showed the greatest elongation in water and in 10^{-4}M ABA solution, although in the latter the growth was markedly reduced (Fig. 26. (i) & (ii)). However, this does not account for the differences in length of the transport segments shown in Table 3

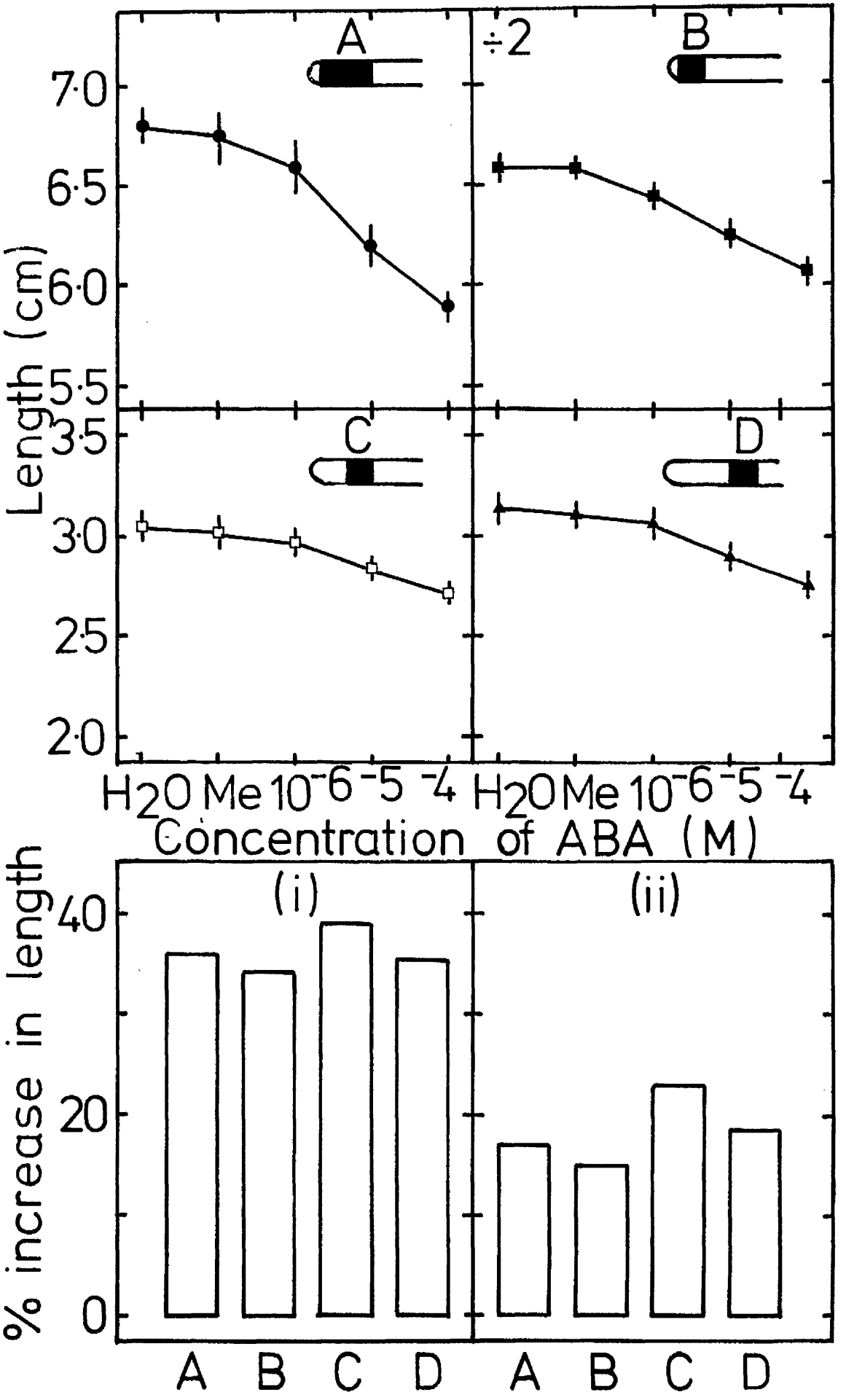
since zone C is virtually common to all three segment types. Thus it appears that when studied in isolation, different zones of the coleoptile do grow at slightly different rates, but in longer segments or intact coleoptiles the pattern may be modified somewhat. The inhibitory effects of abscisic acid on the growth of different zones are similar.

FIGURE 25

The final length (cm x 5) of isolated zones of tissue from Zea mays coleoptiles after 24 hours (at 25°C in the light) in distilled water, methanol control solution (500p.p.m.) and increasing concentrations of ABA solution. The zones were excised at different distances from the coleoptile apex : Zone A 1 - 11mm; Zone B 1 - 6mm; Zone C 6 - 11mm; Zone D 11 - 16mm. The values plotted are means of twenty replicates, twice the standard errors are given as bars. The units for the vertical axis for zone B are those of zone A axis divided by two.

FIGURE 26

Histograms showing the percentage increase in length over the original length of tissue zones A, B, C, and D in (i) distilled water, (ii) 10^{-4}M ABA. Experimental details are as in Figure 25.


A
÷2
B
C
D
7·0
6·5
6·0
5·5
3·5
3·0
2·5
2·0
Length (cm)
H2O Me 10-6 -5 -4 H2O Me 10-6 -5 -4
Concentration of ABA (M)
(i)
(ii)
40
20
0
% increase in length
A B C D A B C D

(xi) Chromatographic analysis of radioactive extracts from coleoptile segments - supplied with ABA-2-^{14}C.

In the preceding sections, movement in coleoptile segments supplied with ABA-2-^{14}C has been discussed in terms of radioactive carbon rather than radioactive abscisic acid. Thus far, no evidence has been presented to establish the identity of the radioactive moiety extracted from the coleoptile tissue. It has been reported that applied ABA-2-^{14}C is metabolised in plant tissues, (Milborrow, 1968). Therefore it was decided to investigate, using chromatographic procedures, the metabolism of ABA-2-^{14}C in coleoptile tissue; to attempt to determine the proportion of radioactivity extracted from segments after a given transport period that was still part of the ABA molecule.

In the first series of experiments coleoptile segments were set up with their basal ends supplied with 10^{-6}M ABA-2-^{14}C in agar blocks (acropetal movement), for 24 hours at 25°C in light and in darkness. At the end of the transport period the segments were divided laterally into two halves, basal and apical, and these tissue halves together with donors and receivers were extracted in 95% ethanol as described in the Methods section, giving eight extracts in all, four from light treatments, four from dark treatments. Selected extracts were analysed using a number of chromatographic techniques as shown in Table 4.

Neither the different grades of Whatman's paper used in chromatograms 1 and 2 nor the distance travelled by the solvent front had any marked effect on the results. Chromatogram 1 was developed for a distance of 10cm, chromatogram 2 for 20cm, and the results were very similar. In these chromatograms, (Figs. 26. 27.)

the peaks of radioactivity from the spots of stock ABA-2-^{14}C and donor block extracts in light and dark were found between RF 0.7 - 0.8. More than 50% in the light and 60% in the dark treatments of the total radioactivity was recovered in this RF zone, and over 80% was recovered between RFs 0.7 and 0.9. In chromatogram 2 the percentage radioactivity appearing in RF zone 0.7 - 0.8 alone was over 80%. The remaining activity was spread over the length of the chromatogram, possibly indicating overloading of the chromatogram. This single radioactive peak probably represents ABA and suggests there was very little breakdown of ABA-2-^{14}C in the donor blocks. In the tissue extracts of dark - transporting segments approximately 30% of the radioactivity was found at RF 0.7 - 0.8. This value was generally less in extracts from apical tissue, which was further away from the donor block. The major peak of radioactivity in tissue extracts was found at RF 0.6 - 0.7, i.e. 0.1 RF lower than the peak thought to represent ABA-2-^{14}C. In the dark basal tissue extract this peak at RF 0.6 - 0.7 approached 50% of the total radioactivity applied to the chromatogram and in the dark apical tissue it was more than 70%; in one chromatogram (Fig. 27 f), all the radioactivity was recovered between RF 0.6 and 0.7. There was however, no consistent evidence that light during the transport period increased the proportion of the radioactivity recovered from this zone.

Similar results were obtained from the silica gel thin-layer chromatograms. In chromatogram 3 (Fig. 28), 82% of the radioactivity from the stock ABA-2-^{14}C solution was recovered from RF 0.6 - 0.7. This value compared well with the published data of Milborrow (1968) and Smith (1971), using the same solvent system, and probably represents ABA-2-^{14}C. It was found that the RF value of this

peak was consistently 0.1 RF less on thin-layer than on paper chromatograms. Correspondingly, a major peak from tissue extracts was found between RF 0.5 and 0.6. From the extract of dark-transported basal tissue (Fig. 28a) 58% of the radioactivity was recovered at RF 0.6 - 0.7 and 31% was recovered at RF 0.5 - 0.6; while from light basal tissue 27% was recovered from RF 0.6 - 0.7 and 53% from 0.5 - 0.6. Thus the proportion of radioactivity at the RF zone corresponding to ABA-2-^{14}C in tissue extracts was less than in the stock solution, and there is some evidence to suggest that light during the transport period increases breakdown.

These results, obtained from analysis of the chromatograms by liquid scintillation counting, were confirmed by the results from the autoradiographs of thin-layer chromatograms (Fig. 29 (i) & (ii)). The autoradiograph of the stock solution, grossly overloaded in chromatogram 4 but more satisfactory in chromatogram 5, revealed one major radioactive area at RF 0.6 - 0.7 which was probably ABA-2-^{14}C. A second, small area of darkening, clearly seen in chromatogram 5 occurred,at RF 0.4 - 0.5, suggesting a radioactive impurity in the stock solution of ABA-2-^{14}C. The autoradiographs of the donor block extracts, light or dark, were essentially similar. The tissue extracts, however, showed two distinguishable areas of darkening between RF 0.5 and 0.7. The upper area corresponded to the marker spot of ABA-2-^{14}C and the lower spot corresponded to the peak found in the tissue extracts on the previous chromatograms. The upper spot corresponding to ABA-2-^{14}C was less distinct in the light-transported tissue extracts, and had almost disappeared in the apical tissue extracts. Thus this series of experiments established the basic techniques required for the

Table 4. Chromatographic techniques used for the analysis of the radioactivity in tissue, receiver and donor block extracts from transport experiments with Zea mays coleoptile segments. The solvent system used in all instances was: N - butanol: N - propanol: 0.880 Ammonia: distilled water:: 2:6:1:2 v/v.

Chromatogram	Technique	Analysis	Figure
1	Paper No. 3.	Liquid scintillation	26
2	Paper No. 1.	Liquid scintillation	27
3	Silica gel TLC	Liquid scintillation	28
4	Silica gel TLC	Autoradiography	29 (i)
5	Silica gel TLC	Autoradiography	29 (ii)

FIGURE 26

Chromatographic analysis of ethanolic extracts of apical tissue halves, basal tissue halves and agar donor blocks, taken from 50 Zea mays coleoptile segments supplied at the basal ends for 24 hours in light or in darkness with agar blocks containing 10^{-6}M ABA-2-^{14}C. The extracts were spotted onto Whatman No. 3 chromatography paper and the chromatograms developed in n-butanol: n-propanol: 0.880 Ammonia: Distilled water :: 2:6:1:2 v/v. The histograms show the radioactivity recovered from each RF zone, assayed on a liquid scintillation spectrometer, expressed as a percentage of the total recovery.

Histograms a-d are for light-transported segments.
Histograms e-h are for dark-transported segments.

Histograms a, e - basal tissue extracts.
Histograms b, f - apical tissue extracts.
Histograms c, g - donor block extracts.
Histograms d, h - stock solutions of ABA-2-^{14}C maintained in light, darkness.

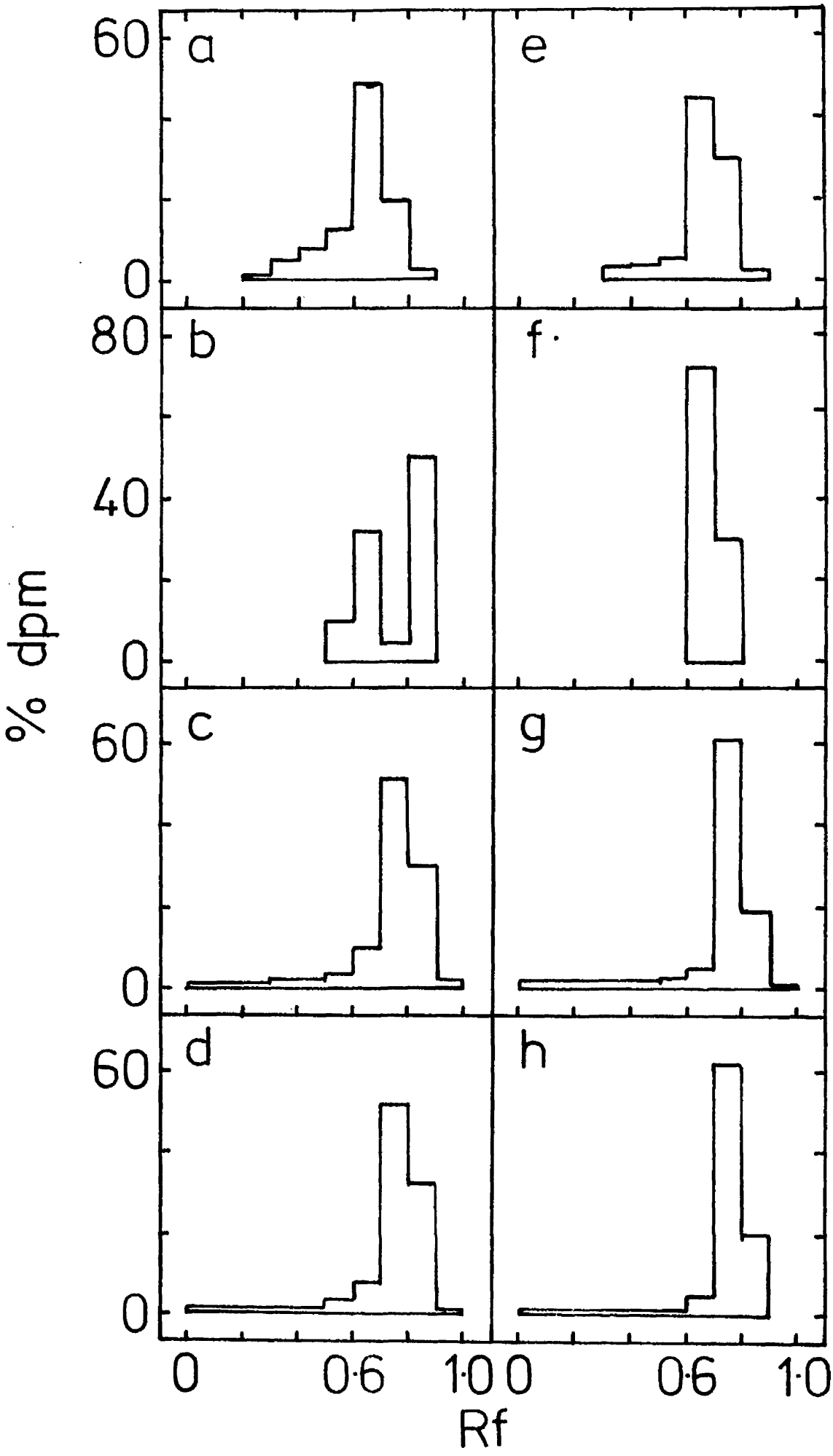

60
0
a
e
80
40
0
b
f
% dpm
60
0
c
g
60
0
d
h
0
0·6
1·0
0
0·6
1·0
Rf

FIGURE 27

As in Figure 26, except that Whatman No. 1 paper was used.

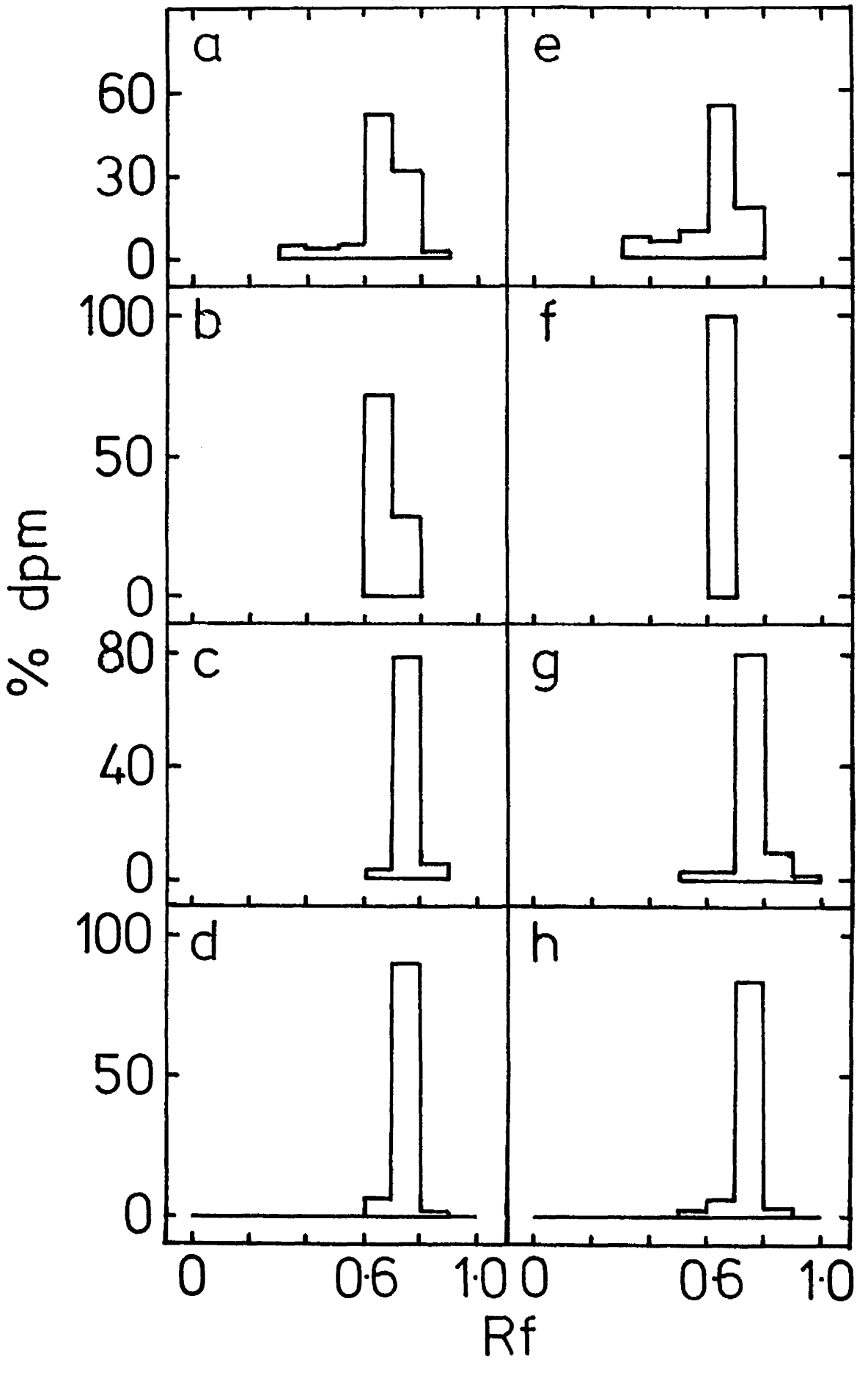

a
e
60
30
0
b
f
100
50
0
% dpm
c
g
80
40
0
d
h
100
50
0
0
0·6
1·0
0
0·6
1·0
Rf

FIGURE 28

The experimental details are as in Figure 26 except that the extracts were spotted onto a 250 μ layer of silica gel on a glass plate and developed in the n-butanol solvent system. The histograms show the radioactivity recovered from each RF zone, assayed on a liquid scintillation spectrometer, expressed as a percentage of the total recovery.

Histogram a - basal tissue extract, dark transport.

Histogram b - basal tissue extract, light transport.

Histogram c - stock ABA-2-^{14}C solution.

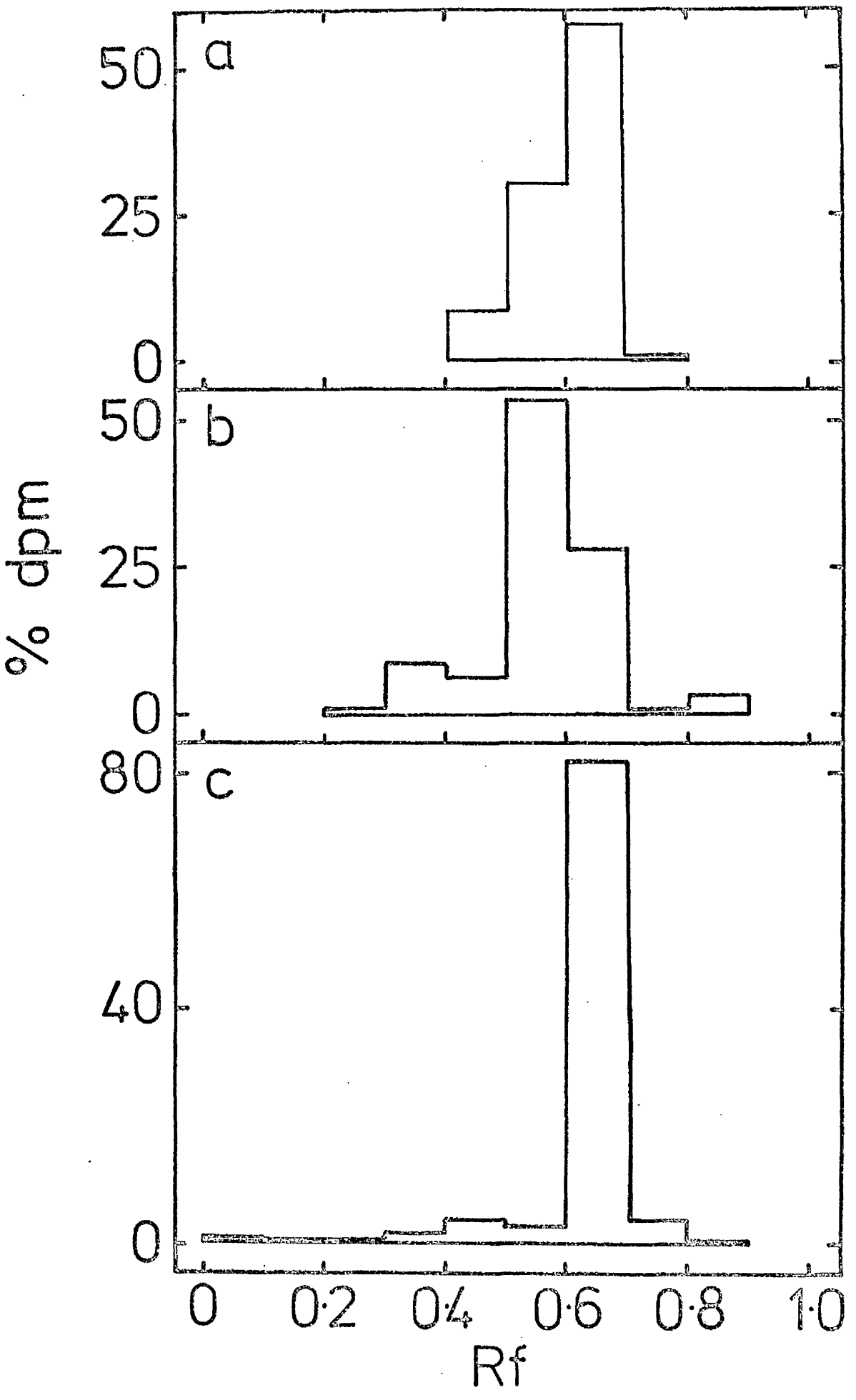
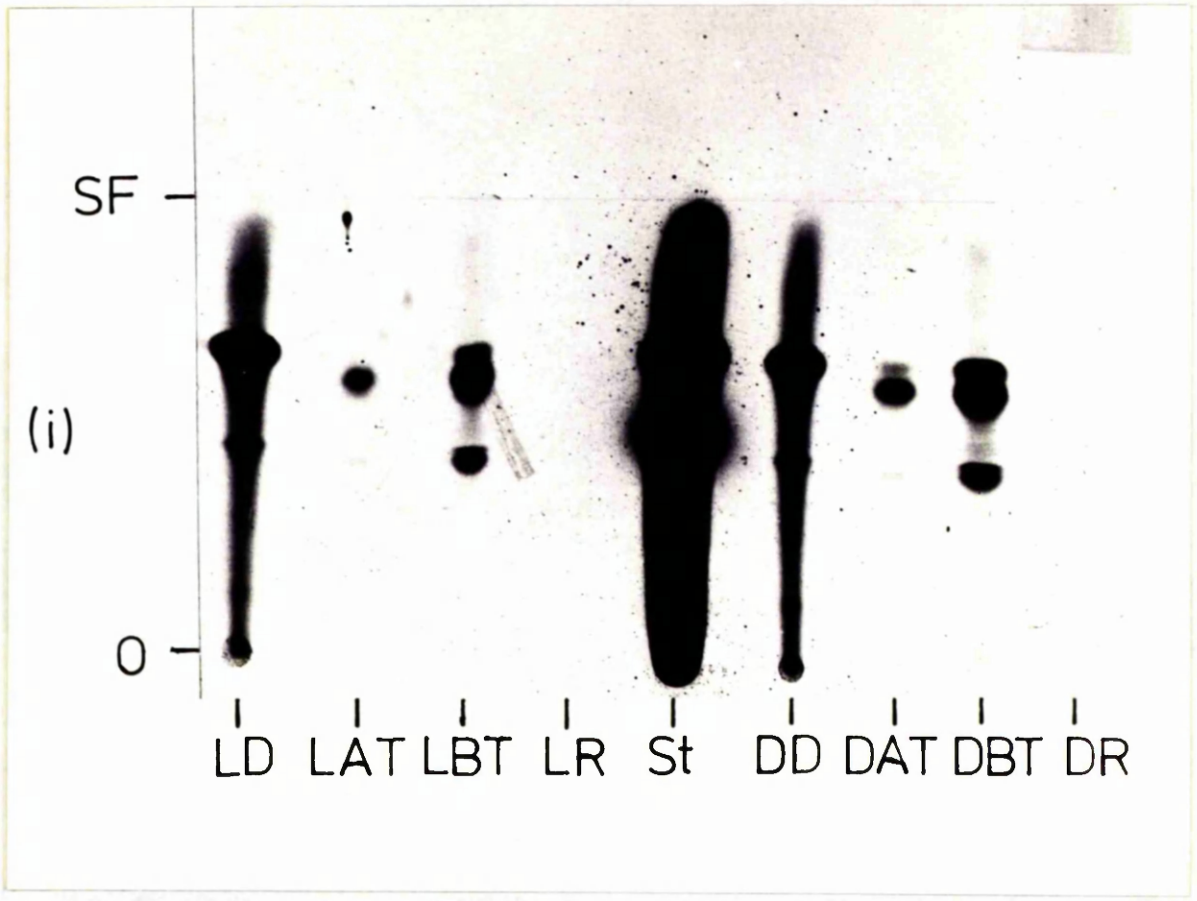

a
b
c
50
25
0
50
25
0
80
40
0
% dpm
0
0·2
0·4
0·6
0·8
1·0
Rf

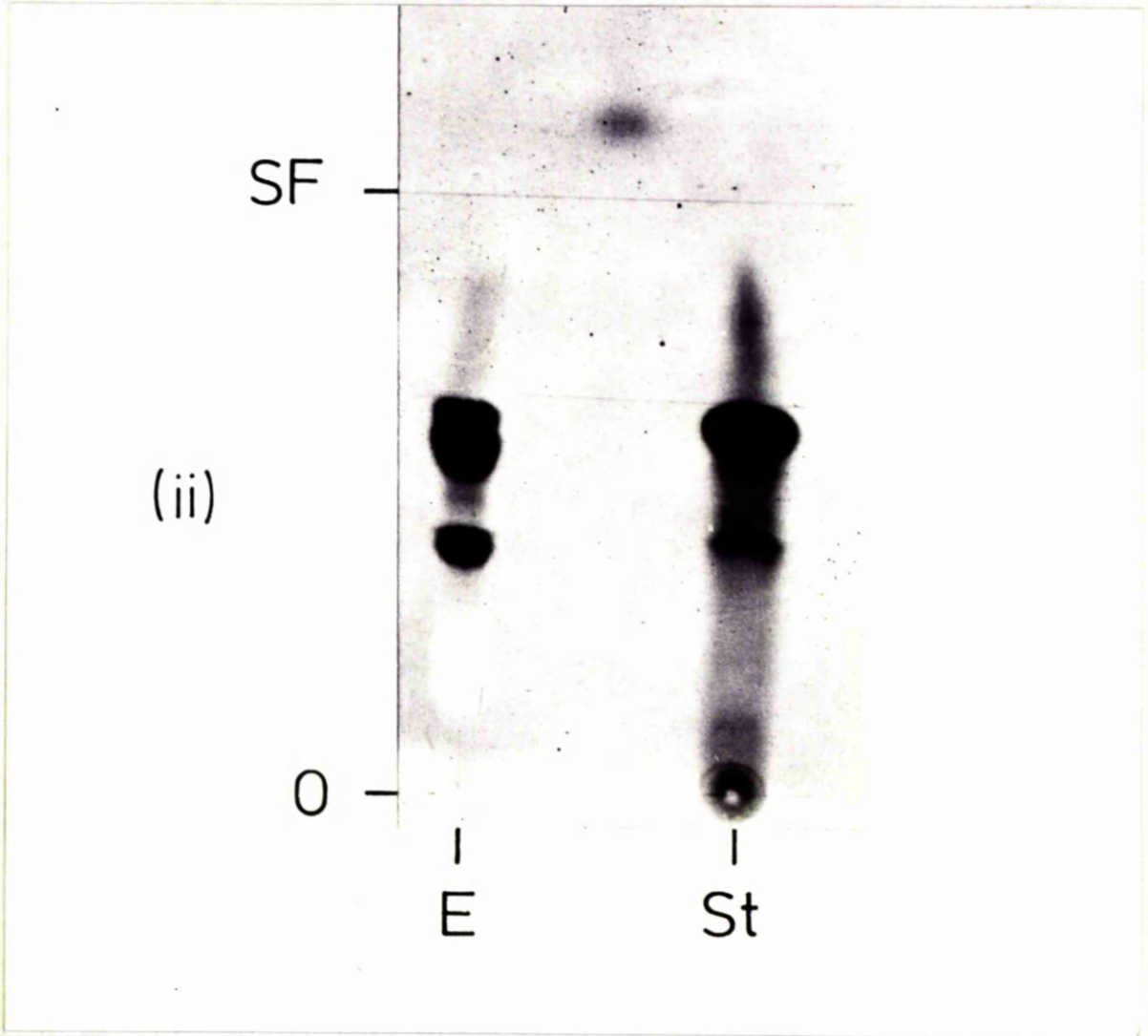
FIGURE 29

Autoradiographs of thin-layer chromatograms prepared as in Figure 28. The plates were exposed for four months to Ilford X-ray film.

(i) Autoradiograph of chromatogram 4. LD - light donors; LAT - light apical tissue; LBT - light basal tissue; LR - light receivers; st - stock ABA-2-^{14}C solution; DD - dark donors; DAT - dark apical tissue; DBT - dark basal tissue; DR - dark receivers.

(ii) Autoradiograph of chromatogram 5, E - tissue extract; st - stock ABA-2-^{14}C.


SF
(i)
0
LD
LAT
LBT
LR
St
DD
DAT
DBT
DR


SF
(ii)
0
E
St

analysis of radioactive extracts and had provided evidence that ABA-2-^{14}C was metabolised or broken down in coleoptile tissue. However, they were subject to three limitations. Firstly, the extracts were made only from acropetally-transporting tissue. Secondly, the extraction and chromatographic procedures were carried out in the light at room temperature such that considerable breakdown could have occurred during these processes. Thirdly, the paper chromatograms prepared for liquid scintillation counting were cut up into ten RF zones, making it difficult to separate peaks of radioactivity that were close together. Consequently a second series of experiments were carried out using similar basic techniques for both acropetal and basipetal transport of ^{14}C in coleoptile tissue. All the extraction and chromatographic procedures were carried out in darkness or dim green light at 20°C and the chromatograms were divided up into twenty zones prior to radioassay. Figure 30 shows the percentage distribution of radioactivity in paper chromatograms of extracts from (i) acropetally, and (ii) basipetally transporting segments and donors in the light. The donor extracts (a and d) show a peak of radioactivity at RF 0.75 - 0.80, corresponding to the peak for ABA-2-^{14}C found in previous experiments using paper chromatograms and the same solvent system. The tissue extracts show a peak of radioactivity at RF 0.60 - 0.65. Between 35 - 60% of the radioactivity recovered occurred in this zone, and in all tissue extracts under 15% of the radioactivity was recovered at the half-RF zone corresponding to ABA-2-^{14}C. The percentage radioactivity at RF 0.60 - 0.65 increased in the tissue halves furthest away from the donor, but there was little or no difference between extracts from acropetally or basipetally-

transporting segments. Figure 31 shows similar results from transport experiments carried out in the dark; there were no marked differences in the distribution of radioactivity on chromatograms of light or dark extracts. In order to separate the two radioactive peaks with greater clarity one chromatogram containing a spot of tissue extract and a marker spot of ABA-2-^{14}C was developed over 40cm in 2:6:1:2 and divided up into 1cm strips. This detailed analysis clearly distinguished two radioactive peaks in the tissue extract; a major peak at RF 0.675 and a smaller one at RF 0.775, the latter corresponding exactly with the peak obtained from the marker spot of ABA-2-^{14}C (Fig.32). The area under the first peak was three times greater than the area under the second peak.

A final chromatogram containing the same tissue extract and marker spot of ABA-2-^{14}C was developed in a different acidic solvent system, viz; 5:1:2.2 :: n-butanol: acetic acid: distilled water v/v (Fig. 33). Chromatography of the stock ABA-2-^{14}C revealed a major peak of radioactivity between RFs 0.90 - 1.0. The major peak of radioactivity from the tissue extract occurred at a slightly lower RF, between RF 0.85 - 0.90. While not providing very clear separation this result suggests that the two peaks from the tissue extract chromatogrammed in 2:6:1:2 were not artifacts of the solvent system.

In summary, all these results suggest that on the basis of chromatographic analysis, a greater proportion of the radioactivity extracted from coleoptile tissue supplied with ABA-2-^{14}C for 24 hours, is attached to some moiety distinct from ABA-2-^{14}C.

FIGURE 30

Chromatographic analysis of the radioactivity in ethanolic extracts of Zea mays coleoptile segments supplied with donor blocks containing 5 μM ABA-2-^{14}C for 24 hours in the light at 25°C. All extraction procedures were carried out in the dark or under dim green light. Whatman No. 1 paper was used for chromatography and the solvent system was : n-butanol: n-propanol: 0.880 ammonia: distilled water :: 2:6:1:2 v/v. Each histogram shows the radioactivity in half-RF zones of the chromatogram of an extract, expressed as a percentage of the total radioactivity recovered from the chromatogram.

(i) Acropetally-transporting segments; (a) donor block extracts
(b) basal tissue extracts
(c) apical tissue extracts

(ii) Basipetally-transporting segments; (d) donor block extracts
(e) apical tissue extracts
(f) basal tissue extracts.

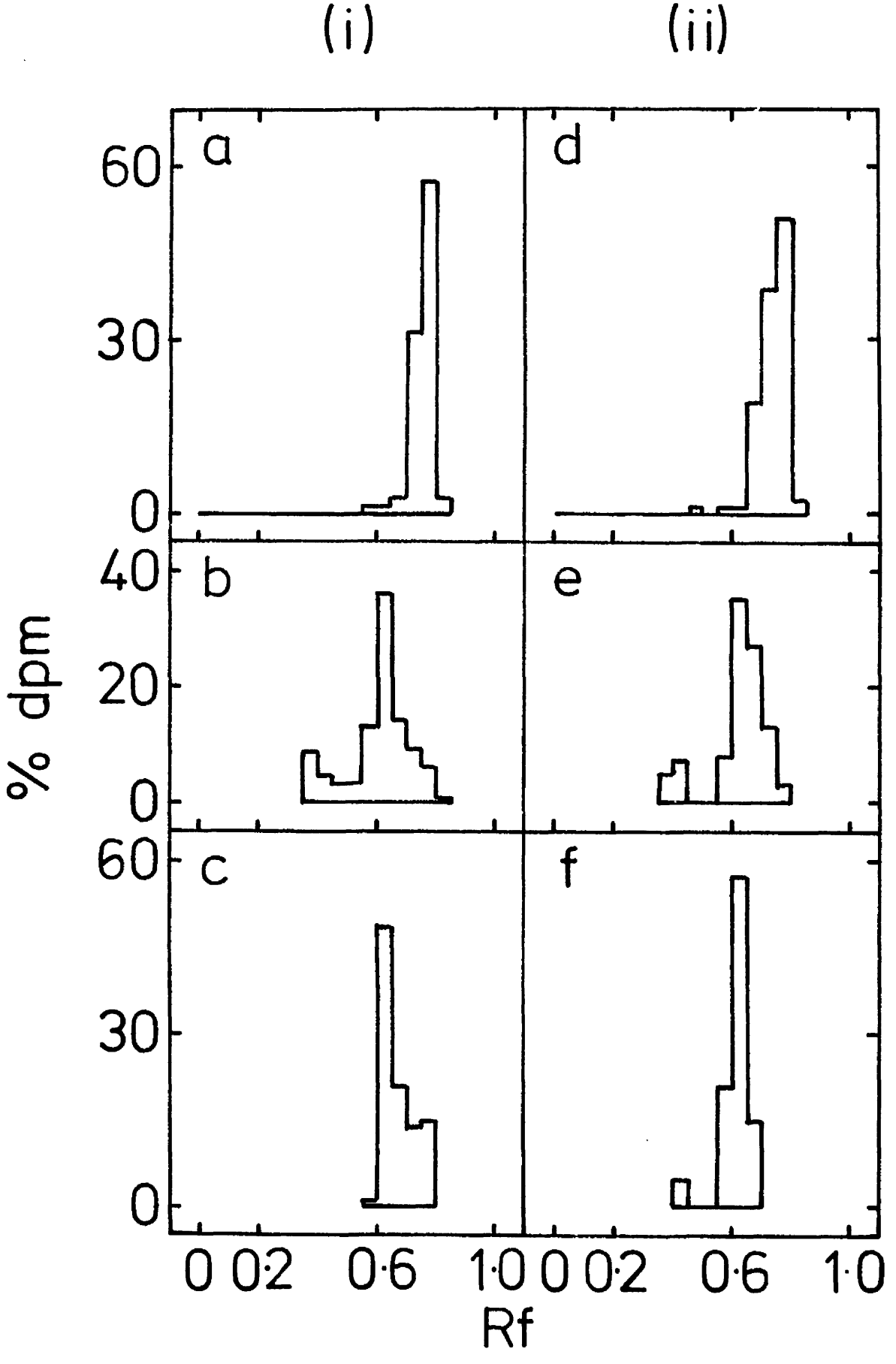

(i)
(ii)
a
b
c
d
e
f
60
30
0
40
20
0
60
30
0
% dpm
0 0·2 0·6 1·0
0 0·2 0·6 1·0
Rf

FIGURE 31

As in Figure 30 except that the transport period was in darkness.

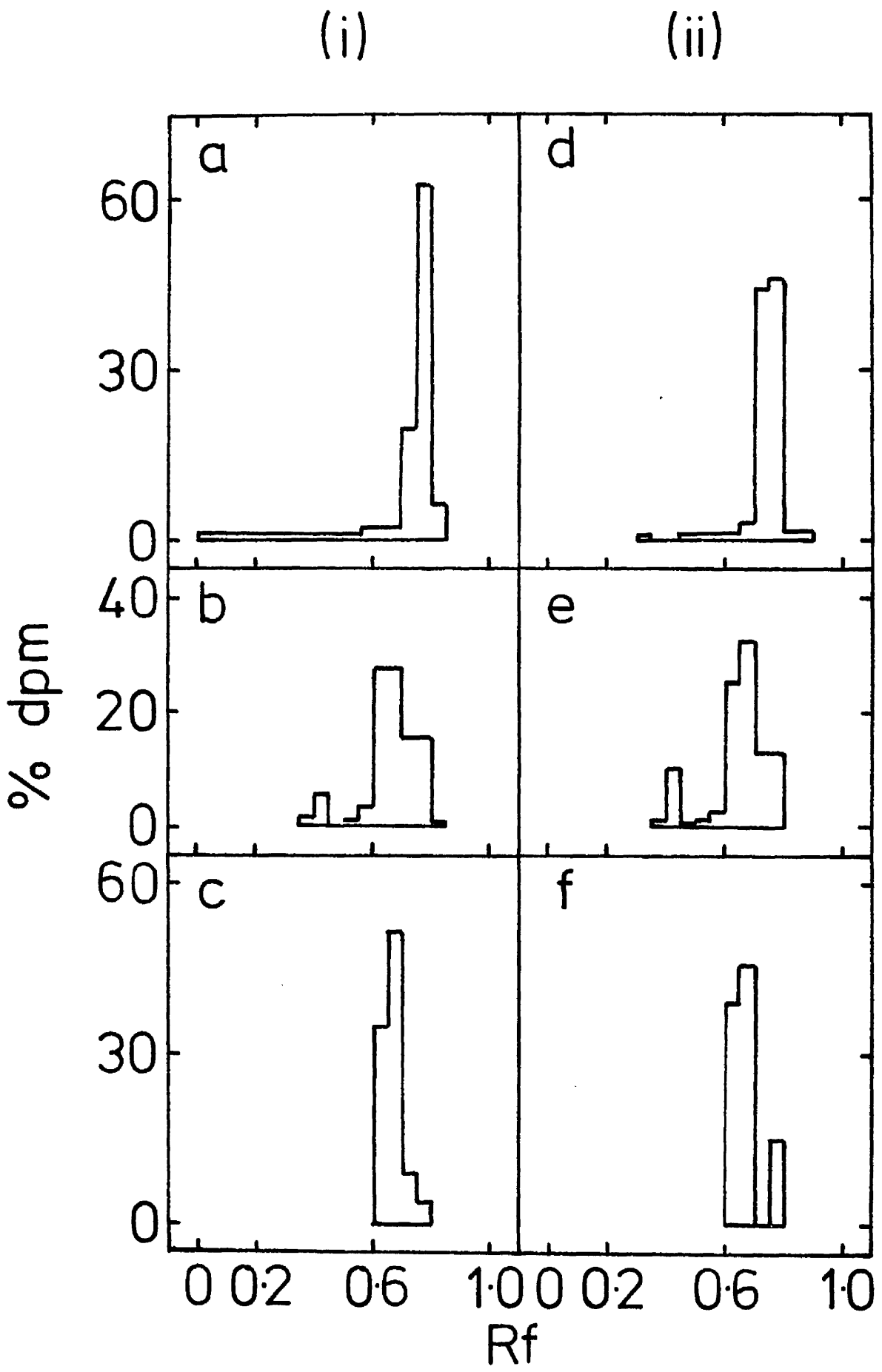

(i)
(ii)
a
b
c
d
e
f
60
30
0
40
20
0
60
30
0
% dpm
0 0·2 0·6 1·0 0 0·2 0·6 1·0
Rf

FIGURE 32

Chromatographic analysis of radioactivity in ethanolic extracts of Zea mays coleoptile segments supplied basally with donor blocks containing 5μM ABA-2-^{14}C for 24 hours in the light at 25^{o}C. All extraction procedures carried out in darkness or in dim green light. Chromatogram developed for 40cm in 2:6:1:2 and divided into 1cm strips for radioassay. Histograms show the percentage radioactivity in each strip.

(a) Coleoptile tissue extract

(b) Marker spot of ABA-2-^{14}C.

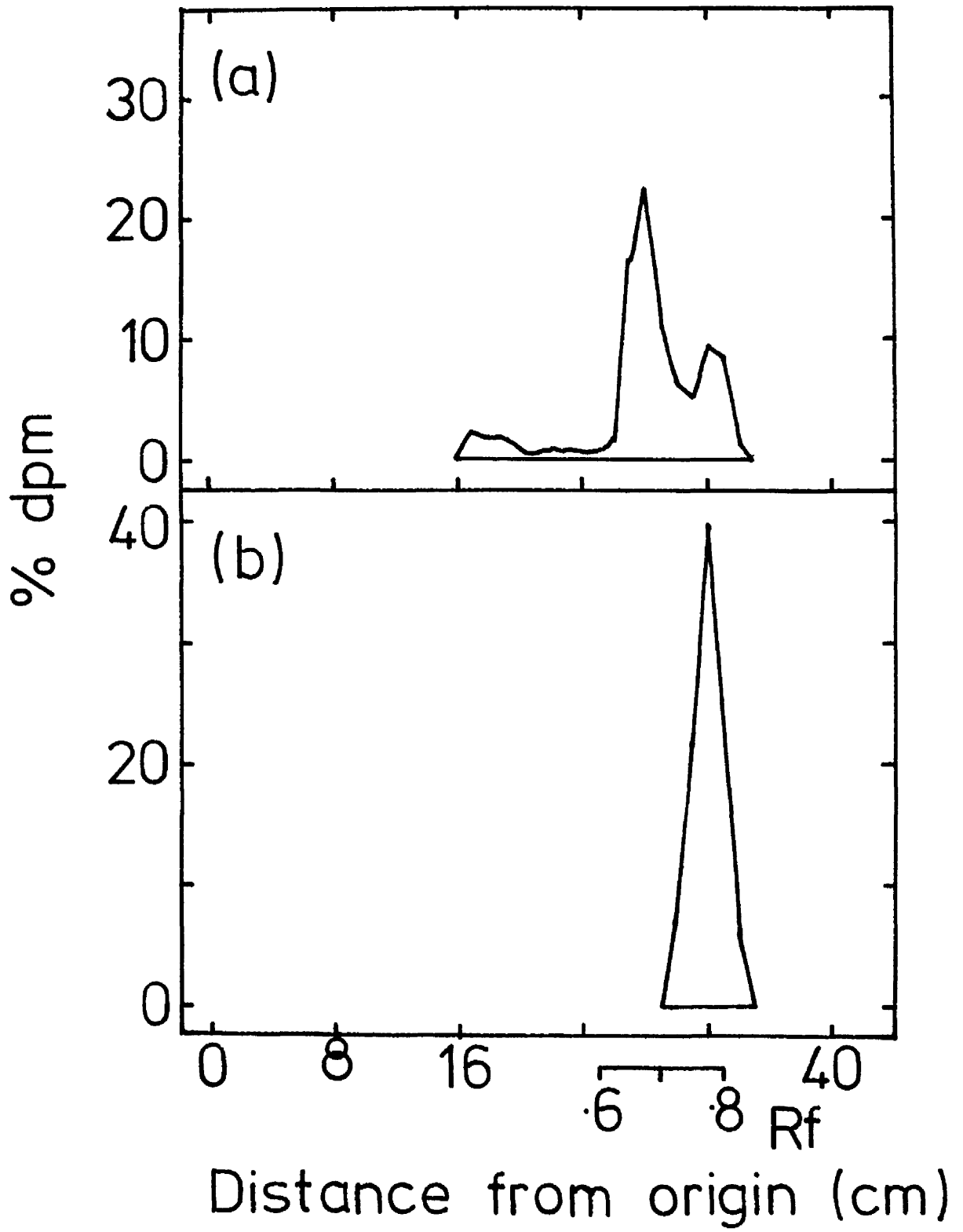
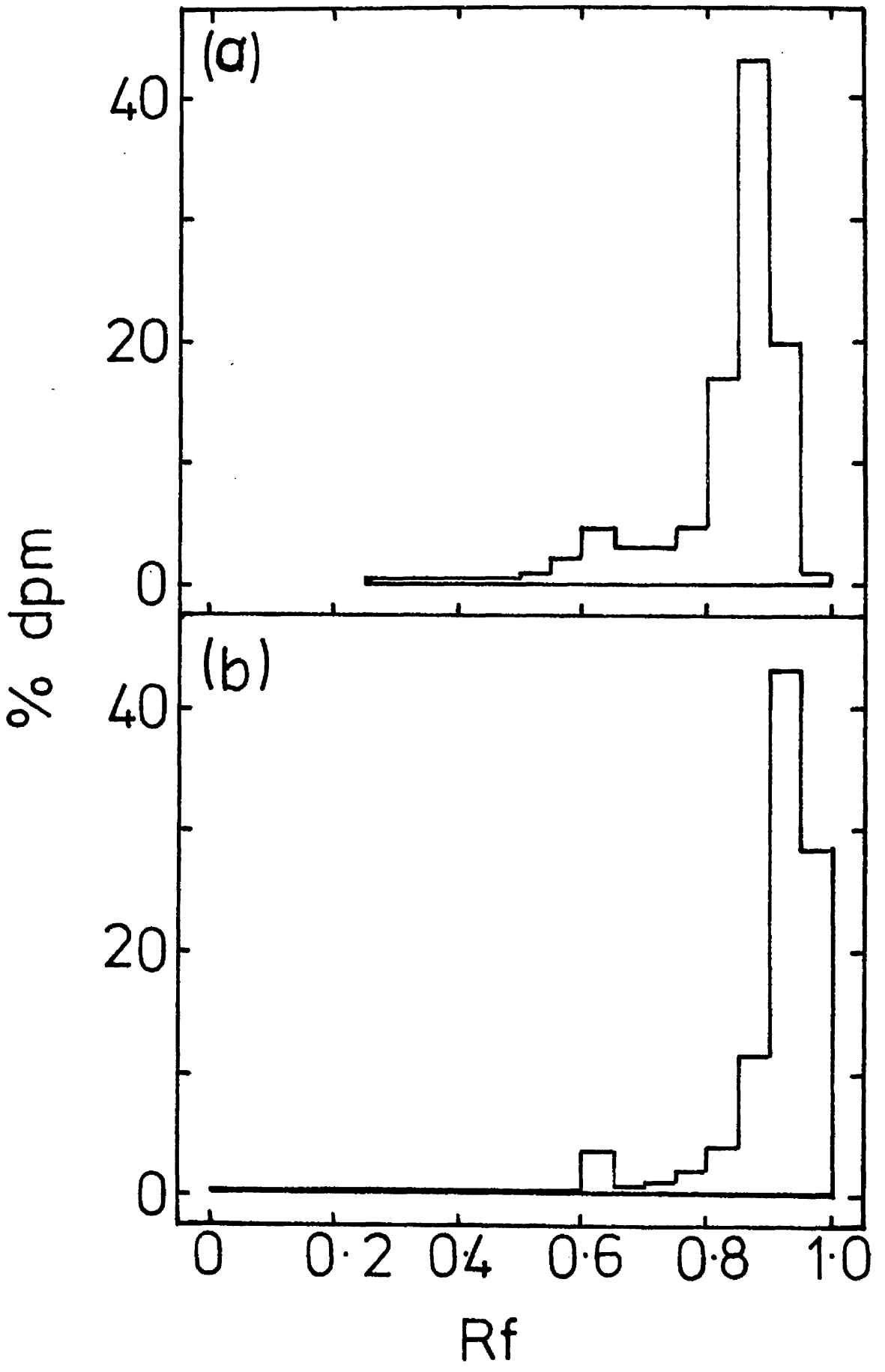

(a)
30
20
10
0
(b)
40
20
0
% dpm
0
8
16
40
·6
·8
Rf
Distance from origin (cm)

FIGURE 33

Chromatographic analysis of radioactivity in ehtanolic extracts of Zea mays coleoptile segments supplied basally with donor blocks containing 5μM ABA-2-^{1}C for 24 hours in the light at 25°C. All extraction procedures carried out in darkness or in dim green light. Chromatogram developed in n-butanol: acetic acid: distilled water :: 5:1:2.2 v/v and divided into half-RF zones for radioassay. Histograms show the percentage radioactivity in each zone.

(a) Coleoptile tissue extract.

(b) Marker spot of ABA-2-^{14}C.


(a)
(b)
40
20
0
% dpm
0
0·2
0·4
0·6
0·8
1·0
Rf

IB Movement and metabolism of ABA-2-^{14}C in Zea root segments

(1) Uptake and movement of ^{14}C in root segments in the dark.

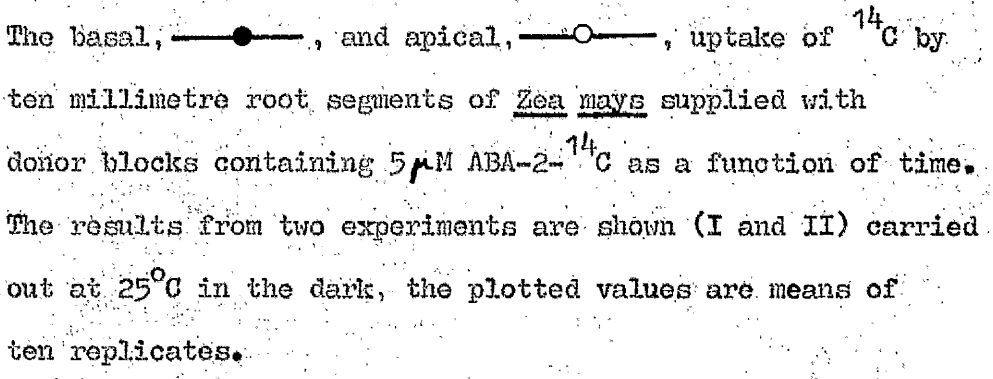
The uptake and movement of ^{14}C were studied in root segments supplied apically or basally with 5 M ABA-2-^{14}C in donor blocks at 25°C in the dark. Ten millimetre, three day-old roots cut 1mm from the apex were used, with 10 roots per holder, divided into two groups of five for counting. The total uptake of ^{14}C into the tissue was considered first, as a function of time. The results of two experiments are shown in Figure 34. The uptake of ^{14}C was greater from the basal end than the apical end in both experiments, but there was considerable variation between the experiments. In the first experiment there was an apparent peak of uptake at 12 hours, whereas in the second the peak was at 18 hours. After the peak in each experiment there was a decline in uptake, followed by an increase. These variations in uptake may reflect a rhythm of uptake, although to establish this would require further experimentation and sophisticated analysis.

The movement of ^{14}C in the tissue was then considered. As with coleoptiles there was very little movement of ^{14}C into receiver blocks after 24 hours; although in the second experiment there was some accumulation after 48 hours, this was very variable. As a result, polarity of movement was studied by analysing accumulation in the tissue. By using more replication over long time periods it may be possible to consider receiver block data. The movement of ^{14}C through the root segments was analysed as in coleoptiles by cutting the segment up into 5 x 2mm sections after the transport period and counting these sections in groups of five.

Figure 35 shows the accumulation of ^{14}C in sections 3 - 5 of acropetally and basipetally transporting segments. In both experiments a clear, significant (to 0.001P) acropetal polarity was established by 12 hours after application of donor blocks. There was however, considerable variation in accumulation over the time periods considered, which mirrored the variations in uptake. This would suggest that movement through the segment was not independent of uptake into the first section of the segment.

When considered on a percentage basis (Fig. 36), the polarity of acropetal movement and accumulation into sections 3 - 5 was even more marked after 12 hours. However, after 24 hours the basipetal movement of ^{14}C expressed as a percentage of total ^{14}C, into sections 3 - 5 had increased, and by 36 hours was similar to the acropetal accumulation. This finding may relate to the root segments becoming less metabolically active after this period at 25°C.

FIGURE 34



The basal, —●—, and apical, —○—, uptake of ^{14}C by ten millimetre root segments of Zea mays supplied with donor blocks containing 5 μM ABA-2-^{14}C as a function of time. The results from two experiments are shown (I and II) carried out at 25°C in the dark, the plotted values are means of ten replicates.

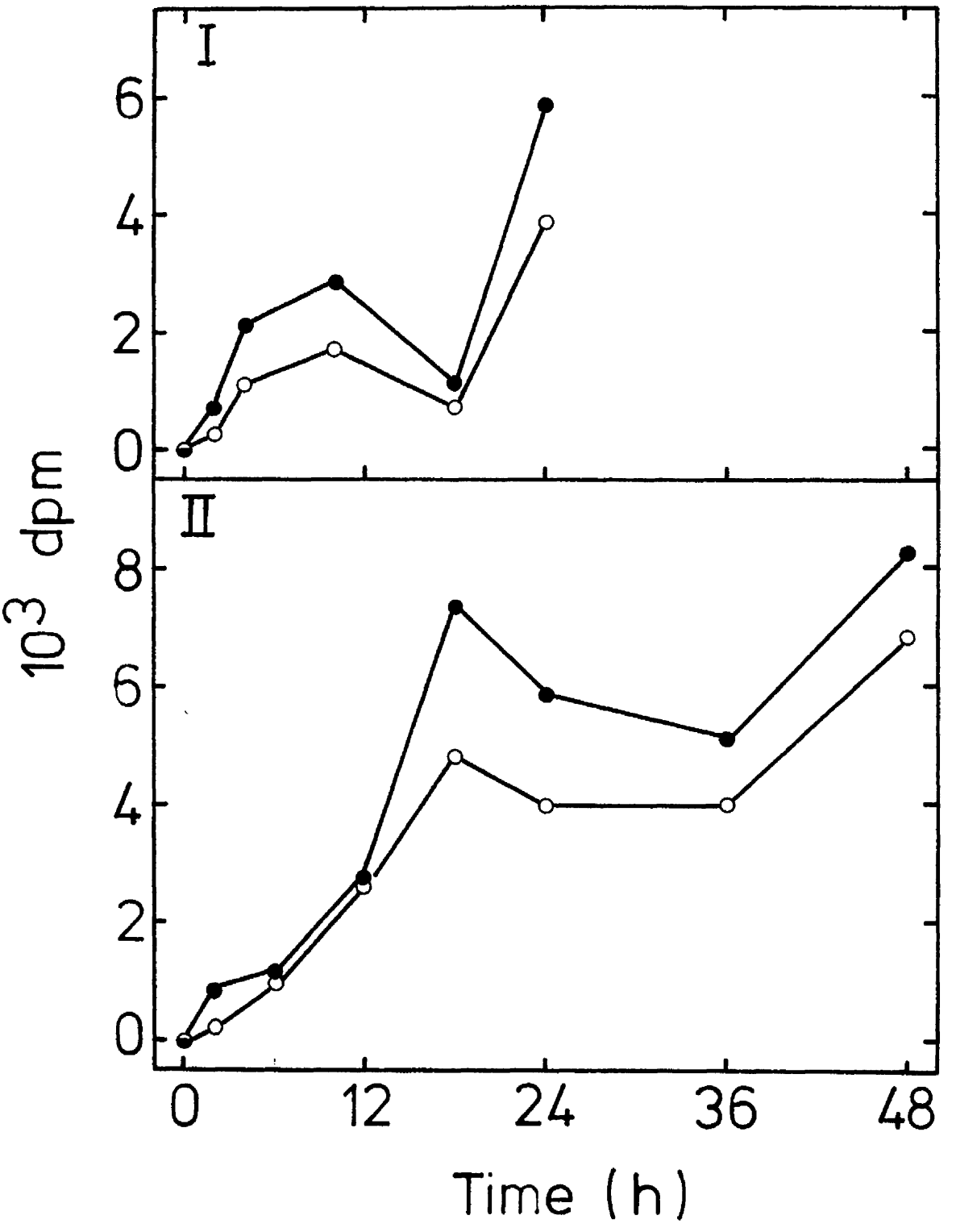

I
II
6
4
2
0
8
6
4
2
0
10^3 dpm
0
12
24
36
48
Time (h)

FIGURE 35

The acropetal, —●— , and basipetal, —○— , accumulation of ^{14}C into sections 3, 4 and 5 of ten millimetre root segments of Zea mays supplied with donor blocks containing 5μM ABA-2-^{14}C as a function of time. Experimental details as in Figure 34.

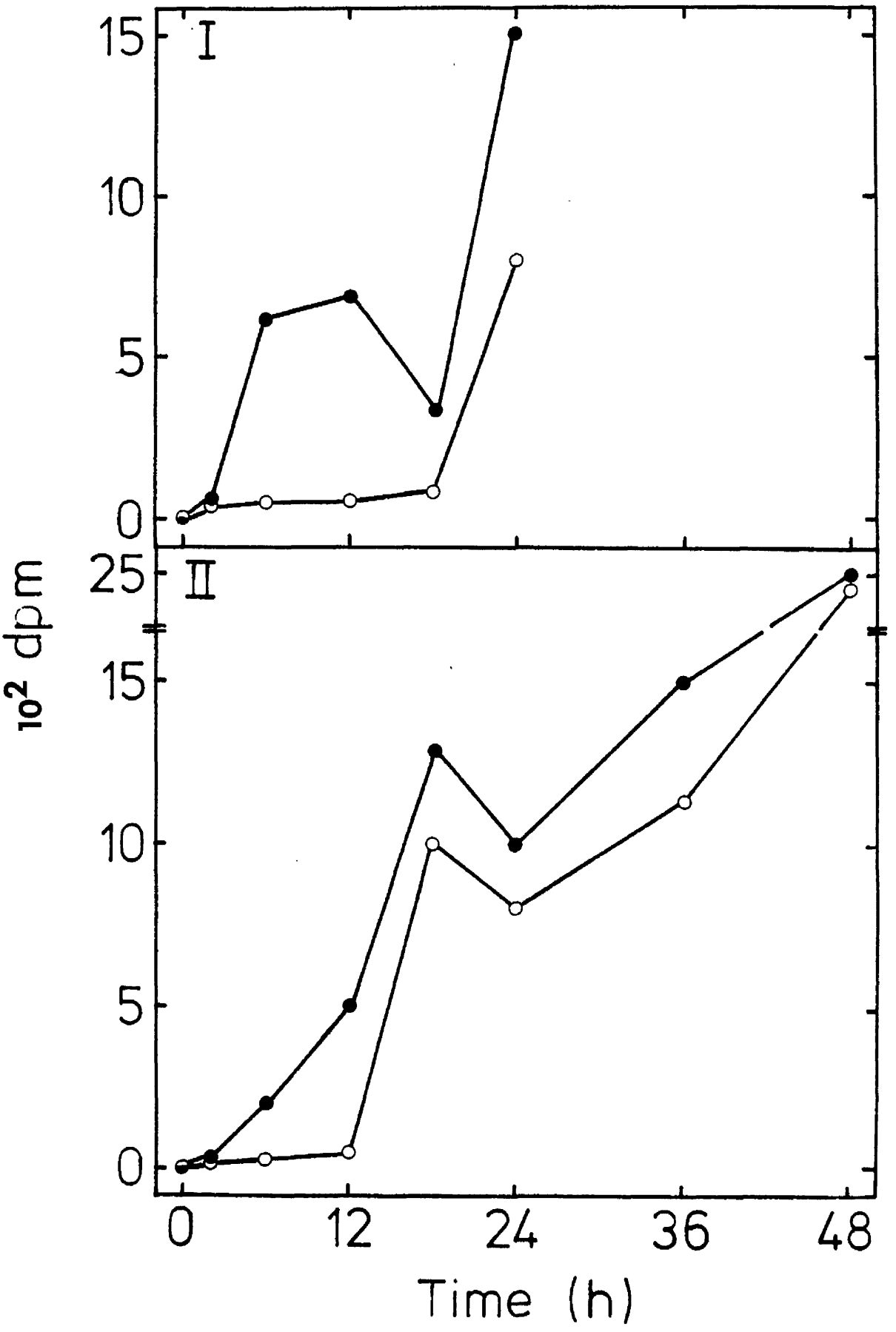
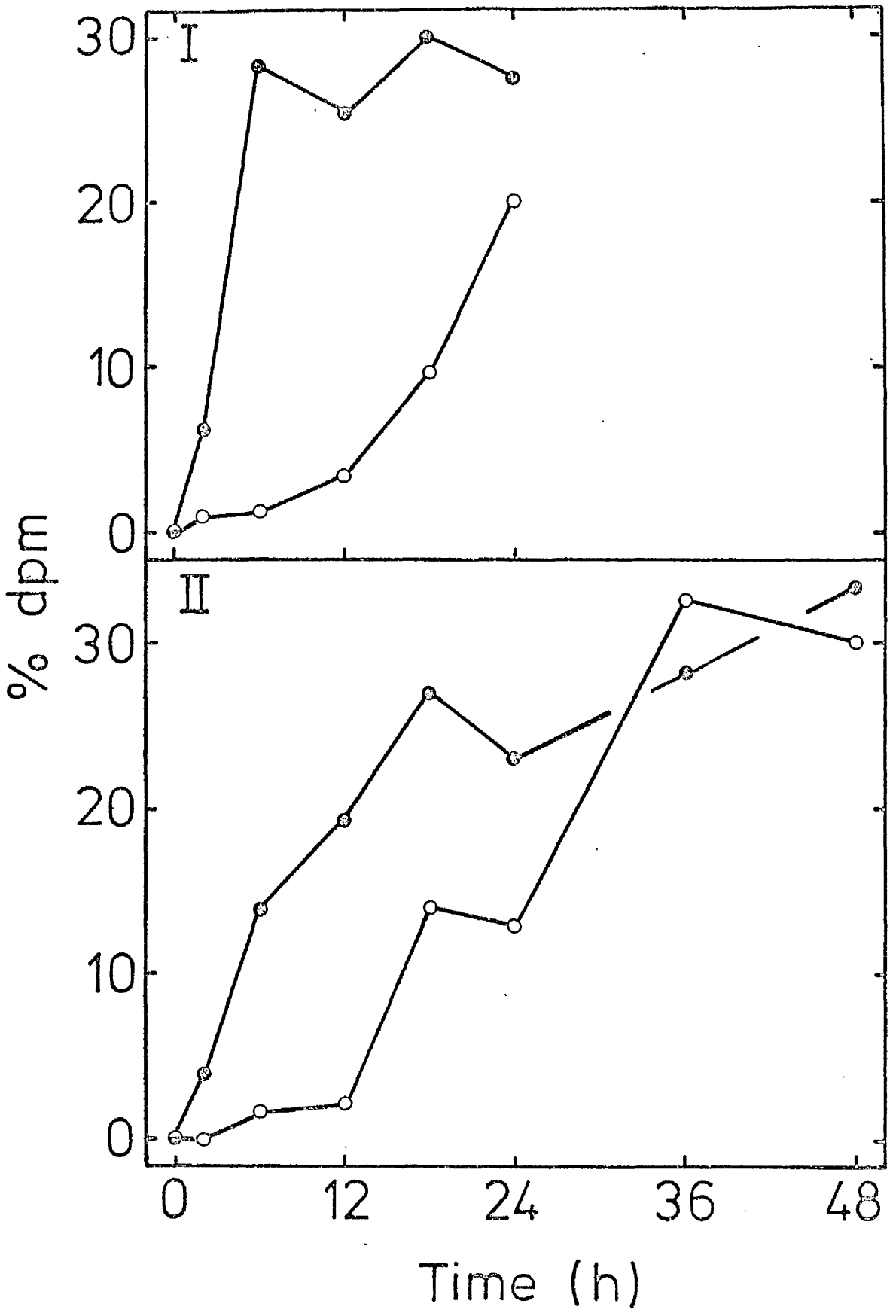

I
II
15
10
5
0
25
15
10
5
0
0
12
24
36
48
Time (h)
10^2 dpm

FIGURE 36

The acropetal, —●—, and basipetal, —○—, accumulation of ^{14}C into sections 3, 4 and 5, expressed as a percentage of the total uptake by ten millimetre root segments of Zea mays, as a function of time. Experimental details as in Figure 34.


I
II
30
20
10
0
30
20
10
0
% dpm
0
12
24
36
48
Time (h)

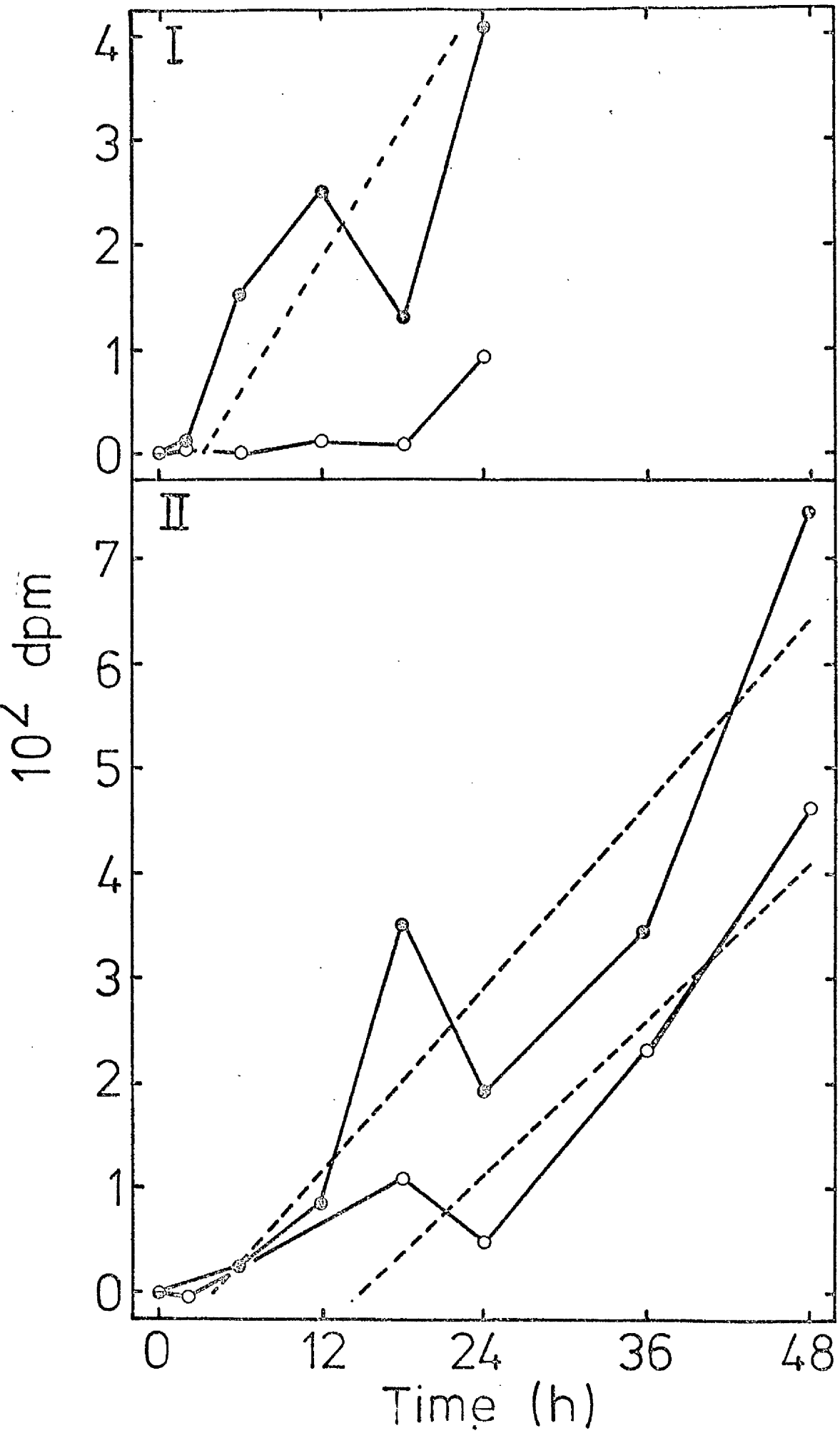
(ii), Velocity of ^{14}C movement in root segments.

The velocity of ^{14}C movement in 10mm root segments has been examined by analysing the accumulation of ^{14}C in the 5th section of the segment. The segments were supplied with 5μM ABA-2-^{14}C at 25°C in the dark. Figure 37 shows the accumulation of ^{14}C in the 5th section of acropetally and basipetally transporting segments in two experiments as a function of time. The points of the acropetal movement plot are widely scattered, making the fitting of the line by eye, difficult. Indeed, when the points are connected by straight lines, the presence is revealed of a peak at 12 to 18 hours followed by a trough after 24 hours. However, the intercept on the 'X' axis of the back extrapolation is probably between 2 and 4 hours, giving a calculated velocity for acropetal movement of ^{14}C of 2 - 4mm h^{-1}. In the basipetal direction accumulation of ^{14}C in significant amounts in the 5th section did not occur until after 18 hours, giving a maximum calculated velocity for basipetal movement of 0.4mm h^{-1}. Further experimentation is required to verify these values, but these initial results suggest that the rate of acropetal movement of ^{14}C when ABA-2-^{14}C is supplied to root segments is much greater than the rate of basipetal movement of ^{14}C.

Comparison of fluxes must await further data.

FIGURE 37

The acropetal, ——●——, and basipetal, ——○——, accumulation of ^{14}C into section 5 of ten millimetre root segments of *Zea mays* supplied with donor blocks containing 5μM ABA-2-^{14}C. The results from two experiments are given (I and II), ten replicates per point, carried out in the dark at 25°C. The dotted lines, giving intercepts on the time axis, were fitted by eye.


I
II
4
3
2
1
0
7
6
5
4
3
2
1
0
10^2 dpm
0
12
24
36
48
Time (h)

(iii) The effect of light on the uptake and movement of ^{14}C in root segments.

Root segments, 10mm in length, were set up as previously described with 5μM ABA-2-^{14}C applied to the apical or basal ends of the segments in the light at 25°C. Figure 38a shows the uptake of ^{14}C into the root tissue as a function of time; the data from Figure 34 (I) are re-plotted for comparison. The pattern of uptake in light and darkness over 24 hours was very similar. A decline in uptake at 18 hours occurred both in the light and dark treated segments. Thus, unless the cycling in uptake is caused by external factors, these results could indicate a rhythm of uptake of ^{14}C into root segments over 24 hours. Further experimentation over longer time periods is required to substantiate this suggestion.

The polarity of movement of ^{14}C in the light has also been investigated. The experiment was carried out as in the dark, analysing the accumulation of ^{14}C into sections 3, 4 and 5 of the root segments. Figure 38b shows that the accumulation of ^{14}C in these sections was again very similar to that obtained in the dark, a marked acropetal accumulation occurring after 12 hours, declining by 18 hours and increasing again after 24 hours. The basipetal accumulation increased sharply after 18 hours, causing a decrease in the polarity of movement after that time. Plotted on a percentage basis in Figure 38c there was a clear acropetal polarity established after 6 hours transport. The accumulation of ^{14}C into section 5 alone, is also plotted, (Fig. 39) and, as with the dark treated segments, there was a large variation in acropetal accumulation, reaching a peak after 12 hours, and declining by 18 hours.

The straight line fitted to these points clearly mis-represents what was actually occurring in the tissue, but gives an approximate acropetal rate of movement of 2 - 4 mmh^{-1}. This again is similar to the dark treated segments. The basipetal rate was approximately 0.6 - 0.7 mmh^{-1}, slightly higher than the rate in the dark.

These results, which again were complicated by an apparent rhythm of uptake, suggest that light does not alter the basic characteristics of ^{14}C movement in root segments supplied with ABA-2-^{14}C in donor blocks at 25°C.

FIGURE 38

(a) The basal, ——●——, and apical, ——○——, uptake of ^{14}C by ten millimetre Zea mays root segments supplied with donor blocks containing 5μM ABA-2-^{14}C in the light at 25°C, as a function of time. Plotted values are means of ten replicates, and the data from Figure 34 (I) are re-plotted for comparison.

(b) The acropetal, ——●——, and basipetal, ——○——, accumulation of ^{14}C in sections 3, 4 and 5 of Zea mays root segments supplied with 5μM ABA-2-^{14}C in the light at 25°C as a function of time. Means of 10 replicates.

(c) As in (b) but radioactivity plotted as a percentage of the total uptake.

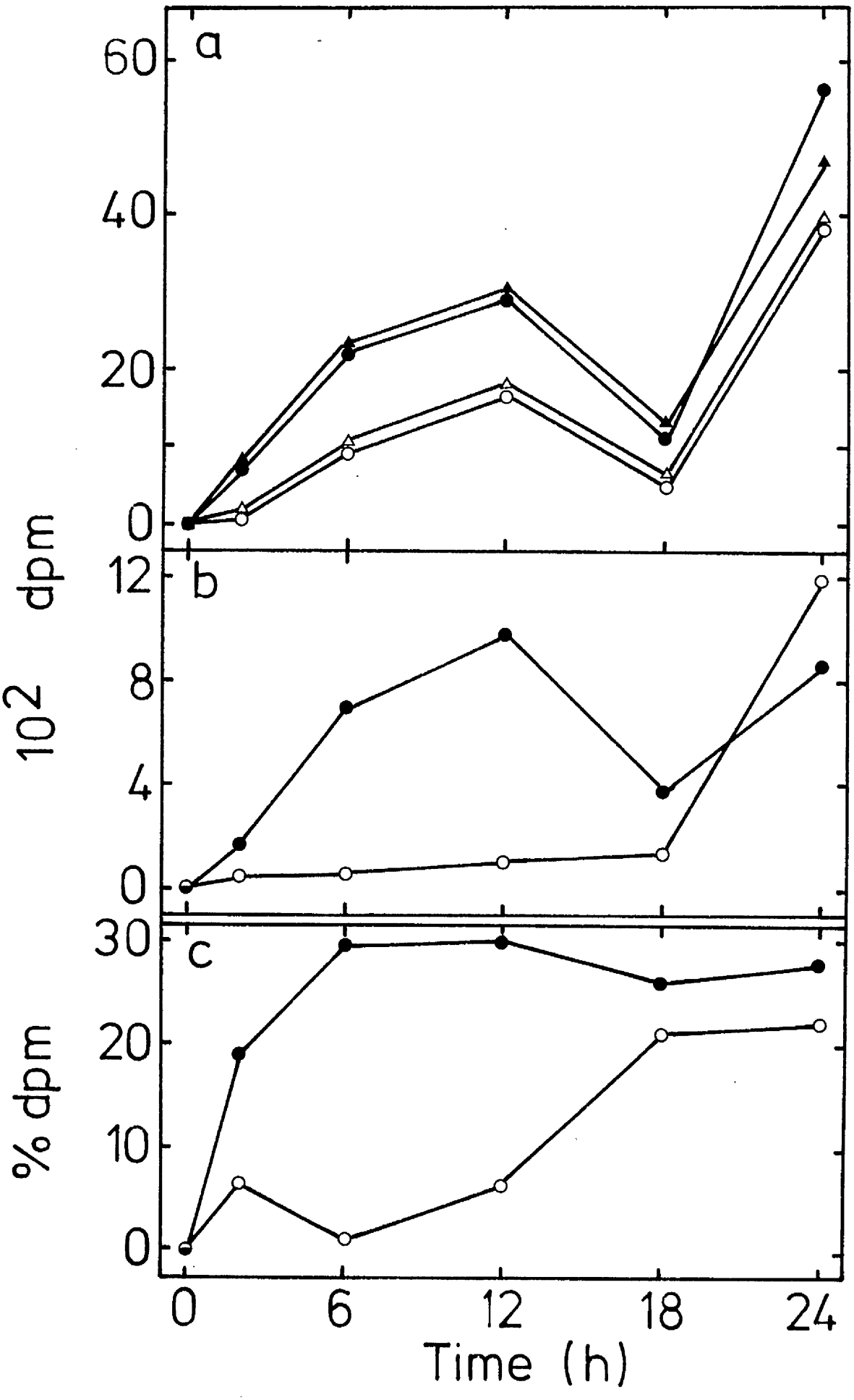
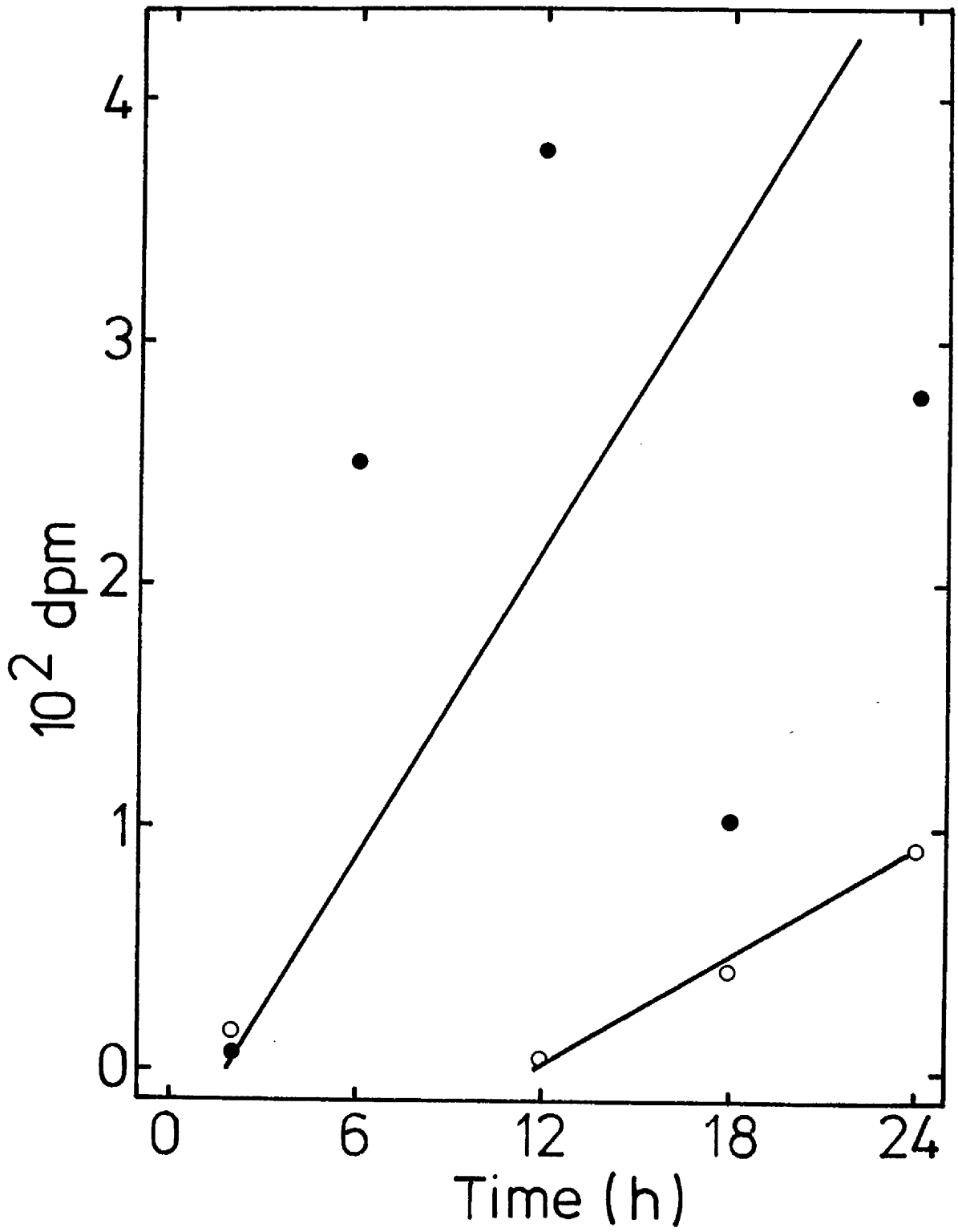

a
b
c
60
40
20
0
12
8
4
0
30
20
10
0
10² dpm
% dpm
0
6
12
18
24
Time (h)

FIGURE 39

The acropetal, —●—, and basipetal, —○—, accumulation of radioactivity in section 5 of Zea mays root segments. Experimental details as in Figure 38; straight lines are fitted by eye.


4
3
2
1
0
10^2 dpm
0
6
12
18
24
Time (h)

(iv) The effect of temperature on the uptake and movement of ^{14}C in root segments.

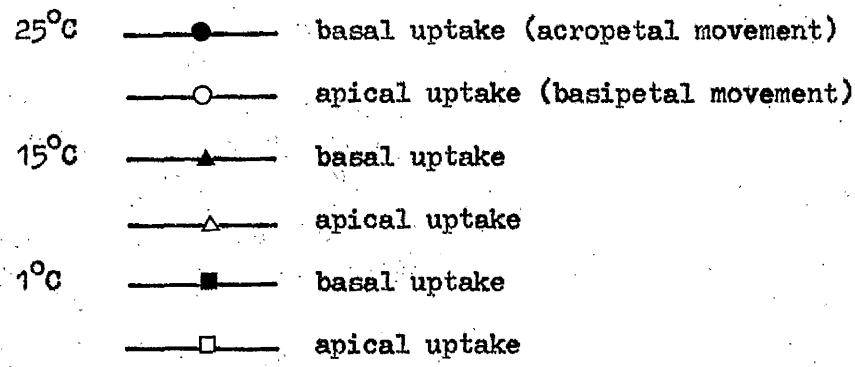
In any study of the relationships between the movement of a substance in tissue, and the physiological and metabolic activity of that tissue, the effects of temperature must be considered. Thus, as with coleoptile segments, the effects of three temperatures, 1, 15 and 25°C were studied on the uptake and movement of ^{14}C in root segments of Zea mays supplied with donor blocks containing 5μM ABA-2-^{14}C in the dark.

The effect of temperature on total uptake was considered first, as a function of time (Fig. 40). At 25°C there were typical cyclic variations in uptake over 24 hours, with basal uptake always greater than apical uptake, but by 48 hours this difference had disappeared; in fact apical uptake was slightly greater than basal uptake. At 15°C basal uptake was always greater than apical uptake, although both increased steadily with time and by 48 hours they exceeded the uptake at 25°C, and apical uptake was only slightly lower than basal uptake. There was no evidence of rhythmic variations in uptake at 15°C. At 1°C the rate of uptake was much lower than at 15°C or 25°C, although basal uptake was always greater than apical uptake. Both increased with time basal uptake showing more variation than apical uptake. There was some suggestion of a rhythmic process with peaks of uptake at 12, 24 and 48 hours. These results suggest that lowering the temperature to 1°C decreases both basal and apical uptake over 24 hours; at 15°C, after an early depression, the basal and apical uptake are as great, or greater, than at 25°C after 48 hours.

The accumulation of radioactivity in sections 3 - 5 of root segments as a percentage of the total uptake, was also considered at the three temperatures, as a function of time (Fig. 41). At 25°C, acropetal % accumulation of ^{14}C in sections 3 - 5 was initially greater than basipetal accumulation, showing an acropetal polarity after 12 hours. But by 24 hours this polarity was abolished and by 48 hours there was a suggestion of basipetal polarity. Variations in accumulation again reflected variations in uptake. At 15°C a clear acropetal polarity was established by 12 hours and maintained over the 48 hour period, with much less variation. At 1°C there was again an acropetal polarity but there was considerable variation in acropetal accumulation of ^{14}C which reflected the variation in uptake. These results suggest that lowering the temperature from 25°C does not markedly alter the basic characteristics of ^{14}C movement in root segments, but does allow the acropetal polarity to be maintained over 48 hours. At 15°C there is less variation in polarity than at the other two temperatures. It is of interest that both uptake and movement of ^{14}C in root segments were not markedly or persistently reduced by lowering the temperature from 25°C to 15°C, and even at 1°C, uptake and movement of ^{14}C were maintained, albeit at a lower level.

FIGURE 40

The basal and apical uptake of ^{14}C by ten millimetre root segments of Zea mays supplied with 5 μMABA-2-^{14}C at three different temperatures, as a function of time. The values plotted are means of twenty replicates.



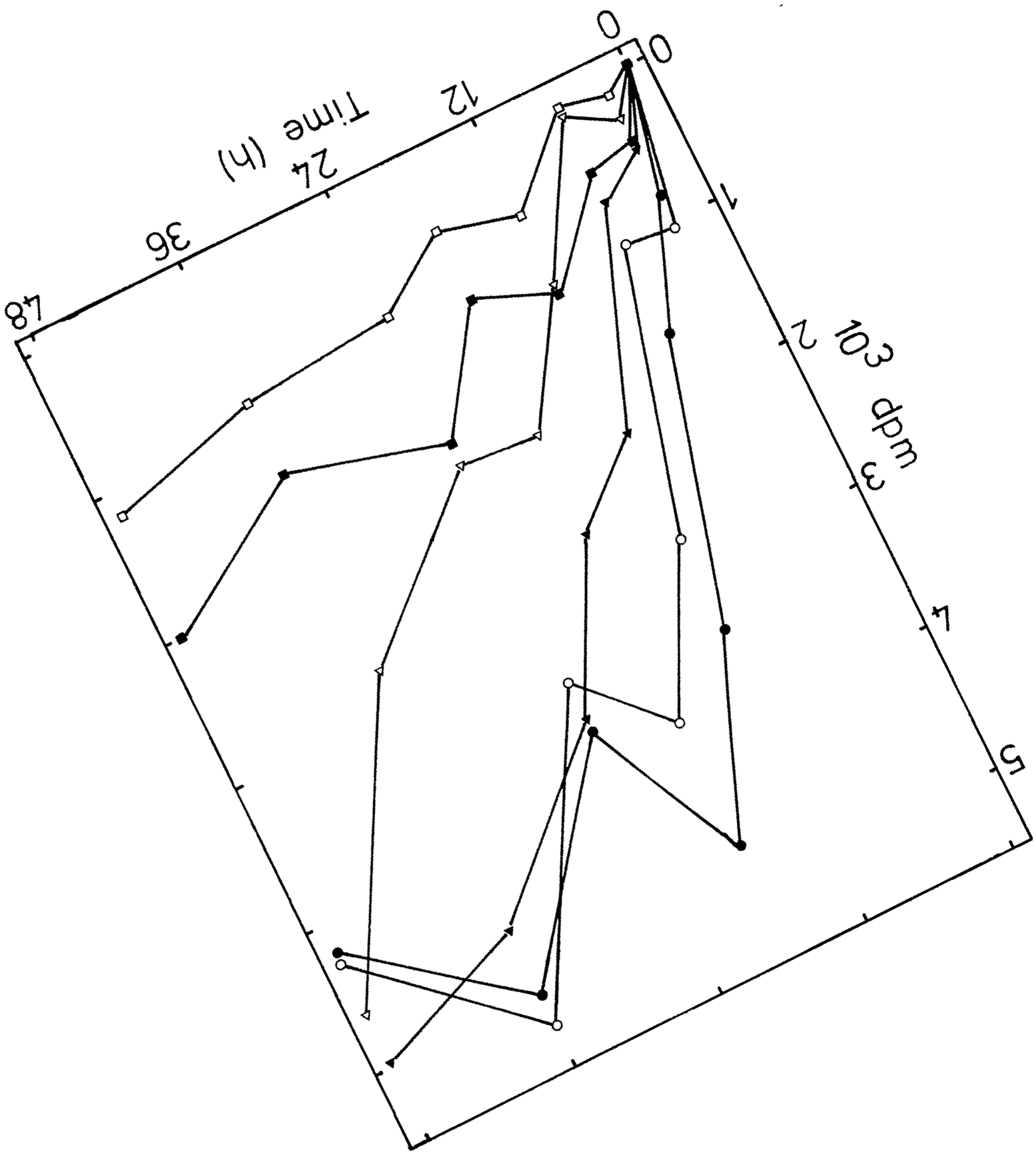
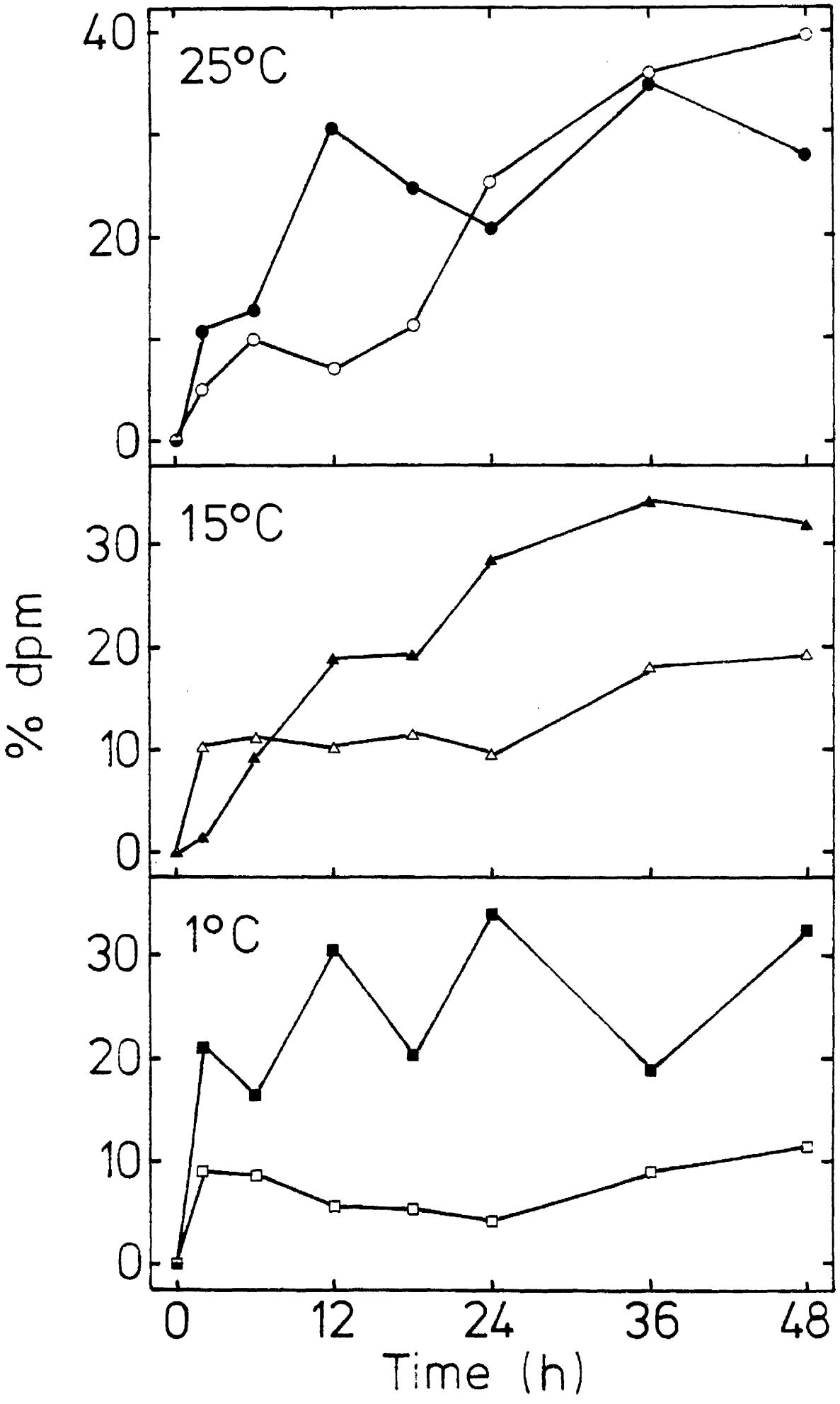

0
12
24
36
Time (h)
0
87
90
1
2
3
4
5
10³ dpm

FIGURE 41

The accumulation of ^{14}C, expressed as a percentage of the total uptake, into sections 3 - 5 of 10mm root segments of Zea mays supplied with donor blocks containing 5μMABA-2-^{14}C at three different temperatures, as a function of time. Plotted values are means of twenty replicates, symbols as in Figure 40.


25°C
15°C
1°C
40
20
0
30
20
10
0
30
20
10
0
% dpm
0
12
24
36
48
Time (h)

(v) Chromatographic analysis of radioactive extracts from root segments supplied with ABA-2-^{14}C.

As with the coleoptile experiments, some attempt must be made to identify the radioactive moiety extracted from the root tissue.

The procedures used were similar to those adopted for coleoptile tissue. Root segments were divided up into apical and basal halves after a 24 hour transport period in light and in darkness and these and the donor blocks, were extracted in ethanol. The extracts were analysed using paper chromatography; Whatman No. 1 paper was used and the solvent system was n-butanol: n-propanol: 0.880 ammonia: distilled water :: 2:6:1:2 v/v. The chromatograms were radioassayed using a liquid scintillation spectrometer and the distribution of radioactivity down the chromatograms plotted as histograms (Fig. 42).

The peak of radioactivity from the donor block extracts in light and darkness corresponded with the peak from stock ABA-2-^{14}C (cf. Fig. 28). From the tissue extracts there were no qualitative differences in the distribution of radioactivity on the chromatograms between light and dark or acropetally and basipetally treated segments; the quantitative differences were probably due to variation in technique. In the extracts from tissue halves nearest the donor blocks, the major peak of radioactivity coincided with the peak in donor block extracts, between Rf 0.7 - 0.8. In the extracts of tissue halves furthest from the donor blocks, very little or no radioactivity occurred in this zone, and a major peak occurred at Rf 0.6 - 0.7.

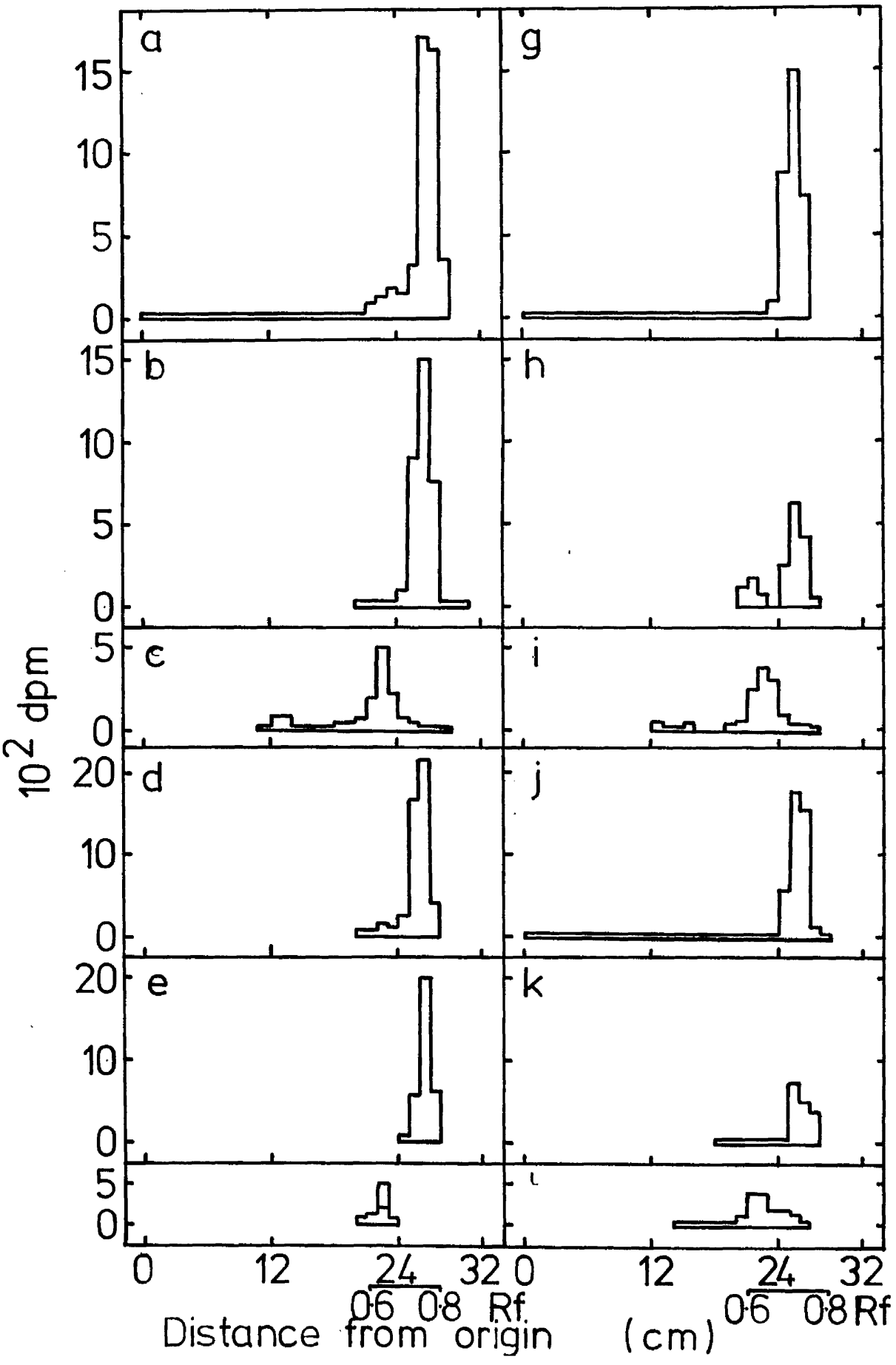
This peak appeared to correspond with peaks found in coleoptile tissue extracts (cf. Fig. 28) and was apparently distinct from the peak corresponding to ABA-2-^{14}C. Thus the metabolism of ABA-2-^{14}C in root segments appeared, on the basis of chromatographic analysis, to be similar to that in coleoptile segments, except that the peak corresponding to ABA-2-^{14}C disappeared almost completely from extracts of tissue halves furthest from the donor blocks This suggests that most, if not all of the radioactivity extracted from that tissue was attached to some moiety distinct from ABA-2-^{14}C.

FIGURE 42

Chromatographic analysis of radioactive extracts of root segments of *Zea mays* supplied with donor blocks containing 5μ MABA-2-^{14}C for 24 hours at 25°C in light or in darkness. The histograms show the distribution of ^{14}C in 1cm strips from the origin to the solvent front. The solvent system used to develop the paper chromatograms was n-butanol: n-propanol: 0.880 ammonia: distilled water :: 2:6:1:2 v/v. All extraction and chromatographic procedures were carried out in the dark or dim green light at 20°C.

Light transport
a - apical donor block extract
b - apical tissue half "
c - basal " " "
d - basal donor block "
e - basal tissue half "
f - apical " " "

Dark transport
g to l - as for a - f.


a
g
b
h
c
i
d
j
e
k
f
l
15
10
5
0
20
10
0
12
24
32
0·6
0·8
Rf
10^2 dpm
Distance from origin (cm)

IC Movement of ABA-2-^{14}C in whole seedlings of Zea mays.

Since the movement of ABA-2-^{14}C had been studied in coleoptile and root segments of Zea mays it was a logical development to study the movement of ABA-2-^{14}C in whole seedlings of Zea mays. However time and other priorities allowed only one set of preliminary experiments to be carried out, using one method of application, viz: an aqueous solution of 1μMABA-2-^{14}C supplied to the primary root of three to four day-old seedlings.

The seedlings were set up as described in the methods section with the tip of the primary root immersed in a 1μMABA-2-^{14}C solution at 25^{o}C in the light. After a given transport period seedlings were removed, each seedling was dissected into root, fruit and shoot and the radioactivity in each part assayed.

The total uptake by the seedlings increased with time (Fig. 43), over a period of 24 hours. The water loss from the system, i.e. solution plus seedlings, (corrected for loss by evaporation from the surface of the solution), was also measured as a function of time. This should have given an estimate of the amount of water or solution taken up and transpired by the seedlings with time. This showed a similar pattern to the uptake of radioactivity. While this does not prove that the radioactivity was taken up in solution with the water molecules in the transpiration stream, it suggests some relationship between the two.

The distribution of ^{14}C in the seedlings as a function

of time, is given as the dpm in root, fruit or shoot parts (Fig. 44a) and as percentage dpm in root, fruit or shoot (Fig. 44b). Over 85% of the radioactivity taken up remained in the root, even after 24 hours; under 8% accumulated in the shoot and under 7% in the fruit. The uptake of radioactivity by the root increased rapidly with time.

Figure 45 (i) shows the rate of water loss from a control system, containing only water, and a treatment system containing 10^{-6}M ABA. (A system comprised of a petri dish containing $8cm^3$ of solution into which were dipped the primary roots of five seedlings.) The rate of water loss decreased during the experiment, and was generally less in the treatment system, suggesting that 1μM ABA may reduce the rate of water loss, i.e. uptake and/or transpiration, by the seedlings. Figure 45 (ii) shows the change in mean relative water content of control seedlings during the experiment, which fell slightly, suggesting that the experimental conditions favoured excessive transpiration.

Thus these experiments, which though of a limited nature, do show that radioactivity is taken up by primary roots of Zea mays seedlings when supplied with ABA-2-^{14}C solution, but only a small proportion of the uptake is moved out of the root. The uptake of radioactivity may be related to the uptake of water by the root.

FIGURE 43

The uptake of radioactivity by the primary root of a Zea mays seedling, —○—, and the water loss from the experimental system (corrected for surface evaporation), —●—, as a function of time at 25°C in the light. The plotted values for uptake in dpm are the means of fifteen replicates, and the values for water loss are the means of three replicate experimental systems (a 'system' comprises of a petri dish of solution containing five seedlings).

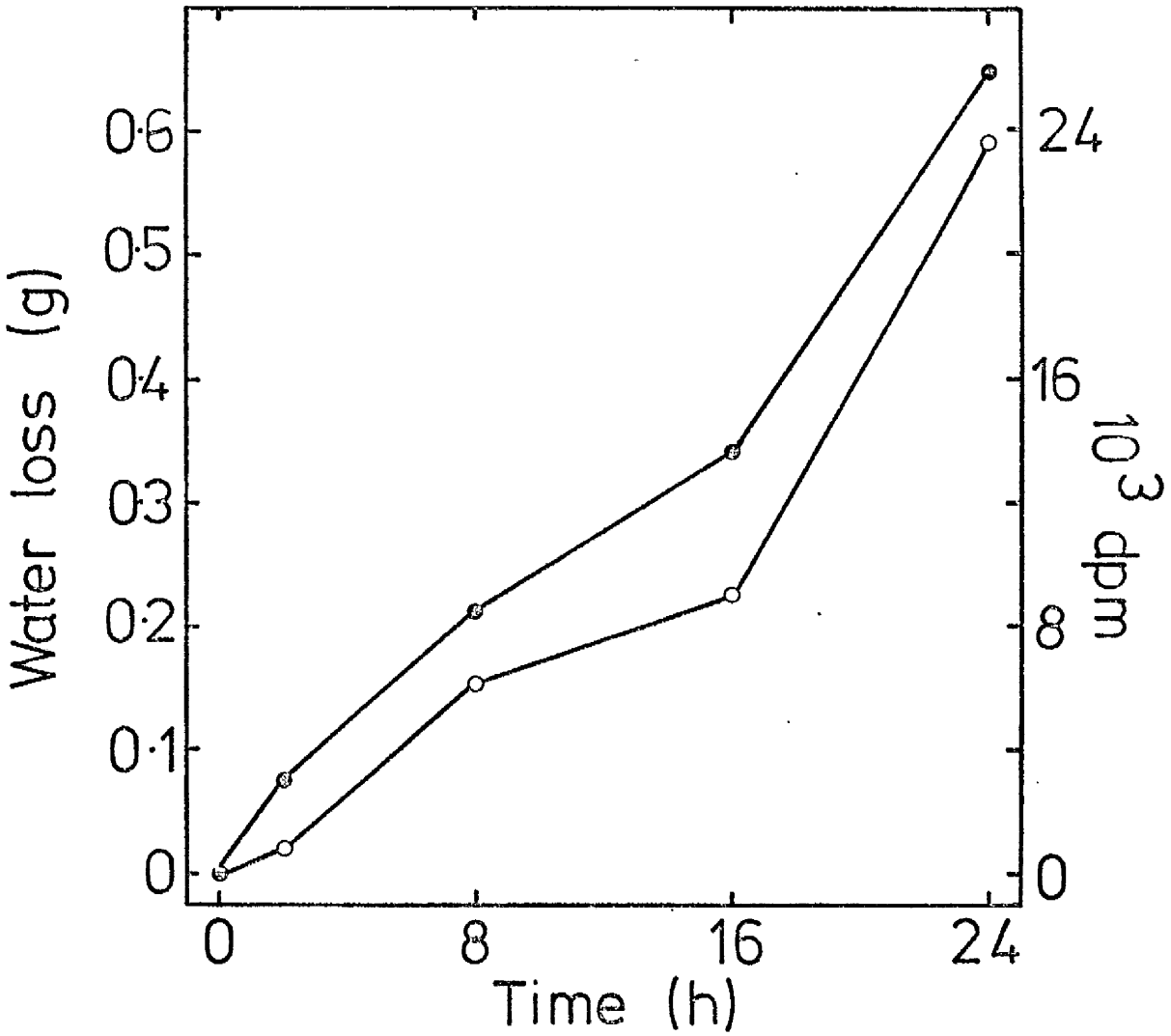

Water loss (g)
0·6
0·5
0·4
0·3
0·2
0·1
0
24
16
8
0
10³ dpm
0
8
16
24
Time (h)

FIGURE 44

(a) The uptake of ^{14}C into the root, —■—, fruit, —▲—, and shoot, —●—, of a Zea mays seedling as a function of time at 25°C in the light, supplied with 1 μM ABA-2-^{14}C solution at the primary root. Plotted values are means of fifteen replicates.

(b) As in Figure 44a., but with the distribution of ^{14}C expressed as a percentage of the total uptake.

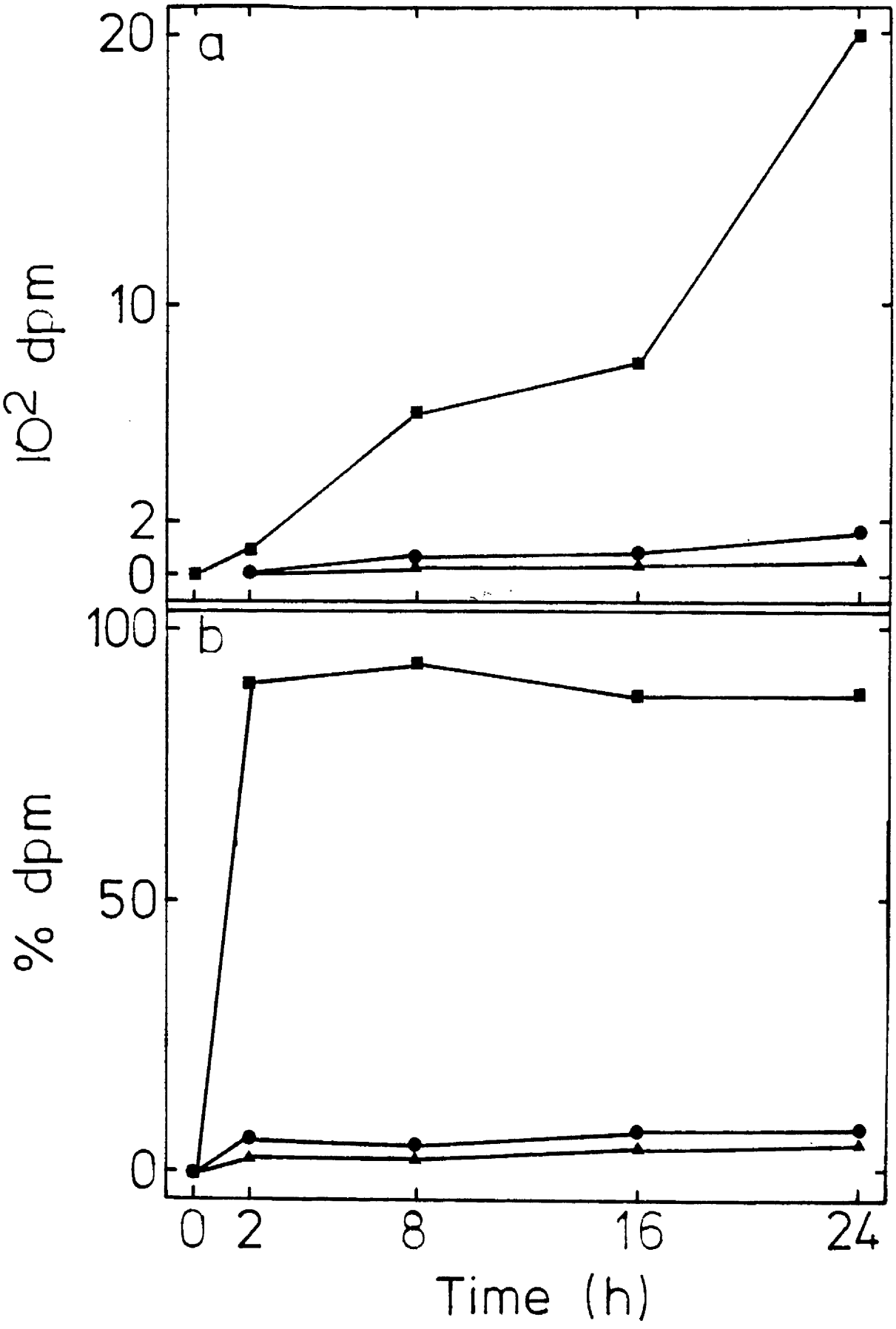
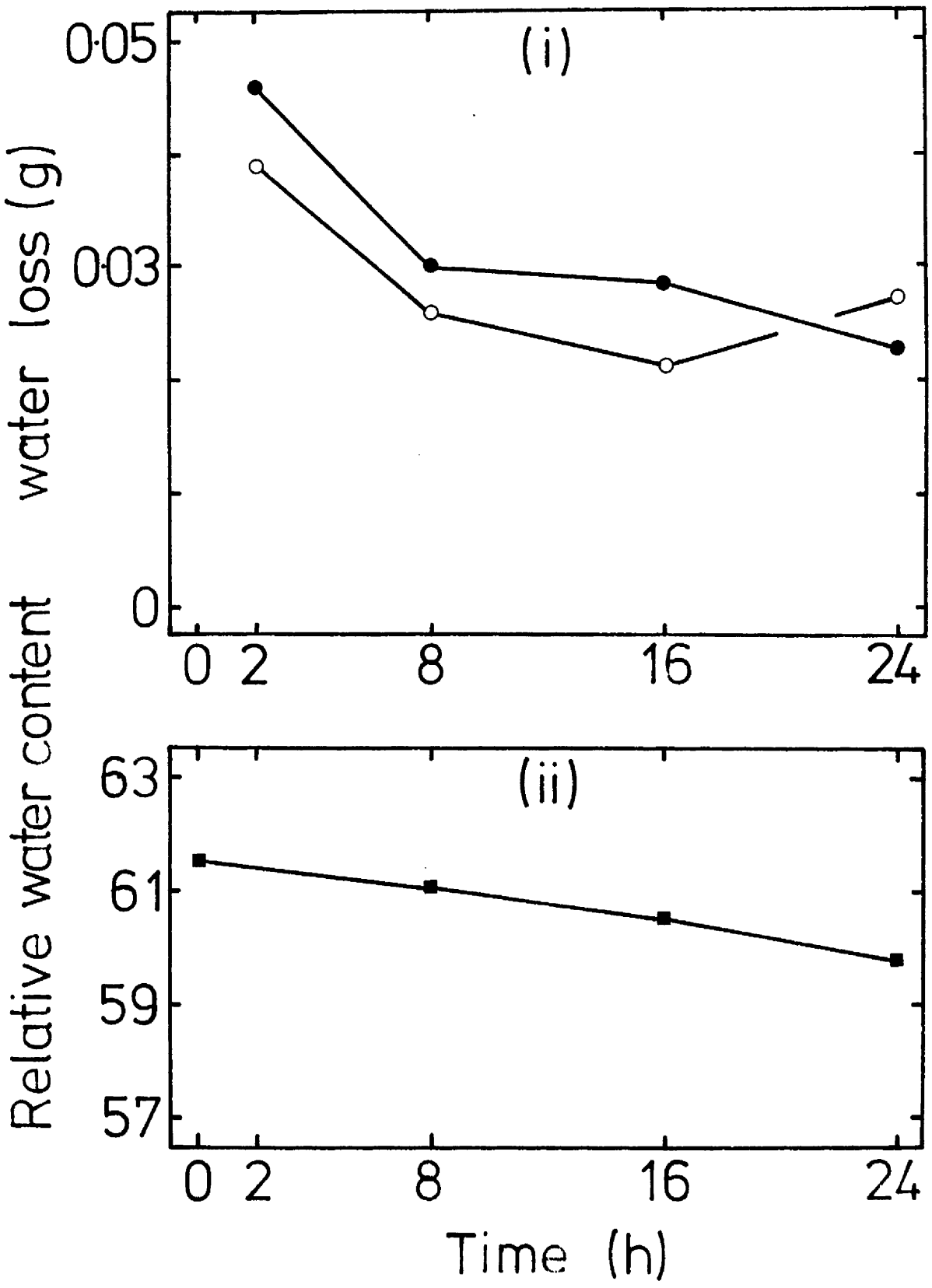

a
b
20
10
2
0
100
50
0
10^2 dpm
% dpm
0
2
8
16
24
Time (h)

FIGURE 45

(i) Rate of water loss from a system containing water, ——●——, and a system containing 1 μMABA-2-^{14}C solution, ——○——, as a function of time at 25°C in the light. Plotted values are means of three replicates.

(ii) The relative water content (percentage) of a control seedling as a function of time at 25°C in the light. Plotted values are means of five replicates.


(i)
0·05
0·03
0
water loss (g)
0 2 8 16 24
(ii)
63
61
59
57
Relative water content
0 2 8 16 24
Time (h)

SECTION II Studies on Phaseolus vulgaris

IIA Movement and metabolism of ABA-2-^{14}C in Phaseolus vulgaris plants

(i) Uptake and movement of ^{14}C in petiole segments.

Section IIA is primarily concerned with the movement of ABA-2-^{14}C in whole plants of P. vulgaris. However, since a relatively detailed study had been made of the movement of ABA-2-^{14}C in isolated segments of Zea mays, it was of interest to carry out a related study in isolated segments of P. vulgaris. Also information gained from the use of isolated segments might enable movement of ABA-2-^{14}C in whole plants to be more clearly understood. In whole plants one intention was to examine the export of ABA-2-^{14}C from leaves. Information on the movement of ABA-2-^{14}C through petioles was considered to be relevant to the later study on the export from leaves. For these reasons it was decided to study the movement of ABA-2-^{14}C through petiole segments of P. vulgaris.

Segments 10mm in length, were excised from the middle regions of primary leaf petioles on three week-old P. vulgaris plants. The segments were set up in a conventional donor block - tissue - receiver block system, in groups of five, at 25^{o}C in the light. At the end of a transport period the segments were halved laterally, and the tissue halves, donor blocks and receiver blocks were prepared for radioassay using liquid scintillation spectrometry. To retain the normal segment terminology the distal end of the petiole segment was termed the apical end, the proximal end was termed the basal end, and the terms acropetal and basipetal were used accordingly. 5μM ABA-2-^{14}C was supplied in the agar donor blocks. There was no significant difference between apical and

basal uptake over 6 hours (Fig. 46a). Uptake was more rapid than in coleoptile segments (cf. Fig. 1), especially over the first hour.

Movement of ^{14}C through the tissue was also considered. Although some radioactivity accumulated in the receiver blocks after the longer time period, certainly more so than with coleoptiles, there was greater variation between replicates, and the amount in the blocks did not increase linearly with time. It is possible that greater replication may have yielded meaningful results, but this was not attempted. Instead, accumulation in the tissue was studied and Figure 46b shows the accumulation of ^{14}C into the tissue halves furthest from the donor; i.e. movement through 5mm of tissue into 5mm of 'receiving' tissue. Radioactivity was detected in this receiving tissue after only 20 minutes, and accumulated rapidly after one hour. There was no significant difference between acropetal and basipetal accumulation after six hours. Extrapolating the linear portions of the graphs back to the time axis gives rates of movement of ^{14}C in excess of $5mmh^{-1}$ in both directions, a rate considerably higher than that in coleoptile segments. When expressed as a percentage of the total uptake, the accumulation in the tissue halves furthest from the donors still shows no clear polarity (Fig. 46c).

Thus, the movement of ^{14}C in petiole segments of P. vulgaris supplied with ABA-2-^{14}C was different to that in coleoptile segments of Zea mays. Both uptake and movement occurred at a faster rate over the time periods considered and there was no clear polarity of ^{14}C movement.

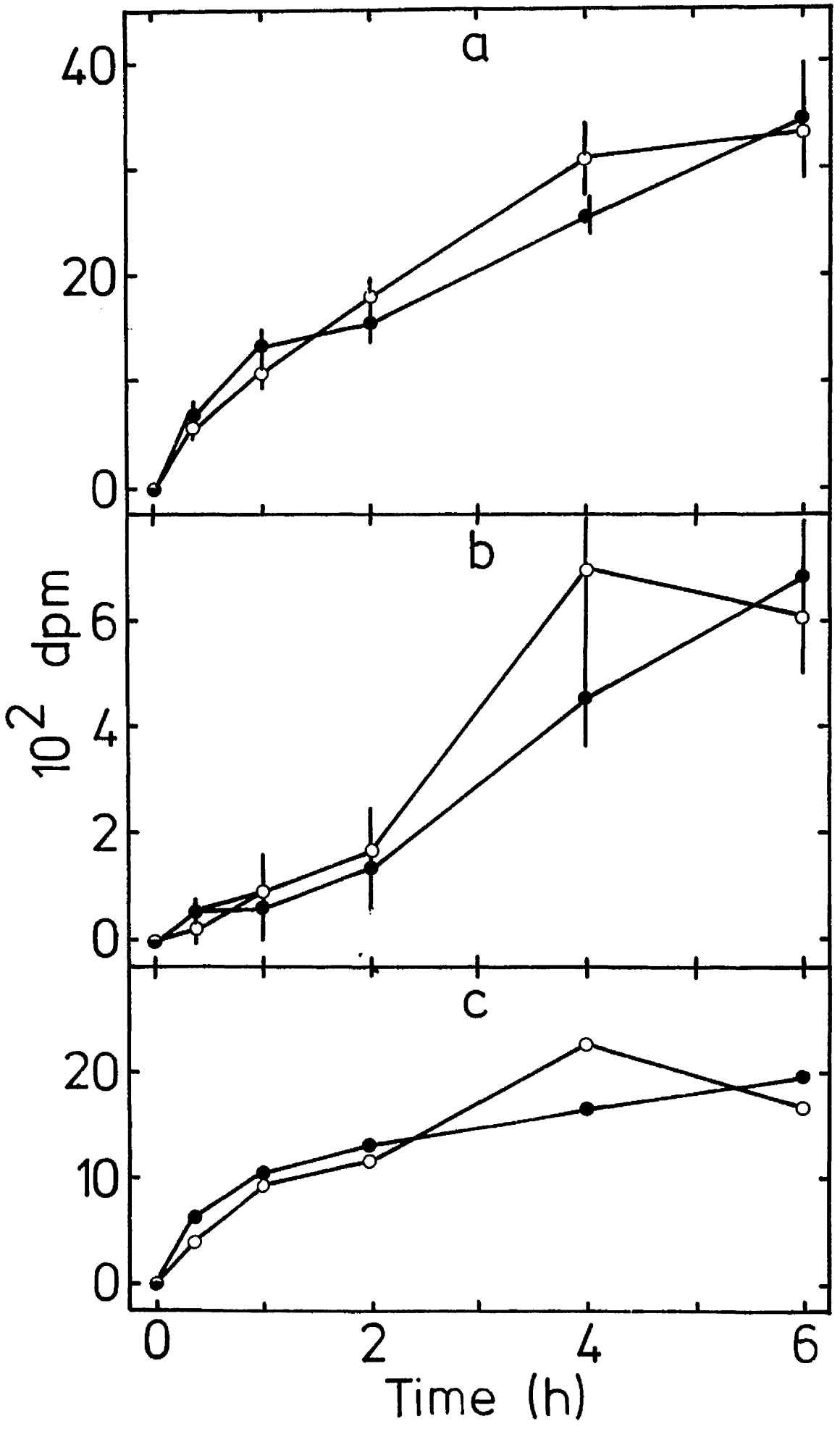
FIGURE 46

The uptake and movement of ^{14}C in 10mm petiole segments of P. vulgaris supplied with donor blocks containing 5 μMABA-2-^{14}C at 25°C in the light as a function of time. The plotted values are means of fifteen replicates, twice the standard errors given as vertical bars.

(a) Basal, —●— , and apical, —○— , uptake of ^{14}C by petiole segments.

(b) Basipetal, —●— , and acropetal, —○— accumulation of ^{14}C in the tissue halves furthest from the donors.

(c) Basipetal, —●— , and acropetal, —○— , accumulation of ^{14}C in the tissue halves furthest from the donors, expressed as a percentage of the total uptake.


a
b
c
40
20
0
6
4
2
0
20
10
0
10^2 dpm
0
2
4
6
Time (h)

(ii) A comparison of four methods of application of ABA-2-^{14}C to a primary leaf of P. vulgaris.

As a first step in the investigation of ABA-2-^{14}C movement in whole P. vulgaris plants, a comparison was made of four application methods. This was carried out to assist in the selection of the most suitable method for use in the subsequent series of experiments. Selection was based on the following criteria.

1

(a) Interest was mainly centred on the export of abscisic acid from leaves and thus only methods of foliar application were considered.

(b) The method must allow efficient uptake; i.e. as high a proportion of radioactivity applied as possible must be taken up. The concentration of ABA-2-^{14}C that could be applied in aqueous solution to a small area of leaf was limited for physiological and practical reasons. Thus efficient uptake was necessary to give measureable and meaningful levels of radioactivity in the tissue away from the donor area.

(c) The method must cause the least possible damage to the plant.

(d) The uptake allowed by the method must be as reproducible as possible.

(e) The radioactivity which is not taken up over the time period considered must be readily recoverable and accounted for.

(f) The precise area of application must be known in order to study distribution of radioactivity in the leaf of application itself. Hence, spray application methods were eliminated.

(g) The method must be relatively simple and quick to carry out to lessen the possible effects of excessive handling of the plants.

The four methods chosen for comparison, carried out as described in the Methods section, are shown in Table 4. Approximately 10,000 dpm were supplied to two week-old plants by each method and plants were harvested after 24 hours at 25°C, 16h photoperiod.

Table 4. A comparison of four methods of application of ABA-2-^{14}C to a primary leaf of two week-old P. vulgaris plants. Approximately 10,000 dpm were applied in each method and the plants harvested after one day at 25°C, 16 hour photoperiod.

Method	ABA-2-^{14}C Conc.	Uptake	% transported	% Distribution Shoot above	Donor	Shoot below	Root
1. Agar block to unabraded leaf	5 μM	305	33	48	25	10	17
2. Agar block to abraded leaf	5 μM	6603	29.4	52	18	12	17
3. Capillary tube into mid-rib	10^{-5}M	1041	68.8	65.3	20	6.7	8.0
4. Injection into mid-rib base	10^{-5}M	1508	62.3	15.3	78.9	2.3	3.5

Method 2 allowed the greatest uptake by the plant; over 66% of the applied radioactivity was taken up and nearly 30% of that uptake moved away from the area of application. Without abrasion (Method 1) uptake was very much lower, suggesting that an intact cuticle was a considerable barrier to uptake. Methods 3 and 4 were less efficient in terms of uptake than Method 2, but a far higher proportion of the radioactivity taken up was transported away from the area of application (68.8% and 62.3% respectively). Using the last two methods, especially method 4, it was difficult to assess the amount of ^{14}C that was not taken up into the tissue. Considerable evaporation of the application solution occurred from the leaf surface surrounding the point of application, probably depositing solid ABA-2-^{14}C, and making subsequent recovery more difficult. This may have been the reason for the high value for the percentage of radioactivity remaining in the donor leaf after 24 hours. Using the agar block method, although the block did dry out slightly during the experiment, it could be easily removed for radioassay at the end of the experiment. The pattern of percentage distribution of ^{14}C in the plant after 24 hours was similar for each method except method 4, where a high proportion of radioactivity remained in the donor leaf (see above). Apart from that result, the greatest % dpm always appeared in the part of the shoot above the leaf of application, and smaller proportions were recovered from the lower parts of the shoot and roots.

The criteria given above are best met by method 2, viz: efficient uptake, donor block easily recoverable, little damage caused to the plant, donor area known precisely, relatively quick and simple. Reproducibility was a problem, the degree of abrasion

being difficult to standardise. Too much abrasion and extensive damage would occur to the underlying cells; too little and the uptake would be impeded. By trial and error and standardising procedures as far as possible, the problem of variability could be partially overcome. Instead of using abrasion, a surface active agent could have been added to the donor blocks to improve penetration, but this was not attempted. A particular advantage of method 2 was that a relatively low concentration of ABA-2-^{14}C (5μM) was applied to a relatively large surface area of tissue; the radioactivity may have moved more naturally into the transport system(s). In methods 3 and 4 however, a higher concentration (10^{-5}M) was applied to a small region of damaged tissue, possibly causing adverse physiological effects, and the radioactivity may have been introduced artificially into the transport system(s).

Thus for the remaining series of experiments method 2 was used; i.e. application of an agar donor block containing 5μM ABA-2-^{14}C to a lightly abraded area, adjacent to the mid-rib of a primary leaf of P. vulgaris. The block was always placed at a standard distance from the leaf base. To reduce drying out of the block during the transport period it was covered with a small sheet of transparent polythene.

(iii) Distribution of ^{14}C in P. vulgaris following foliar application of ABA-2-^{14}C.

To study the movement of ABA-2-^{14}C in whole plants of P. vulgaris, Method 2 (see above) was used for the application of ABA-2-^{14}C to a primary leaf, or the first trifoliate leaf, of 15 and 24 day-old plants. The plants were harvested for radioassay after 24 and 48 hours at 25°C, 16 hour photoperiod. The distribution of ^{14}C was expressed as a percentage of the total ^{14}C moved away from the area of application, or as dpm g^{-1} (Table 5). Some of this work has already been reported (Hocking etal., 1972).

Twenty-four hours after primary leaf application to a 15 day-old plant, which had at least one expanding trifoliate leaf, over 58% of the radioactivity was recovered from the shoot above the donor leaf, mostly in the expanding leaflets. Only 10% remained in the donor leaf away from the area of application. 16.4% of the ^{14}C was recovered in the roots; with plants of this age there were no developed root nodules. On a fresh weight basis the accumulation of radioactivity in the upper shoot was more than ten times greater than in any other part of the plant. This result shows that the radioactivity was exported from the donor leaf to regions of active growth, notably the expanding trifoliate leaves (White, pers. comm).

Twenty-four hours after the application of ABA-2-^{14}C to the primary leaf of 24 day-old plants, a smaller proportion of ^{14}C (26.5%) was recovered from the upper shoot than from 24 day-old plants; very little ^{14}C accumulated in the first trifoliate leaf, which was now fully expanded. Over 30% of the ^{14}C was recovered from

Table 5. The distribution of radioactivity in 15 day-old and 24 day-old plants of *P. vulgaris* following application of 5×10^{-6} M ABA-2-^{14}C to a primary leaf. Means of ten replicates, S.E's calculated using angular transformation.

		Shoot Above	Donor Leaf	Shoot Below	Roots	Nodules
(a) Primary leaf application						
15 day-old						
24h	% dpm	58.6	10.4	15.2	16.4	-
		±2.4	±2.5	±1.3	±3.5	
	dpm g^{-1}	2224	-	213	70	-
24 day-old						
24h	% dpm	26.5	34.0	10.8	12.7	18.1
		±3.4	±3.1	±2.1	±1.6	±3.0
	dpm g^{-1}	28	82	59	53	442
48h	% dpm	49.1	18.6	12.8	7.5	11.7
		±7.4	±7.7	±4.6	±0.5	±2.6
(b) First trifoliate leaf application						
15 day-old						
24h	% dpm	0.16	98.33	0.44	1.07	
24 day-old						
24h	% dpm	73.2	7.2	5.5	7.1	6.9

the root system, 18% from the nodules alone, which were increasing in number and size in plants of this age. On fresh weight basis the accumulation in the nodules was very marked. After 48 hours, proportionately less radioactivity was recovered from the donor leaf and root system and nearly 50% was recovered from the upper shoot. Thus, while initially a high proportion of the ^{14}C taken up was exported from the donor leaf towards the root system, after a longer time period some of the radioactivity may have been redistributed to the growing regions of the shoot.

The distributions of ^{14}C 24 hours after application of ABA-2-^{14}C to the first trifoliate leaf of 15 day-old and 24 day-old plants were very different (Table 5 (b)). In the younger plants over 98% of the ^{14}C remained in the donor leaf, whereas in the 24 day-old plants only 7.2% was recovered from the donor leaf, and 73.2% was recovered from the upper parts of the shoot. In the 15 day-old plants the first trifoliate leaf was still expanding and there was probably a net influx of material into the leaf; thus it is possibly not surprising that there was little export of ^{14}C. However, in the 24 day-old plants the leaf was full expanded and probably exporting assimilates. This result, therefore, suggests that the radioactivity may have been moving with the assimilate flow towards regions of active growth. This possibility was investigated further by the use of steam-girdling techniques.

(iv) The effects of steam-girdling the stem of P. vulgaris and of prolonged darkness on ^{14}C distribution.

The pattern of distribution of applied ABA-2-^{14}C in Phaseolus vulgaris plants reported in the previous section, represents the movement of ^{14}C from an exporting leaf to a number of different growth centres. Since this movement may depend on the continued translocation of sugars in the phloem, a study was made of the effect of steam-girdling the stem, above and below the leaf of application, on the distribution of ^{14}C in 24 day-old plants (Table 6). The stem was steam-girdled 24 hours before the application of ABA-2-^{14}C to a primary leaf. Steam-girdling the stem below the leaf of application totally abolished the movement of radioactivity to parts of the plant below the girdle, while movement of radioactivity past a girdle above the leaf of application was markedly reduced. For a week after such treatment the leaves above the steam-girdle remained fully turgid. These results suggest that the movement of ^{14}C in P. vulgaris, supplied with ABA-2-^{14}C to the primary leaf, depends on the living cells in the tissues and is possibly associated with the translocation of sugars in the phloem.

The possible relationship between the movement of assimilate and of an applied substance can be further clarified by investigating the effects of maintaining the plants in prolonged darkness before and during the experiment. In prolonged darkness, photosynthesis will cease and the export of photosynthetic assimilate should decrease and eventually stop, as the supply is exhausted.

Table 6. The effect of steam-girdling on the movement of radioactivity in P. vulgaris following the application of 5×10^{-6}M ABA-2-^{14}C to a primary leaf. Means of 5 replicates.

	% dpm moving past steam-girdle	
	24 hours	48 hours
Girdle above leaf of application	1.0	4.5
Control*	20.0	30.0
Girdle below leaf of application	0	0
Control*	27.0	24.0

* Estimated dpm moving past the area of stem where the girdle was applied in treated plants.

Plants were kept in darkness at 25°C for 2.5 days prior to the application of ABA-2-^{14}C to a primary leaf. The distribution of ^{14}C in these plants after 24 hours was compared to that in control plants maintained in a 16 hour photoperiod (Table 7). The total recovery of ^{14}C, as a percentage of that applied, was greater from the plants maintained in darkness, 90.5%, as compared with 79.4% for the controls. This could be interpreted as more radioactivity being lost, perhaps as $^{14}CO_2$, in the light than in darkness. However, uptake is significantly (to 0.001P) reduced in dark-treated plants compared to the controls, and the difference in recovery may be explained in terms of a more efficient extraction from an agar donor block, than from tissue. The very highly significant effect

Table 7. The effect of prolonged darkness on the movement of radioactivity in P. vulgaris plants kept in darkness at 25°C for 2.5 days prior to application of 5×10^{-6}M ABA-2-^{14}C to a primary leaf. Plants harvested after 24 hours transport in the dark. Controls carried out in the light in the usual way.

	% dpm			
	% (of total applied ^{14}C) recovered.	% (of total applied ^{14}C) taken up.	% (of uptake) moved away from donor area (% transported)	% (of transported ^{14}C) moved out of donor leaf.
Light	79.4	78.4±5.8	21.5±1.6	76.6±3.5
Darkness	90.5	27.0±4.0	75.8±2.8	0.05±0.03
't' value for difference	-	6.105	18.3	13.90
Significance level.	-	0.001	0.001	0.001

of light on uptake suggests that some stage of the uptake process may be light dependent. Of the radioactivity taken up, very little (0.05%) was exported from the donor leaf in dark-treated plants compared with the controls (76.6%). This difference which was significant to 0.001P, suggests that the export of ^{14}C from the donor leaf supplied with ABA-2-^{14}C may be very clearly related to the continued export of photosynthetic assimilates in the phloem. This is on the assumption that export of assimilates had ceased after 2.5 days in darkness, although no direct evidence of this was sought.

At the same time the distribution of radioactivity throughout the donor leaf itself, away from the area of application, was not inhibited in dark-treated plants. 75.8% of the uptake was distributed throughout the leaf away from the donor area in dark-treated plants compared with 21.5% in control plants. The magnitude of this difference may be partly a reflection of the difference in uptake and the method of data presentation. The area of application in the control plants had a far greater loading of radioactivity than in dark-treated plants and so the actual amount of ^{14}C moved away, as opposed to the percentage, was similar in both cases.

Thus, to summarize the most important results from these experiments, both the effects of steam-girdling the stem and of maintaining the plants in darkness suggest that the export of ^{14}C from leaves of P. vulgaris supplied with ABA-2-^{14}C depends on the continued translocation of assimilates, possibly in the phloem.

(v) A comparison of ^{14}C distributions following application of IAA-1-^{14}C, IAA-2-^{14}C, sucrose-^{14}C, ABA-2-^{14}C to a primary leaf or a trifoliate leaf of P. vulgaris.

A comparison of the movement of abscisic acid with other growth substances or natural assimilates would aim to show whether the movement of exogenous ABA in P. vulgaris possessed unique characteristics or was basically similar to the movement of a hormone such as IAA or an applied translocatable assimilate such as sucrose. Such information would be important in any assessment of the physiological significance of the movement of ABA-2-^{14}C in P. vulgaris.

ABA-2-^{14}C, IAA-1-^{14}C, IAA-2-^{14}C and Sucrose-^{14}C were applied in the usual way to a primary leaf or the first trifoliate leaf of fifteen day-old plants of P. vulgaris. The plants were harvested for radioassay after 24 hours at 25°C, 16h photoperiod. The total radioactivity applied in each case was known, and the recovery, uptake and distribution of ^{14}C were compared (Table 8). Recovery of 95% ethanol-soluble radioactivity varied considerably following application of the tracers to a primary leaf, but was less variable following trifoliate leaf application. Recovery of ^{14}C from ABA-2-^{14}C was greatest in both cases (79.4 and 65.2% respectively) when compared to the other tracers. In one case (sucrose-^{14}C to a primary leaf) recovery was less than 50%. These low recoveries made the data more difficult to interpret since the nature of the losses was not fully understood. It is probable that the use of a more sophisticated extraction technique could have improved the recoveries. If most of the loss were due to inefficient extraction which was relatively constant for any one tracer then this problem may not be too serious, but must always be borne in mind.

Table 8. A comparison of ^{14}C uptake in P. vulgaris 24 hours after application of ABA-2-^{14}C, IAA-1-^{14}C, IAA-2-^{14}C or Sucrose-^{14}C to (a) a fully expanded primary leaf (b) an expanding trifoliate leaf. Means of 10 fifteen day-old plants.

Substance.	Total ^{14}C applied.	% (of total ^{14}C) recovered.	% (of total applied ^{14}C) taken up.	% (of uptake) moved away from donor area (% transported).	% (of transported ^{14}C) moved out of donor leaf.
(a) Primary leaf					
ABA-2-^{14}C	13609	79.4	78.4	21.5 ± 1.6	76.57 ± 3.5
IAA-1-^{14}C	11398	53.5	53.2	14.31 ± 3.8	51.81 ± 7.8
IAA-2-^{14}C	12185	57.0	54.4	8.1 ± 1.2	60.72 ± 5.9
Sucrose-^{14}C	14411	44.5	43.8	72.0 ± 2.3	90.2 ± 1.52
(b) Trifoliate leaf					
ABA-2-^{14}C	10966	65.2	64.5	46.2 ± 7.8	1.28 ± 0.62
IAA-1-^{14}C	10267	50.1	49.5	35.3 ± 6.8	12.48 ± 2.1
Sucrose-^{14}C	11598	57.7	56.8	35.8 ± 5.1	0.42 ± 0.22

The percentage of the applied dpm taken up in each case was similar but slightly lower than the % recovery showing that nearly all the remaining radioactivity was recovered from the tissue. The values for IAA-1-^{14}C and IAA-2-^{14}C were very similar, showing that decarboxylation of the side chain at the C-1 position was not a problem in this instance. The percentage of the uptake which moved away from the donor area (% transported) showed marked variation with tracer and with point of application. The % transported following ABA-2-^{14}C applications to a primary leaf was half that following trifoliate leaf application and conversely the % transported following sucrose-^{14}C application to a primary leaf was double that following trifoliate leaf application. In both cases IAA was the least mobile compound.

The most interesting comparison, however, was that concerning the percentage of the transported ^{14}C which was moved out of the donor leaf i.e. the % ^{14}C exported (Table 8 column 6 and Table 9). Following primary leaf application, the ^{14}C from sucrose-^{14}C was the most mobile, over 90% being exported from the donor leaf in 24 hours. This was significantly greater (to 0.01P) than the export of ^{14}C following ABA-2-^{14}C application, and these were both significantly greater (to 0.01P) than export of ^{14}C following radioactive IAA application. There was no significant difference between the export of ^{14}C following application of IAA-1-^{14}C or IAA-2-^{14}C. After trifoliate leaf application of ABA-2-^{14}C and sucrose-^{14}C there was very little export of ^{14}C from the donor leaf ($< 1.5\%$). However, there was a significantly (0.001P) greater export of ^{14}C following IAA-1-^{14}C application compared to either ABA-2-^{14}C or sucrose ^{14}C. All three tracers exhibited a significant difference between the export of ^{14}C from a primary leaf and export

Table 9. The radioactivity (in % dpm) moved out of the donor leaf 24 hours after the application of ABA-2-^{14}C, IAA-1-^{14}C, IAA-2-^{14}C or Sucrose-^{14}C to a primary leaf (P) or trifoliate leaf (T) of 15-day-old plants of P. vulgaris. Means of eight or ten plants. Standard errors and 't' values calculated using angular transformation

% dpm moved out of donor leaf

ABA-2-^{14}C		IAA-1-^{14}C		IAA-2-^{14}C		Sucrose-^{14}C	
P	T	P	T	P	T	P	T
76.57	1.28	51.81	12.48	60.72	-	90.2	0.42
±3.5	±0.62	±7.78	±2.1	±5.9	-	±1.52	±0.22

SIGNIFICANCE TESTS

Means compared	't' value	Level of significance (probability)
ABA - IAA-1 (P)	3.03	0.01
ABA - Sucrose (P)	3.71	0.01
IAA-1 - Sucrose (P)	5.61	0.001
IAA-1 - IAA-2 (P)	0.76	Not significant
ABA - IAA-1 (T)	5.20	0.001
ABA - Sucrose (T)	1.32	Not significant
IAA-1 - Sucrose (T)	5.79	0.001
ABA (P) - ABA (T)	24.36	0.001
IAA-1 (P) - IAA-1 (T)	5.57	0.001
Sucrose (P) - Sucrose (T)	67.50	0.001

from a trifoliate leaf, the latter export being much less. Thus there were markedly significant quantitative differences between tracers in their export of radioactivity. Following primary leaf application ^{14}C from sucrose-^{14}C was the most mobile, while following trifoliate leaf application ^{14}C from IAA-1-^{14}C was the most mobile. In general, the

export of ^{14}C from a small, rapidly expanding leaflet of a trifoliate leaf of P. vulgaris (the average length of the donor leaflet increased by 9.8mm during the experiment) was very much lower than the export from a mature primary leaf.

The distribution of the transported ^{14}C 24 hours after primary leaf application of the tracers is shown in Table 10 (a). There were marked differences in the patterns of distribution of the three tracers. In addition to the differences in the donor leaf, the proportion of ^{14}C in the shoot above the donor leaf following ABA-2-^{14}C application was considerably greater than for either sucrose-^{14}C or radioactively-labelled IAA. Conversely, the proportion of ^{14}C in the roots was least following ABA-2-^{14}C, greater for IAA-2-^{14}C and IAA-1-^{14}C and greatest following sucrose-^{14}C application. Overall, over 50% of the transported ^{14}C moved above the donor leaf following ABA-2-^{14}C application and only 26% moved below; i.e. there was a net acropetal export; while following sucrose-^{14}C application over 50% ^{14}C moved down the plant and only 35% moved above the donor leaf, i.e. there was a net basipetal export.

Table 10 (b) shows the distribution of ^{14}C following trifoliate leaf application. Whereas more than 98.5% of the ^{14}C from ABA-2-^{14}C and Sucrose-^{14}C remained in the donor leaf, over 12% of the ^{14}C from IAA-1-^{14}C was exported basipetally and 1.3% moved acropetally. This suggested a distinctive basipetal polarity for the export of ^{14}C from IAA-1-^{14}C applied to an expanding trifoliate leaf of a 15 day-old bean plant.

Table 10. A comparison of ^{14}C distribution in P. vulgaris, 24 hours after application of ABA-2-^{14}C, IAA-1-^{14}C, IAA-2-^{14}C or Sucrose-^{14}C to (a) a fully expanded primary leaf (b) and expanding trifoliate leaf. Means of 10 fifteen-day-old plants.

Substance	Shoot above		Donor leaf		Shoot below		Roots	
	% dpm	$dpmg^{-1}$	% dpm	$dpmg^{-1}$	%dpm	$dpmg^{-1}$	% dpm	$dpmg^{-1}$
(a) Primary leaf								
ABA-2-^{14}C	50.6 ± 2.9	2780 ± 371	23.5 ± 3.5	-	12.0 ± 0.8	226 ± 35	13.3 ± 1.6	108 ± 16.3
IAA-1-^{14}C	14.5 ± 4.5	167 ± 29	48.1 ± 7.8	-	16.8 ± 3.1	96 ± 28	20.3 ± 4.6	41 ± 3.8
IAA-2-^{14}C	20.4 ± 1.7	218 ± 56	39.2 ± 5.9	-	17.0 ± 1.5	75 ± 17	23.2 ± 3.4	36 ± 5
Sucrose-^{14}C	35.5 ± 4.5	3272 ± 217	9.83 ± 1.5	-	21.4 ± 3.3	837 ± 181	33.1 ± 2.8	520 ± 54
(b) Trifoliate leaf								
ABA-2-^{14}C	0.08 ± 0.07	3.4 ± 3.0	98.7 ± 0.62	-	0.33 ± 0.2	17 ± 15	0.84 ± 0.5	9.9 ± 9.7
IAA-1-^{14}C	1.29 ± 0.4	250 ± 115	86.5 ± 2.5	-	6.04 ± 1.5	88.0 ± 26.1	6.19 ± 1.9	25.4 ± 6.2
Sucrose-^{14}C	0.05 ± 0.04	-	99.56 ± 0.2	-	0.06 ± 0.05	3.9 ± 2.8	0.41 ± 0.25	5.2 ± 3.1

(vi) The effects of steam-girdling the stem of P. vulgaris on the ^{14}C distributions of four tracers applied to a primary leaf.

To complete the comparison of the movement of ABA-2-^{14}C, Sucrose-^{14}C and ^{14}C - labelled IAA in P. vulgaris, the effects of steam-girdling the stem were considered. However, instead of studying the distribution over the whole plant, only the effects of steam-girdling on the accumulation of ^{14}C in root nodules of 24 day-old plants were investigated. This approach not only provides a reasonable indication of the effects of steam-girdling but also allows accumulation of radioactivity in these structures to be studied further.

Twenty-four day-old plants were steam-girdled at the lower part of the stem, just above the cotyledonary node, 24 hours prior to the application of the tracer. After a 4 day transport period for both treated and non-girdled control plants the nodules on each root system were harvested, weighed and radioassayed. The accumulation of ^{14}C, on a fresh weight basis, in the nodules on control plants over 4 days is shown in Figure 47(a). The accumulation of ^{14}C from ABA-2-^{14}C was significantly (0.001P) greater than ^{14}C from Sucrose-^{14}C, which in turn was significantly (0.001P) greater than the accumulation of ^{14}C from IAA-^{14}C. Steam girdling prevented this accumulation of ^{14}C in each case almost completely (Fig. 47 (b)). The differences between steam-girdled and control plants were all significant (0.001P). These results show that the movement of ^{14}C from ABA-2-^{14}C, Sucrose-^{14}C or ^{14}C - IAA, applied to the primary leaf, into root nodules of P. vulgaris is dependent in all cases, on the living cells in the stem tissue. Moreover, in view of the fact that the

amounts of radioactivity applied for each tracer were similar, the results suggest that root nodules selectively accumulate ^{14}C from ABA-2-^{14}C to a greater extent that ^{14}C from Sucrose - ^{14}C and to a much greater extent than ^{14}C from IAA-^{14}C.

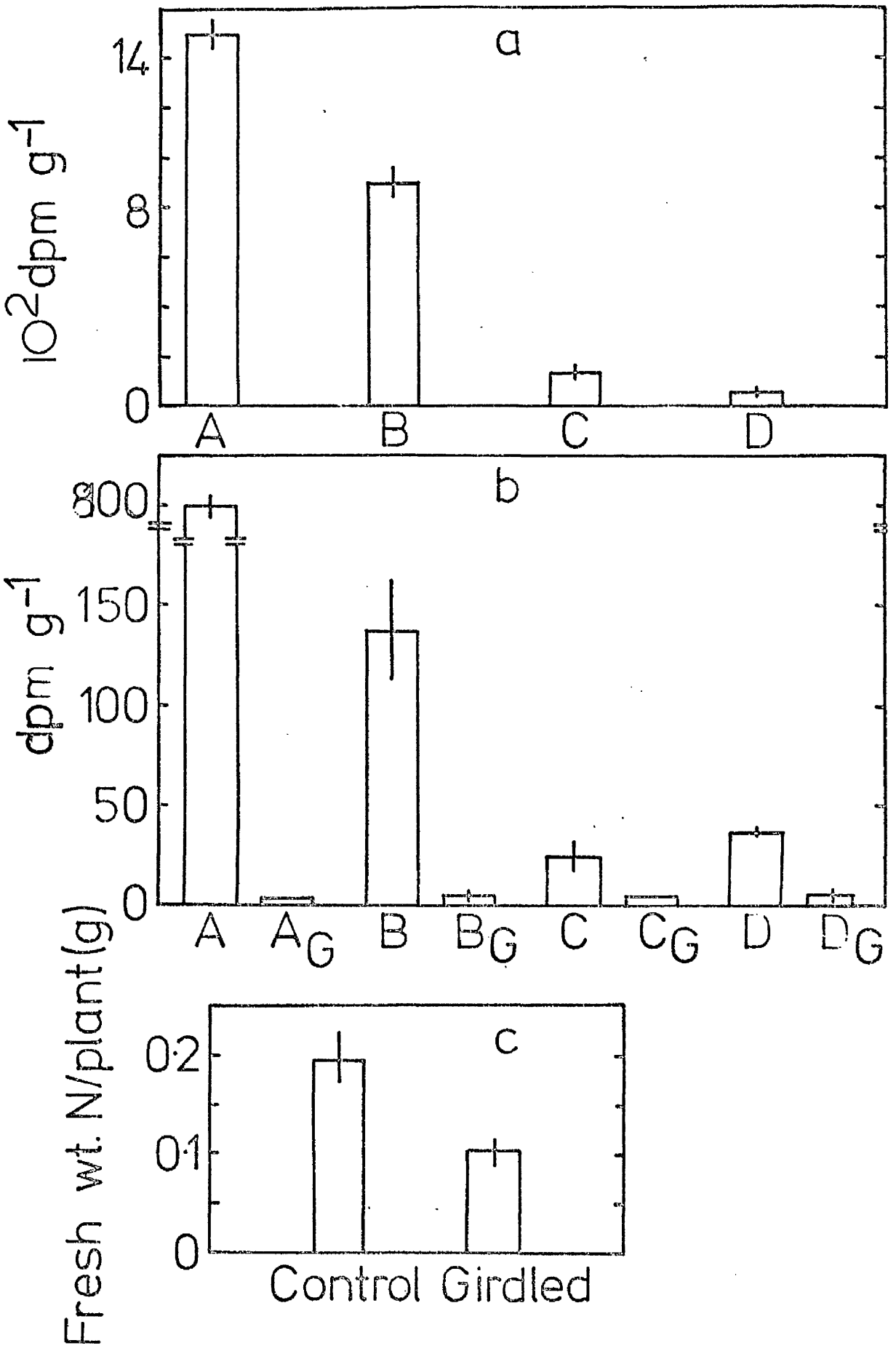
Figure 47(c) shows the effect of steam-girdling the stem on root nodule production in a bean. The fresh weight of nodules plant^{-1} was significantly (0.001P) reduced in girdled plants over 4 days. This suggests that the continued flow of assimilates from the shoot is necessary for normal nodule production.

FIGURE 47

(a) The accumulation (10^2 x dpm g^{-1}) of radioactivity in root nodules of 24 day-old P. vulgaris plants, 4 days after application of; A ABA-2-^{14}C, B Sucrose-^{14}C, C IAA-1-^{14}C, D IAA-2-^{14}C. Histograms show the means of ten replicates, vertical bars representing twice the S.E's.

(b) The effect of steam-girdling the stem (indicated by the suffix G) on the accumulation of ^{14}C in root nodules of P. vulgaris plants, 4 days after application of the tracers, as above.

(c) The effect of steam-girdling the stem on the production of root nodules. (Fresh weight nodules $plant^{-1}$). The nodules of control and girdled plants were weighed 4 days after the steam girdles were applied. Means of ten plants, vertical bars representing twice the S.E's.


a
14
8
0
10^2dpm g^{-1}
A
B
C
D
b
800
150
100
50
0
dpm g^{-1}
A
A_G
B
B_G
C
C_G
D
D_G
c
0·2
0·1
0
Fresh wt. N/plant(g)
Control
Girdled

(vi) Metabolism of IAA-1-^{14}C, IAA-2-^{14}C and ABA-2-^{14}C in the whde plants of P. vulgaris.

In previous sections movement in P. vulgaris following tracer application has been discussed only in terms of ^{14}C movement. At some stage in the investigation it was obviously important to determine whether the radioactive carbon was still attached to the original molecule. Therefore, chromatographic analysis of the radioactivity in tissue extracts was carried out.

Agar donor blocks containing IAA-1-^{14}C or IAA-2-^{14}C were applied to primary leaves of 24 day-old plants. After 24 hours in a 16 hour photoperiod at 25°C, the donor blocks were moved and the area under the donor block was removed, extracted in ethanol, and the ethanol extracts analysed on paper chromatograms developed in 5:1:2.2 and radioassayed. This particular tissue was used as it was known to contain a relatively high concentration of radioactivity and could be analysed without extensive extraction and purification procedures. The results from this analysis however may not accurately represent tissue extracts taken from other parts of the plant. For tissue extracts, donor extracts and stock solutions, using IAA-1-^{14}C (Fig. 48) or IAA-2-^{14} (Fig.49), histograms were constructed showing the amount of radioactivity in half Rf zones, as a percentage of the total ^{14}C on the chromatogram.

The peaks of radioactivity in the IAA-1-^{14}C donor block extract and stock solution chromatograms were at Rf 0.90 - 0.95, and over 90% of the radioactivity was recovered between Rf 0.85 - 0.95. For the tissue extract, under 10% of the radioactivity was

recovered at this Rf, and a distinct peak occurred at Rf 0.75 - 0.80. A less distinct peak was found at Rf 0.60 - 0.65.

The main peak of radioactivity in the IAA-2-^{14}C donor block extract and stock solution chromatograms occurred at Rf 0.85 - 0.90 and is therefore similar to IAA-1-^{14}C. In the tissue extract there was no clearly defined peak at this Rf as there was with IAA-1-^{14}C, but nonetheless, about 12% of the ^{14}C was recovered at Rf 0.85 - 0.90. The major peak in the tissue again occurred at Rf 0.75 - 0.80, but the third peak at Rf 0.60 - 0.65 was not so evident. Thus apart from that peak, the results from IAA-1-^{14}C and IAA-2-^{14}C were similar, showing that only a small proportion ($<$ 12%) of the radioactivity recovered in ethanol extracts from the tissue co-chromatographed with stock IAA-1-^{14}C or IAA-2-^{14}C. This suggested that considerable breakdown or metabolism of the IAA molecule had occurred, and it was not limited to the decarboxylation of the side chain. However this result does not exclude the possibility that the smaller amounts of radioactivity that could be detected in tissue away from the donor area were ~~not~~ still as ^{14}C-IAA.

A similar experiment was carried out using ABA-2-^{14}C. Figure 50 (a) shows an autoradiograph of chromatograms of a crude ethanolic tissue extract and a stock solution of ABA-2-^{14}C. Chromatograms of duplicate extracts are shown in histogram form following radioassay by liquid scintillation spectrometry (Fig. 50 b and d, tissue extract and stock ABA-2-^{14}C respectively) together with a donor extract (c). The only major peaks of radioactivity in (c) and (d) were at Rf 0.75 - 0.85 which probably represent ABA-2-^{14}C. Approximately 35% of the

radioactivity in the tissue extract, was recovered at that Rf, and a second peak also occurred at Rf 0.65 - 0.70. These chromatograms were similar to those obtained from ethanolic extracts of coleoptile tissue supplied with ABA-2-^{14}C for 24 hours (cf. Fig. 32). These results suggest that although a substantial proportion of the radioactivity recovered in 95% ethanolic extracts co-chromatographed with ABA-2-^{14}C, there was evidence of considerable labelling of a chromatographically distinct moiety.

This aspect was investigated more thoroughly using purified extracts of tissue taken from different parts of the plant. ABA-2-^{14}C was supplied to the primary leaves of 40 plants which were harvested after 24 hours. They were then divided into a number of parts prior to extraction; viz:-

1. apices
2. first trifoliate leaves
3. second " "
4. donor blocks
5. primary leaves
6. upper stems
7. lower stems
8. primary roots
9. lateral roots

The tissues were extracted in 80% ethanol, reduced to the aqueous phase, partitioned with ether and the ether extracts analysed using paper (Fig. 51) and thin-layer (Fig. 52) chromatograms developed in 2:6:1:2. This simple purification procedure removed a large proportion of the contaminating material, leaving only the ether-soluble component, which should have included ABA. All the extracts were chromatographed on the same sheet of paper or thin-layer plate to make comparisons more accurate.

On paper, the stock solution of ABA-2-^{14}C gave a major peak at Rf 0.7 - 0.8, typical of previous results (cf. Fig. 26). In order to ascertain that impurities in the tissue extracts were

not modifying the Rf characteristics if any ABA-2-^{14}C present, a stock solution was mixed with an unlabelled ethanolic tissue extract. This mixture was passed through the same purification procedure and analysed, giving exactly the same result of one single major peak at Rf 0.7 - 0.8. Extracts 1 and 2 gave a single major peak coinciding with the peak of the stock solution (Fig. 50). Extracts 3, 7, 8 and 9 showed a major peak at Rf 0.6 - 0.7, while extract 6 gave a broad peak at Rf 0.6 - 0.8. Extract 4 co-chromatogrammed with stock ABA-2-^{14}C, showing there was little or no breakdown in the donor blocks. The results from Extracts 3, 6, 7, 8 and 9 suggest that there was substantial breakdown of ABA-2-^{14}C in the tissue away from the donor area, and that most, if not all, of the radioactivity recovered in ethanolic extracts was attached to some moiety chromatographically distinct from ABA-2-^{14}C.

These results for Extracts 1 and 2 are difficult to explain. Analysis of the thin-layer chromatograms suggests that they may be misleading. Extract 2 on thin-layer gave similar results to 3, 5, 7, 8 and 9 (Fig. 52), showing a major peak at Rf 0.5 - 0.6, compared with the stock solution which gave a single major peak at Rf 0.6 - 0.7. Extract 2 however, also gave 25% recovery at Rf 0.6 - 0.7 showing that a substantial proportion of ABA-2-^{14}C was probably present. Extracts 8 and 9 also showed peaks of between 30-40% at Rf 0.6 - 0.7, corresponding to ABA-2-^{14}C, although they had not given corresponding peaks on paper. Extract 6, which showed a broad peak at Rf 0.6 - 0.8 on paper, gave a major peak at 0.6 - 0.7, again corresponding to ABA-2-^{14}C on thin-layer plates.

Overall, chromatography of most of the extracts, either on paper or thin layer plates gave a radioactive peak at 0.1 Rf less than the peak corresponding to ABA-2-^{14}C. The conflicting results may have arisen because the two 'peaks' were close together, such that when the chromatogram was analysed in single Rf zones, they overlapped. If the chromatograms were analysed in more detail these peaks could be separated (cf. Fig. 32, 50). Despite this, some of the extracts, notably 1, 2 and 6, all from the upper parts of the plant did give substantial recoveries of ^{14}C at the same Rf as ABA-2-^{14}C, suggesting that at least some of the radioactivity had been distributed as ABA-2-^{14}C.

In a concluding experiment ethanolic extracts were made of tissue from the donor leaf area of plants supplied with ABA-2-^{14}C for 1 and 5 days. Duplicate extracts were analysed on thin-layer plates developed in 2:6:1:2 and chloroform: methanol: water :: 75:22:3 v/v. Expressed in histogram form (Fig. 53) the stock solution gave a peak at Rf 0.5 - 0.6 with both solvent systems (Fig. 53 a, d,). In the chloroform system however, a second, smaller peak occurred at Rf 0.0 - 0.1. The tissue extract after one day gave a broad peak at Rf 0.3 - 0.5 in 2:6:1:2 (Fig. 53c) and a narrow peak at Rf 0.3 - 0.4 and a smaller peak at Rf 0.0 - 0.1 in 75:22:3 (b). The five day extract gave the same broad peak in 2:6:1:2 (f) and two peaks in 75:22:3, one at Rf 0.3 - 0.4 and another at Rf 0.1 - 0.2 (c). In both tissue extracts using both solvent systems, under 7% of the radioactivity was recovered at the Rf corresponding to ABA-2-^{14}C. Apart from the unidentified peak found using 75:22:3, there was little difference between 1 and 5 day extracts. In general these results suggest that there is substantial metabolism or breakdown of applied ABA-2-^{14}C in P. vulgaris.

FIGURE 48

Chromatographic analysis of (a) stock IAA-1-^{14}C, (b) donor blocks and (c) tissue extracts from P. vulgaris plants maintained under 16 hour photoperiods at 25°C and supplied with IAA-1-^{14}C to a primary leaf for 24 hours. Ethanolic extracts were spotted onto Whatman No. 3 chromatography paper and the chromatograms were developed in n-butanol: acetic acid: distilled water :: 5:1:2.2 v/v. The histograms show the radioactivity recovered from each half - Rf zone of the chromatogram, assayed using liquid scintillation spectrometry, expressed as a percentage of the total recovery.

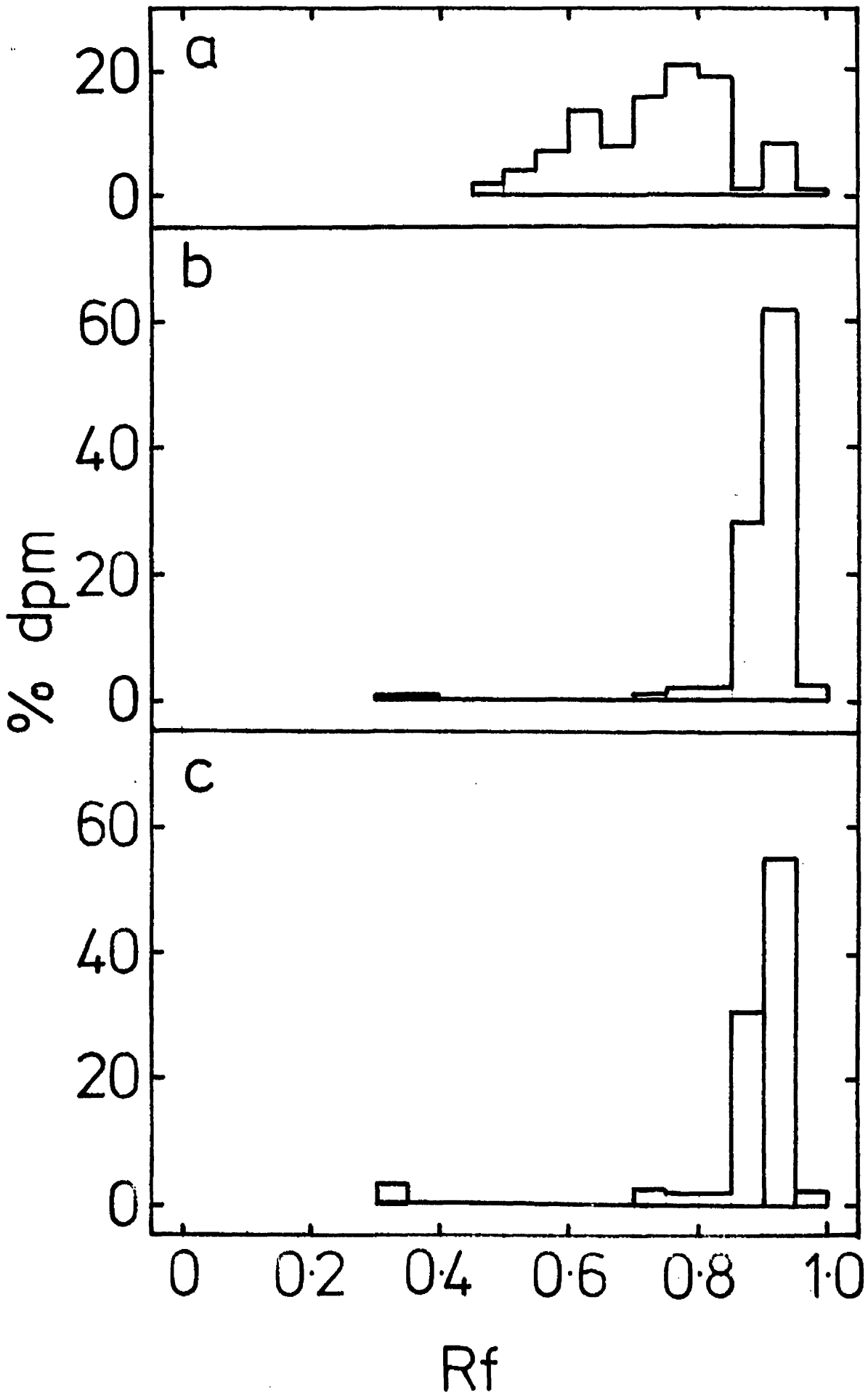

a
b
c
20
0
60
40
20
0
60
40
20
0
% dpm
0
0·2
0·4
0·6
0·8
1·0
Rf

FIGURE 49

As in Figure 48, except that IAA-2-^{14}C was used.

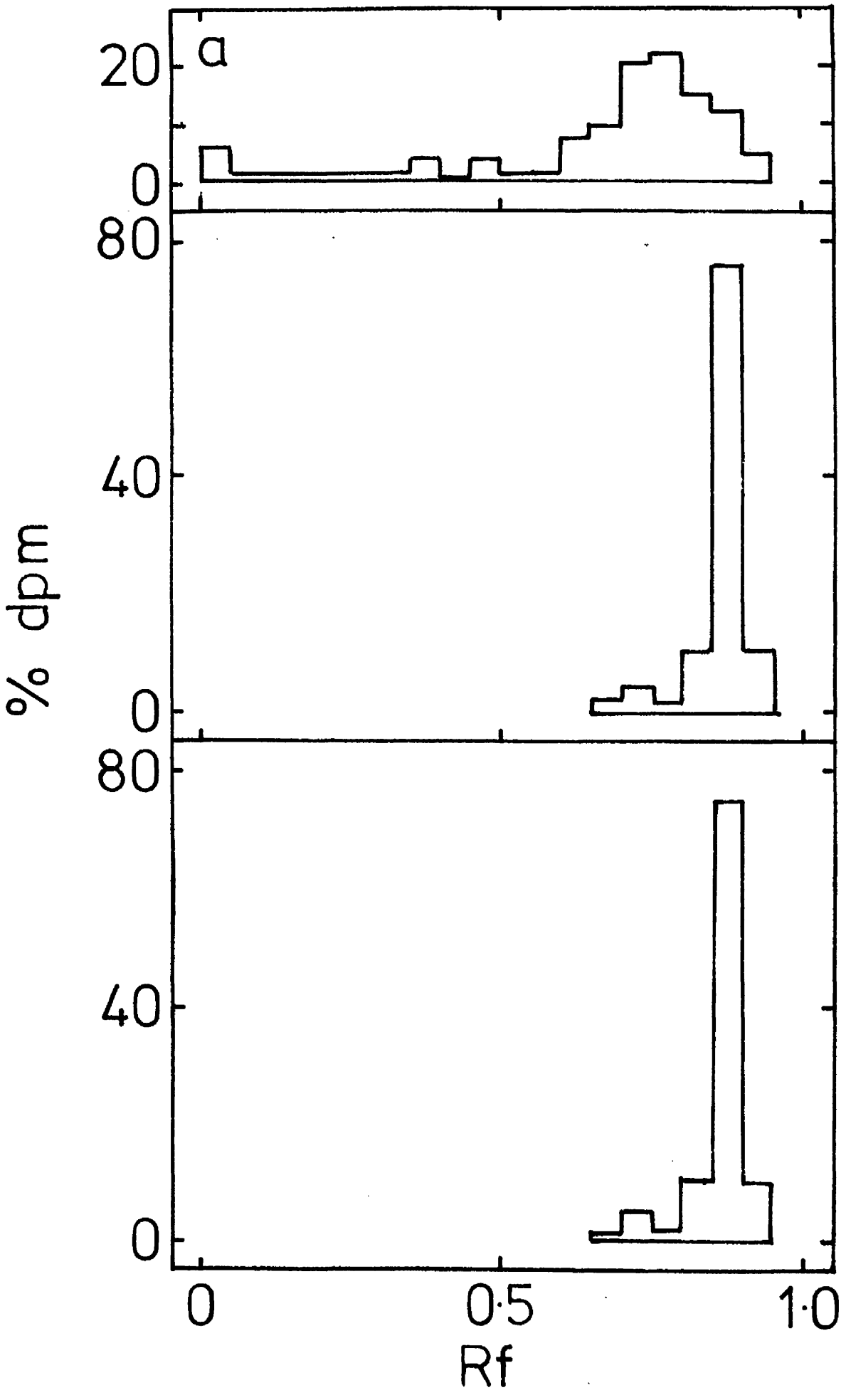

a
20
0
80
40
0
80
40
0
% dpm
0
0·5
1·0
Rf

FIGURE 50

Chromatographic analysis of stock ABA-2-^{14}C (histogram d), donor block extracts (c) and tissue extracts (b) from *P. vulgaris* plants maintained under 16 hour photoperiods at 25°C and supplied with ABA-2-^{14}C to a primary leaf for 24 hours. The extracts were spotted onto Whatman No. 3 chromatography paper, and the chromatograms developed in n-butanol: n-propanol: 0.880 ammonia: distilled water :: 2:6:1:2 v/v. Histograms b, c and d show the radioactivity recovered from each half - Rf zone of the chromatograms, assayed by liquid scintillation spectrometry, expressed as a percentage of the total radioactivity recovered from each chromatogram.

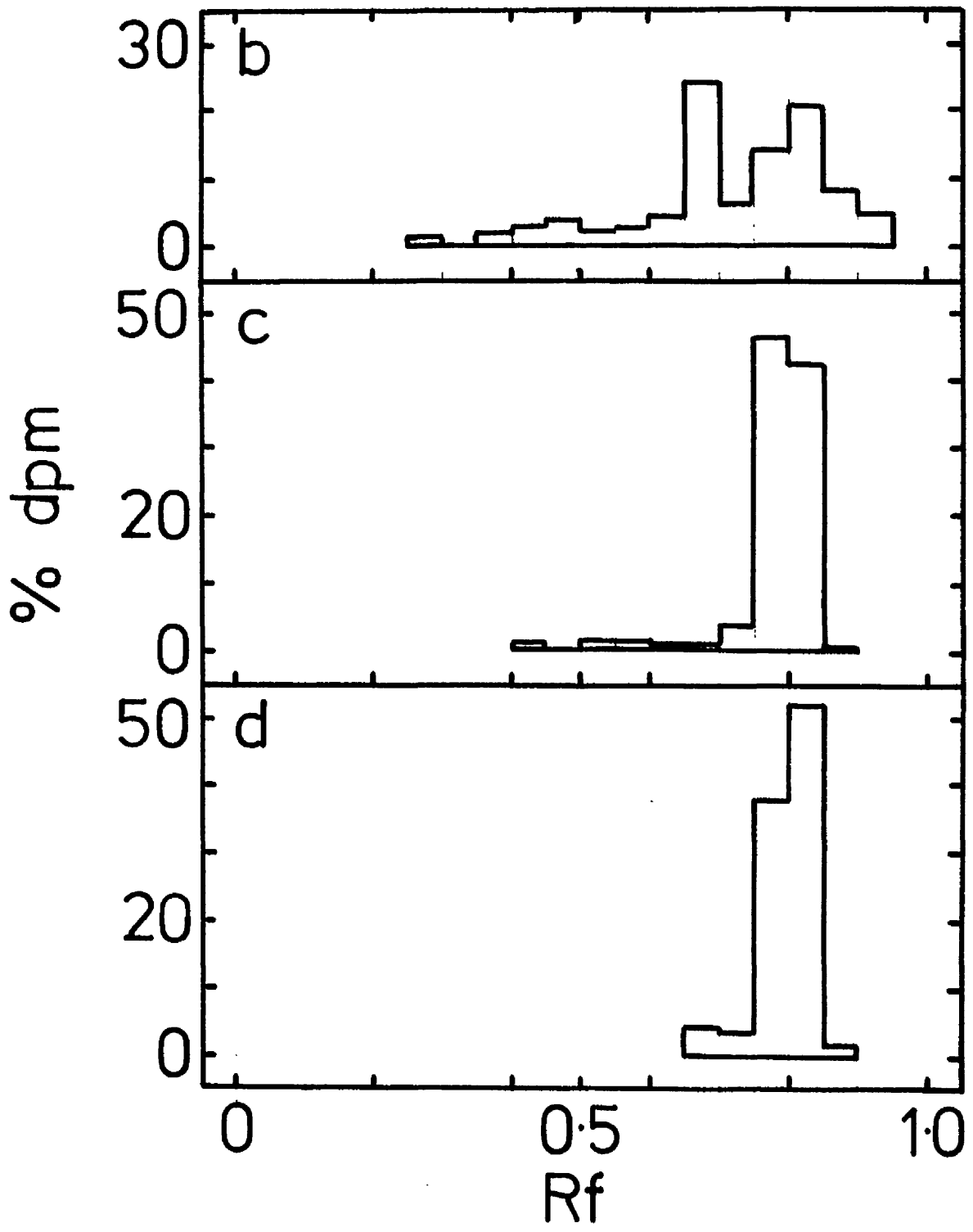

30
b
0
50
c
20
0
% dpm
50
d
20
0
0
0·5
1·0
Rf

FIGURE 51

Chromatographic analysis of stock ABA-2-^{14}C, donor blocks and tissue extracts from P. vulgaris plants supplied with ABA-2-^{14}C to a primary leaf for 24 hours. Ethanolic extracts (80% ethanol) of blocks and tissue were reduced to the aqueous phase and extracted with ether at pH 3.5. The ether extracts were spotted onto Whatman No. 3 chromatography paper and the chromatograms developed in n-butanol: n-propanol: 0.880 ammonia: distilled water :: 2:6:1:2 v/v. The histograms show the radioactivity recovered from each Rf zone of the chromatograms, assayed by liquid scintillation spectrometry, expressed as a percentage of the total radioactivity recovered from each chromatogram.

Extracts

Stock : stock ABA-2-^{14}C

Stock + chl. : " " plus ethanolic chlorophyll extract.

Histogram 1 : apices

" 2 : first trifoliate leaves

" 3 : second trifoliate leaves

" 4 : donor blocks

" 6 : upper stems

" 7 : lower stems

" 8 : primary roots

" 9 : lateral roots

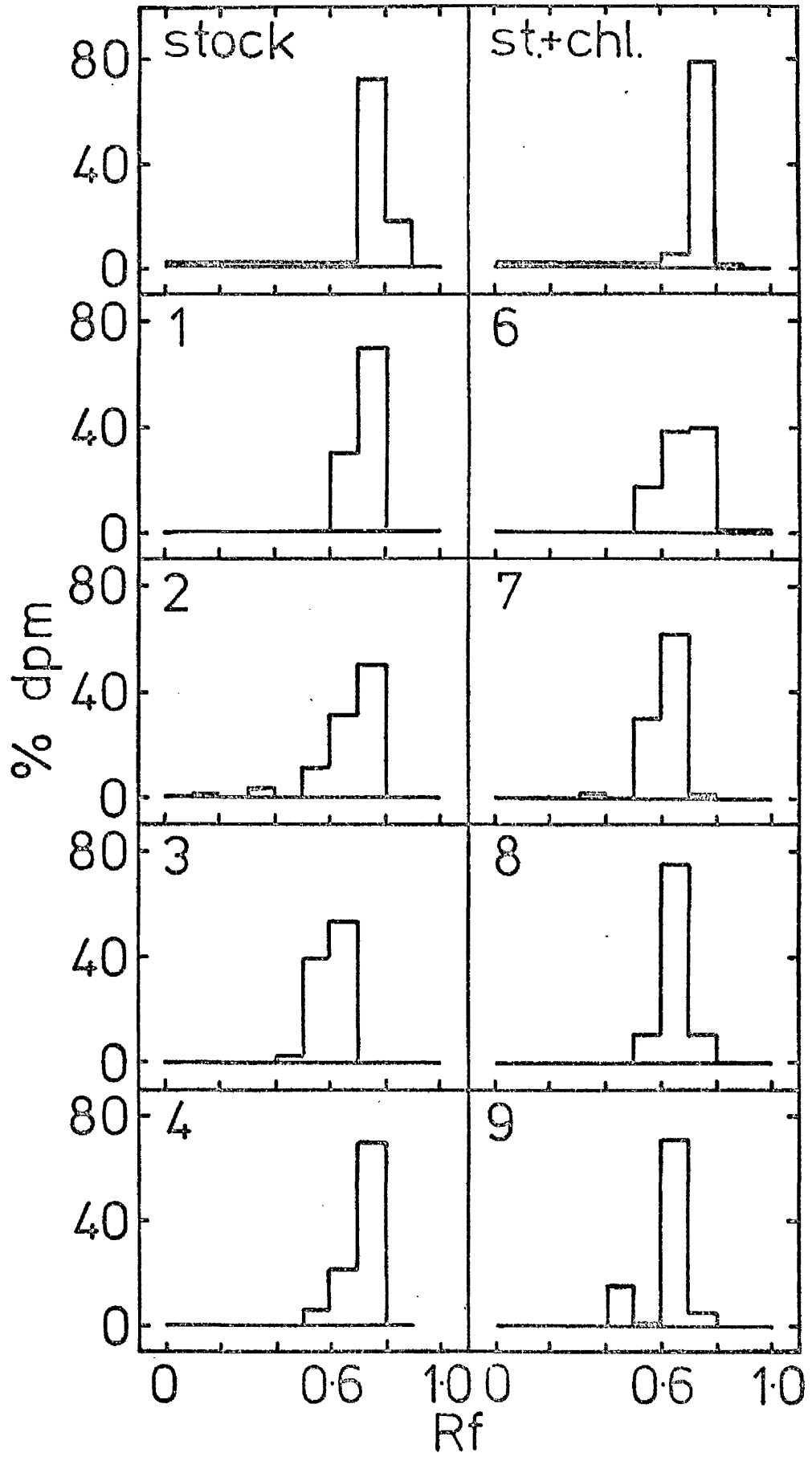

stock
st.+chl.
1
6
2
7
3
8
4
9
80
40
0
% dpm
0
0·6
1·0
Rf

FIGURE 52

As in Figure 51, except that thin-layer chromatography (250 μ silica gel) was used to analyse the extracts.

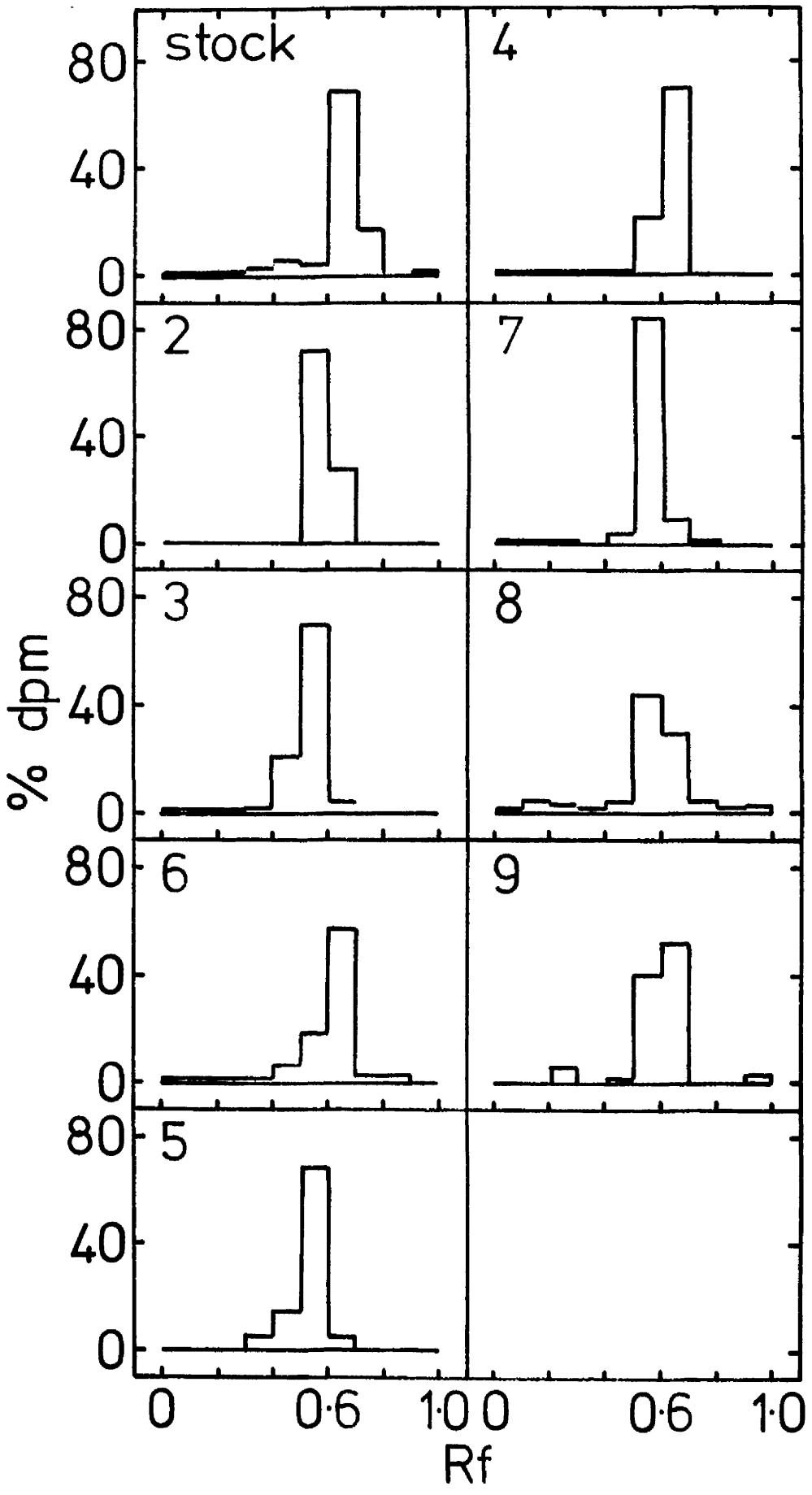
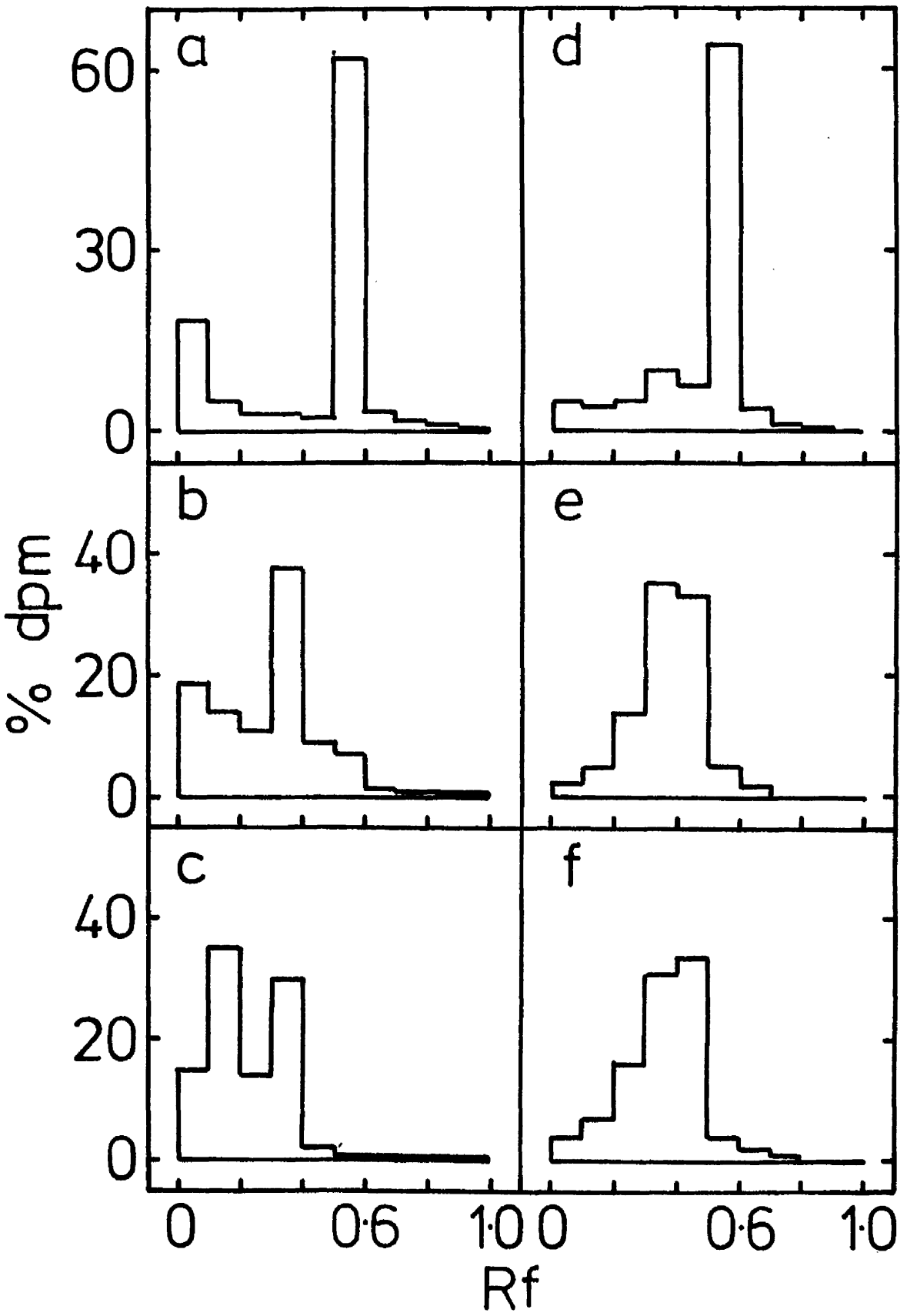

stock
4
2
7
3
8
6
9
5
80
40
0
% dpm
0
0·6
1·0
Rf

FIGURE 53

Thin-layer chromatography (250 μ silica gel) of stock ABA-2-^{14}C (a and d) and ethanolic tissue extracts from P. vulgaris plants supplied with ABA-1-^{14}C for 1 (b,e) and 5 (c f) days. Replicate chromatograms were developed in chloroform: methanol: water :: 75:22:3 v/v (a - c) and n-butanol: n-propanol: ammonia: water :: 2:6:1:2 v/v (d - f). The histograms show the radioactivity recovered from each Rf zone of the chromatograms, assayed using liquid scintillation spectrometry, expressed as a percentage of the total radioactivity recovered from each chromatogram.


a
d
b
e
c
f
60
30
0
40
20
0
40
20
0
% dpm
0
0·6
1·0
0
0·6
1·0
Rf

IIB Studies on the physiological effects of ABA in relation to its movement and metabolism in P. vulgaris.

(i) Physiological effects, uptake, movement and metabolism of ABA in hydroponically-cultured plants of P. vulgaris

During the study of the movement of ^{14}C in bean following foliar application of ABA-2-^{14}C, no visible effects on growth were observed. However, the possible effects of applied ABA on growth of whole bean plants are obviously of interest. During the transport studies only very small amounts of ABA were applied and consequently gross effects on growth were neither expected nor indeed wanted, since these might have affected the transport characteristics. It was shown that uptake of ^{14}C through an unabraded leaf surface was very poor so it was unlikely that foliar application of larger quantities of ABA to unabraded leaves would result in any marked effects on growth. Instead, an alternative method of application was sought. Spraying the leaves with ABA solution containing a surface-active agent was not considered, largely because it was thought to be difficult to quantify. An initial attempt at the hydroponic culture of P. vulgaris plants was successful using a simple Long Ashton culture solution. As a result, it was decided to study the effects of supplying ABA in solution to the roots of hydroponically cultured bean plants, on a readily-measured parameter of growth.

The stem lengths of P. vulgaris plants growing in Long Ashton culture solution containing 10^{-5}M ABA solution are shown in Figure 54. In Expt. I fresh culture/ABA solution was supplied every five days as indicated by the arrows. Control

plants received the appropriate concentration of methanol solution. At the start of the experiment (day 1), there was no significant difference between the mean heights of control and treatment plants. Sixteen days after the first application of ABA there was a significant difference (0.01P) between the mean heights of treatment and control plants. By this time the treatment plants had ceased to elongate.

In experiments II and III only one application of culture/ABA solution was made, at the time indicated. After ten days (Expt. II) or sixteen days (Expt. III), there was no significant difference between the mean heights of control and treatment plants.

The rates of increase in height (cm day^{-1}) for the plants in these experiments are shown in Figure 55. In Experiment I the rate of extension growth of both control and treatment plants declined rapidly from the onset of the experiment, due probably to ontogenetic drift, and there was no marked effect on rate of the first application of ABA. It was not until after the third application that there was any significant difference in rate between control and treatment plants.

In Experiment II the growth rates of both control and treatment plants declined rapidly from the onset of the experiment before the application of ABA, and there was no marked effect of ABA on growth rate throughout the experiment. In Experiment III the application of ABA was made at day 1, and the growth rate of the treatment plants declined more than the control plants such that there was a significant difference

(0.01P) in rate 3 days after the first application. However, since the mean height of the treatment plants was initially greater, this did not result in a significant difference in height. The difference in rate declined with time and after 7 days there was no significant difference between the two rates. Thereafter the rates of both sets of plants remained relatively steady.

Thus considering the three experiments together there was some evidence that ABA did reduce the rate of stem extension, but the effects were neither very rapid (Expt. I) nor long-lasting (Expt. III) nor were they consistent (Expt. II).

During Experiment I it was noticed that the control plants in their flasks were lighter than the treatment plants, suggesting that the former were losing more water. ABA has been implicated in the regulation of water relations of plants (Jones and Mansfield, 1972), and accordingly this loss in weight was measured in Experiments II and III.

The loss in weight of the culture flask plus plant was recorded for both control and treatment plants during Experiments II and III and corrected for loss by evaporation from the flask alone. These values should give a measure of the difference in water loss from control and treatment plants, i.e. the difference in transpiration, assuming that any other cause of weight change affected treatment and control plants equally. In both experiments the water loss from the control plants was significantly greater than the loss from treatment plants (Fig. 56), 5 or 6 days after the application of ABA to the culture solution. The rate of water loss (g day^{-1}) shows the effect more clearly (Fig. 57). Both rates

were variable over the time period, since the experiments were carried out in a greenhouse subject to fluctuations in temperature, but the rate of water loss from the treatment plants was significantly less than from control plants 24 hours after the application of ABA. This difference was greatest on the second day after application and thereafter declined. These results suggest that ABA has an inhibiting effect on the uptake and/or movement and/or transpiration of water in P. vulgaris, when supplied to the roots of hydroponically-cultured plants.

These physiological effects of ABA alone provide strong evidence that ABA is taken up from solution by roots of Phaseolus vulgaris.

To investigate this possibility further, a number of treatment plants in Experiment II were supplied with both unlabelled ABA and with ABA-2-^{14}C at the same final concentration of 10^{-5}M, so that the uptake of radioactivity could be followed during the experiment. Replicate plants were harvested after one day and then every two days, for radioassay, to give both uptake (dpm g^{-1}) and distribution (% dpm) of ^{14}C in roots and sheet, as a function of time. Uptake (Fig. 58 i) by roots was linear with time. There was no detectable accumulation of ^{14}C in the shoot after one day, but thereafter accumulation was also linear with time, but at a lower rate than in roots. After 7 days 68% of the total ^{14}C was recovered from the roots and 32% (400 dpm g^{-1}) from the shoot (Fig. 58 ii). This experiment shows that ^{14}C was being taken up by the roots and being transported to the shoots during the experiment.

Information on the stability of ABA in the culture solution and its fate following uptake is a prerequisite in explaining the relative ineffectiveness of ABA on stem elongation in Experiments II and III when only one application of ABA was made to the culture solution.

The stability of ABA in culture solution was ascertained by sampling the culture solutions at regular intervals from the radioactive treatment plants in Experiment II. These samples were analysed by paper chromatography using 2:6:1:2 as the solvent system (Fig. 59). The original stock solution gave a single major radioactive peak at Rf 0.75 - 0.80, and analysis of culture solution samples after, 1, 3 and 5 days gave very similar results. These results showed that, at least after 5 days, there was very little chromatographically-detectable breakdown of ABA-2-^{14}C in the culture solution.

The metabolism of ABA-2-^{14}C taken up by the plants during Experiment II was also investigated. Samples of shoot and root tissue were taken at regular intervals and extracted in ethanol. The ethanolic extracts were analysed by paper chromatography using the 2:6:1:2 solvent system (Fig. 60). Stock ABA-2-^{14}C gave a single major peak at Rf 0.75 - 0.85. The root extract after 3 days (Fig. 60 b) showed a major peak at Rf 0.75 - 0.80 corresponding to ABA-2-^{14}C, but after 5 and 7 days the proportion of ^{14}C recovered at that Rf declined markedly. A number of minor peaks were detected but these varied from extract to extract. In shoot extracts after only 3 days the percentage of ^{14}C recovered at the Rf corresponding to ABA-2-^{14}C was less than 10%. In extracts taken after 5 and 7 days a peak of radioactivity was recovered at Rf 0.6 - 0.7, which was

similar to the peak found earlier in extracts of bean and coleoptile tissue. In all the extracts there was a considerable spread of radioactivity over the length of the chromatogram and as a result no clear pattern of breakdown emerged. It was clear, however, that only a very small percentage of the radioactivity recovered in ethanolic extracts of tissue co-chromatographed with ABA-2-^{14}C. This suggested that considerable breakdown of ABA-2-^{14}C had occurred in both roots and shoots during the experiment and very little ABA-2-^{14}C accumulated in the tissue, despite the fact that it was supplied continuously. It should be noted that these results were obtained from Experiment II where there was no significant effect of applied ABA on the stem extension of Phaseolus vulgaris plants. Further experimentation in this area is required.

FIGURE 54

The effects on stem length of supplying 10^{-5}M ABA to the culture solution of hydroponically-cultured plants of P. vulgaris. The stem heights of control, —●— , and treatment, —○— , plants from three experiments (I, II and III) are shown as a function of time. The plotted values are means of fifteen replicates, the vertical bars representing twice the standard error of the mean. The arrows indicate the time of application of ABA to the Long Ashton culture solution. The plants were maintained under long days in a greenhouse.

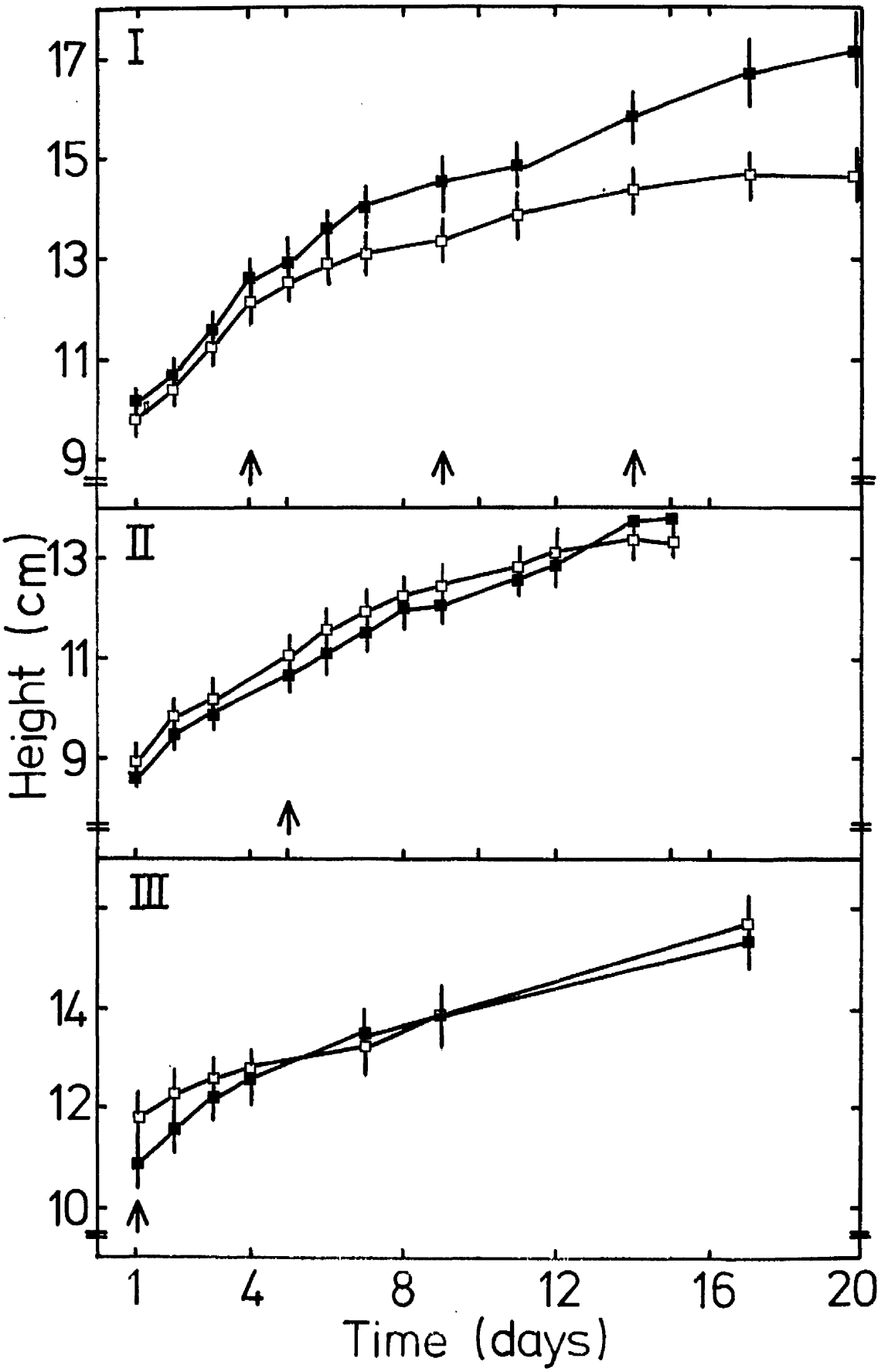

I
II
III
17
15
13
11
9
13
11
9
14
12
10
Height (cm)
1
4
8
12
16
20
Time (days)

FIGURE 55

The effects on the rate of stem elongation of supplying 10^{-5}M ABA to the culture solution of hydroponically-cultured plants of P. vulgaris. The rates of height increase (cm day^{-1}) of control, —▲— , and treatment, —△— , plants from three experiments are shown as a function of time. Experimental details are as in Figure 54.

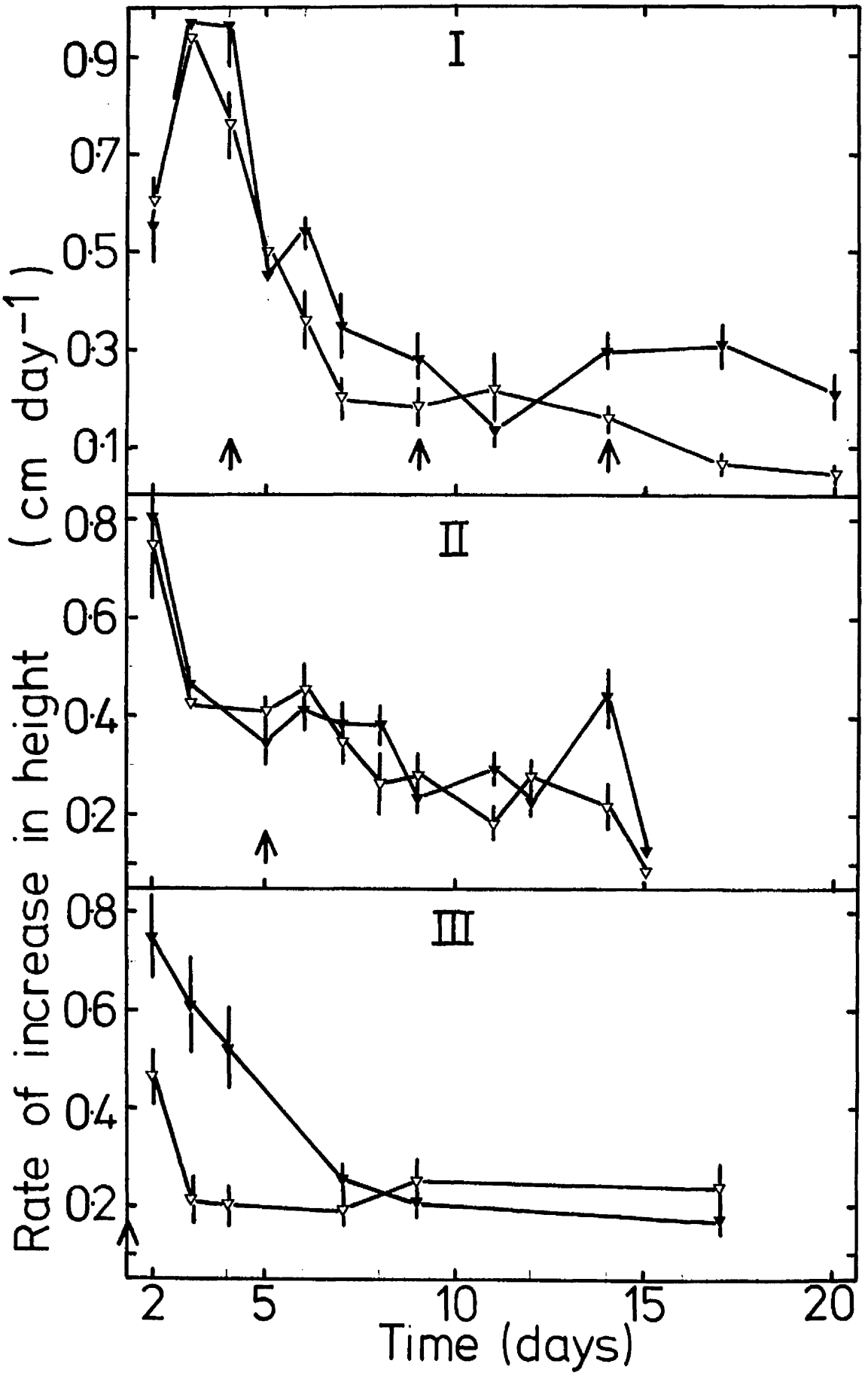

I
II
III
0·9
0·7
0·5
0·3
0·1
0·8
0·6
0·4
0·2
0·8
0·6
0·4
0·2
Rate of increase in height (cm day⁻¹)
2
5
10
15
20
Time (days)

FIGURE 56

The effects of supplying 10^{-5}M ABA to the culture solution on the weight loss from hydroponically-cultured plants of P. vulgaris. The losses in weight are shown from control plants and flasks, ——▲—— , and treatment plants and flasks, ——△—— , as a function of time, for two experiments (II and III). The plotted values are means of fifteen replicates, the vertical bars representing twice the standard errors. The arrows indicate the time of application. The plants were maintained under long days in a greenhouse.

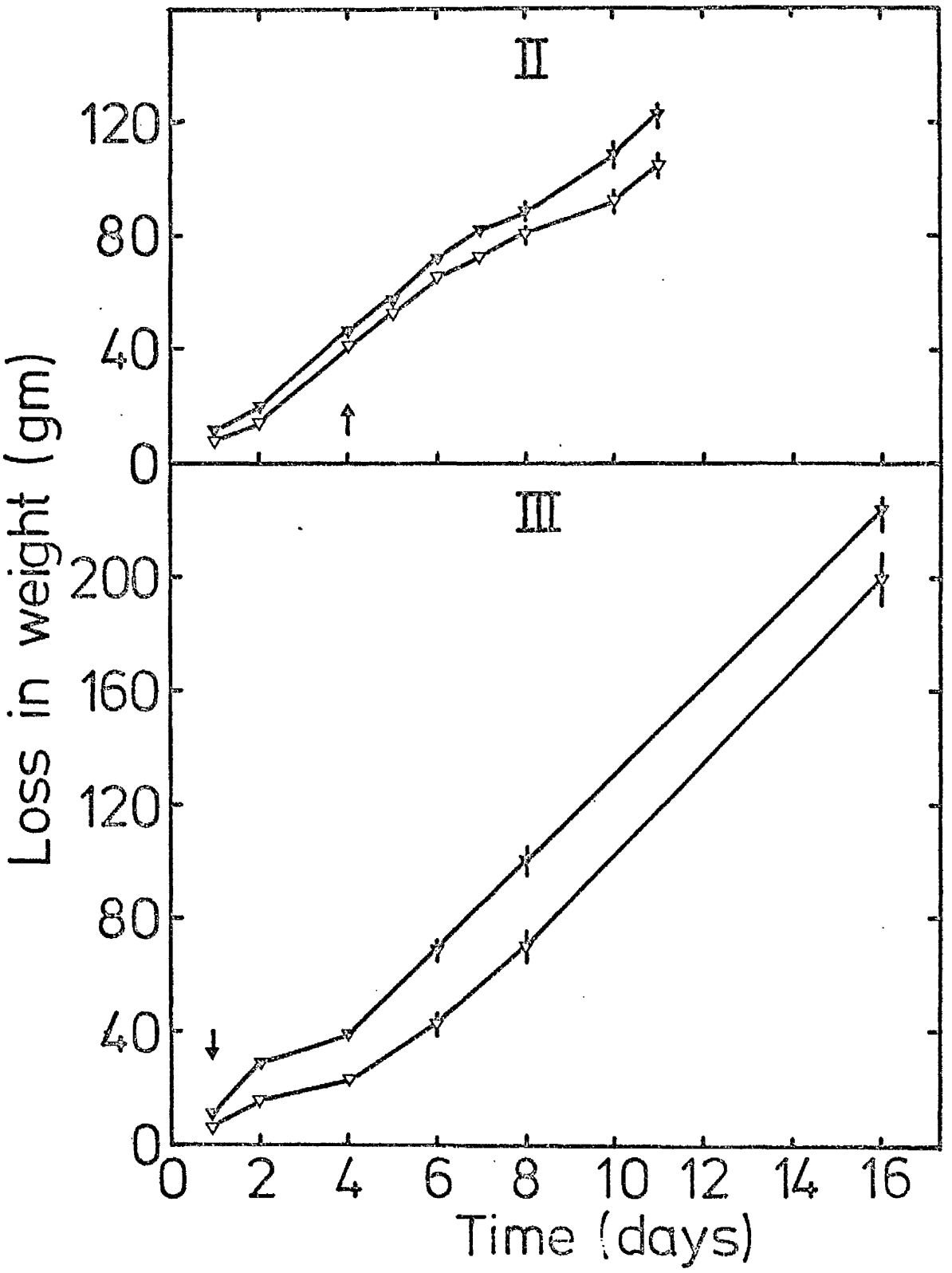

II
III
120
80
40
0
200
160
120
80
40
0
0
2
4
6
8
10
12
14
16
Loss in weight (gm)
Time (days)

FIGURE 57

The effects of supplying 10^{-5} M ABA to the culture solution on the rate of weight loss from hydroponically-cultured plants of P. vulgaris. The rates of weight loss (gm day^{-1}) are shown from control plants plus flasks, ——▲—— , and treatment plants plus flasks, ——△—— , are shown as a function of time. Experimental details are as in Figure 56.

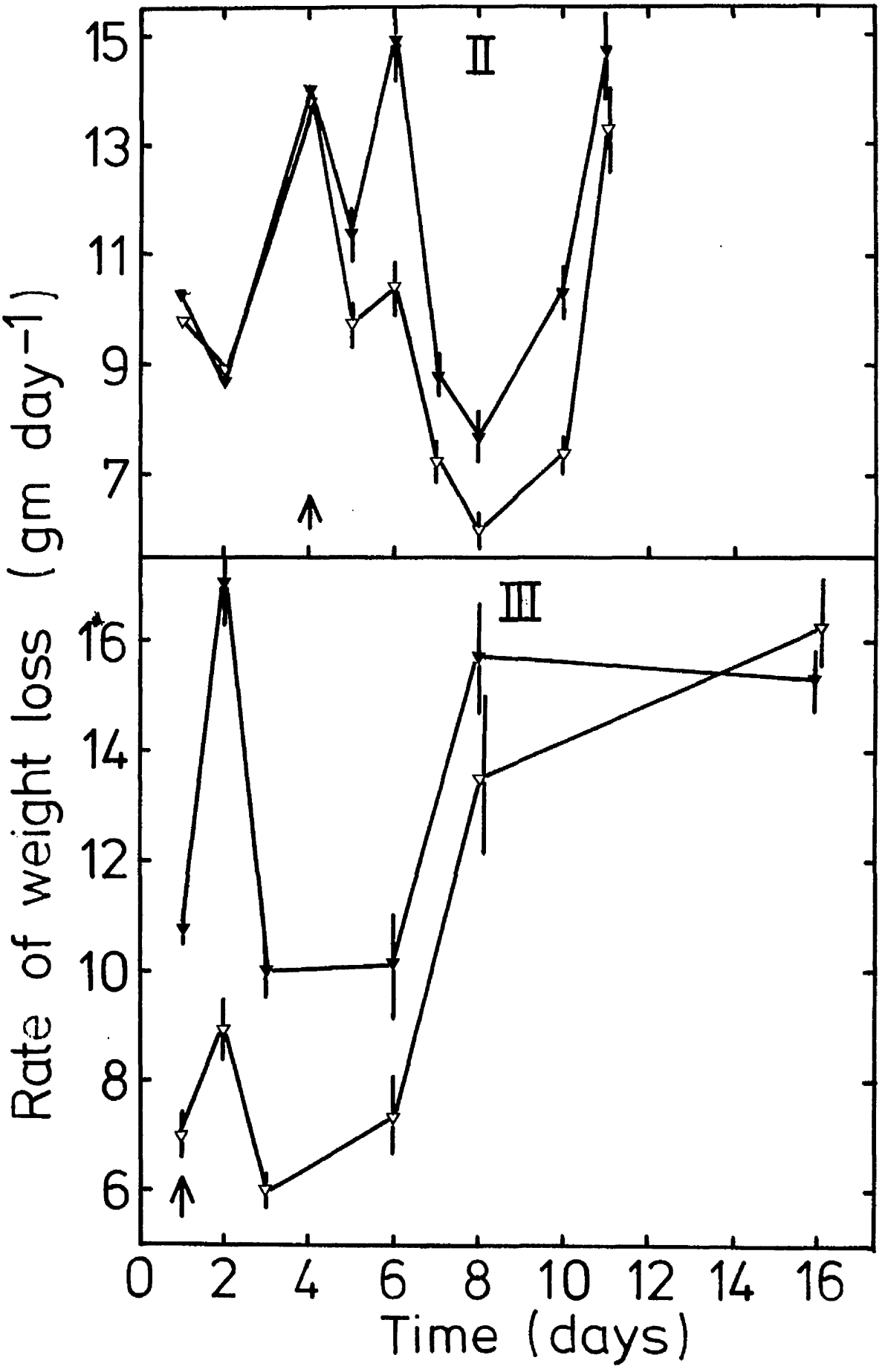

II
III
15
13
11
9
7
16
14
12
10
8
6
0
2
4
6
8
10
12
14
16
Time (days)
Rate of weight loss (gm day-1)

FIGURE 58

(i) Uptake of ^{14}C by P. vulgaris plants following application of 10^{-5}M ABA-2-^{14}C to the culture solution of hydroponically-cultured plants maintained under long days in a greenhouse. Uptake (dpm g^{-1}) into roots, —○—, and shoots, —●—, is shown as a function of time after application. Plotted values are means of five replicates.

(ii) Percentage distribution of ^{14}C in P. vulgaris plants (—○—, roots, —●—, shoots) following application of 10^{-5}M ABA-2-^{14}C to the culture solution of hydroponically-cultured plants.

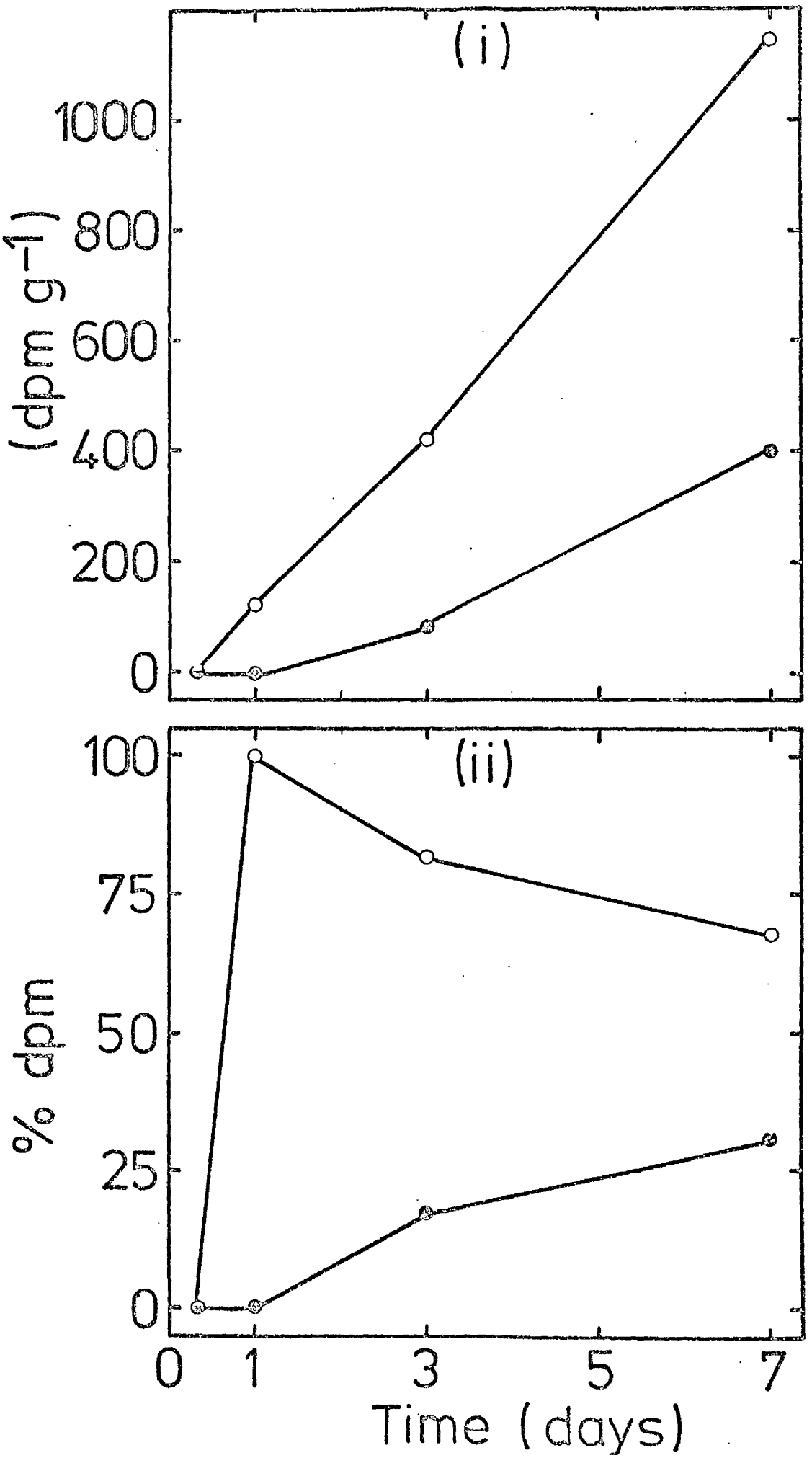

(i)
1000
800
600
400
200
0
(dpm g-1)
(ii)
100
75
50
25
0
% dpm
0
1
3
5
7
Time (days)

FIGURE 59

Chromatographic analysis of the radioactivity in 5 cm^3 samples of Long Ashton culture solution containing the roots of P. vulgaris plants. The solutions were supplied originally with ABA-2-^{14}C at a final concentration of $10^{-5}M$. The samples were applied to paper chromatograms which were developed in the 2:6:1:2 solvent system. The histograms show the chromatographic analysis, expressed as the percentage ^{14}C in each half - Rf zone, of (a) stock ABA-2-^{14}C and of culture solutions (b) immediately, (c) 1 day, (d) 3 days and (e) 5 days after application of ABA-2-^{14}C.

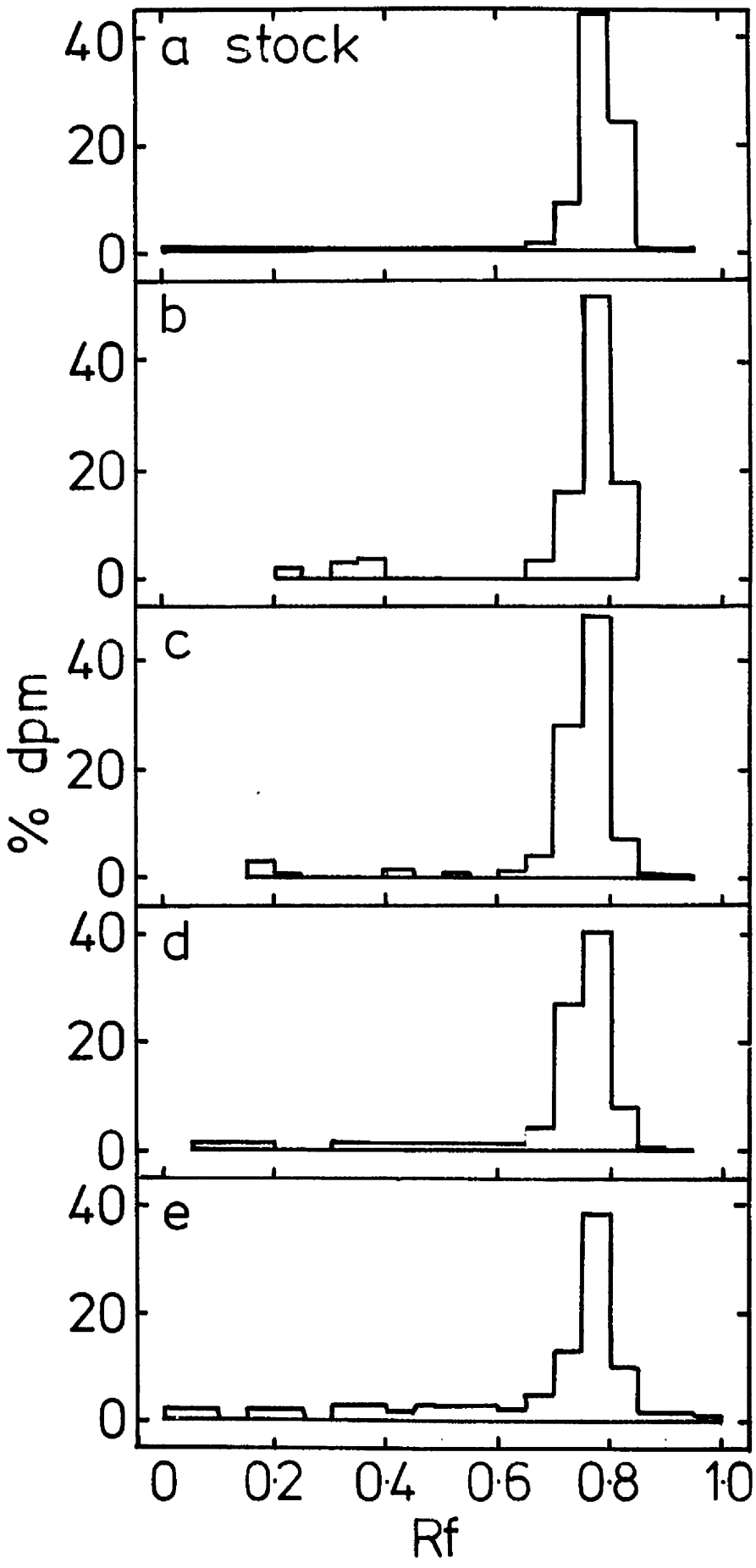
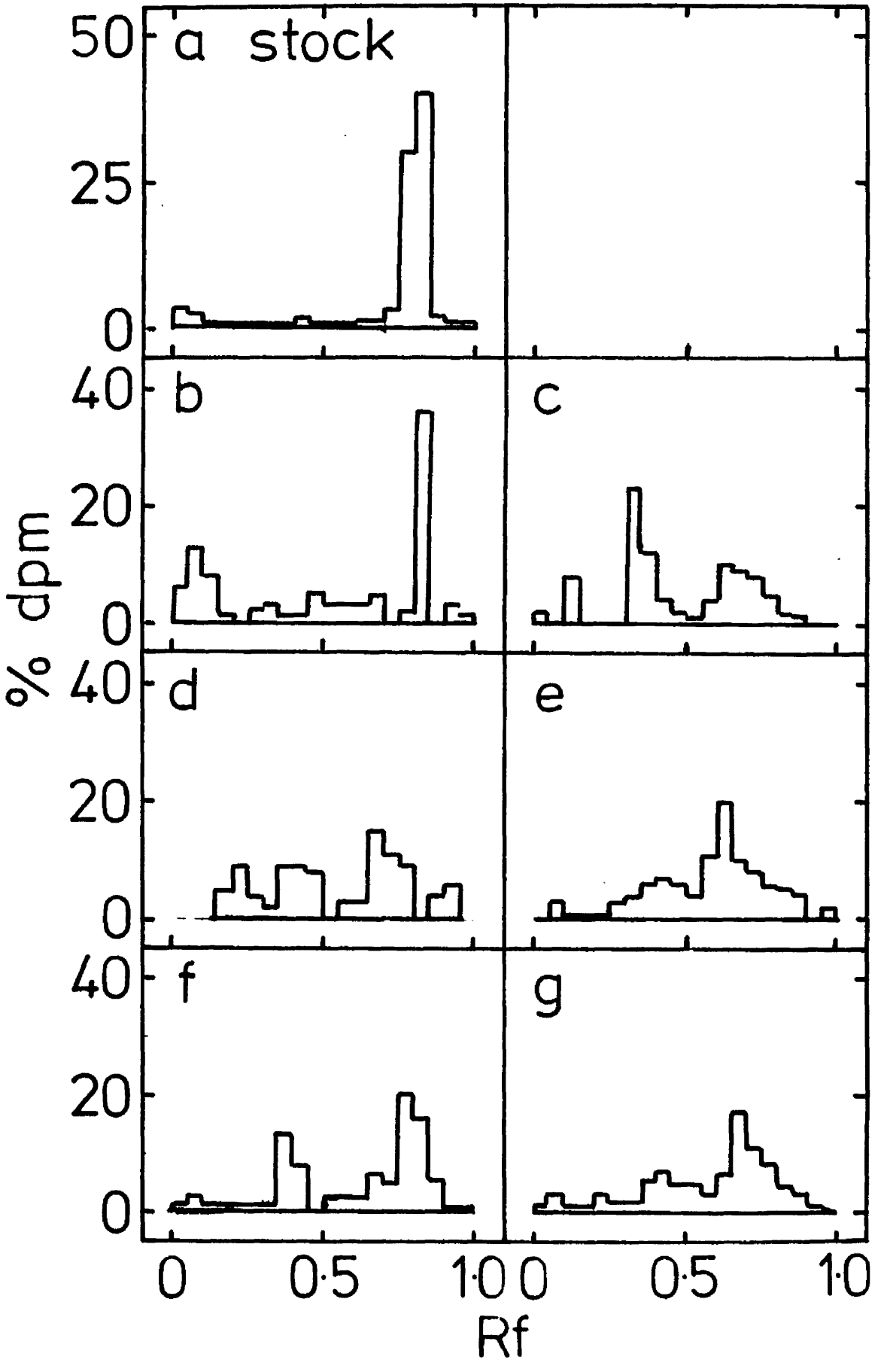

a stock
b
c
d
e
40
20
0
% dpm
0
0·2
0·4
0·6
0·8
1·0
Rf

FIGURE 60

Chromatographic analysis of the radioactivity in tissue extracts from P. vulgaris plants supplied with ABA-2-^{14}C at a concentration of 10^{-5}M to the culture solutions. Ethanolic extracts of root and shoot tissue were applied to paper chromatograms which were developed in 2:6:1:2. The histograms show the analysis, expressed as the percentage ^{14}C in each half - Rf zone, of:- (a) ABA-2-^{14}C stock solution; root tissue extracts taken (b) 3 days, (d) 5 days and(f) 7 days after application of ABA-2-^{14}C, and shoot tissue extracts taken (c) 3 days, (e) 5 days and (g) 7 days after application of ABA-2-^{14}C. During the experiment the plants were maintained under long days in a greenhouse.


a stock
b
c
d
e
f
g
50
25
0
40
20
0
% dpm
0
0·5
1·0
Rf

(iii) Metabolism of ABA-2-^{14}C in P. vulgaris plants during periods of imposed water stress.

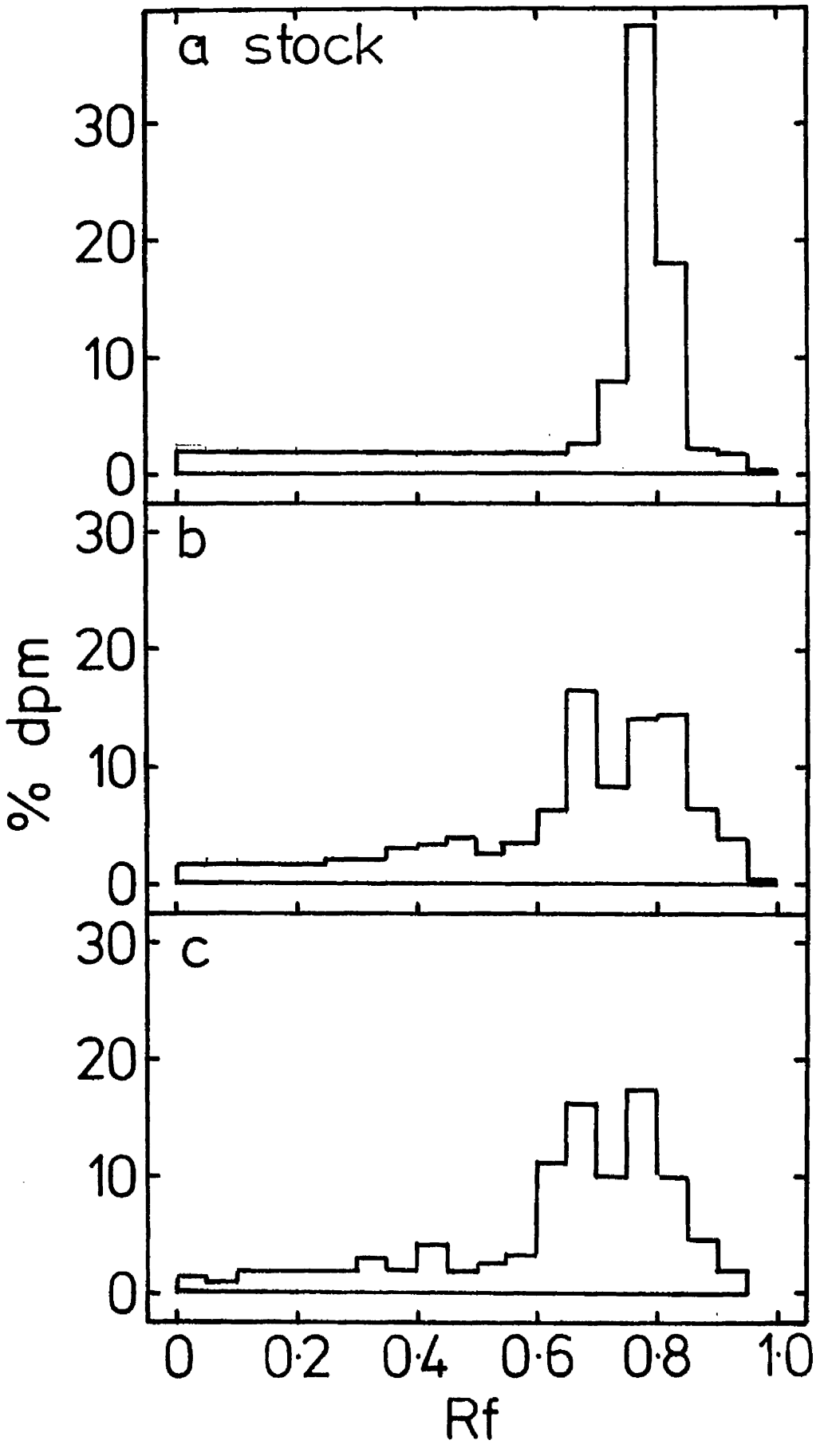
The endogenous levels of ABA change during periods of water stress (e.g. Wright & Hiron, 1972) and applied ABA is known to affect the rate of transpiration (e.g. Mizrahi etal., 1971) In addition it was shown in the previous experiment that ABA applied to the roots of hydroponically-cultured bean plants reduces the rate of water loss. Thus the metabolism of ABA in plants during periods of water stress is of considerable interest. This has been investigated by an analysis of the metabolism of ABA-2-^{14}C in plants during periods of imposed water stress.

Donor blocks containing 5μM ABA-2-^{14}C were applied to ten hydroponically-cultured plants of P. vulgaris. After 1 day, half the plants were stressed by the addition of mannitol (31g litre^{-1}) to the culture solution. The other five plants were left as controls. After a further 24 hours the agar blocks were removed and the donor leaves of stressed and unstressed plants extracted with ethanol. The extracts were analysed on paper chromatograms developed in the butanol system; the chromatograms were divided into half-Rf zones for radioassay. Histograms expressing the recovery of ^{14}C in each zone as a percentage of the total, for stock ABA-2-^{14}C, stressed and unstressed tissue extracts, are shown in Figure 61. Stock ABA-2-^{14}C gave a single, major peak at Rf 0.75 - 0.80, and over 54% of the total ^{14}C was recovered from Rf 0.75 - 0.85. In unstressed (b) and stressed (c) tissue extracts, there were two distinct peaks; one at Rf 0.75 - 0.85 corresponding to ABA-2-^{14}C and the other at Rf 0.65 - 0.70 corresponding to the peak found in earlier experiments (cf. Fig. 32).

The percentage radioactivity recovered from Rf 0.75 - 0.85 was 27% and 27.5% for unstressed and stressed extracts respectively. Thus although the percentage of ^{14}C recovered at the zone corresponding to ABA-2-^{14}C was halved in tissue extracts, there was little or no difference between the stressed or unstressed tissue extracts. This suggests that the metabolism of applied ABA-2-^{14}C in P. vulgaris was not markedly affected by imposed water stress over a period of twenty-four hours. The imposition of stress during this period was clearly shown by a marked wilting of the leaves and stem of the treated plants.

FIGURE 61

The effect of water stress on the metabolism of applied ABA-2-^{14}C in *P. vulgaris*. 5μM ABA-2-^{14}C was supplied to a primary leaf of the hydroponically-cultured plants. After 1 day half the plants were stressed with 31 g litre^{-1} mannitol, the other plants were left as unstressed controls. After another day the plants were harvested and extracted in ethanol. Samples of the extracts were applied to paper chromatograms which were developed in 2:6:1:2. The histograms show the percentage radioactivity recovered from each half-Rf zone in chromatograms of (a) stock ABA-2-^{14}C, (b) unstressed tissue extracts and (c) stressed tissue extracts. During the experiment the plants were maintained under long days in a greenhouse.


a stock
b
c
30
20
10
0
% dpm
0
0·2
0·4
0·6
0·8
1·0
Rf

(iv) A comparison of the effects of ABA, GA_3, IAA and mannitol on the growth of hydroponically cultured P. vulgaris plants

Having discovered that ABA had only a small, if any, effect on stem extension when applied to the roots of bean plants growing in water culture, a comparison of the effects of other growth substances applied in a similar way, was of interest.

Solutions of ABA, GA_3, IAA and mannitol at a final concentration of 10^{-5}M were applied to the culture flasks of hydroponically-cultured bean plants. The height of each plant was measured over a period of 16 days, together with the loss in weight of the flask plus plant. Throughout the experiment there were no significant differences between the mean heights of plants treated with ABA, IAA, mannitol and the controls (Fig. 62). The mannitol treatment was included to study any possible effects of adding 10^{-5}M of a non growth-active but osmotically active solute to the solution in the culture flask.

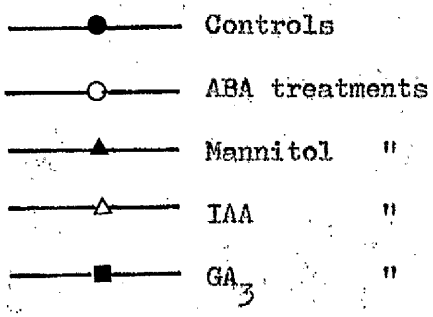
After 2 days the mean height of the plants treated with GA_3 was significantly (0.001P) greater than the control. After 16 days the mean height of the GA_3 treated plants was 100cm, whereas the mean height of the controls was 15.3cm. These results show that GA_3 has a marked and rapid effect on the stem extension of hydroponically-cultured plants of P. vulgaris, when supplied as a solution to the roots

The loss in weight from flasks and plants treated with ABA solution was less than from the controls for the duration of the experiment, the difference being significant (0.001P) after 3 days (Fig. 63). There were no significant differences between

the mean weight losses of the other treatment plants and controls for the duration of the experiment. On the assumption that the difference in weight loss represented the difference in water loss, this suggested that only ABA had a significant effect on the water relations of P. vulgaris plants.

FIGURE 62

The effects of ABA, IAA, GA_3 and mannitol on the stem extension of *P. vulgaris* plants as a function of time. 10^{-4}M solutions were added to the culture flasks of bean plants to give final concentrations of 10^{-5}M, and plant heights were recorded over the next 16 days. The values plotted are means of twenty replicates, the vertical bars representing twice the standard errors. The plants, initially two weeks old, were maintained under long days in a greenhouse.



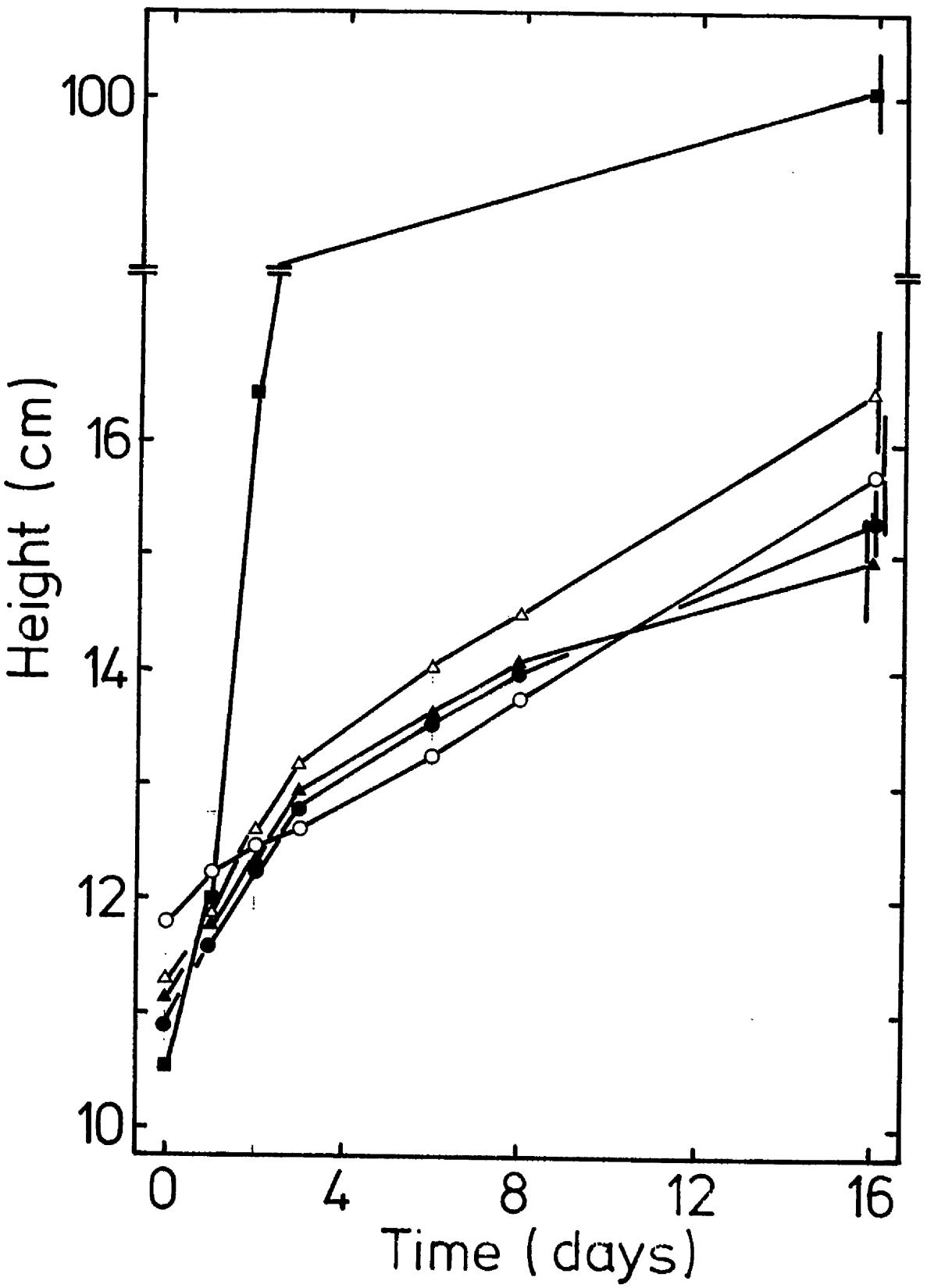
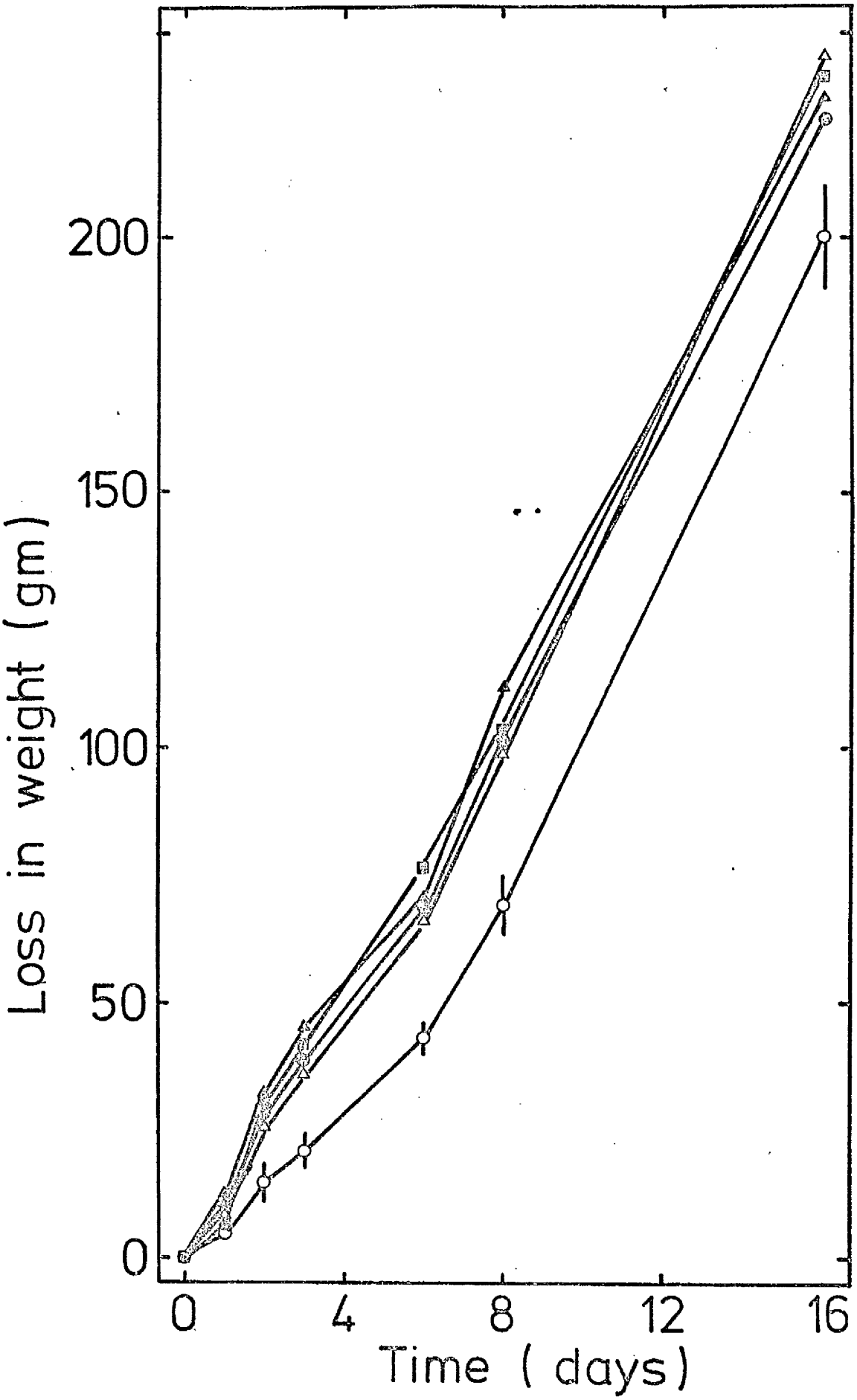

100
16
14
12
10
Height (cm)
0
4
8
12
16
Time (days)

FIGURE 63

The effect of ABA, IAA, GA_3 and mannitol on the loss in weight from hydroponically-cultured bean plants. Experimental details are as in Figure 62. The weights of plants plus culture flasks were recorded over the 16 day period.


200
150
100
50
0
Loss in weight (gm)
0
4
8
12
16
Time (days)

SECTION III Studies on seedlings of deciduous tree species.

IIIA The uptake, movement and metabolism of ABA-2-^{14}C in deciduous tree seedlings.

(i) A comparison of five methods of application of ABA-2-^{14}C to seedlings of Alnus glutinosa

The characteristics of uptake and subsequent distribution of a substance are probably dependent to some extent on the locus and method of application. Initially therefore, a comparison was made of five methods of applying ABA-2-^{14}C, including two different loci, to three month-old seedlings of Alnus glutinosa. The methods used were:-

1. Application of a small agar donor block containing 5μM ABA-2-^{14}C to an unabraded leaf surface.

2. Application of a small agar donor block containing 5μM ABA-2-^{14}C to a lightly abraded leaf surface.

3. Injection of 10mcm^3 of 10^{-5}M ABA-2-^{14}C into the distal end of a leaf petiole.

4. Application of 10mcm^3 of 10^{-5}M ABA-2-^{14}C to a rolled-up leaf in small vial containing 1cm^3 of water such that the leaf was completely wetted.

5. Application of 10mcm^3 of 10^{-5}M ABA-2-^{14}C to the culture flask, containing 50cm^3 of solution, of a hydroponically cultured plant.

For methods 1, 2 and 3 the uptake was considered only after 24 hours. For methods 4 and 5, which from preliminary experiments were shown to allow less rapid penetration, uptake was considered after 24, 48 and

72 hours. At the end of a transport period the plants were harvested for radioassay as for bean plants, except in methods 4 and 5. In the former method the donor leaf had to be washed free of surplus ABA-2-^{14}C solution and hence percentage recovery was not determined. In method 5 percentage recovery was not determined as the roots had to be washed, and distribution of ^{14}C was only considered for shoot and roots.

Table 11 shows firstly the approximate amount of radioactivity applied in each method. In methods 3, 4 and 5, using ABA-2-^{14}C solution, the actual amount applied was kept constant rather than the concentration, which was much less in methods 4 and 5. The percentage recovery was highest for method 1 since most of the ^{14}C remained in the agar block, from which it could be easily recovered. The recovery for method 2 was considerably lower than for method 3.

The percentage uptake by an unabraded leaf was too low for the method to be of any practical use. Percentage uptake following petiole injection was higher than uptake by an abraded leaf. This might have been expected, but in fact was the reverse of the result found for uptake by P. vulgaris, where five times more solution was applied. Uptake by the plants following methods 4 and 5 was much lower. Although the concentration of the radioactivity in the application solution was much lower after dilution, than in method 3, the solution was applied to a much larger surface area; clearly there was insufficient compensation for dilution. Uptake by either method 4 or 5 after 72 hours was only 10%.

Table 11. A comparison of five methods of applying ABA-2-^{14}C to 3 month-old seedlings of Alnus glutinosa.

Method		dpm applied	% of applied dpm recovered	% of applied dpm taken up	% of uptake transported
1. agar block to unabraded leaf.		9239	99.0	1.1	39.3
2. agar block to abraded leaf.		9239	67.4	64.1	46.7
3. petiole injection.		2000	85.0	80.5	88.0
4. leaf vial application	24h	2000	-	0	0
	48h	2000	-	6.8	66.0
	72h	2000	-	10.2	20.0
5. addition to culture solution	24h	2000	-	6.4	22.1
	48h	2000	-	8.4	12.5
	72h	2000	-	10.4	11.1

The percentage of the uptake transported i.e. radioactivity moved away from the donor area, was again highest with method 3. Although the uptake was observed to increase with time with methods 4 and 5, the percentage moving into the shoot actually decreased with time.

Thus in terms of efficiency, i.e. uptake for amount applied, methods 2 and 3 were the most satisfactory. Method 3 gave the highest percentage uptake but was less easy to carry out. The diameter of a petiole from a 3 month-old Alder seedling is very small,

and considerable damage could be inflicted during application. Consequently reproducibility was a problem and also the solution may have been injected directly into the vascular tissue, possibly affecting its subsequent transport. On the other hand, method 2, which was used extensively for studies with P. vulgaris, was satisfactory from most aspects, except, possibly reproducibility. Method 4 might be useful were the concentration of radioactivity in the application solution increased. It involved no damage to the plant although it placed the leaf in an unnatural environment. Method 5 was the only method of root application tested. At the concentration used it was not very effective in supplying radioactivity to the shoot, but this may be improved by increasing the concentration of ABA-2-^{14}C in the culture solution. This would again involve large amount of ABA-2-^{14}C. The method was easy to carry out but required the plants to be grown in liquid culture. The plants were easy to harvest. Reproducibility was a problem in both methods 4 and 5, because the surface area of tissue in contact with the solution could vary considerably.

Table 12 compares the distribution of radioactivity in the plants following application by the five methods. Methods 2 and 3 gave similar distribution whereas in method 1 a greater percentage of the ^{14}C remained in the donor leaf. Using method 4 the percentage remaining in the donor leaf increased with time. After 72 hours the percentage accumulation of ^{14}C in the shoot above the donor leaf was very low compared to methods 2 and 3 (an observation of considerable significance in a later section).

Following root application the accumulation in the shoot was much lower than with foliar application, and the percentage accumulation in the shoot decreased with time.

Table 12. Distribution of ^{14}C in *A. glutinosa*, expressed as a percentage of the transported ^{14}C, 24 hours after application of 5 µM ABA-2-^{14}C by five different methods.

Method	% distribution (% dpm)			
	Shoot above	donor leaf	shoot below	roots
1. agar block to unabraded leaf	25.9	45.9	28.2	0
2. agar block to abraded leaf	60.0	25.8	9.4	4.7
3. petiole injection	51.2	37.1	8.9	2.8
4. leaf vial 24h	0	0	0	0
spplication 48h	17.6	33.1	33.8	15.4
72h	3.9	81.8	5.4	8.9
5. application to 24h		22.1		77.9
culture soln. 48h		12.5		87.5
72h		11.1		88.9

These results show that the method and locus of application of ABA-2-^{14}C have a considerable effect on the uptake and distribution of radioactivity in *Alnus glutinosa*.

(ii) The distribution of ^{14}C in A. glutinosa, Acer pseudoplatanus, and Betula pubescens following foliar application of ABA-2-^{14}C.

A primary aim in this investigation was to elucidate the characteristics of ABA movement in deciduous tree species in order to assess the possible role of the movement in the control of bud dormancy. To this end, the uptake and movement of applied ABA-2-^{14}C has been studied in young seedlings of A. glutinosa and to a lesser extent in Acer pseudoplatanus and Betula pubescens. Particular emphasis has been placed on the export and distribution of ABA-2-^{14}C following foliar application.

First, the distributions of ^{14}C in A. glutinosa, Acer pseudoplatanus and B. pubescens were compared, 24 hours after application of ABA-2-^{14}C to fully expanded leaf. From the results of the previous section application method 2 was used, an agar block containing 5μM ABA-2-^{14}C to an abraded leaf, and the plants were harvested in the normal way. To study distribution the plants were divided into four parts, viz: leaf of application; shoot above the leaf of application; shoot below the leaf of application; roots. The radioactivity in each part was expressed as the percentage of the total ^{14}C recovered from the plant, excluding the donor area (Table 13). The distribution of ^{14}C in A. glutinosa and B. pubescens after 24 hours was very similar, with most of the ^{14}C, 63.3% and 59.6% respectively, occurring in the shoot above the leaf of application. The percentage ^{14}C recovered from the roots was much lower than was recovered from P. vulgaris in a similar experiment.

Table 13. Distribution of ^{14}C in three woody species, 24 hours after application of an agar block containing 5 μM ABA-2-^{14}C to an abraded, expanded leaf. Means of five plants maintained at 20°C under a 16 hour photoperiod.

	% dpm		
	Alnus glutinosa	Betula pubescens	Acer pseudoplatanus
shoot above leaf of application	63.3	59.6	20.6
leaf of application	19.2	22.8	52.0
shoot below	16.2	16.7	20.7
roots	1.3	0.9	6.7

In Acer pseudoplatanus the distribution of ^{14}C was dissimilar, with only 20.6% of the radioactivity occurring in the shoot above, a far greater percentage being recovered from the leaf of application.

The distribution of ^{14}C in A. glutinosa and Acer pseudoplatanus is shown in more detail in Figure 64. In the former over 5% of the radioactivity was found in the apical region and high percentages were found in the young, actively growing leaves. Very little ^{14}C was recovered from the older, mature leaves below the leaf of application, or in the roots. The distribution in B. pubescens, although not shown, was very similar. In Acer pseudoplatanus, some radioactivity was found in the apical region. The % accumulation in

the young, upper leaves was higher than in the lower leaves, apart from the donor leaf, but this was not so marked as in Alder. A greater percentage ^{14}C was found in the lower parts of the stem and the roots compared to Alder.

One explanation of this difference may be that although the two species were the same age, their morphology was very different. The average seedling of Acer pseudoplatanus had fewer leaves than A. glutinosa , such that the application had to be made to what was the lowest, or first true leaf. With A. glutinosa this was not the case, as can be seen from Figure 64. This highlights the problems, and questions the relevance, of comparing the distribution of ^{14}C in two different species with differing morphology. As with the export of assimilates, the age, size and position of the leaf on the plant together with the development of the vascular traces from the leaf may determine to a large extent the amount and direction of movement and subsequent distribution of an applied substance. A. glutinosa and B. pubescens, when 3 months-old, had very similar morphological characteristics in relation to the number, size and position of leaves, and the distribution of ^{14}C in these species was very similar.

This problem was investigated further in a single species by comparing the export and distribution of ^{14}C following application of ABA-2-^{14}C to an upper, young, large leaf and a lower, older, smaller leaf of A. glutinosa (Table 14). In general the mobility of ^{14}C in the lower donor leaf was much less than in the upper donor leaf. Not only was the % transported halved, but the percentage ^{14}C remaining in the lower donor leaf was almost four times that in the upper donor leaf. A greater percentage radioactivity reached the

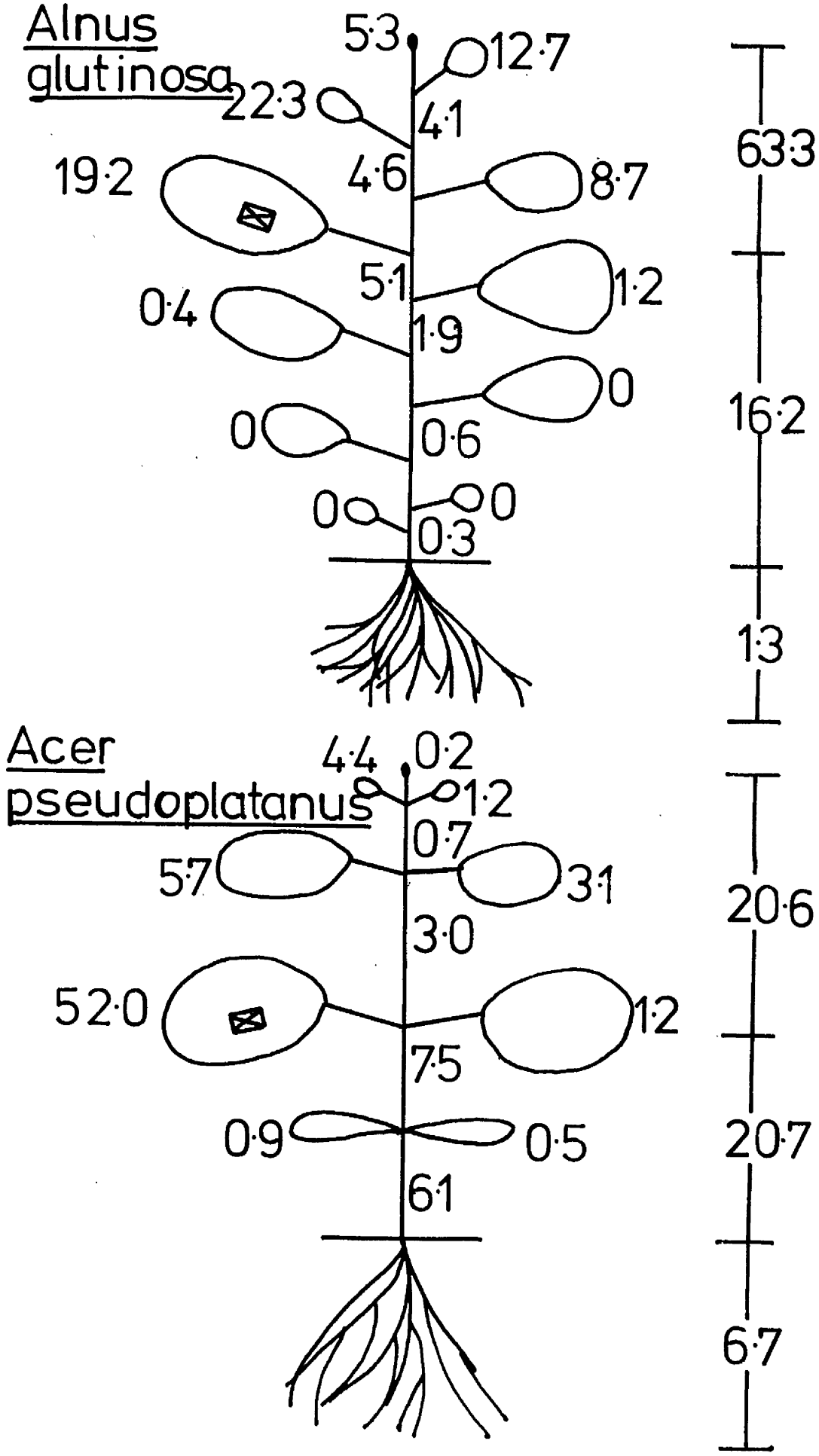
Table 14. A comparison of the export and distribution of ^{14}C 24 hours after application of ABA-2-^{14}C to an upper, young, large leaf and a lower, older, smaller leaf of A. glutinosa.

	% of applied ^{14}C taken up	% of uptake transported	% distribution			
			shoot above	donor leaf	shoot below	root
Uppermost expanded leaf	64.1	46.2	62.9 ±1.1	22.9 ±1.8	12.1 ±1.6	1.4 ±0.3
Lowest available leaf	59.3	23.8	9.8 ±2.3	83.6 ±3.7	3.9 ±0.8	3.9 ±1.1
Significance level difference			0.001	0.001	0.05	N.S.

roots from the lower donor leaf than from the upper donor leaf. Thus, clearly the position, age and size of the leaf of application of ABA-2-^{14}C have critical effects on the export and subsequent distribution of ^{14}C. Consequently in all experiments the leaf of application must be chosen carefully.

FIGURE 64

Distribution of ^{14}C in Alnus glutinosa and Acer pseudoplatanus 24 hours after application of 5μM ABA-2-^{14}C to the leaf indicated. The values given are the percentage of the total counts moved away from the area of application. Plants maintained under 16 hour photoperiods at 20°C. Values are of individual plants, representative of five.


Alnus glutinosa
5·3
12·7
22·3
4·1
19·2
4·6
8·7
5·1
1·2
0·4
1·9
0
0
0·6
0
0
0·3
63·3
16·2
1·3
Acer pseudoplatanus
4·4
0·2
1·2
0·7
5·7
3·1
3·0
52·0
1·2
7·5
0·9
0·5
6·1
20·6
20·7
6·7

(iii) The distribution of ^{14}C in A. glutinosa as a function of time after application of ABA-2-^{14}C.

In the previous section, distribution of ^{14}C was considered for only one time period. In the following experiment, the distribution of ^{14}C in A. glutinosa was studied as a function of time. ABA-2-^{14}C was applied using method 2 to the youngest, fully-expanded leaf of 3 month-old seedlings. Application was made at about 10.00 am and the plants were harvested after 12, 24, 48 and 72 hours. They were maintained on a 16 hour photoperiod, 8.00 am to midnight, at 20°C in a growth cabinet.

The % distribution of ^{14}C is shown in Figure 65a. After 12 hours there was considerable export of ^{14}C from the donor leaf and over 30% and 23% of the radioactivity was found in the shoot above and shoot below the donor leaf respectively. Over the next 12 hours the export of ^{14}C continued and the percentage accumulation in the shoot above doubled; as a result the % ^{14}C in the shoot below the leaf of application fell. After 24 hours virtually all the radioactivity had been taken up from the donor block and so subsequent changes would probably be due to a redistribution of radioactivity. Over the next 24 hours the % dpm in the donor leaf remained the same, while the % dpm in the shoot above fell, and in the shoot below increased. This suggested that some redistribution of radioactivity down the plant had occurred. Between 48 and 72 hours, the distribution of ^{14}C did not change markedly, suggesting that most of the radioactivity was immobilized.

During the experiment the % dpm in the root system

slowly increased, but even after 72 hours the accumulation was less than 6%. However, on a fresh weight basis, dpm g^{-1} (Fig. 65b), the accumulation of ^{14}C in the root system was more marked, after 48 hours it was more than half that in the shoot. Accumulation in root nodules is shown in detail in Figure 66. Expressed as a percentage of the total ^{14}C in the plant, the accumulation of ^{14}C in root nodules reached only 2.2% after 72 hours (Fig. 66a). However, expressed as a percentage of the radioactivity in the root system, the nodules made a significant contribution (Fig.66b), reaching nearly 50% after 12 hours and declining to 33% after 72 hours. Nevertheless the accumulation of ^{14}C in Alder nodules was far less marked than in nodules of P. vulgaris. In addition, it should be remembered that the accumulation of ^{14}C in the roots of B. pubescens, which does not have root nodules, was similar to Alder; so that Alder nodules do not appear to have a marked effect on distribution of ^{14}C in the plant.

FIGURE 65

The distribution of ^{14}C in Alnus glutinosa as a function of time after application of 5μM ABA-2-^{14}C to an upper, expanded leaf of plants maintained under a 16 hour photoperiod at 20°C. The distribution is expressed as (a) percentage of the total radioactivity moved away from the donor area into: the shoot above the donor leaf, —●—, the donor leaf, —○—; shoot below the donor leaf, —▲—; roots, —■—; (b) the dpm mg^{-1} fresh weight in shoots, —●—; roots, —▲—; root nodules, —■—. Values are means of five replicates, the vertical bars representing twice the standard error.

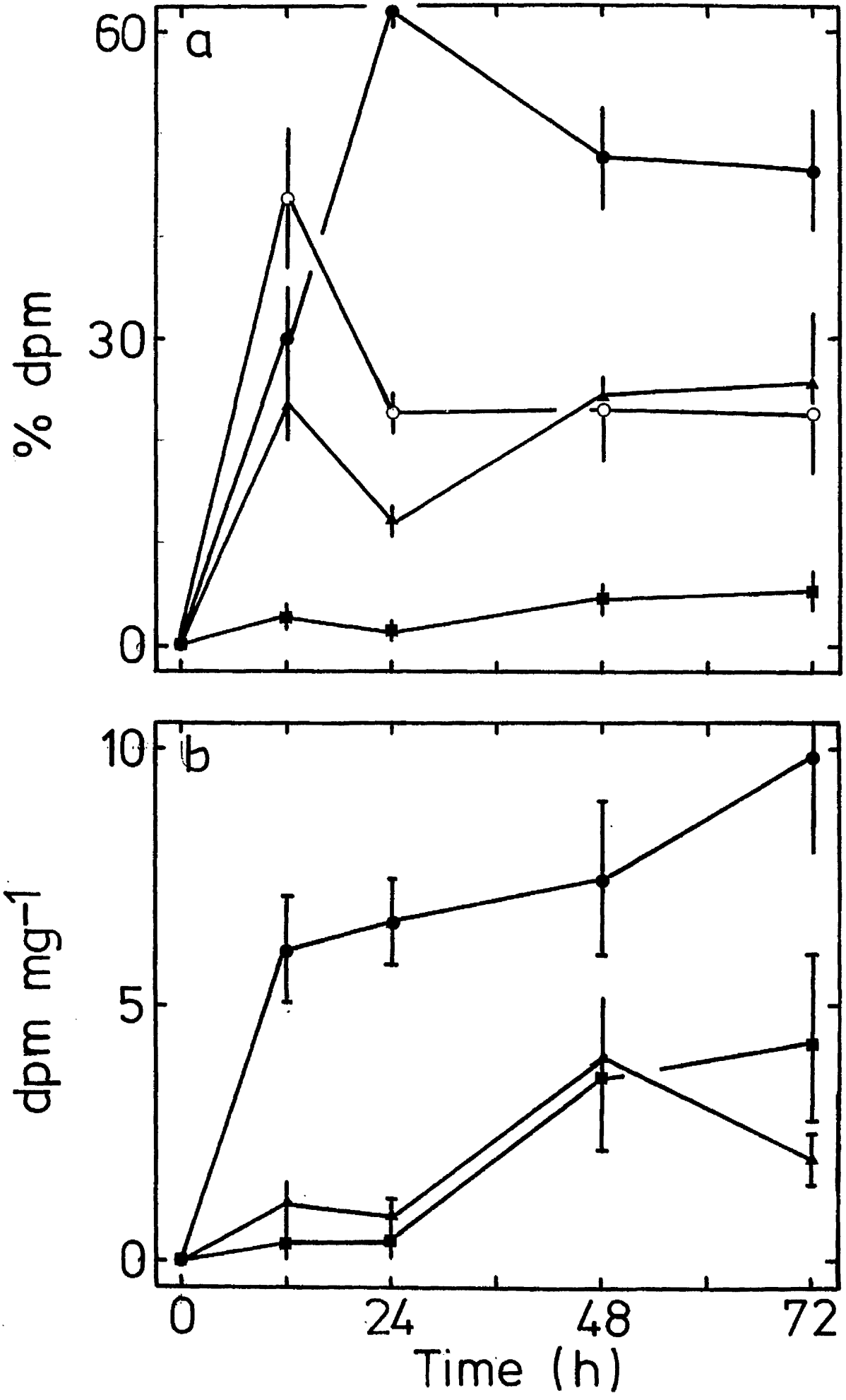
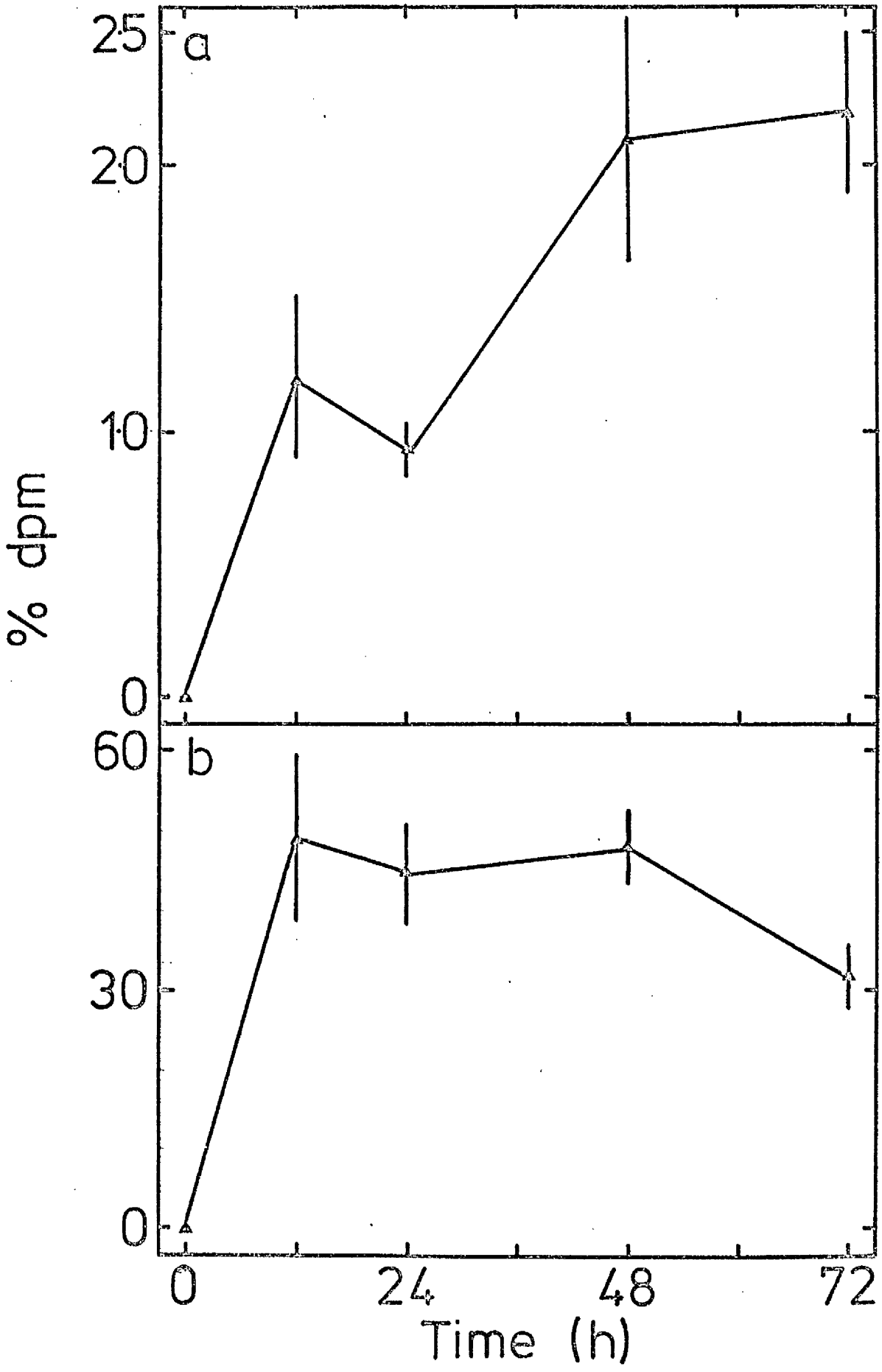

a
60
30
0
% dpm
b
10
5
0
dpm mg⁻¹
0
24
48
72
Time (h)

FIGURE 66

The accumulation of ^{14}C in root nodules of Alnus glutinosa as a function of time after application of ABA-2-^{14}C to an upper, expanded leaf of plants maintained under 16 hour photoperiods at 20°C. The accumulation in nodules is expressed as: (a) a percentage of the total radioactivity recovered from the plant; (b) a percentage of the radioactivity recovered from the root system. Values given are means of five replicates, vertical bars representing twice the standard errors.


25
20
10
0
a
% dpm
60
b
30
0
0
24
48
72
Time (h)

(iv) A comparison of the distribution of radioactivity following application of Sucrose-^{3}H and ABA-2-^{14}C to *A. glutinosa*

In a previous section the similarity was noted between the export of assimilates and the export of radioactivity from leaves of *A. glutinosa* supplied with ABA-2-^{14}C. Consequently it was decided to compare the distributions of radioactivity following the application of ABA-2-^{14}C and a radioactive tracer of a transportable assimilate such as sucrose-^{3}H.

Method 2 was used to apply the radioactive tracers to the youngest, fully-expanded leaf of 3 month-old *A. glutinosa* plants. After 24 hours the plants were harvested in the usual way for radioassay. The distribution of radioactivity, either tritium(^{3}H) or carbon(^{14}C), was expressed as a percentage of the total radioactivity recovered (Fig. 67a). The radioactivity from sucrose-^{3}H application was more mobile than the radioactivity from ABA-2-^{14}C. With the former, only 10% of the radioactivity remained in the donor leaf, and over 70% was recovered from the shoot above the donor leaf. The pattern was similar for both tracers, with more radioactivity moving above the donor leaf than below it, although 9% of the radioactivity from sucrose-^{3}H was recovered from the roots, compared with 2% for ABA-2-^{14}C.

Figure 67b shows the radioactivity recovered from the root nodules as a percentage of the total radioactivity in the plant; five times more radioactivity from sucrose-^{3}H accumulated in nodules than from ABA-2-^{14}C. Similarly, the percentage of the total root system radioactivity in the root nodules was greater for sucrose-^{3}H than ABA-2-^{14}C (Fig. 68c).

Thus, although the overall pattern of distribution was similar for sucrose-^{3}H and ABA-2-^{14}C, there was quantitative differences due to the greater mobility of the sugar in the tissues.

FIGURE 67

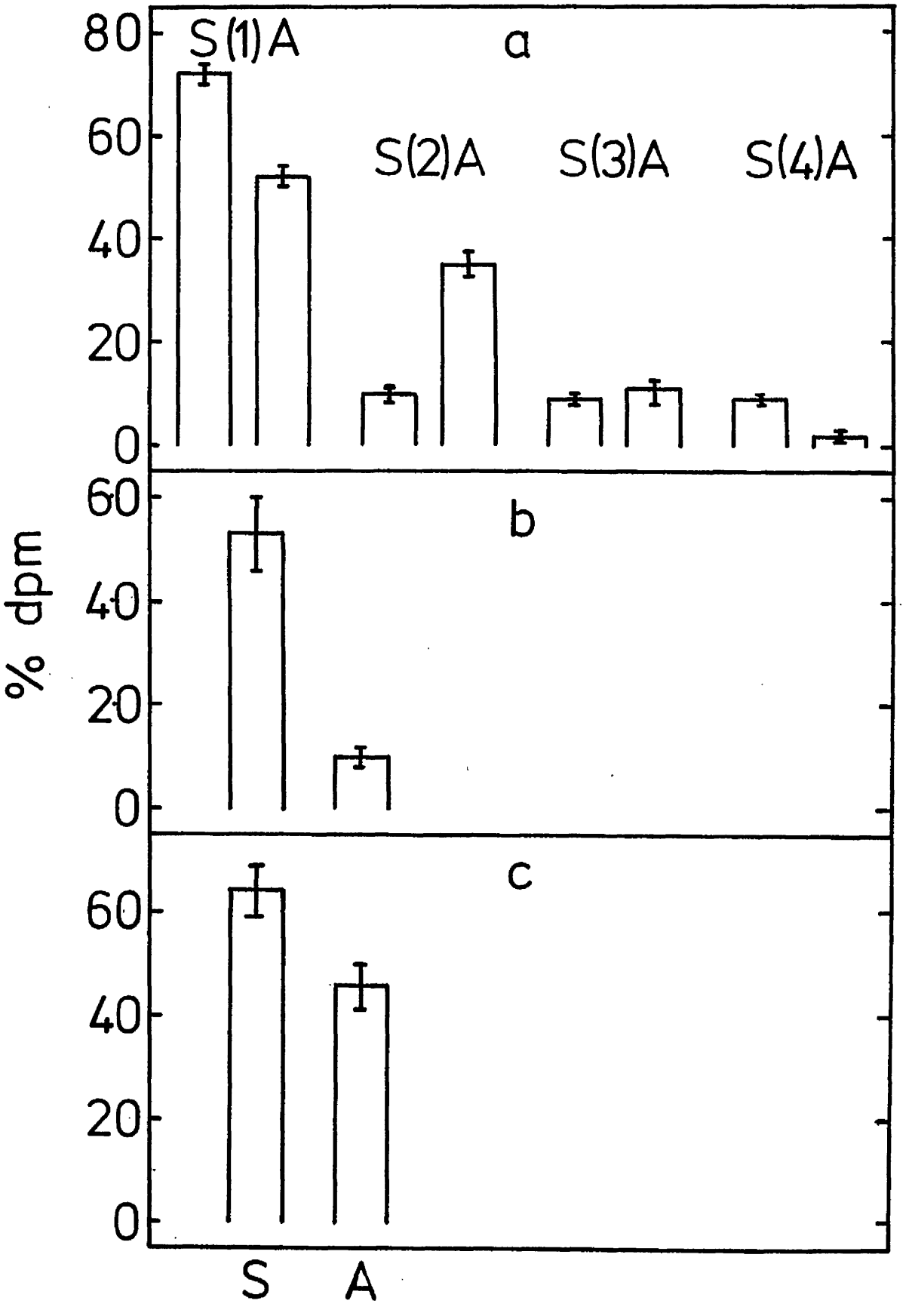
A comparison of the distribution of radioactivity in Alnus glutinosa, 24 hours after application of 10^{-8}M sucrose-^{3}H (S) or 5μM ABA-2-^{14}C (A) to an upper, expanded leaf of plants maintained under 16 hour photoperiods at 20°C.

(a) Percentage distribution of radioactivity in: (1) the shoot above the donor leaf; (2) the donor leaf; (3) the shoot below; (4) roots.

(b) The accumulation of radioactivity in root nodules as a percentage of the total radioactivity recovered from the plant.

(c) The accumulation of radioactivity in root nodules as a percentage of the radioactivity in the root system.

The vertical bars represent the standard errors of the means, which were from 5 replicates.


80
60
40
20
0
S(1)A
a
S(2)A
S(3)A
S(4)A
60
40
20
0
b
% dpm
60
40
20
0
c
S
A

(v) Metabolism of ABA-2-^{14}C in A. glutinosa.

As in the studies using other species, uptake and movement in A. glutinosa has been discussed only in terms of radioactivity. It is important to attempt to elucidate the identity of the mobile radioactive moiety and, using the radioactive tracer, to study the extent to which ABA is metabolised in the tissue.

For studies on ABA metabolism in A. glutinosa, 3 month-old hydroponically-cultured plants were supplied with culture solutions containing 10^{-5}M ABA-2-^{14}C for two days. This concentration of ABA-2-^{14}C allowed extensive uptake of radioactivity to occur. Plants were harvested and shoots and roots were extracted separately in 80% methanol. The methanol extracts were reduced to the aqueous phase and extracted with ether at pH 3.0, with ether at pH 8.0 and finally with water-saturated n-butanol at pH 8.0. Samples were taken at each step for analysis. Chromatographic analysis was carried out on Whatman No. 3 paper, developed in one of three solvent systems:-

1. n-butanol: n-propanol: Ammonia: Water :: 2:6:1:2 (v/v).
2. n-butanol: Acetic acid: Water :: 5:1:2.2 (v/v).
3. Isopropanol: Ammonia: Water :: 8:1:1 (v/v).

The results of the analyses are given as histograms showing the % dpm in half-Rf zones down the chromatogram (Figs. 68, 69, 70). The Rf values of the major peaks of radioactivity in each chromatogram together with the % dpm recovered at the Rf corresponding to ABA-2-^{14}C are shown in Table 14.

Table 14. Rf values of the major peak of ^{14}C and the % dpm recovered at the Rf of ABA-2-^{14}C in the chromatograms of A. glutinosa extracts; details as in Figures 68, 69, 70, 71.

A - Rf of major peak of ^{14}C

B - % dpm recovered from the half-Rf zone corresponding to the Rf of ABA-2-^{14}C

Extract	Solvent System					
	2 : 6 : 1 : 2		5 : 1 : 2.2		8 : 1 : 1	
	A	B	A	B	A	B
ABA-2-^{14}C	0.775	57	0.925	75	0.755	43
Acidic ether shoot	0.70	3	0.825	2	0.65	10
Acidic ether root	0.70	8	0.80	2	0.60	5
Basic ether shoot	0.825	10	0.65	0	0.85	7
Basic ether root	0.825	6	0.80	0	0.85	6
Culture soln.	0.775	14	0.90	30	0.75	11
Final aqueous shoot	0.475	1				
Final aqueous root	-	10				
n-butanol shoot	0.80	31				

The basic solvent systems (1, 3) gave similar results, the Rf of ABA-2-^{14}C was only different by 0.025 Rf. The acidic solvent system (2) showed a similar pattern of breakdown although the Rf values were different. There was little difference between shoot and root extracts. The % dpm recovered at the Rf corresponding to ABA-2-^{14}C from the chromatograms of all the ether extracts, except the culture solutions, was less than 10%. This included the acidic

ether extracts which should have contained the ABA-2-^{14}C had it been present in the original extract.

The analyses of the final aqueous and n-butanol extracts are shown in Figure 71 and summarized in Table 14. The major peak found at Rf 0.45 - 0.50 in the aqueous shoot extract did not occur in any other extract using the same solvent system (2:6:1:2). The n-butanol extract was interesting in that it gave a major peak corresponding to ABA-2-^{14}C. If correct, this is difficult to explain since most, if not all the ABA-2-^{14}C present in the original extract should have been removed during the ether partitioning. One possibility is that the ABA-2-^{14}C was conjugated with some moiety making it ether insoluble until it was extracted by water-saturated n-butanol which is a highly effective solvent. However this result was only obtained once, and therefore requires confirmation.

The results show that very little radioactivity was recovered as ABA-2-^{14}C in alcoholic extracts of A. glutinosa tissue supplied for two days with ABA-2-^{14}C. There appeared to be two or possibly three chromatographically distinct radioactive products in the tissue extracts. One was ether soluble at pH 3.0 and occurred at Rf 0.65 - 0.70 in 2:6:1:2. The second was ether-soluble at pH 8.0 and occurred at Rf 0.825 in 2:6:1:2. The third was ether insoluble and occurred at Rf 0.45 - 0.50 in 2:6:1:2.

FIGURE 68

Metabolism of ABA-2-^{14}C in Alnus glutinosa. Paper chromatography of shoot and root extracts taken two days after application of 10^{-3}M ABA-2-^{14}C to the culture solutions of hydroponically-cultured plants maintained under 16 hour photoperiods at 20°C. Chromatograms were developed in n-butanol: n-propanol: 0.880 ammonia: water :: 2:6:1:2 v/v. The histograms show the percentage radioactivity recovered from each half-Rf zone in chromatograms of:-

(a) stock ABA-2-^{14}C solution

(b) acidic ether extract of shoots

(c) acidic ether extract of roots

(d) basic ether extract of shoots

(e) basic ether extract of roots

(f) culture solution.

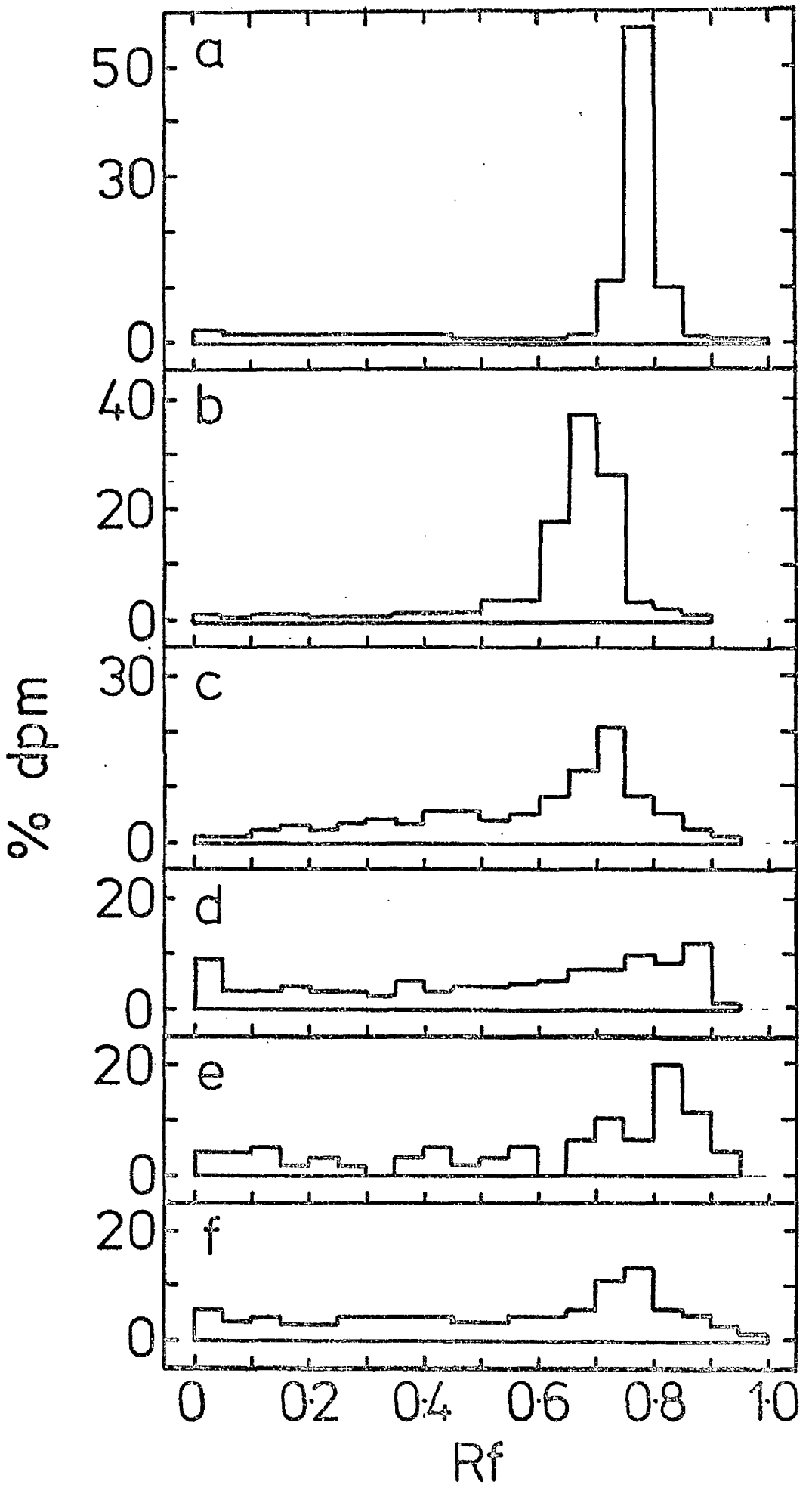

a
b
c
d
e
f
50
30
0
40
20
0
30
0
20
0
20
0
20
0
% dpm
0
0·2
0·4
0·6
0·8
1·0
Rf

FIGURE 69

Metabolism of ABA-2-^{14}C in Alnus glutinosa (cont.). Paper chromatography of shoot and root extracts using n-butanol: acetic acid: water :: 5:1:2.2 v/v., as the solvent system. Details are as in Figure 68.

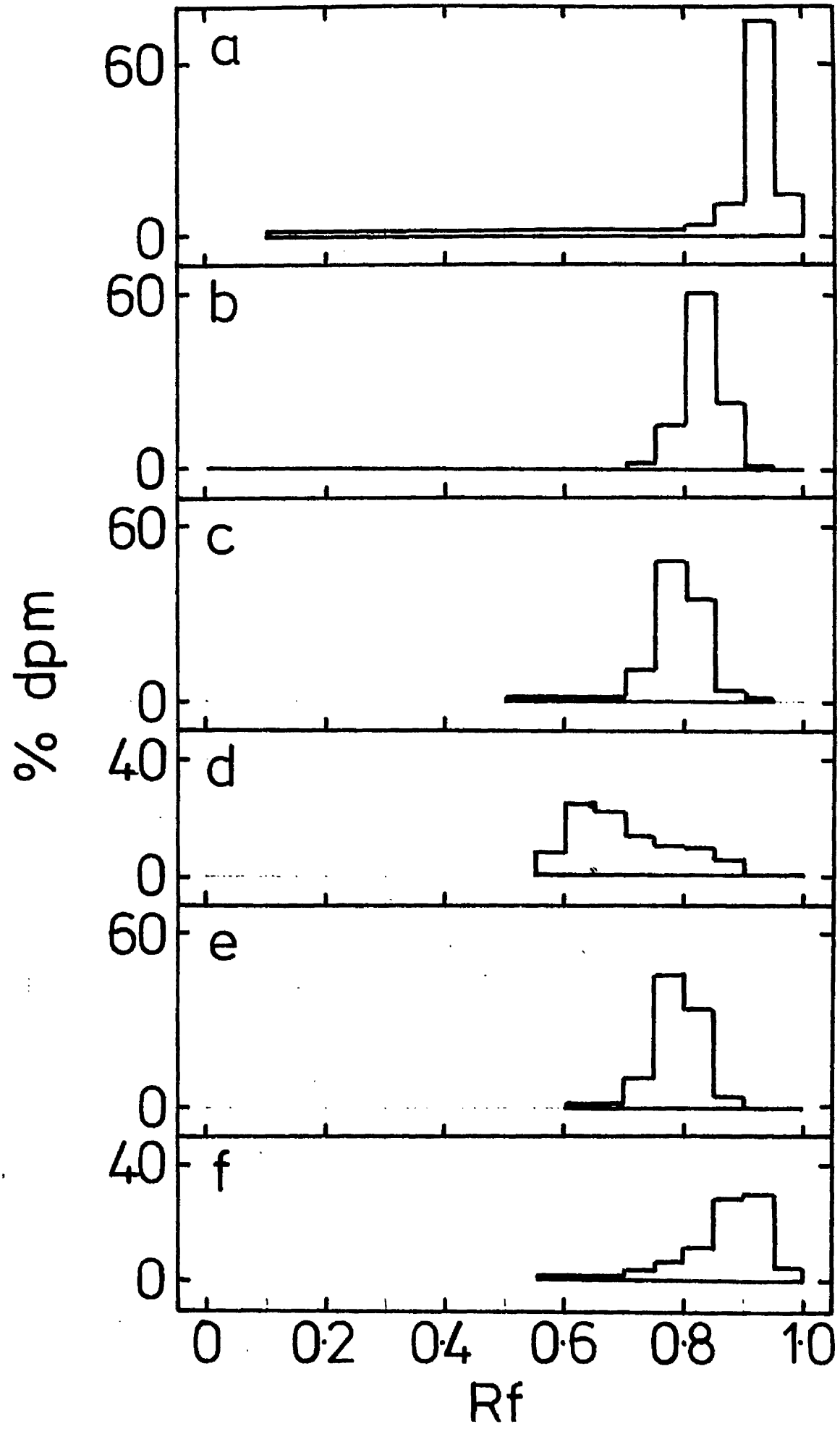

a
b
c
d
e
f
60
0
40
% dpm
0
0·2
0·4
0·6
0·8
1·0
Rf

FIGURE 70

Metabolism of ABA-2-^{14}C in Alnus glutinosa (cont.). Paper chromatography of shoot and root extracts using isopropanol: ammonia: water :: 8:1:1 v/v, as the solvent system. Details are as in Figure 68.

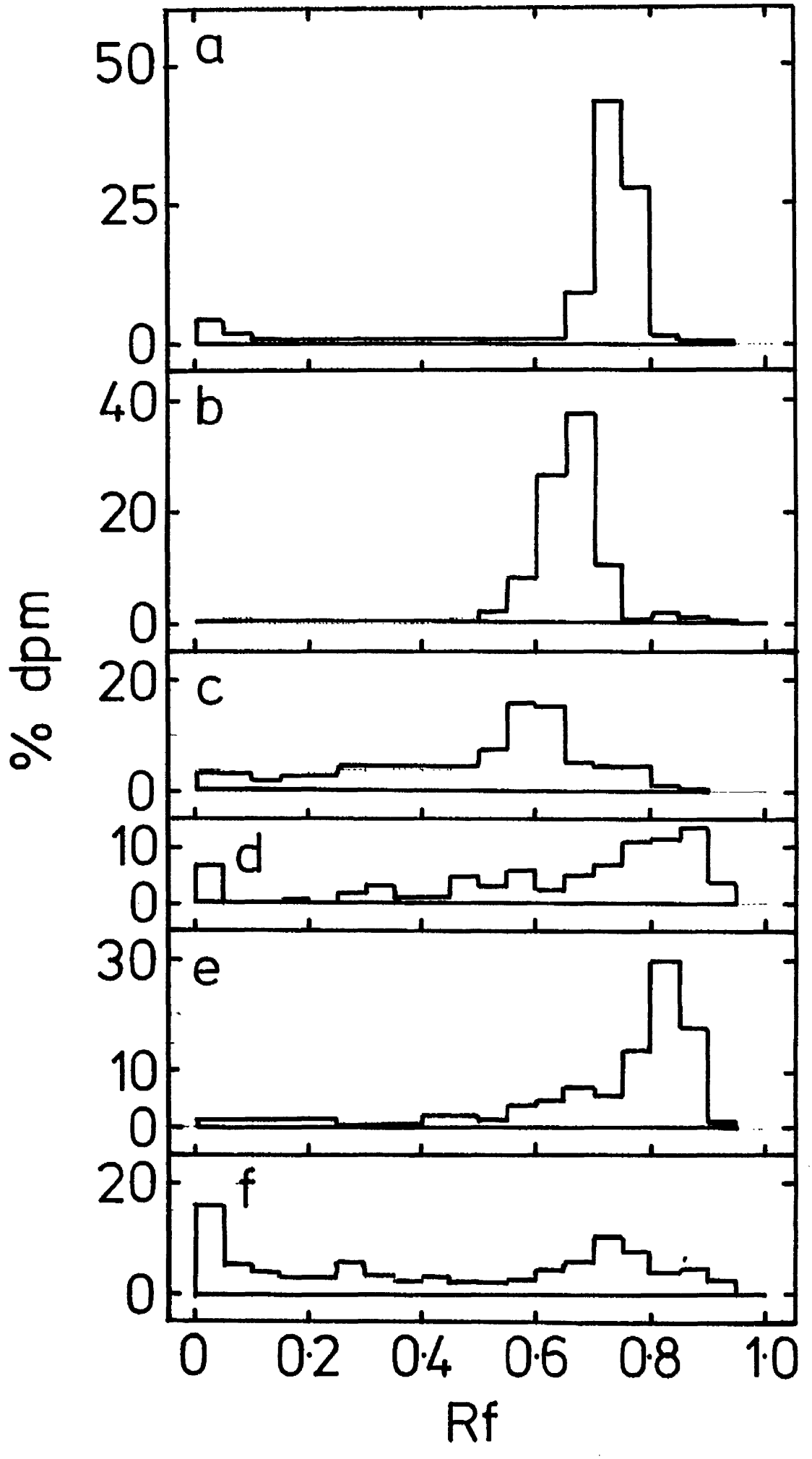

a
b
c
d
e
f
50
25
0
40
20
0
20
0
10
0
30
10
0
20
0
% dpm
0
0·2
0·4
0·6
0·8
1·0
Rf

FIGURE 71

Metabolism of ABA-2-^{14}C in *Alnus glutinosa* (cont.). Paper chromatography, using 2:6:1:2, of:-

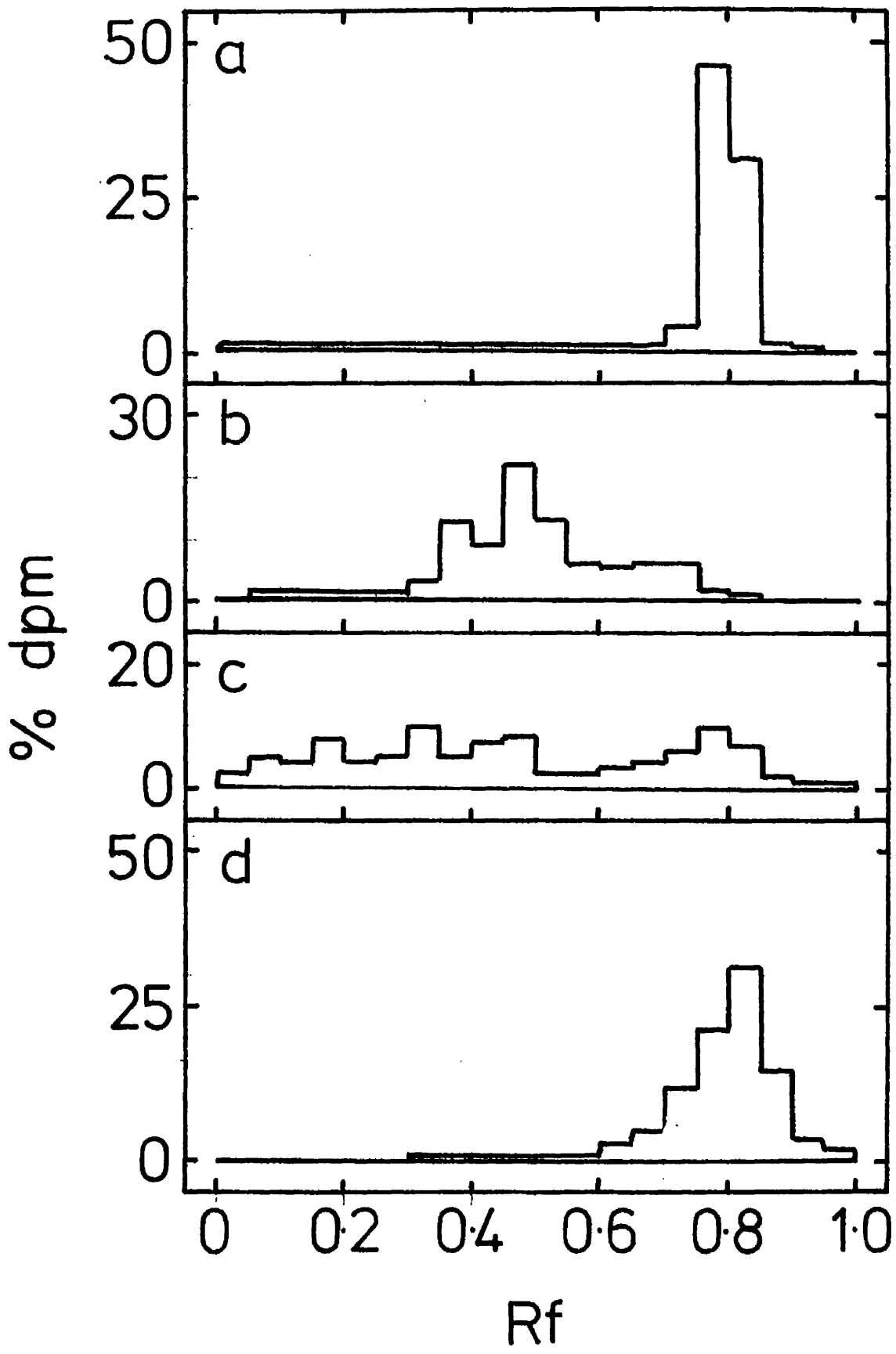
(a) stock ABA-2-^{14}C solution

(b) aqueous extract of shoot tissue

(c) aqueous extract of root tissue

(d) saturated n-butanol extract of final aqueous extract of shoot tissue.

Details are as in Figure 68.


50
a
25
0
30
b
0
20
c
0
50
d
25
0
% dpm
0
0·2
0·4
0·6
0·8
1·0
Rf

III B Studies on the role of ABA in the control of bud dormancy in A. glutinosa and B. pubescens.

(i) The photoinduction of bud dormancy in A. glutinosa and B. pubescens.

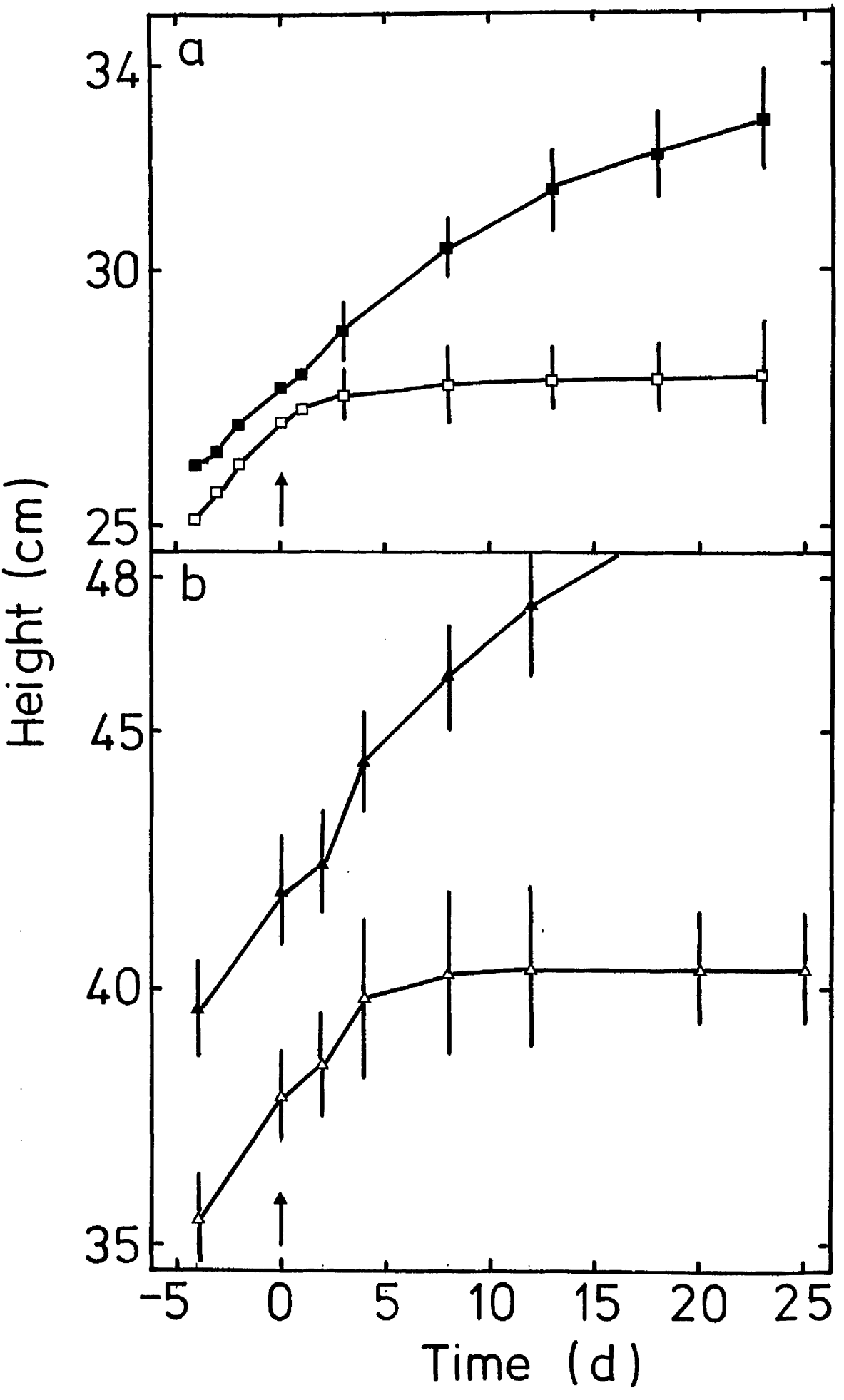
In all the woody species investigated so far, day length has a marked effect on the formation of the dormant state. Short days promote or induce the cessation of growth and the formation of resting buds (Waring, 1969). As the first step in the study of the role of ABA in controlling bud dormancy, the photoinduction of bud dormancy in the experimental plants under artificial conditions was examined, so that the characteristics of the response under these particular conditions could be elucidated.

Five month-old plants of A. glutinosa and B. pubescens were placed in a growth cabinet at 20°C under a 16 hour photoperiod (8.00 am - midnight). The photoperiod consisted of 8 hours full intensity light plus 8 hours of supplementary light from two fluorescent tubes. The heights of the plants were measured over a four day period and then half the plants of each species were transferred to an adjacent cabinet sharing the same temperature controlling mechanism on an 8 hour photoperiod of full intensity light. The heights of the two sets of plants were measured at regular intervals for the following 25 days (Fig.72). Between the second and third day (A. glutinosa), or the fourth and eighth day (B. pubescens) the rate of height increase of the plants under the eight hour photoperiod was reduced. By the thirteenth day (A. glutinosa) or the twelfth day (B. pubescens) stem elongation had ceased in the short day plants, while the long day plants continued to grow at a steady rate. Resting buds, both at the apex

and at the lateral axes were beginning to form by this time in plants maintained under short days. In Alnus glutinosa the formation of resting buds was characterised by a reddening of the bud scales. By the twentieth day all the buds were dormant.

FIGURE 72

The photoperiod induction of bud dormancy in (a) Alnus glutinosa, —■—, long days, —□—, short days; (b) Betula pubescens, —▲—, long days, —△—, short days. The long photoperiods were comprised of 8 hours full intensity light plus 8 hours supplementary light, while the short photoperiods were of 8 hours full intensity light only. Both sets of plants were maintained at 20°C. The arrows indicated the beginning of the short day treatments. The values given are the means of thirty replicates, twice the standard errors given as vertical bars.


a
b
34
30
25
48
45
40
35
Height (cm)
-5
0
5
10
15
20
25
Time (d)

(ii) The effect of root application of ABA on the growth of A. glutinosa in long and short days.

Having determined that short days would induce bud dormancy in A. glutinosa under the experimental conditions used, the next step was to study the effects of ABA on the growth of A. glutinosa under the same conditions.

It was found that application of ABA solution to the culture flasks of hydroponically cultured bean plants had certain physiological effects (Section IIB (i)). In addition it was found when comparing methods of applying ABA-2-^{14}C to A. glutinosa, that this method of application allowed some uptake of ^{14}C even though the ABA-2-^{14} was supplied at a very low concentration (Table 11).

It was decided to carry out a preliminary series of experiments designed to (a) test the effectiveness of root application of ABA solution and (b) determine the most suitable and meaningful parameter(s) for assessing the effect of ABA on the growth of A. glutinosa.

Twenty plants were set up at 20°C under 16 hour photoperiods and ten were supplied with ½ strength Crone's culture solution 10^{-5}M ABA. The other ten plants were controls supplied with Crone's solution and an appropriate concentration of methanol. The following parameters of growth were measured for both control and treatment plants over periods of 15, to 48 days.

1. Height
2. Node number
3. Internode length (second internode from apex)
4. Leaf length (first expanding leaf)

It was proposed to take fresh and dry weights at the end of the experiment but in the end this was not done. Parameters 2, 3 and 4 were only measured over the first fifteen days. Application of ABA/culture solution was made at day 1. The culture solutions were renewed at 14 day intervals.

The effect of ABA on plant height, expressed as the percentage increase over the original height (Fig. 73a), was neither marked nor rapid, but after 25 days there was a clear difference between treatment and control plants. This difference was not significant because of the large variation around the mean. This trend continued throughout the experiment and the difference was significant (to 0.05P) after 35 days, but the treatment plants were still growing after 48 days. Thus the application of ABA had reduced the rate of height increase from 3% day^{-1} to 2.25% day^{-1}.

The effect of ABA on node number (Fig. 73b) showed a similar, but less marked trend, over 15 days, reducing slightly the rate of increase in node number.

The effect of ABA on the percentage increase in internode length (Fig. 74a, 2nd internode from apex) was more marked, causing a significant inhibition (to 0.05P) after 5 days, and this difference was maintained over the fifteen day period. By that time that particular internode had ceased to elongate. The effect on leaf expansion showed the same trend, but the differences were not significant (Fig. 74b).

Thus the application of ABA to the roots of hydroponically-cultured plants of A. glutinosa under long days had an inhibiting effect, albeit slight, on all the parameters of growth measured. Improved replication may have made the differences

FIGURE 73

The effects of ABA, supplied to the culture solution of hydroponically cultured plants of A. glutinosa growing in long days, on: (a) percentage increase in height; (b) node number; —●—, control plants; —○—, treatment plants. The treatment plants were supplied with ABA at a final concentration of 10^{-5}M, the control plants with the appropriate amount of methanol. The values plotted are means of 10 replicates; the vertical bars represent twice the standard errors, calculated using angular transformation when percentage values were involved.

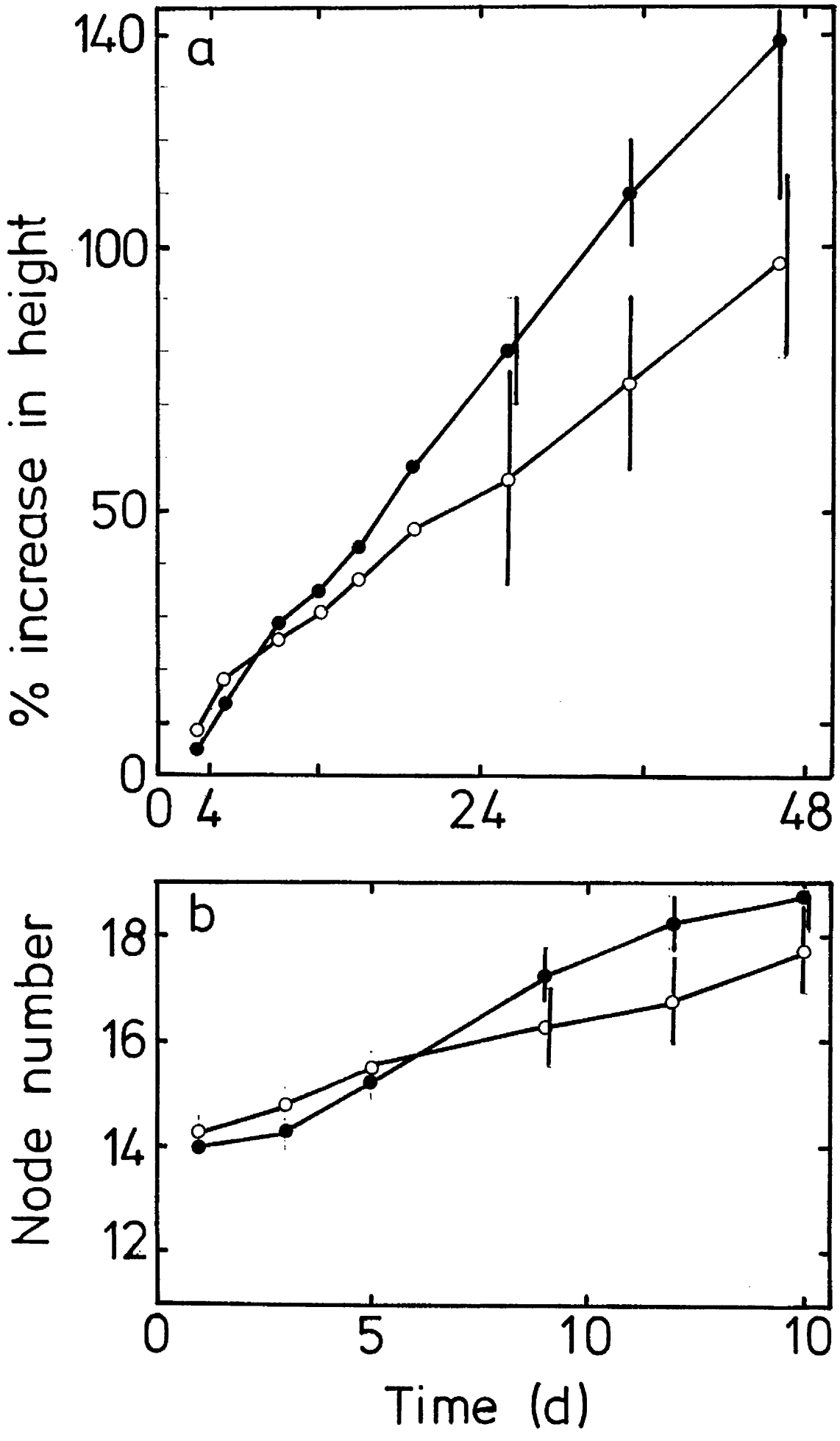
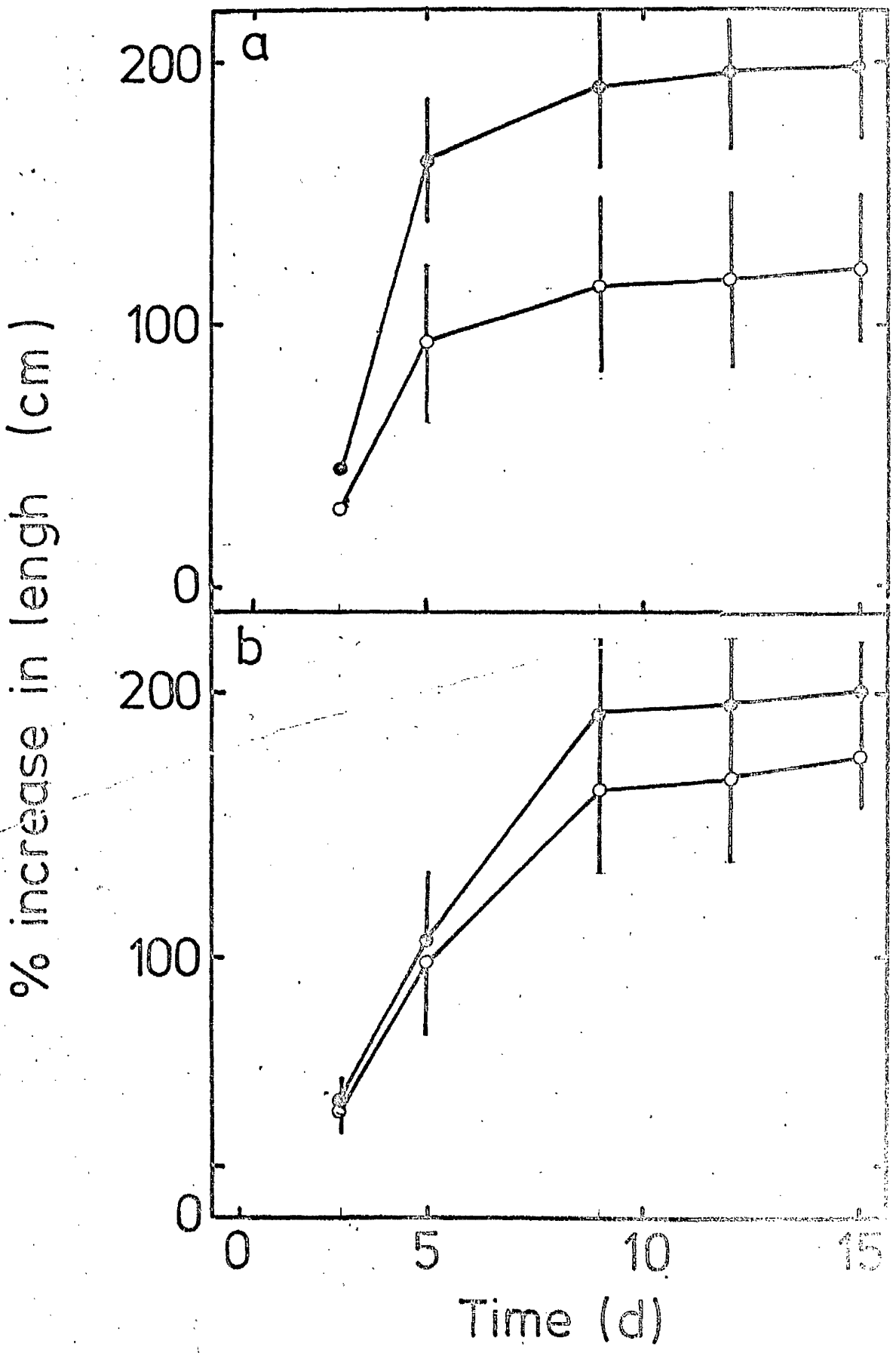

a
140
100
50
0
% increase in height
0
4
24
48
b
18
16
14
12
Node number
0
5
10
10
Time (d)

FIGURE 74

The effects of ABA, supplied to the culture solution of hydroponically cultured plants of A. glutinosa growing in long days, on: (a) percentage increase in internode length, second internode from the apex; (b) percentage increase in the length of the leaf lamina, first expanding leaf; —●—, control plants; —○—, treatment plants. The treatment plants were supplied with ABA at a final concentration of 10^{-5}M, the control plants with the appropriate amount of methanol. The values plotted are means of 10 replicates; the vertical bars represent twice the standard errors, calculated using angular transformation.


a
b
200
100
0
200
100
0
0
5
10
15
% increase in lengh (cm)
Time (d)

more significant. Perhaps the most useful parameters to show the effect on growth were plant height and internode length, which are obviously related parameters. The problem with the latter is that any one internode only elongates for a limited period, and over a long term experiment a number of internodes would have to be measured. Thus to obtain a continuous record of the effect of ABA on the growth of A. glutinosa, the most convenient parameter to measure would be plant height.

Having established a suitable method of application and a suitable parameter of growth, the effect of ABA on the growth of A. glutinosa in long and short days was examined. Plants were set up as before, with the following treatments.

1. Long days + methanol (Control)
2. Long days + 10^{-5}M ABA
3. Short days + methanol (Control)
4. Short days + 10^{-5}M ABA

The lighting regimes were: long days - 8 hours full intensity plus 8 hours supplementary; short days - 8 hours full intensity. Thirty plants were used per treatment, and plant heights were measured at five day intervals. Application of the ABA and introduction of the short day plants to short days occurred at day 0, as indicated by the arrow (Fig. 75). The ABA was renewed after eight days.

The response of the long and short day control plants was typical. In short days there was a reduction in the rate of height increase after 3 days, and growth had virtually ceased after 13 days. In long days the plants continued to grow at a relatively steady

rate, declining slightly over 23 days. ABA caused a significant (0.01P) reduction in the rate of height increase compared to the controls in long days after eight days. This difference was maintained, if not slightly increased, for the duration of the experiment, although the plants were still growing after 23 days at the rate of 0.06 cm day^{-1} compared to the controls at 0.12 cm day^{-1}. ABA had little effect on short day plants. The difference between the two curves was due mainly to the control plants growing at a faster rate prior to the application of ABA and the introduction of short days. Thereafter the two curves were almost parallel. This is shown clearly when the rate of increase in height per day is plotted against time (Fig. 76 (i)). The growth rate of the short day treatment plants was, for some unknown reason, significantly higher than the other plants prior to the application of ABA and short days. This rate fell rapidly and one day after application there was no significant difference between the growth rates of the two sets of short day plants; a situation which continued for the rest of the experiment. The growth rate of the long day + ABA plants was significantly lower than the growth rate of the long day control plants three days after application, having initially been higher than the control plants. This difference in growth rate was significant (to 0.01P) for the rest of the experiment.

The time when an apical bud became dormant was also recorded during the experiment (Fig. 76 (ii)). Clearly the determination of when a bud becomes dormant is difficult without using some arbitrary criterion. For Alnus glutinosa the characteristic reddening of the bud scales was taken as the criterion for dormancy, since this could be easily recorded, although almost certainly the bud would have been developing a

FIGURE 75

The effects of photoperiod and ABA on the increase in height (cm) of hydroponically cultured plants of A. glutinosa, over a period of 23 days at 20°C. The treatments were as follows:

—■—, long days (8 hours full intensity light plus 8 hours supplementary).

—▲—, long days plus ABA.

—□—, short days (8 hours full intensity light).

—△—, short days plus ABA.

The ABA was supplied to the culture solutions at a final concentration of 10^{-5}M. The arrows indicate the time of application. The short day treatments began at day 0. The values plotted are means of thirty replicates, the vertical bars representing the standard errors.

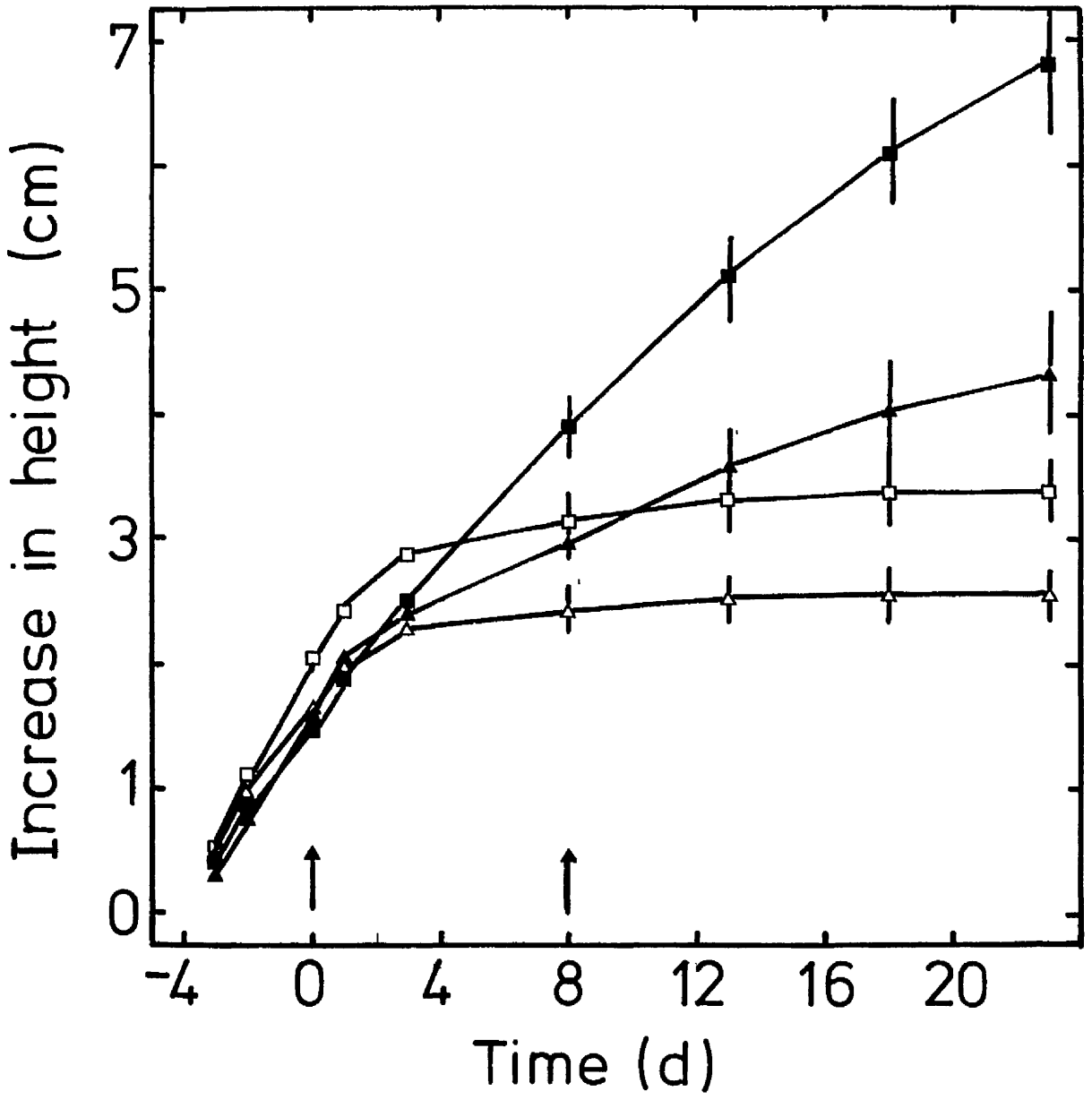

Increase in height (cm)
7
5
3
1
0
-4
0
4
8
12
16
20
Time (d)

FIGURE 76

The effects of photoperiod and ABA on, (i) the rate of height increase (cm day^{-1}) and (ii) the percentage of dormant buds, of hydroponically cultured plants of *A. glutinosa*. The experimental details are as in Figure 75. A bud was considered dormant when it developed a characteristic red colouration.

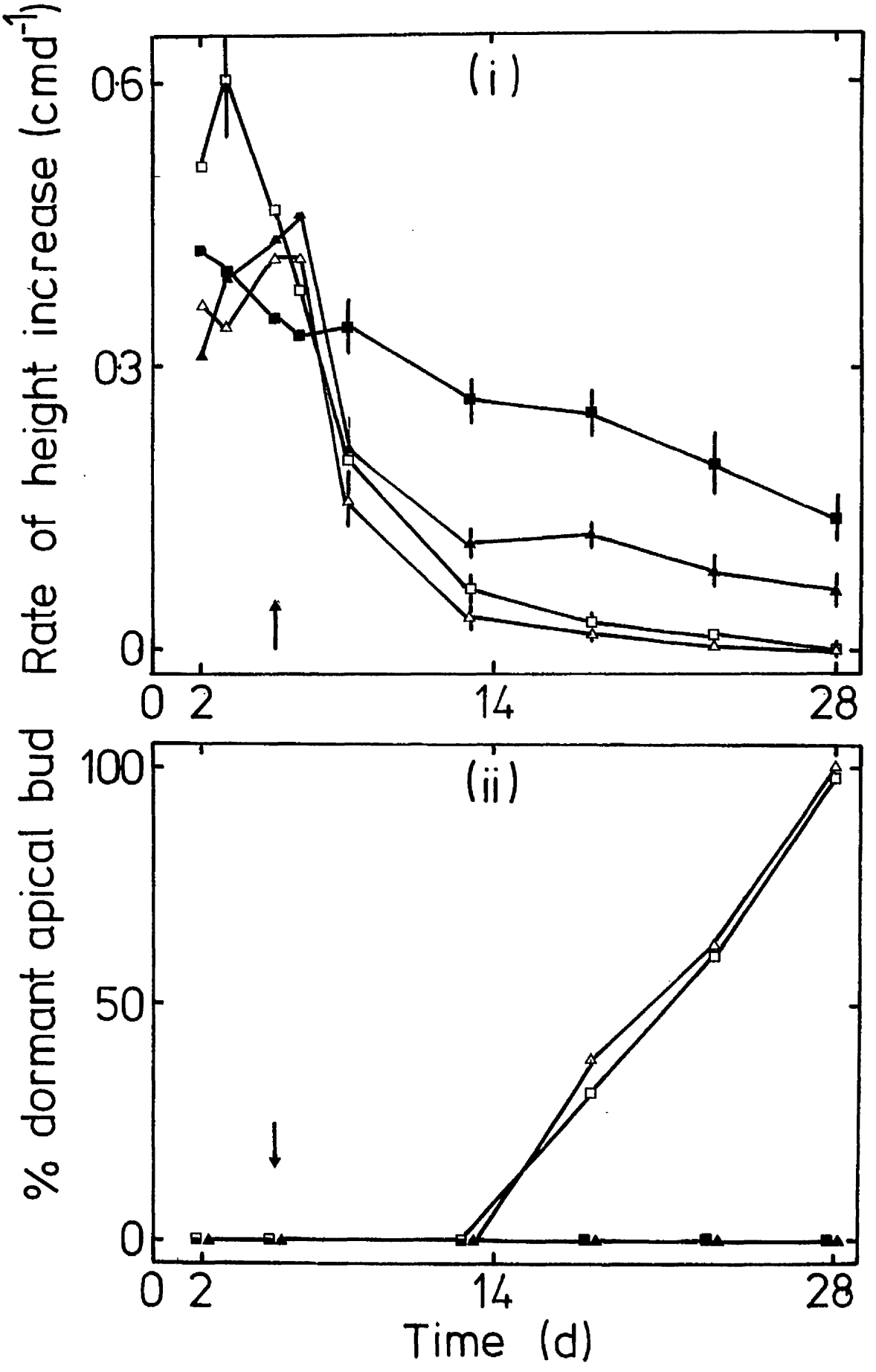

(i)
0·6
0·3
0
0 2 14 28
Rate of height increase (cmd⁻¹)
(ii)
100
50
0
0 2 14 28
% dormant apical bud
Time (d)

PLATE 1 Close up of the apical bud of a 5 month-old plant of Alnus glutinosa maintained under short days (8 hour photoperiods) for 23 days.

PLATE 2 Close-up of the apical bud of a 5 month-old plant of Alnus glutinosa maintained under long days (16 hour photoperiods) continuously.

PLATE 3 Close-up of the apical bud of a 5 month-old plant of Alnus glutinosa maintained under long days and supplied continuously with 10^{-5} M ABA to the roots.

resting structure before this colour change occurred. Using this criterion, 32% of the apical buds became dormant 13 days after the introduction of short days. They were all dormant after 23 days. There was little or no difference between control and ABA treated plants. No buds became dormant under long days, either with or without ABA(see Plates 1, 2, 3).

These results suggest that exogenously applied ABA does not induce bud dormancy in Alnus glutinosa growing under 16 hour photoperiods, when applied at a concentration of 10^{-5}M to the roots of hydroponically cultured plants, over a period of 23 days. However, it appears to have a marked effect, which is evident three days after the initial application, on stem elongation, in these plants. ABA has little effect on the growth of plants maintained under 8 hour photoperiods.

On day eight of the above experiment, 1 cm^3 of 10^{-5}M ABA-2-^{14}C was added to the culture solutions of 5 plants in both of the ABA treatments. At the end of the experiment these plants were harvested for radioassay. Samples were also taken for chromatographic analysis. The uptake and distribution of ^{14}C in these plants are shown in Table 15

The uptake of ^{14}C was greater in long day than in short day plants. This was probably a reflection of the fact that the short day plants had virtually stopped growing by day 8 (Fig. 75). However the % distribution of ^{14}C between shoots and roots of long and short day plants was very similar. The concentration of ^{14}C on a fresh weight basis was greater in the apices of long day plants than short day plants, but this was

Table 15. The uptake and distribution of ^{14}C, 15 days after application of ABA-2-^{14}C to the culture solutions of hydroponically-cultured plants of A. glutinosa maintained under long and short days. Means of five replicates.

	Total Uptake	% Distribution		Distribution in dpm g^{-1} fr.wt.			
		Shoot	Roots	Apex	1st leaf	Roots	Nodules
LD	4781	47.6	52.4	860	1236	4306	709
SD	2700	46.7	53.3	686	1605	2830	508

reversed in the first or youngest expanding leaf. The accumulation of ^{14}C in the roots and nodules was greater in long day plants.

The results of the chromatographic analyses of ethanolic extracts from the radioactive treatment plants as shown in Table 16. The major peak in the apical extracts from long and short day plants did not correspond with ABA-2-^{14}C, and only 12.5% (short day) and 4.7% (long day) of the ^{14}C was recovered from the Rf zone of ABA-2-^{14}C. Thus, even though some radioactivity had accumulated in the apices of long and short day plants most of it appeared to be chromatographically distinct from ABA-2-^{14}C.

These results do not provide any evidence that the effects of short days on the growth of A. glutinosa are associated with the movement, accumulation or metabolism of ABA.

Table 16. Chromatographic analysis of ethanolic extracts from *A. glutinosa* plants growing in long or short days, supplied with ABA-2-^{14}C for 15 days. Application was made to the culture solution. Paper chromatography using n-butanol: n-propanol: ammonia: water :: 2:6:1:2 (v/v).

Extract	Rf of major peak	% dpm recovered at ABA Rf zone
ABA-2-^{14}C	0.75 - 0.80	56.5
Apex SD	0.65 - 0.70	12.5
Apex LD	0.60 - 0.65	4.7
Culture Soln. SD	0.70 - 0.80	57.0
Culture Soln. LD	0.70 - 0.80	62.0

(iii) The effect of leaf application of ABA on the growth of A. glutinosa and B. pubescens.

The experiments were carried out to re-examine the possible role of ABA in the hormonal control of bud dormancy, an hypothesis which is based, in part, on the experiments of Eagles (Eagles, 1963) (Eagles and Wareing, 1964) and more recently, El-Antably et al., (1967). The experimental techniques used in the present experiment are similar to those of Eagles except that pure ABA solutions were applied to leaves, instead of tissue extracts and plants of Alnus glutinosa were used in addition to B. pubescens. Moreover, the availability of radioactively-labelled abscisic acid has enabled the fate of the applied ABA to be followed more closely. The experiments were carried out in a growth cabinet with a 16 hour photoperiod at 20°C. Duplicate experiments were carried out for each species, with 15 replicates per treatment. In each experiment application of ABA was made by immersing a rolled-up, upper, expanded leaf in a small vial containing water or a solution of ABA. As the plant grew during the experiment further applications were made to the next fully expanded leaf; the day of application is indicated by an arrow on the graph. Plant height and node number were recorded at regular intervals. A set of plants were left untreated as controls. Graphs were plotted of height against time and increase in node number against time for A. glutinosa (Figs. 77, 78, 79) and B. pubescens (Fig. 80, 81). The final % increase in height and increase in node number together with the statistics are given in Table 17.

In the first experiment with A. glutinosa (Figs. 77, 78)

three concentrations of ABA were used, namely, 2×10^{-4}M, 10^{-5}M and 5×10^{-7}M, together with a water treatment and a control. The increase in height was approximately linear with time, and there were no marked differences between treatments. The % increases in height at the end of the experiment (Table 17) showed that all the treatments increased more than the control, especially the lower concentrations of ABA, but none of the differences between treatment and control means were significant. The node number for each treatment increased relatively linearly with time and again all the treatments increased more than the control. After 24 days, the mean increase in node number for the 5×10^{-7}M and 10^{-5}M treatments were significantly greater (0.01P and 0.05P respectively) than the control. Thus in this experiment there was no evidence of any inhibition of growth by ABA, and some evidence of promotion of growth at the lower concentration of ABA.

In the second experiment only water and 2×10^{-4} treatments were compared (Fig. 79). Although the mean height of the ABA treated plants was lower than the water treatment, the difference was similar for most of the experiment, i.e. the curves were not diverging at an appreciable rate. A similar pattern occurred with the increase in node number, but again the differences were not significant.

Thus in these two experiments with A. glutinosa there was no evidence of a marked inhibition of growth by the continual application of ABA to leaves.

In the two experiments with B. pubescens (Figs. 80. 81), the effects of 2×10^{-4}M ABA and water treatments were compared

Table 17. The % increase in height and the increase in mean node number at the end of experiments on the effect of ABA on the growth of A. glutinosa and B. pubescens. Values given are means of fifteen replicates, ± standard errors. Student 't' values were calculated on the difference between control and treatment means. % values were transferred using angular transformation.

	Treatments				
	Control	H_2O	$2x10^{-4}$M	10^{-5}M	$5x10^{-7}$M ABA
A. Glutinosa Expt. I					
% increase in height	36.4	37.5	39.9	44.7	48.4
(% cm ± S.E.)	±4.2	±5.8	±3.5	±6.0	±6.4
't' value	-	0.15	0.65	1.17	1.61
Increase in node number	4.3	5.1	4.4	5.0	5.25
	±0.23	±0.40	±0.25	±0.24	±0.25
't' value	-	1.70	0.36	2.13*	3.16**

A. glutinosa Expt.II	H_2O	$2x10^{-4}$M
% increase in height	99.1 ± 7.5	88.7 ± 8.0
't' value	-	0.95
Increase in node number	4.8 ± 0.37	3.75 ± 0.2
't' value	-	1.72

Table 17 continued.../

Table 17. continued...

B. pubescens I	Control	H_2O	$2x10^{-4}M$
% increase in height	61.2 ± 6.8	66.8 ± 5.2	57.61 ± 5.0
't' value	-	0.70	0.45
Increase in mean number	7.8 ± 0.27	8.3 ± 0.51	8.0 ± 0.39
't' value	-	0.98	0.43

with the control. The results were less variable than in A. glutinosa. There were no significant differences in height or mean node number between the treatments and the control after 22 days. Over this period there was no evidence that leaf-applied ABA induced, or promoted, the formation of a dormant apical bud.

These results suggest that ABA does not have any effect on the growth of B. pubescens, as measured by stem extension, when applied at a concentration of $2 \times 10^{-4}M$ to an entire, fully expanded, upper leaf.

In the experiments with A. glutinosa, replicate plants were supplied with $5 \times 10^{-7}M$ ABA-2-^{14}C in exactly the same way and at the same time as the other treatments. These plants were harvested at regular intervals for radioassay of uptake and distribution of ^{14}C (Table 18). Samples were also taken for chromatographic analysis of the radioactivity (Table 19).

FIGURE 77

The effect of leaf-applied ABA on the growth of 3 month-old plants of A. glutinosa. Plant height is plotted as a function of time, Experiment I. The following solutions were applied to rolled-up, upper, expanded leaves, at times indicated by the arrows:

—▲— , control, no application.

—●— , distilled water.

—○— , 2×10^{-4} M ABA.

—△— , 10^{-5} M ABA.

—■— , 5×10^{-7} M ABA.

Fifteen plants were used for each treatment, and the plotted values are mean heights, with the vertical bars representing twice the standard errors. The plants were maintained under 16 hour photoperiods at 20°C

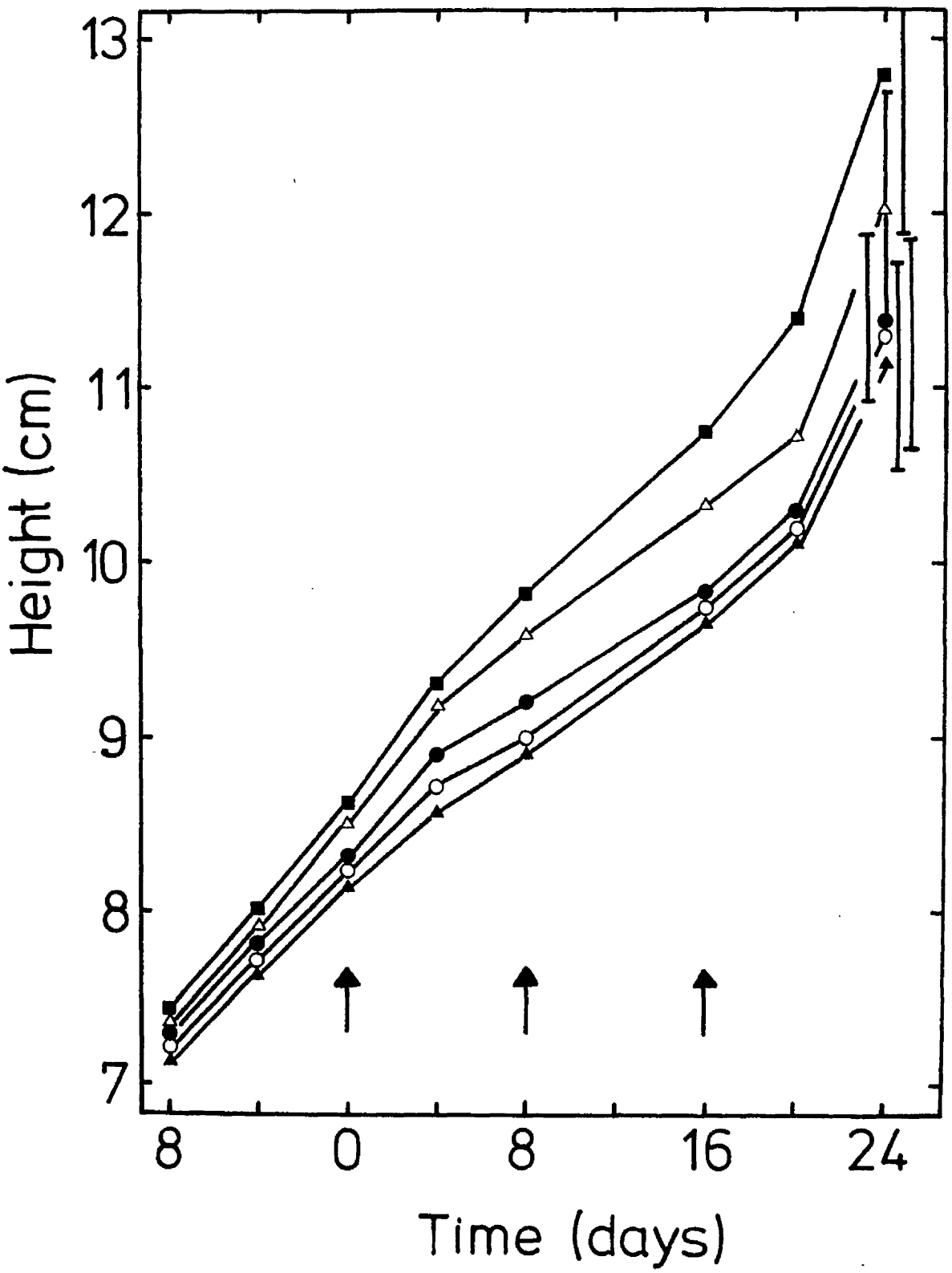

13
12
11
10
9
8
7
Height (cm)
8
0
8
16
24
Time (days)

FIGURE 78

The effect of leaf-applied ABA on the growth of 3 month-old plants of Alnus glutinosa (continued). Increase in node number is plotted as a function of time. Experiment I. Details are as in Figure 77.

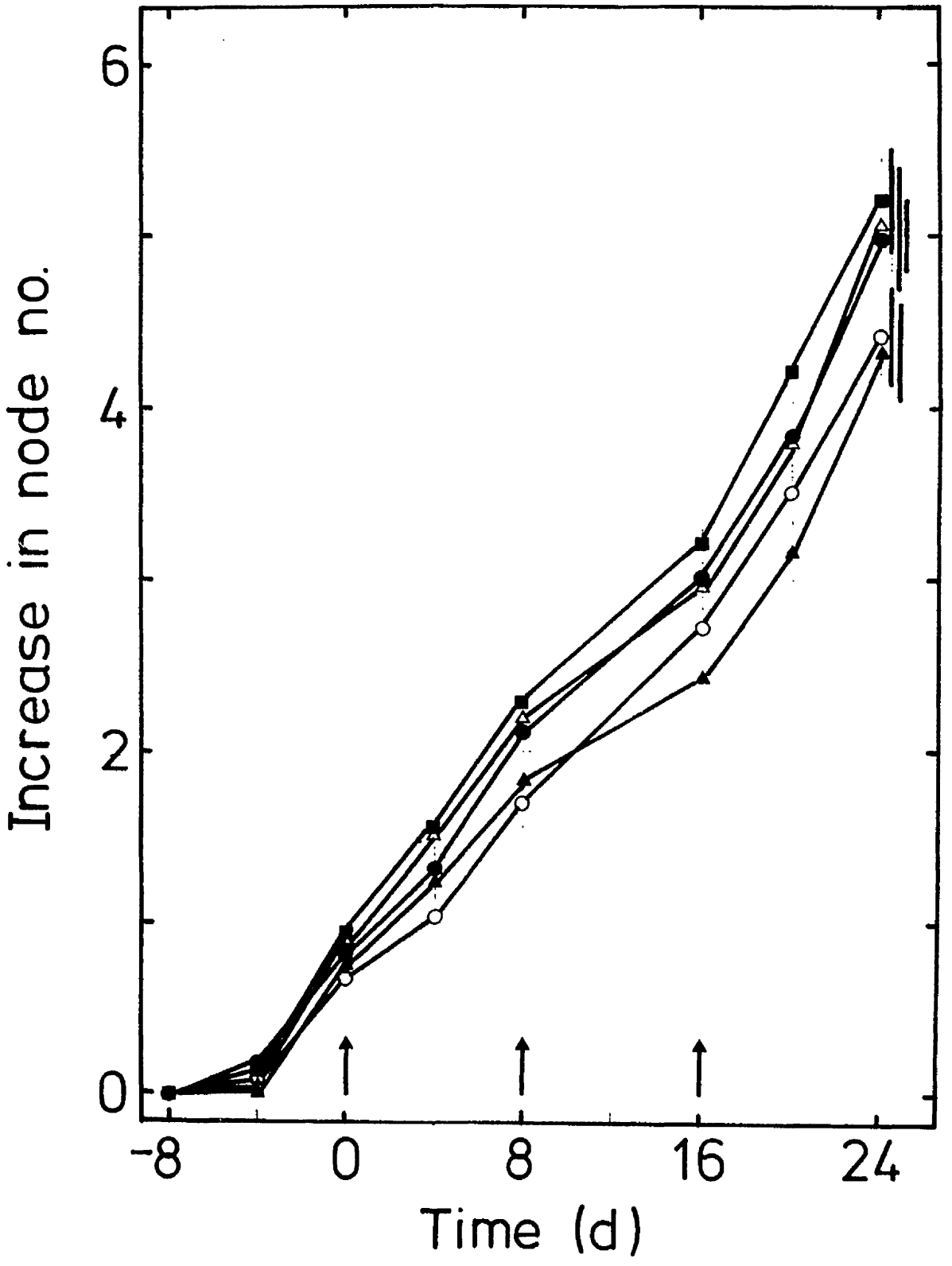

Increase in node no.
6
4
2
0
-8
0
8
16
24
Time (d)

FIGURE 79

The effect of leaf-applied ABA on the growth of 3 month-old plants of Alnus glutinosa (continued). Height (a) and increase in node number (b) are plotted as functions of time, Experiment 2. The following solutions were applied to rolled-up, upper, expanded leaves, at times indicated by the arrows:

—●— , distilled water.

—○— , 2×10^{-4} M ABA

Fifteen plants were used for each treatment, the plotted values are means, with vertical bars representing twice the standard errors. The plants were maintained under 16 hour photoperiods at 20°C.

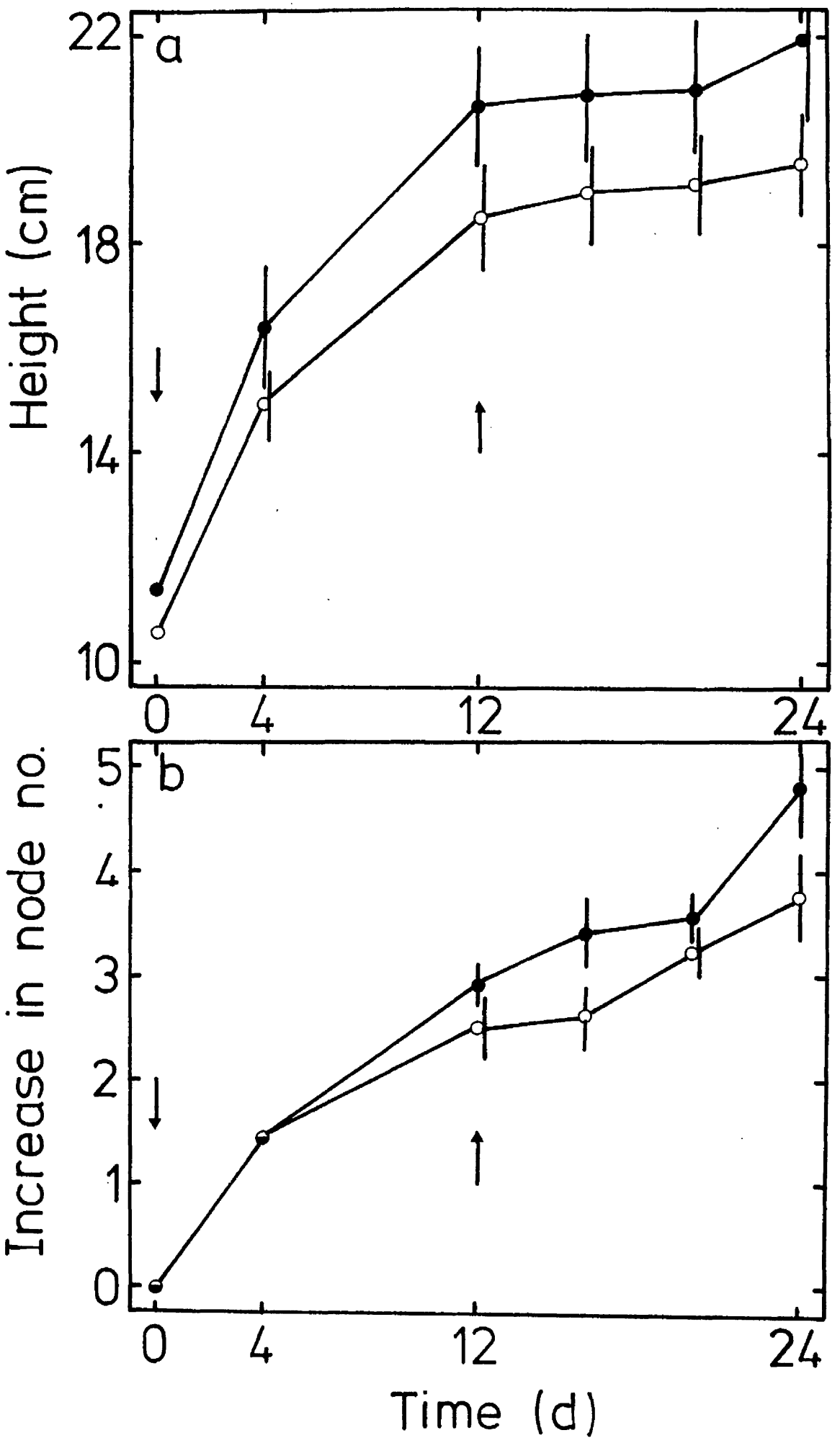

a
b
Height (cm)
Increase in node no.
Time (d)
22
18
14
10
5
4
3
2
1
0
0
4
12
24

FIGURE 80

The effect of leaf-applied ABA on the growth of 3 month-old plants of B. pubescens. Plant height (i) and increase in node number (ii) are plotted as functions of time, Experiment I. The following solutions were applied to upper, rolled-up, expanded leaves, at times indicated by the arrows:

—○— , control, no application.

—●— , distilled water.

—▲— , 2×10^{-4} M ABA

Fifteen plants were used for each treatment, the plotted values are means with the vertical bars representing twice the standard errors. The plants were maintained under 16 hour photoperiods at 20°C.

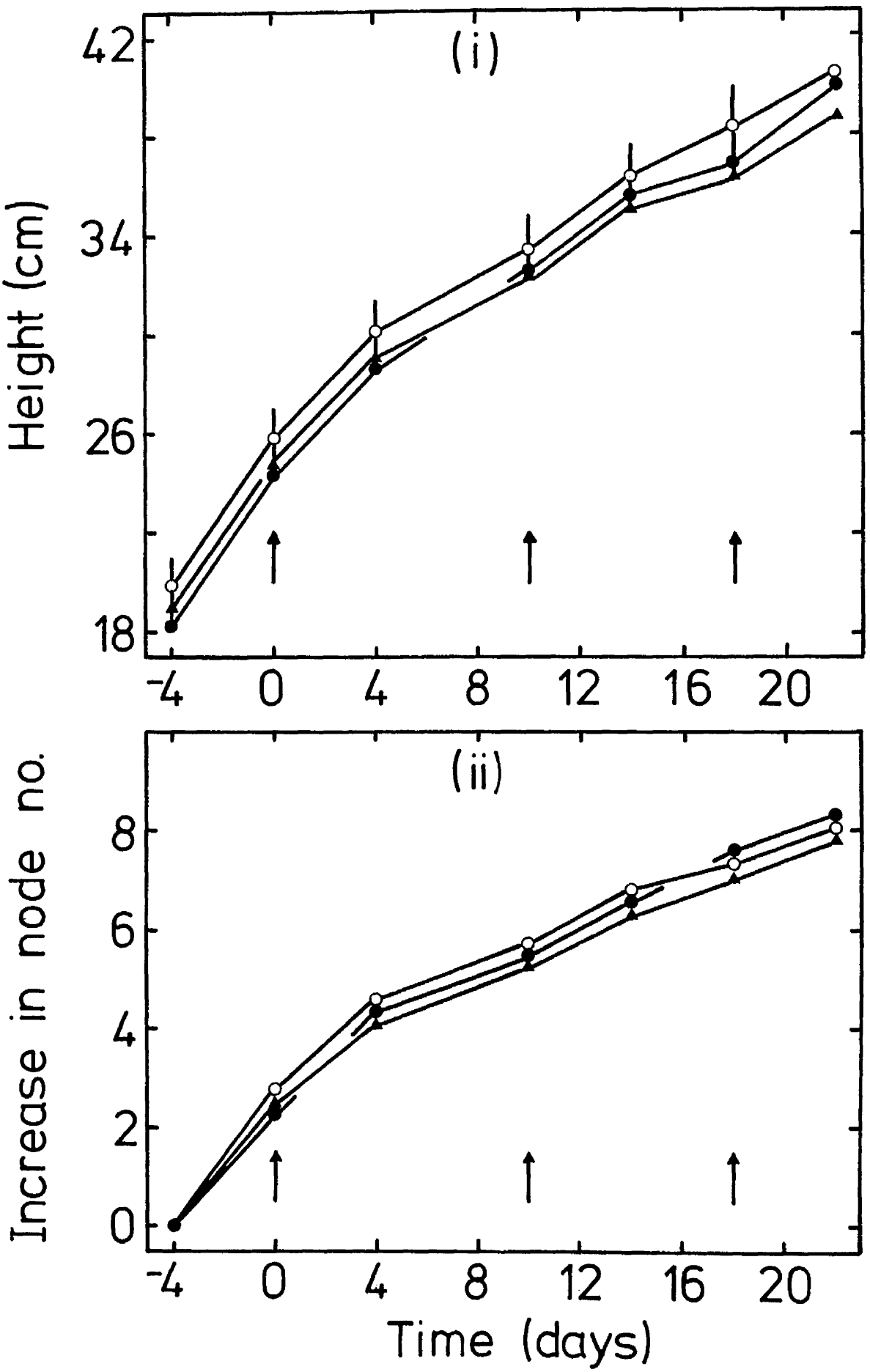

(i)
Height (cm)
42
34
26
18
-4
0
4
8
12
16
20
(ii)
Increase in node no.
8
6
4
2
0
-4
0
4
8
12
16
20
Time (days)

FIGURE 81

The effect of leaf-applied ABA on the growth of 3 month-old plants of B. pubescens (continued). Plant height (i) and increase in node number (ii) are plotted as functions of time, Experiment 2. The details are as in Figure 80.

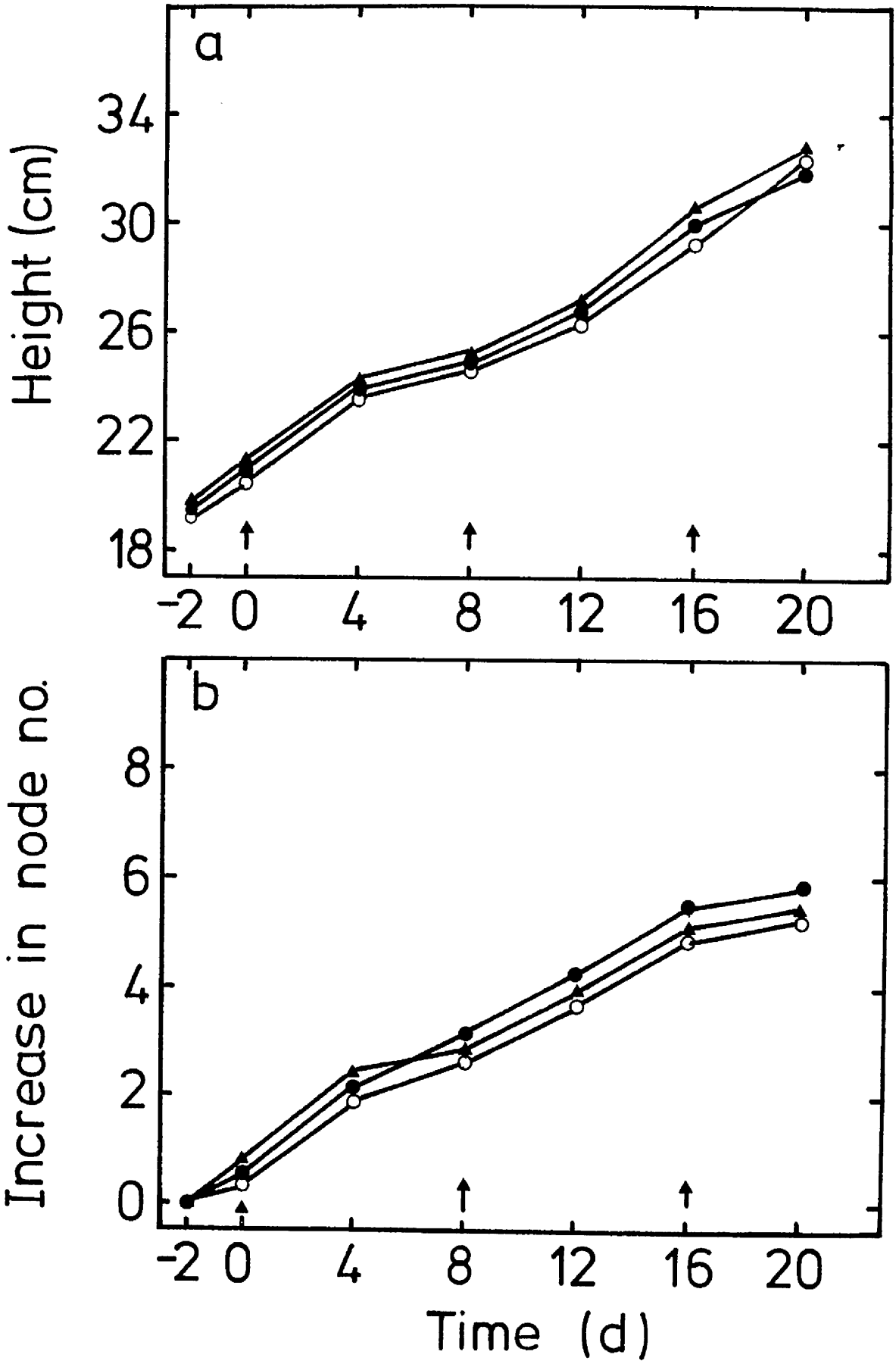

a
Height (cm)
34
30
26
22
18
b
Increase in node no.
8
6
4
2
0
-2
0
4
8
12
16
20
Time (d)

Table 18. The uptake and distribution of ^{14}C in Alnus glutinosa after supplying 5 x 10^{-7}M ABA-2-^{14}C to successive upper, fully expanded leaves. Radioactivity is expressed as dpm, % dpm (of total uptake or of total exported) and dpm g^{-1} fresh weight. Values are means of five replicates. Plants were maintained under 16 hour photoperiods at 20°C.

Days after application	Uptake	% exported	Distribution											
			Shoot above		(Donor leaf) First		Second		Third		Shoot below		Roots	
			%	dpm g^{-1}	%	dpm g^{-1}	%	dpm g^{-1}	%	dpm g^{-1}	%	dpm g^{-1}	%	dpm g^{-1}
4	3675	18.35	11.25	1847	81.6	30321	-		-		5.45	397	1.6	58
8	2824	8.03	5.3	241	91.9	8833	-		-		1.83	.26	0.26	15
16	6513	4.4	3.0	803	74.2	45087	21.4	16039	-		1.3	97	0	0
24	12853	3.6	3.3	737	48.5	58220	34.9	48572	15.6	18419	0.3	60.1	0.03	6.0

Table 19. Chromatographic analysis of ethanolic extracts from *A. glutinosa* growing in long days at 20°C, supplied with 5×10^{-7}M ABA-2-^{14}C solution. Application was made to an upper expanded leaf. The solvent system used was; n-butanol: n-propanol: ammonia: water :: 2:6:1:2 (v/v).

Extract	Rf of major peak	% dpm recovered at ABA Rf
ABA-2-^{14}C	0.75 - 0.85	73.0
8 day apex	0.70 - 0.75	9.5
8 day donor leaf	0.75 - 0.80	35.9
8 day ABA-2-^{14}C	0.75 - 0.80	63.1
24 day apex	0.70 - 0.75	12.3
24 day donor leaf	0.75 - 0.80	44.4

The trend of uptake of ^{14}C increased over the 24 days of the experiment. Uptake and distribution were complicated by the fact that after 8 and 16 days further applications of ABA-2-^{14}C were made to different leaves. The uptake after 8 days was inexplicably lower than after 4 days. The % of the uptake exported from the donor leaf(ves) decreased with time; i.e. the more that was taken up, the more that was immobilised in the donor-leaf(ves). The distribution in the plant was dominated by the increasing percentage remaining in the donor leaf, the percentage ^{14}C decreasing in most parts of the plant with time. The % ^{14}C in the apex reached 11.25 after the first four days, a concentration of 1847 dpm g^{-1}. But at successive harvests this declined to approximately 3.0%, i.e. 700-800 dpm g^{-1}. Nonetheless the accumulation in the apical region was markedly higher than elsewhere,

especially the roots where accumulation was very low. This was possibly a reflection of the application being made to an upper expanded leaf which might show a greater tendency to export to the upper regions of the plant.

The results from the chromatographic analysis show that only a small proportion of the radioactivity recovered from the apical region of the plants showed the same Rf characteristics as ABA-2-^{14}C. After 24 days the percentage was 12.3%. The percentage of the radioactivity in the donor leaf extracts which co-chromatographed with ABA-2-^{14}C was much higher, reaching 44.4% after 24 days. This was probably due to the very high uptake of ^{14}C into these tissues, i.e. only a certain proportion of the uptake could be metabolised or broken down by the tissue; when this value is exceeded ABA-2-^{14}C will accumulate. The major peak of radioactivity in the apical regions occurred at Rf 0.7 - 0.75, 0.05 to 0.1 Rf lower than ABA-2-^{14}C in 2:6:1:2, a result which was consistently found for tissue extracts.

Thus these results show that a small percentage of the radioactivity applied to the leaves did accumulate in the apical regions. Of the radioactivity that was recovered from the apical region, a small, but possibly significant amount was chromatographically similar to ABA-2-^{14}C

DISCUSSION

The importance of elucidating the pattern of endogenous hormone movement in the study of the hormonal control of growth correlations has been established. Much of what is known about the transport of hormones has been learnt by studying the movement of exogenously applied hormones or growth substances in either isolated sections of plant tissue or intact plants. This approach, which has been adopted for this present study, is most meaningful when the movement of the applied substance truly reflects the movement of the endogenous hormone under natural conditions. While this ideal situation may only rarely be achieved, the relationship between the applied and the endogenous substance is often sufficiently close for useful information to be obtained. If hormone transport in the plant can be considered to occur in certain tissues, then any substance which enters those tissues may reveal some of their transport potential. Consequently the exogenous application of natural or synthetic non-natural compounds can provide useful information about, for example, the movement of substances in phloem or xylem vessels.

Fundamental problems can arise, however, when using this approach. Firstly, the method of application or introduction of the substance into the system can involve many variables such as locus of application, concentration, formulation, timing of application, etc., and accordingly may influence the subsequent movement of the substance. Therefore, it may be difficult to isolate the problems of uptake from those of movement. If uptake markedly influences movement, then complexities

arise which do not occur in a natural situation and which question the relevance of using an application technique to gain information on the movement of endogenous substances. On the other hand, there are many instances where interest is centred primarily on the fate of applied substances per se., e.g. the fate of applied herbicides or other growth controlling chemicals, where these problems of relationships with endogenous substances are not important. Consequently, provided the uptake problems are borne in mind, studies of this type provide useful information for both agricultural-chemical technology and hormone physiology.

A second problem of fundamental importance concerns the use of isolated plant segments. Studies of the movement of substances in these tissues may not yield reliable information on the movement in intact plants. While some of the characteristics of IAA movement in segments, for example, may reflect those in intact plants, this may not be true for a substance which moves mainly in a vascular system. This problem may primarily arise from the mis-interpretation of results. A technically sound experiment may be invalidated by the nature of the conclusions drawn from it. Difficulties are bound to arise if, to take an extreme case as an example, inferences drawn from a study on the movement of a synthetic substance in coleoptile segments were to be applied directly to the movement of an endogenous hormone in a deciduous tree. On the other hand, studies on the movement of applied ABA in Zea coleoptile segments may yield valuable information on the movement of endogenous ABA in intact Zea seedlings.

In addition to these general problems concerned with

fundamental concepts, many other complexities may arise when dealing with specific instances. The choice of tracer, for example, may be important. When using a radioactively labelled molecule as a tracer it is assumed, with reasonable justification, that the radioactive molecule behaves in the same way as the unlabelled molecule. Ideally the label should remain attached to the original molecule, or when breakdown occurs the fate of the radioactive moiety should be followed, so that it is known what the radioactivity actually represents. Similarly the choice of experimental material is important; the physiological state, developmental stage and age of the plant must be considered. If isolated segments are used the choice of tissue, length of segment, shape of segment, timing of the excision, etc., may all be important in influencing the uptake, movement and metabolism of the applied substance. Coupled to these factors, the effects of environmental parameters must be considered. Not only is it important to standardize conditions, but also the analysis of the effects of various parameters may assist in characterising the transport system.

A number of these aspects have been considered in this study. In Section IA the results of studies on the movement of ABA-2-^{14}C in coleoptile segments of Zea mays are presented. The length of segment used was set somewhat arbitrarily at 10mm, excised one millimetre from the apex. The choice of this segment has markedly influenced the results obtained. Using the donor/segment/receiver system, applying ABA-2-^{14}C in agar donor blocks, the uptake of radioactivity by the segment, which resembles a truncated cone or frustum in shape, is greater from the basal end than from the apical end.

This difference is possibly a function of cross-sectional area of tissue in contact with the donor block. This result contrasts with the movement of IAA in Zea coleoptile segments, where despite the difference in cross-sectional area, apical uptake of IAA-1-^{14}C was greater than basal uptake (Christie and Leopold, 1965). A similar study on IAA movement in Avena coleoptile segments again showed greater apical uptake than basal uptake (Goldsmith, 1966a). With ABA-2-^{14}C the difference in uptake inevitably complicates a comparison of acropetal and basipetal movement since the former is supplied by a greater loading of radioactivity in the section of tissue adjacent to the donor block. Despite this, however, basipetal movement, as measured by the percentage accumulation of radioactivity in the tissue away from the donor block, is greater than acropetal movement. The degree of polarity of transport can be expressed as the ratio of basipetal to acropetal transport, but with isolated segments this ratio has little fundamental significance since it depends on the experimental conditions (Goldsmith, 1969), especially the shape of the segment used. It is sufficient to say that for this type of segment, under the stated conditions, movement of ^{14}C following ABA-2-^{14}C applications shows a slight, net, basipetal polarity. The movement of IAA-1-^{14}C in Zea coleoptile segments also shows a basipetal polarity, but this is much more marked, or even absolute over short time periods (Hertel and Leopold, 1963). The distributions of radioactivity down a segment after a given transport period following ABA-2-^{14}C or IAA-1-^{14}C application to the apical end are, however, very different. Using ABA-1-^{14}C the accumulation of radioactivity decreases rapidly down the segment giving an almost logarathmic

curve, typical of a diffusion process (Fig. 5). With IAA, marked accumulation of radioactivity occurs in the basal end of the segment and the receivers (Goldsmith and Thimann, 1962).

A distinctive characteristic of ^{14}C movement in coleoptile segments following ABA-2-^{14}C application is that there is very little exodus of radioactivity into the receiver block in either direction. Similar observations have been reported (e.g. Milborrow, 1968), and these findings again contrast with IAA movement, where substantial accumulation of ^{14}C occurs in the receiver blocks in the basipetal direction (Goldsmith and Thimann, 1962). The amount of ^{14}C-IAA transported basipetally through sections is the same whether the receiver is a block of agar or more of the section. Christie and Leopold (1965) suggest that exit of IAA from the base of the segments is a metabolically dependent step. If this is an active process it may also be specific for certain molecules such as IAA, and may not allow the passage out of ABA molecules. Alternatively the ABA may be broken down and/or immobilised by the time it reaches the base of the segment, a possibility that will be considered in more detail later. Clearly however, the rate of movement is important in this respect.

Following the accumulation of ^{14}C through an 8mm segment into a 2mm section of tissue acting as a receiver, the basipetal rate of movement, as calculated by the intercept method is 1.0 - 1.3 mmh^{-1}, while the acropetal rate is 0.5 - 0.8 mmh^{-1}. These rates are considerably slower than the rate of IAA movement in Zea coleoptile segments, where rates of 10 - 15 mmh^{-1} have been reported (Hertel and Leopold, 1963; Goldsmith, 1967a). The rate of basipetal movement of ABA in

Zea is also slower than that reported in other tissues (e.g. Milborrow, 1968; Ingersoll and Smith, 1971), but is similar to the rate of movement of 2-4-D in petiole segments of P. vulgaris (McCready, 1963). Went and White (1939) have recorded the basipetal velocities of 6.7 mmh^{-1} and 5.3 mmh^{-1} for indolebutyric acid and anthracene acetic acid respectively through Avena coleoptile segments, and cis-cinnamic acid showed no movement at all during the times tested. Thus the movement in either direction of ^{14}C in Zea coleoptile segments supplied with ABA-2-^{14}C has a lower velocity than most other plant growth substances in similar tissues.

This slow rate of movement makes the use of isolated tissue segments more complex. Longer time periods are required for meaningful analysis during which time the physiological condition of the segments may be changing. During these long periods the cut surfaces of the segments may partially 'reseal', making the passage in or out of the segment more difficult. This may be a further explanation for the lack of movement of ^{14}C into receiver blocks. Also considerable growth of the segments occurs during the transport period, not only making analysis more complex but possibly influencing hormone movement. It is probably more valid to study movement of substances in isolated segments only over shorter time periods.

As shown in Figures 7, 8 and 9 the inversion of transporting segments with respect to gravity has no significant effect on the uptake and movement of ^{14}C or the growth of Zea coleoptile segments supplied with ABA-2-^{14}C for 24 hours. Similar results have been found for the movement of

IAA in some tissues (van der Weij, 1932; Skoog, 1938; Pilet, 1965), although Little and Goldsmith (1967) found that inversion of oat coleoptiles inhibited the basipetal transport of IAA by 66 per cent, but acropetal transport was promoted, possibly leading eventually to a loss of polarity. They also found that elongation was decreased by inversion. Hertel and Leopold (1963) found that inversion reduced basipetal transport of IAA in Zea coleoptile segments by 10 - 20 per cent. Naqui and Gordon (1966) using a similar system found that inverting the segments 5 hours prior to, as well as during the transport period, was required to reduce the capacity for basipetal transport and to a lesser degree the velocity of basipetal movement. Clearly the effects of inversion on ABA-2-^{14}C and IAA-1-^{14}C movement are dissimilar, an observation which may be significant with regard to the hormonal control of plant georesponses.

The effects of donor concentration on the movement of radioactivity following ABA-2-^{14}C application did not produce any evidence for a rate limiting step or a specific transport site. Uptake, acropetal and basipetal movement were all proportional to concentration of the donor over the range considered. There was always a concentration gradient from the donor through the tissue in the direction of movement, a distribution pattern highly characteristic of a diffusion process. Such findings together with a relatively low rate of movement and the absence of a marked polarity have been used in some instances to suggest that diffusion may play a dominant role in the movement of radioactivity in coleoptile segments. However the effects of changing the ambient temperature and imposing anoxic conditions suggest some participation of a

metabolically-dependent step, especially in the uptake process. Both acropetal and basipetal movement were reduced to a similar degree, corresponding with the reduction in uptake such that the slight basipetal polarity was maintained, even at $10^{o}C$ or under nitrogen; i.e. there was no evidence that the slight basipetal polarity was a function of active, aerobic metabolism. Thus, this evidence, which is limited, may suggest that the acropetal and basipetal movement occurs via a similar mechanism and that the observed differences in movement may be more a function of the shape and growth of the segment.

Two lines of evidence support this suggestion. Firstly using segments excised from older coleoptiles, which show a lower rate of extension growth, the polarity of movement is less clear, although uptake is still largely a function of cross-sectional area of tissue in contact with the donor block. Secondly and more importantly, considering uptake and movement on a fresh weight basis and using segments excised from different regions of the coleoptile produces different results (Table 3). These results clearly show that the more closely the segment resembles a uniform cylinder of tissue, the more any differences between apical and basal uptake and acropetal and basipetal movement are abolished. The differences have not been completely resolved, since even with segments excised 6mm from the apex the tissue is not cylindrical in shape. In addition when movement is considered in terms of concentration of radioactivity in the tissue i.e. dpm g^{-1}, an acropetal polarity emerges. If diffusion is a major process in this movement then concentration may be a more relevant parameter than net accumulation and the acropetal polarity measured in this way may reflect the greater

concentration gradient in the acropetal direction. The fact that the front of radioactivity moves at a faster rate basipetally than acropetally may be of little relevance, especially since the flux of ^{14}C movement was found to be similar in both directions.

Whether or not the slight basipetal polarity as measured by accumulation ~~it~~ is important, as a matter of interest it is difficult to envisage what effect segment shape may have on movement. It is reasonable to argue that more than just shape changes in segments excised at different distances from the coleoptile apex. Certainly the rate of cell elongation may vary and for this reason the growth was considered of different zones of tissue taken from 1 to 16mm from the apex. However the results suggest no immediately obvious relationship between zones of elongation and movement of radioactivity. This may well merit further study, and it remains to be seen whether other non-morphological factors are involved. Of relevance here are experiments which have been carried out using agar cylinders to simulate the shape of a tissue segment (e.g. Yeomans and Audus, 1964). It is difficult, however, to assess the significance of such attempts, since the detailed structure of a tissue segment is so different from a homogeneous mass of agar, whatever its shape. No results published thus far have provided any real evidence that shape alone does influence transport.

While the shape of the segment or some other unknown factor may explain the difference between acropetal and basipetal accumulation of ^{14}C, these problems are secondary to that of determining the actual mechanism of ^{14}C movement in coleoptile segments supplied with ABA-2-^{14}C. As discussed above a straight choice between 'active', metabolically-dependent transport and

passive transport by diffusion (even so-called 'passive' transport may require metabolic energy, see Goldsmith, 1969) is not possible on the basis of the available evidence. The effects of temperature and anoxic conditions suggest at least one metabolically dependent step. The basipetal movement of ^{14}C as measured by accumulation in the tissue showed a Q10 of approximately 2, which indicates metabolic involvement, but such determinations can be misleading (Chang and Jacobs, 1972). The results of kinetic studies can also be difficult to interpret. The fact that the rate of movement of ^{14}C in either direction following ABA-2-^{14}C application is much lower than the published values for basipetal movement of IAA in similar tissues, does not necessarily mean that the former is more likely to be due to diffusion. It has long been held, however, that the rate of basipetal movement of IAA in coleoptile segments is far higher than could be attributed to diffusion (Went, 1928; van der Weij, 1932; Goldsmith, 1966a). Consequently, for this and other reasons, basipetal movement of IAA in coleoptile segments is considered to be active, while the much slower acropetal movement is thought to be typical of a diffusion process. Similar conclusions have been reached for the movement of IAA in other tissues (McCready, 1963, 1968). More recently Chang and Jacobs (1972) have reported that the rates of 'diffusion' of IAA through killed (boiled) sections of Coleus petioles are at least as high, if not higher, than the rate of basipetal transport through living tissue. Also they could not distinguish IAA movement through living or killed tissue on basis of IAA flux or Q1o values. Polarity of movement, however, was lost in boiled tissues. They concluded that their results show that auxin polarity in living tissue may derive mostly

from a metabolically induced block in acropetal movement. Wilkins and Scott (1968), also using boiled tissue segments, reported that the normal acropetal polarity of IAA movement in Zea roots was abolished, if not reversed, in boiled sections, although there was little information on the effects on rates of movement. The rate of movement of IAA through agar cylinders, presumably occurring by diffusion, is also much higher than basipetal IAA movement through coleoptile segments and other tissue segments (Larsen, 1955; Yeomans and Audus, 1964; Chang and Jacobs, 1972). As stated above an agar cylinder is probably not an accurate simulation of a tissue section; the problem is really that of determining what the true rate of free diffusion of a molecule through living tissue should be. Even if movement appears to be non-metabolically dependent, the observed rate of movement may not reflect the rate of free diffusion. Goldsmith (1966b), for example, has shown that in oat coleoptiles, basipetal transport recycles a proportion of the acropetally moving auxin, which probably masks the true rate and flux of acropetal movement.

Clearly kinetic studies alone are unlikely to determine the nature of a transport mechanism. If this were possible, it would be tempting to suggest that the low rate of ^{14}C movement in coleoptile segments supplied with ABA-2-^{14}C was due, not to diffusion, but to a form of metabolically-induced inhibition of movement in either direction. This suggestion is confounded by other data, however, for the apparent suppression of metabolism by low temperature or anoxic conditions does not increase the observed rates of movement. Because the concentration gradient of ^{14}C through the tissue is highly suggestive of a diffusion process and also because of a lack of marked polarity

of movement in tissue known to be capable of polarizing hormone movement, the metabolically dependent step may be associated more with the uptake of radioactivity. Suppression of metabolism would decrease uptake and this alone may reduce subsequent movement by reducing the concentration gradient in the tissue. This effect may be so marked that it may be impossible to distinguish a separate effect on movement per se. This inability to disassociate movement from uptake confounds further analysis of the transport mechanism. The use of pulse-labelling techniques might resolve this problem (Goldsmith, 1967a,b), but these are unlikely to be successful with such a slow-moving substance. In the absence of such data it is more reasonable to suggest that both basipetal and acropetal movements of ^{14}C through coleoptile segments supplied with ABA-2-^{14}C, occur largely by diffusion. The demonstration of a metabolically dependent component, distinct from uptake and possible involving an inhibition of movement, requires further experimentation.

Two important points must be made at this stage. Firstly the results only refer to net uptake and movement of ^{14}C. No account has been made of losses of radioactivity due to either breakdown and subsequent loss of ^{14}C during transport or loss during recovery of the ^{14}C from the tissue. Since the ABA is labelled at the 2-carbon of the side chain, rather than at the carbonyl carbon, loss of radioactivity as $^{14}CO_2$ is unlikely. Secondly the results discussed thus far refer to the uptake and movement of ^{14}C not ABA-2-^{14}C, to which the radioactivity was originally attached. Studies on the metabolism of ABA-2-^{14}C in Zea coleoptiles during transport show that only a small proportion of the radioactivity is recovered from the tissue as ABA-2-^{14}C. On the basis of chromatographic analysis the proportion in tissue

halves furthest from the donor block is less than 15%. In tissue halves nearer the donor, the proportion of ^{14}C which co-chromatographs with ABA-2-^{14}C never exceeds 30%. Only one major radioactive product, chromatographically distinct from ABA-2-^{14}C, has been detected in the coleoptile tissue extracts. This product appears to be chromatographically similar to Metabolite C (Milborrow, 1968), which was recovered, together with a Metabolite B, from tomato shoots, french bean and sycamore petiole sections supplied with ABA-2-^{14}C, and subsequently identified as 6'-hydroxymethyl ABA (Milborrow, 1969). This derivative is thought to undergo re-arrangement to give phaseic acid, which has been isolated from seeds of Phaseolus multiflorus by MacMillan and Price (1968). In a more recent publication (Milborrow, 1972), the author reports that five subsequent attempts to re-isolate Metabolite C have given phaseic acid, so the significance of the former seems to be in doubt. Milborrow also expresses doubts on whether phaseic acid is, in fact, a naturally-occurring degredation product of ABA or is merely an artifact formed during the extraction processes. He suggests it may be the latter. Phaseic acid, however, has also been tentatively identified in cotton fruit (Davis et al., 1972) and was found to accelerate abscission in the excised nodes of cotton seedlings. Thus further work is necessary to elucidate the role of phaseic acid in plants. Similarly, further experimentation is required to confirm whether the radioactive product obtained from coleoptile tissue in the present study is Metabolite C, or phaseic acid, or some other moiety, or even an artifact of extraction. Also it is important to determine if the metabolite has any physiological activity.

A number of other metabolites of ABA have been recovered from tissues supplied with ABA-2-^{14}C. Metabolite B, abscisyl-β-D-glucopyranoside (Milborrow, 1970), has also been characterised by Koshimizu (1968). The glucose ester appears to be stable in the plant but it is rapidly hydrolysed by expressed plant juices. Milborrow (1972) suggests that ABA may be transported as the glucose ester in petiole segments of P. vulgaris, but that the enzymes at the cut surfaces hydrolyse the ester as it passes into the receiver block. No evidence was found for the presence of Metabolite B in the extracts of coleoptile tissue. In embryonic bean axes, Walton and Sandheimer (1972) have reported two further metabolites (M1 and M2) which are apparently distinct from the two reported by Milborrow. McWha and Hillman (1973) have reported a metabolite from lettuce fruits which appears to be chromatographically distinct from any of those previously reported. Further information on the identification of these metabolites is required before the metabolism of ABA in plant tissues can be fully understood.

From the results of the metabolism of ABA-2-^{14}C in coleoptile segments after a 24 hour transport period, five possibilities can be suggested:- (i) Substantial breakdown/metabolism of ABA-2-^{14}C occurs prior to uptake by enzymes at the cut surfaces of the segments; the metabolite and ABA-2-^{14}C remaining are taken up and are moved away, the proportion of ABA-2-^{14}C remaining in the tissue decreasing with time and distance away from the donor. This would mean that the observed uptake and movement characteristics represent both ABA-2-^{14}C and a metabolite, making meaningful analysis more difficult. However, the fact that there is no breakdown of ABA-2-^{14}C in donor blocks

in contact with the cut surfaces renders this possibility unlikely. (ii) The ^{14}C is taken up as ABA-2-^{14}C and is then metabolised, the product being the primary mobile component, such that the proportion of ABA-2-^{14}C would decrease with distance from the donor. This would mean that at least the uptake characteristics represented those of ABA. (iii) The metabolism of ABA-2-^{14}C begins as soon as the substance enters the tissue and continues slowly for the duration of the transport period, the amount of ABA-2-^{14}C remaining being mainly a function of time and original concentration of ABA-2-^{14}C applied. This would imply a non-specific transport system, moving either molecule freely. If movement does occur by diffusion this is quite a strong possibility. (iv) The ^{14}C is taken up as ABA-2-^{14}C and a small proportion moves away from the donor area. As it moves it is metabolised and immobilised. This would mean that the observed transport characteristics would represent largely those of ABA. More evidence, especially from time course experiments is required to substantiate this possibility. Obviously it is of major importance to establish the identity of the mobile component(s). Until this is achieved it must be assumed that the observed transport characteristics do not wholly represent those of ABA. (v) The radioactive product may be an artifact of extraction. This is unlikely since no such breakdown occurred in extracts of donor blocks taken through the same procedure. Even simple alcoholic extracts showed the presence of the radioactive product. It should be pointed out, however, that not all the problems of extraction were resolved. There was some evidence of binding of radioactivity to particulate cell fractions during certain procedures, and some radioactivity remained in the aqueous phase after repeated ether extractions. It is possible that these

radioactive fractions represent metabolite B, although this was never separated out chromatographically. These complexities require clarification.

Section IB of the results is concerned with the uptake, movement and metabolism of ABA-2-^{14}C in subapical segments of Zea mays roots. These studies were made more complex by persistent variations in uptake that occur both within and between experiments. The existence of an uptake rhythm was neither confirmed nor eliminated since more sophisticated techniques are required.

Similar variations in uptake, also unsubstantiated, have been observed during the transport of 2,-4,D in root segments of pea (Wilkins, pers. comm). Despite these complexities, which in the absence of more detailed data, will not be considered further, a number of trends are clear. (i) Basal uptake is greater than apical uptake, possibly proportional to the cross-sectional area of tissue in contact with the donor. (ii) There is an acropetal polarity of ^{14}C movement as measured by both net accumulation and % accumulation in tissue halves further from the donor after 12 hours transport. This polarity declined until by 36 hours, it was abolished. (iii) There is little consistent movement of ^{14}C into receiver blocks. (iv) The rate of acropetal movement of ^{14}C can be estimated at between 2 - 4 mmh^{-1}, which is considerably higher than the basipetal rate over the first 24 hours. (v) Light does not alter the basic characteristics of ^{14}C movement. (vi) Lowering the temperature from 25°C to 15°C has little effect on uptake and movement of ^{14}C in root segments; the segments are more active over longer transport periods at the lower temperature. Lowering the

temperature further to 1°C, reduces both uptake and movement, but acropetal polarity of ^{14}C movement is maintained.

(vii) The metabolism of ABA-2-^{14}C in roots is essentially similar to that in coleoptiles, there being evidence of extensive breakdown of the applied ABA-2-^{14}C.

A comparison of these results with those obtained from coleoptile segments is of particular interest. Root segments, which also resemble a frustum in shape (Wilkins and Scott, 1968a), show a similar pattern of ^{14}C uptake to that in coleoptiles; i.e. greater uptake of ^{14}C occurs at the end of the segment with the greater cross-sectional area of tissue in contact with the donor block. The relationship between area and uptake, however, is not necessarily directly proportional. Polarity of ^{14}C movement in root segments is markedly acropetal over 24 hours, while coleoptile segments show only a slight basipetal polarity as measured by net accumulation of ^{14}C. This result confounds, to some extent, the suggestion that polarity in coleoptile segments may be a function of segment shape, because in roots the situation is reversed; i.e. movement is polarised towards the smaller end of the segment. In a study of IAA movement in roots, Wilkins and Scott (1968a) found that, over six hours, the polar flux of ^{14}C through root segments of a number of different genera, e.g. corn (frustum), wheat, sunflower pea (all virtually cylindrical), appeared to bear no relationship to the shape of the segment. If, on the other hand, the movement of ^{14}C in roots occurs <u>via</u> a totally different mechanism to that in coleoptiles, the fact that data referring to segment shape are not consistent may be irrelevant. The acropetal rate of movement of ^{14}C in roots is more than twice the basipetal movement of ^{14}C in coleoptiles. While this may indicate different

transport mechanisms, such a conclusion would be unjustified on the basis of this evidence alone. Many factors, e.g. anatomy of the tissues, could account for this difference. The effects of temperature on ^{14}C movement are markedly different in roots compared to coleoptiles. In the latter, lowering the temperature from 25°C to 15°C or 10°C does have a marked effect on uptake and movement of ^{14}C, suggesting that coleoptiles function less efficiently at lower temperatures compared to roots. This may be because roots are better adapted to functioning at lower ambient temperatures than those normally experienced by shoots. 25°C is perhaps an unnatural temperature at which to carry out experiments on Zea mays roots. Clearly the characteristics of uptake and movement of ^{14}C in root segments following ABA-2-^{14}C application are completely different to those in coleoptile segments.

There are no previous reports of ABA movement in roots, but many similar studies have been carried out on the movement of IAA in sub-apical root segments of Zea mays and other species. In nearly all of the studies movement of IAA was strongly acropetal (e.g. Pilet, 1965; Kirk and Jacobs, 1968; Scott and Wilkins, 1968b; Hillman and Phillips, 1970; Aasheim and Iversen, 1971). There are some reports of less clearly marked acropetal movement; Faber (1936), Hertel and Leopold (1963), Pilet (1964) and Iverson and Aasheim (1970) reported movement in both directions for a number of different species, while Yeomans and Audus (1964) found little movement in Vicia roots.

The work of Wilkins and co-researchers on IAA movement in roots is of particular relevance, because not only were Zea mays root segments used, but some of the techniques employed are very

similar to those used in present study. IAA transport, as measured by accumulation of ^{14}C in agar receivers, is active metabolically (Wilkins and Scott, 1968b) and is light (Scott and Wilkins, 1969) and temperature sensitive (Wilkins and Cane, 1970). The small basipetal transport component is thought to be accounted for by virtue of physical diffusion (Wilkins and Scott, 1968b). A more recent paper, (Wilkins et al., 1972) however, reveals some differences between the movement of ^{14}C following IAA-1-^{14}C or ABA-2-^{14}C application. This paper reports the uptake and movement of ^{14}C from IAA-1-^{14}C in the tissue as well as in receiver blocks. Basal uptake exceeded apical uptake over 16 hours, but after 12 hours apical uptake declined such that the ratio between basal and apical uptake was not constant. From this observation the authors conclude that the difference between apical and basal uptake cannot be ascribed to the difference in cross-sectional area of the apical and basal ends of the segments as otherwise the ratio would have remained constant with time. Using ABA-2-^{14}C in the present study, over longer transport periods, more variation in uptake is observed, but the difference between apical and basal uptake is approximately similar, over 48 hours. It would be of interest to see if uptake of IAA showed similar, marked, variations with time over longer transport periods.

Using IAA-1-^{14}C there is a marked acropetal polarity of ^{14}C movement as measured in the receiver blocks and in the tissue halves further away from the donors, but on a percentage basis the accumulation in the tissue does not show an acropetal polarity. Using ABA-2-^{14}C, however, there is a marked acropetal polarity in the tissue even when expressed on a percentage basis after 12 hours, though this declined by 24 hours. The difference between IAA and

ABA movement over the short transport periods, may be due to the fact that with ABA-2-^{14}C very little radioactivity moves into receiver blocks, giving rise to the effect of increasing the apparent polarity of accumulation observed in the tissue. In the study on IAA movement the authors suggest that only a small part of the radioactivity applied, that which reaches the receiver blocks unchanged, appears to be located in the polar transport system, while the bulk is not. The polarity found in the movement of the bulk radioactivity within the segment may be related to the polarity of IAA uptake from the donor blocks. The latter part of this conclusion may also apply to the movement of radioactivity from ABA-2-^{14}C. Thus, apart from exit into receivers, the mechanism(s) of movement of ^{14}C in Zea mays root segments may be basically similar following application of IAA-1-^{14}C or ABA-2-^{14}C. The net velocities of acropetal movement of radioactivity are similar at 2-4 mmh^{-1}. Although there are quantitative differences, the effects of temperature on ^{14}C movement are similar. In addition, with both IAA-1-^{14}C and ABA-2-^{14}C there is substantial breakdown and labelling of metabolic products in the root tissue, so the movement of radioactivity in the tissue does not wholly represent the movement of the original labelled molecules.

The results of the studies on the uptake and movement of ^{14}C in petiole segments of Phaseolus vulgaris (Section IIA (i)), following application of ABA-2-^{14}C, conflict with other reports. Whereas in the present study no consistent polarity of movement is observed, Milborrow (1968) reported a 3:1 basipetal polarity in french bean petioles and Dorffling and Bottger (1968) also found a basipetal polarity of ABA-like activity in young Coleus stem sections. Milborrow's report, however, gives no

details of the experiments carried out or any transport data and Dorffling and Bottger were essentially studying the movement of ABA-like activity rather than ABA per se. More recently, Ingersoll and Smith (1971) have carried out a detailed study of the movement of radioactively-labelled ABA in cotton petioles. They found no evidence of polar movement of ABA in this tissue. They also report that the velocity of movement in cotton is independent of segment length and donor concentration and is inhibited by metabolic inhibitors such as DNP. They suggest that the movement of ABA in cotton is an active, metabolically dependent process. These aspects have not been investigated in this present study with petioles, but studies on coleoptiles indicate the existence of a metabolically dependent step. Movement in coleoptiles, however, is not independent of donor concentration. Also the rate of movement of ABA in cotton (22.6 mmh^{-1}) is far higher than the rate of movement in coleoptiles (0.5 - 1.5 mmh^{-1}) or bean petioles (5 mmh^{-1} approx.). The difference in movement velocities may be caused by differences in metabolism and binding of ABA. While little or no breakdown of applied ABA occurs in cotton tissues (Ingersoll and Smith, 1970), there is evidence in this study and elsewhere (Milborrow, 1968) that substantial breakdown of applied ABA occurs in both coleoptile and bean tissue. If the breakdown or metabolic products have different movement or binding characteristics, clearly movement will be affected. It remains to be seen if this is the major difference between the movement of ABA in different tissues.

The rate of movement of ABA in cotton is also higher than that of IAA in similar systems (McCready and Jacobs, 1963; Smith and Jacobs, 1968), at between 6 - 7 mmh^{-1}. This rate of IAA

movement is very similar to the rate of movement of ^{14}C in bean petioles, found in this present study, although a consistent basipetal polarity of IAA has been reported in bean petioles and other tissue segments (McCready and Jacobs, 1963; Leopold, 1963; Goldsmith, 1969). Most of the studies on IAA movement in tissue segments, especially bean petioles, have used receiver block data to assess polarity of movement (e.g. McCready, 1968). As with root segments, the polarity of IAA movement in bean petiole segments may be largely a function of the movement of IAA into receiver blocks. Using ABA-2-^{14}C, movement through bean petioles into receivers is neither marked, nor consistent so analysis of movement by this means is not possible. Interestingly, with cotton petioles, ABA does appear to move freely into receivers, but still no polarity is found (Ingersoll and Smith, 1971). Kaldeway (1968) has maintained that because the cells at the end of the segment have been damaged and probably constitute a 'dead layer' the last step in the transport process must occur by diffusion. Consequently the movement of radioactivity through this layer would depend on the concentration gradient across it. Therefore the concentration of radioactivity in the cells adjacent to this layer is of particular importance, and if this was studied in detail more information on the differences between ABA and IAA movement may be obtained. Alternatively passage through the last living cells into this dead layer may be active and show molecular specificity. There is evidence that for auxin transport this step is metabolically dependent (Christie and Leopold, 1965 a, b; Winter, 1967), but this has not been fully resolved (Goldsmith, 1969).

The movement of ABA in intact plants of Phaseolus vulgaris has been discussed elsewhere (Hocking et al., 1972). An important

aspect, as discussed earlier, is the method of application and this has been carefully considered in this study. The observation that penetration is hindered by an intact cuticle agrees with the results of a recent study on cuticular penetration of ABA (Blumenfeld and Bukovac, 1972). Using isolated cuticles from tomato fruits and leaves of apricot, pear and orange, penetration of ABA was linear with time, greater as the undissociated ion than the dissociated ion, and greater through dewaxed than non-dewaxed cuticles. Significantly less ABA penetrated the tomato fruit cuticle than NAA or 2,4-D. There was no evidence that ABA was altered during transfer across the cuticle. These results show that penetration occurs, but as the authors point out, they do not show whether penetration may be sufficiently fast or slow to be a rate-limiting step in events subsequent to application. In addition the penetration of isolated cuticles may be different to that through intact surfaces where the underlying cells and specialised structures such as stomata (Sargent and Blackman, 1962; Franke, 1964; Jung et al., 1965) and trichomes (Butterfass, 1965) may modify the uptake pattern.

Methods of application have been studied on a number of occasions (e.g. Bachofen, 1962; Little and Blackman, 1963; Crafts and Crisp, 1971) and the method chosen herein probably avoids most of the artefacts commonly considered.

The pattern of distribution of applied ABA-2-^{14}C in Phaseolus vulgaris seedlings represents movement from an exporting leaf to a number of different growth centres. The similarity of the movement of an applied substance to the translocation of nutrients is not unusual (Zimmermann, 1969; Crafts and Crisp, 1971). The effects of steam-girdling the stem

and of sustained dark treatments suggest that the export of ^{14}C depends upon the continued translocation of assimilates. Similar studies on the movement of applied substances and ^{14}C photosynthates have reached similar conclusions (Mitchell and Brown, 1946; Hay and Thimann, 1956; Nelson et al., 1959). Although the identity of the transporting tissues has not been fully established, it is possible that substantial movement occurs in the phloem. One problem here is that little or no radioactivity moved from the leaf of application into the opposite primary leaf. Mullins (1970) studying the transport of ^{14}C-assimilates in seedlings of Phaseolus vulgaris of a similar age, found that the primary leaves are connected through a vascular anastomosis at the node, and ^{14}C photosynthate moves from one primary leaf to another, despite the fact that both are exporting assimilates at the same time. As this occurs it is difficult to explain why ^{14}C from ABA-2-^{14}C does not move into the opposite leaf if it is moving with the assimilate flow. Studies with petioles showed no preferential movement of ^{14}C and so the movement of ^{14}C into the leaf is unlikely to be hindered by the petiole. This clearly requires further study. In addition he found that plants of this age (13 - 15 days) there is no direct vascular connection between the primary leaves and the distal tissues, such as the trifoliate leaves. As movement of ^{14}C does occur between primary and trifoliate leaves, this must be via an indirect route. Evidence from the former study shows that assimilate from primary leaves moves down the stem initially and is then transferred to an ascending pathway, the bundles of which traverse the anastomosis at the second node. It is of considerable importance to determine if the movement of ^{14}C from ABA-2-^{14}C follows a similar path.

because this would show how closely the movement of radioactivity follows the path of assimilates.

The comparison of ^{14}C distributions following application of IAA-^{14}C, ABA-2-^{14}C and sucrose-^{14}C to P. vulgaris is of interest. The effectiveness of the application methods is shown by the fact that in every case nearly all the radioactivity recovered had been taken up by the plant. The loss from donors is consistently high, more than 97%, for each tracer. As pointed out in the results section the differences in the recovery of each tracer complicates a direct comparison, and efforts must be made to elucidate the reasons for these differences. It is also difficult to define the limits of uptake, when uptake can be said to have occurred. A considerable proportion of the 'uptake' may be immobilised during the passage through the remnants of the cuticle and the first layer of cell walls, especially with the growth substances where the percentage moving away from the donor area is under 22%. This problem also requires further investigation. Following uptake by a primary leaf radioactivity from sucrose-^{14}C appears to be the most mobile, both in terms of movement away from the donor area and export from the leaf. The final distribution of ^{14}C from primary leaves after 24 hours are also different, i.e. in terms of percentage accumulation, ^{14}C movement from ABA-2-^{14}C showed a net acropetal polarity; ^{14}C movement from sucrose-^{14}C showed net basipetal polarity; ^{14}C movement from IAA-^{14}C was less extensive, but more tended to accumulate in regions below the leaf of application. All could be interpreted as representing movement from a source to the more actively-growing regions except that these regions are different. If the movement of applied sucrose-^{14}C is more closely representative of the flow of

assimilates from an exporting leaf, then the movement of ^{14}C from ABA-2-^{14}C tends to be, to some extent, against the main assimilate flow. This is a quantitative difference, rather than a qualitative one, since a substantial proportion of the sucrose-^{14}C is distributed in a similar way to ^{14}C from ABA-2-^{14}C. Following application to a trifoliate leaf, which is still rapidly expanding and thus cannot be considered as an exporting leaf, only ^{14}C from IAA-^{14}C is exported in any quantity, primarily in a basipetal direction. This result suggests that applied IAA may move in a system in bean plants distinct from that which transports ABA and sucrose, and this movement occurs against the flow of assimilates. However, when only the distribution of radioactivity is considered after a fixed time period, there is little direct information on the way by which the radioactivity reached the point whence it was recovered; or if it had reached its final destination. The overall pattern of distribution indicates a possible transport system(s) and the information from steam-girdling shows that living tissues are involved, but direct evidence is lacking. For this, sampling during transport, or from actual transporting tissues is necessary. Thus there are a number of possible explanations for the different mobilities and distributions observed: (i) the tracers may move, or be moved, in different transport systems; (ii) the tracers may move in the same transport system which shows specific quantitative differences in uptake or entry , rate of movement, degree of binding, degree of leakage, active removal, secretion etc.; (iii) each tracer may move by a number of different ways,, some possibly competing with each other or occurring in opposite directions, and the combination of these ways, which may be different for each tracer, achieves the

final, observed distribution; (iv) the occurrence of re-distribution cannot be overlooked when considering movement over 24 hours; (v) the tracers may be metabolised in varying ways, at varying rates and at varying locations which may also affect the final distribution. As regards the last possibility, the studies on the metabolism of applied ABA-2-^{14}C and IAA-^{14}C in bean plants suggest that only a small proportion of the radioactivity may move as the original molecule, and any distribution of the original molecule may be masked by the distribution of breakdown products. Despite these difficulties, the fact that different distributions of ^{14}C do occur following application of ABA-2-^{14}C and IAA-1-^{14}C to bean plants, is a significant observation in itself, in terms of the hormonal control of plant growth. It indicates that the plant has the ability to control the distribution of endogenous hormones, a vital aspect of the overall hormonal concept.

There would appear to be no reports of applied ABA-2-^{14}C movement in intact plants prior to this study, so no comparisons can be made. Studies on IAA or auxin movement in intact plants are also limited. Most information regarding auxin translocation in intact plants is provided by studies of the distribution of foliarly applied synthetic auxins (Crafts and Yamaguchi, 1958; Slife et al., 1962; Little and Blackman, 1963). Eschrich (1968) showed that a rapid acropetal movement of IAA-^{14}C label occurs in Vicia faba leaves treated with high concentrations of radioactive IAA. The movement is more rapid than phloem translocation and appears to be the result of movement of two water-soluble, phloem immobile IAA metabolites, one of which is indoleacetylaspartic acid. There are many examples of IAA movement in non-vascular, parenchymatous tissue

(Goldsmith, 1969) but movement of auxin has also been observed in veins of tobacco leaves (Avery, 1935) so that both parenchymatous tissue and vascular tissue may be capable of polar transport. More recently, Long and Basler (1973) studied auxin translocation in 'intact' bean plants. They removed the cotyledons of 8 day-old plants and injected IAA and synthetic auxins into the pith of the stem. Marked acropetal translocation was observed both in the phloem, to young shoots, and in the xylem, to the primary leaves. There was also evidence of transfer between xylem and phloem. Leaving aside the possible problems caused by the rather drastic method of application, the results do implicate the involvement of the xylem and phloem in the regulation of auxin translocation.

The physiological responses of bean plants to root application of ABA do not reveal any new phenomena, but they confirm well established observations. As mentioned in the Introduction, there is strong evidence in favour of the involvement of ABA in plant-water relationship. The effectiveness of the root application method is demonstrated, despite the fact that there is substantial breakdown of the applied ABA in the tissue. This extensive metabolism of the applied ABA may explain the lack of a direct or marked effect of the applied ABA on growth, as measured by stem extension. Although movement of ^{14}C into the stem from ABA-2-^{14}C applied to the roots does occur, the rapid breakdown of ABA may prevent sufficient ABA from accumulating in the stem tissues. The effectiveness of root application of substances in altering shoot growth is shown by the marked effect of root-applied GA_3 on stem extension. Nonetheless, sufficient ABA must be taken up unchanged to exert an effect on the rate of water loss, presumably by reducing stomatal aperture.

Studies on the movement of ABA-2-^{14}C in Alnus glutinosa and two other woody species have also included a consideration of application problems. Both the method and particularly the locus of application can have marked effects on subsequent distribution. Using a method similar to that used for P. vulgaris, the distribution of ^{14}C in alder from an upper, expanded leaf resembles the distribution in bean. More ^{14}C accumulates above the leaf of application than below, although the proportion reaching the roots is less. A detailed comparison is not really valid because the plants differ in morphology. Both, however, have root nodules, but the accumulation of ^{14}C on a percentage basis is much greater in bean nodules than those of alder. The accumulation of ^{14}C in the former is such that initial studies have been carried out on the effects of ABA on nitrogen fixation in root nodules. Although the results are too preliminary to be reported here, this is an area which merits further study.

The distribution of ^{14}C in alder over longer transport periods indicates that re-distribution does occur, at least for a certain period. After 2 days most of the radioactivity is immobilised and no further re-distribution occurs. As with bean, the mobility of labelled sucrose in alder, applied in the same way as ABA-2-^{14}C, was greater than the mobility of ^{14}C from ABA-2-^{14}C. The results do not preclude the possibility that movement of ^{14}C in alder does occur with the flow of assimilates. The quantitative differences may be explained in terms of differences in metabolism, binding and immobilisation of the applied substances.

Studies on the metabolism of ABA-2-^{14}C in alder show that, as with bean and corn coleoptiles, there is substantial breakdown of the applied

ABA-2-^{14}C, subject to the limitations discussed previously. Thus the movement of ^{14}C in alder may not wholly represent the movement of ABA-2-^{14}C. Apart from the major radioactive product which has been found in bean and corn tissue as well as alder supplied with ABA-2-^{14}C, there are two additional chromatographically distinct radioactive moieties in alder extracts. These may be artefacts of the extraction process, but one had chromatographic properties similar to Metabolite B (Milborrow, 1968).

After establishing the photoperiodic sensitivity, with regard to bud dormancy, of the two experimental species, the effects of applied ABA on bud dormancy induction in Alnus glutinosa and B. pubescens have been considered. The results provide no evidence that root-applied or leaf-applied ABA induces the formation of resting buds in these species. The effects of root or leaf-applied ABA on the growth of alder have not been reported previously. While root-applied ABA has a slight effect on the growth of alder, as measured by stem extension, this response may be explained by the now established effect of ABA on plant-water relations. A sustained inhibition of water movement and transpiration is likely to reduce plant growth generally (Jones and Mansfield, 1972; Mizrahi and Richmond, 1972). There is little evidence that ABA is involved in the response of alder to short days, a response which involves a cessation of growth as well as the formation of resting buds. Tracer studies show that the ABA is taken up by the plant under long days or short days, and there is no evidence that the distribution of applied ABA is related to the effects of short days. Moreover, the studies on the metabolism of the applied ABA-2-^{14}C in alder suggest that there is extensive breakdown of the applied substance. Again there are

no marked differences between the metabolism of ABA-2-^{14}C in long days or short days. The extensive breakdown of ABA-2-^{14}C in the tissue may provide, however, an explanation of the lack of growth activity of the applied substance. Using leaf application there is no significant effect of ABA on the growth of alder maintained under 16 hour photoperiods at 20°C. In fact there is a tendency for lower concentrations of applied ABA to promote growth. Labelling studies show that only a small proportion of the applied ABA is taken up by the leaf (-ves) and is exported to the upper regions of the stem, including the apical region. Also very little of the ^{14}C present in this region appears to be chromatographically similar to ABA-2-^{14}C. Analysis of the ABA solution in the application vials reveals very little breakdown of the ABA-2-^{14}C occurs before entry. The metabolism of applied ABA in plant tissue is, however, a major area for further research. Much more must be known about the nature of the breakdown products, including their unequivocal identification using GC-MS.

The results of the effects of leaf-applied ABA on the growth of birch are very similar to those on alder and conflict with those of Eagles (1962), Eagles and Wareing (1963), and El-Antably *et al.*, (1967). In this present study using the method as described, there is no significant effect of leaf-applied ABA on the induction of resting buds, or on growth in birch seedlings maintained under long days. These results are particularly relevant to the work of Eagles (1962) because the techniques used were modelled on his. Threee deviations from his technique must be emphasized. Firstly, he applied a tissue extract to the leaves, whereas pure ABA solution was used in this study. This could be a crucial difference between the two experiments. While

the inhibitor extract is now known to contain ABA (Lenton et al., 1971), it may obviously contain other inhibitory factors (see Introduction). It is quite possible that one or more of these factors may be responsible for the observed effects on bud dormancy. Secondly, he maintained the experimental plants under a 14½ hour photoperiod. This was considered to be too close to the critical photoperiod, believed to be just below 14 hours for birch (Kawase, 1961) and so a 16 hour photoperiod was used for this study. It is possible that the daylength has to be near to the critical value before the growth of the plant can be inhibited. Eagles (1962) experimented with plants growing under 18 hour photoperiods and found the effects of the inhibitor application less marked. El-Antably, et al., however, used 18 hour photoperiods in their study and reported inhibition of growth by repeated applications of ABA to the leaves and apical region. Thirdly, Eagles changed the application leaf every 4 days, whereas in the present study this was found to be unnecessary, and so the leaf was changed every 8 days. As regards to the work of El-Antably et al., the main difference here is that they sprayed the apical region daily with 25 ppm ABA solution in addition to leaf applications, while the concentration of ABA they applied to the leaves was half that used in this study. Also the ABA used was obtained from a different source.

From the results of the present study it can be suggested that leaf-applied ABA alone does not induce bud dormancy in birch or alder growing in long days. This leaves two alternatives. Either endogenous leaf ABA does have a role in controlling bud dormancy under natural conditions but applying ABA in long days does not simulate this role because of problems of penetration, movement and metabolism. Or, endogenous leaf ABA does not have a

direct controlling effect on bud dormancy. If the results of this present study are considered in isolation, the first alternative is possible. Tracer studies show that the proportion of ABA taken up by the leaves from solution is low. A comparative study of application methods considered earlier shows that this method of applying ABA-2-^{14}C is far less efficient than using an agar donor block to an abraded leaf surface. Therefore, penetration is a problem. Only a small proportion of the ^{14}C taken up is moved towards the apical region; most of the ^{14}C appears to be immobilised in the application leaf. Moreover only a small proportion of ^{14}C reaching the apex is chromatographically similar to ABA-2-^{14}C. These difficulties may not only explain why the applied ABA does not have any effect on growth of the plants, but more importantly they indicate that applied ABA may not fairly represent the role of endogenous ABA. On the other hand this alternative is rendered less likely by the observation that, using gas-liquid chromatography, no increase in endogenous ABA occurs when birch seedlings are transferred to dormancy inducing conditions (Lenton et al., 1972). On the basis of available evidence the second alternative is more likely, that endogenous leaf-ABA does not have a direct, controlling role in the photoperiodic induction of bud dormancy in birch and alder.

BIBLIOGRAPHY

Aasheim, T. and T.H. Iversen, 1971. Decarboxylation and transport of auxin in segments of sunflower and cabbage roots. II A Chromatographic study using IAA-1-^{14}C and IAA-5-^{3}H. Physiol. Plant. 24, 325-329.

Addicott, F.T., Carns, H.R., Lyon, J.L., Smith, O.E., and J.L. McMeans, 1964. On the Physiology of Abscisins. In Regulateurs Naturels de la Croissance Vegetale, Proc. 5th Intern. Conf. Plant Growth Substances, Gif s/Yvette, 1963, Editions du Centre National de la Recherche Scientifique, Paris, 457-474.

Addicott, F.T. and J.L. Lyon, 1969. Physiology of Abscisic acid and related substances. Ann-Rev. Plant Physiol. 20, 139-64.

Addicott, F.T., Lyon, J.L., Ohkuma, K., Thiessen, W.E., Carns, H.R., Smith, O.E., Cornforth, J.W., Milborrow, B.V., Ryback, G. and P.F. Wareing, 1968. A new name for Abscism II (Dormin). Science, 159, 1493.

Arney, S.E. and D.L. Mitchell, 1969. The effect of abscisic acid on stem elongation and correlative inhibition. New Phytol. 68, 1001-15.

Aspinall, D., Paleg, L.G. and F.T. Addicott, 1967. Abscisin II and some hormone-regulated plant responses. Aust. J. Biol. Sci. 20, 869-82.

Avery,* G.S., Jr. 1935. Differential distribution of a phytohormone in the developing leaf of Nicotiana, and its relation to polarized growth. Bull. Torrey Bot. Club, 62, 313-330.

Bachofen,* R., 1962. Transport und Verteilung von markierten Substanzen, III. Einfluss der futterungs methode auf Absorption und Transport von ^{32}P bei Phaseolus vulgaris. Vierteljahresschr. Naturforsch. Ges. Zurich, 107, 31-39.

Bennett-Clark, T.A. and N.P. Kefford, 1953. Chromatography of the growth substances in plant extracts. Nature, 171, 645-47.

Black, M. and P.F. Wareing, 1959. The role of germination inhibitors and oxygen in the dormancy of light sensitive seeds of Betula spp. J. Exp. Bot. 10, 134-145.

Blumenfeld, A. and M.J. Bukovac, 1972. Cuticular penetration of Abscisic Acid. Planta, 107, 261-268.

Bond, G., 1951. Symbiosis of leguminous plants and nodule bacteria. IV The importance of the oxygen factor. Ann. Bot. Lond. 15, 95.

Bowen, M.R., 1969. The mobility of growth regulators. Ph.D. Thesis, University of Wales.

Bowen, M.R. and G.V. Hoad, 1968. Inhibitor content of phloem and xylem sap obtained from willow (Salix viminalis L.) entering dormancy. Planta, 81, 64-70.

Butterfass, T., 1956. Fluoroskopische Untersuchungen an Keulenhaaren von Vicia Faba L. und Phaseolus coccineus L. Protoplasma, 47, 415-428.

Cathey,* H.M., 1968. Response of some ornamental plants to synthetic abscisic acid. Proc. Amer. Soc. Hort. Sci. 93, 693-98.

Chang, Y.- P. and W.P. Jacobs, 1972. The contrast between active transport and diffusion of Indole-3-acetic acid in Coleus petioles. Plant Physiol. 50, 635-9.

Christie, A.E. and A.C. Leopold, 1965. Entry and exit of indoleacetic acid in corn coleoptiles. Plant Cell Physiol. 6, 737-754.

Cooper, W.C., Rasmussen, G.K., Rogers, B.J., Reece, P.C. and W.H. Henry, 1968. Control of abscission in agricultural crops and its physiological basis. Plant Physiol. 43, 1560-1576.

Cornforth, J.W., Milborrow, B.V., Ryback, G. and P.F. Wareing, 1965a. Identity of sycamore 'dormin' with abscisin II. Nature, Lond. 205, 1269-1272.

Cornforth, J.W., Milborrow, B.V. and G. Ryback, 1965b. Synthesis of (±) abscisin II. Nature, Lond. 206, 715.

Cornforth, J.W. Milborrow, B.V. and G. Ryback, 1966. Identification and estimation of (+) abscisin II ('dormin') in plant extracts by spectropolarimetry. Nature, Lond. 210, 627-628.

Cornforth, J.W. Mallaby R. and G. Ryback, 1968. Synthesis of (+)-(2-^{14}C) Abscisic acid. J. Chem. Soc. London. (C), 1565-1568.

Crafts, A.S. and C.E. Crisp, 1971. Phloem transport in plants. W.H. Freeman and Co., San Francisco.

Crafts, A.S. and S. Yamaguchi, 1958. Comparative tests on the uptake and distribution of labelled herbicides by Zebrina pendula and Tradescantia fluminensis. Hilgardia, 27, 421-454.

Davis, L.A., Lyon, J.L. and F.T. Addicott, 1972. Phaseic acid: Occurrence in Cotton fruit; Acceleration of Abscission. Planta, 102, 294-301.

Davison, R.M. and H. Young, 1973. Abscisic acid content of xylem sap. Planta, 109, 95-98.

Dorffling, K., 1963. Uber das Washstaff-hemmstaff system von Acer pseudoplatanus I. Der Jahresgung der Wachs-und Hemmstaffe in Knospen, Blatten und im Kambium. Planta, 60, 390-412.

Dorffling, K., 1966. Weitere Untersuchungen uber korrelative Knospenhemmung; Anwendung zweizer Biotests mit Knospen bei der Papierchromatographischen Untersuchung von Extrakten aus Pisum Pflanzen. Planta, 70, 257-274.

Dorffling,* K., 1967. (+) -Abscisin II (dormin) im Aetherextrakt von Pisumsprossen. Naturwissenschaften 54, 23-24.

Dorffling, K. and M. Bottger, 1968. Transport von Abscisin-saure in Explanten, Blattstiel-und Internodial-segmenten von Coleus rheueltianus. Planta, 93, 233-42.

Eagles, C.F., 1962. Studies on Dormancy in plants, in relation to daylength and growth regulators. Ph.D. Thesis, University of Wales.

Eagles, C.F. and P.F. Wareing, 1963. Dormancy regulators in woody plants. Nature, Iond. 199, 874.

Eagles, C.F. and P.F. Wareing, 1964. The role of growth substances in the regulation of bud dormancy. Physiol. Plant. 17, 697-709.

El-Antably, H.M.M., Wareing, P.F. and J. Hillman, 1967. Some physiological responses to D, Labscisin (dormin). Planta, 73, 74-90.

Eliasson, L., 1969. Growth regulators in Populus tremula. I. Distribution of Auxin and growth inhibitors. Physiol. Plant. 22, 1288-1301.

Eshrich, W., 1968. Translokation radioaktiv markierter Indolyl-3-essigsaure in Sie brohren von Vicia faba. Planta, 78, 144-157.

Evans, L.T., 1966. Abscisin II: Inhibitory Effect on Flower induction in a long-day plant. Science, 151, 107-108.

Faber,* E.R., 1936. (Auxin transport) Jahrb. Wiss. Bot. 83, 439-69.

Ferguson, T.P. and G. Bond, 1957. Observations on the formation of root nodules in A. glutinosa. Ann. Bot. Lond. 17, 175.

Franke, W., 1964. Role of guard cells in foliar absorption. Nature, (Lond.) 202, 1236-1237.

Glinka, Z. and L. Reinhold, 1971. Abscisic acid raises the permeability of plant cells to water. Plant. Physiol. 48, 103-105.

Goldsmith, M.H.M., 1966. Maintenance of polarity of auxin movement by basipetal transport. Plant Physiol. 41, 749-754.

Goldsmith, M.H.M., 1967a. Movement of pulses of labelled auxin in corn coleoptiles. Plant physiol. 42, 258-263.

Goldsmith, M.H.M., 1967b. Separation of transit of auxin from uptake: average velocity and reversible inhibition by anaerobic conditions. Science, 156, 661-663.

Goldsmith, M.H.M., 1969. Transport of plant growth regulators. In Physiology of plant growth and development, ed. M.B. Wilkins, McGraw-Hill, London, pp. 127-162.

Goldsmith, M.H.M. and K.V. Thimann, 1962. Some characteristics of movement of indoleacetic acid in coleoptiles of Avena I. Uptake, destruction, immobilization and distribution of IAA during basipetal translocation. Plant Physiol. 37, 492-505.

Hemberg, T., 1949a. Significance of growth inhibiting substances and auxins for the rest period of potatoes. Physiol. Plant. 2, 24-36.

Hemberg, T., 1949b. Growth inhibiting substances in the terminal buds of Fraxinus. Physiol. Plant. 2, 37-44.

Hertel, R. and A.C. Leopold, 1963. Versuchezur Analyse des Auxin transports in der Koleoptile von Zea mays L. Planta, 59, 535-562.

Hillman, J.R., 1970. The hormonal regulation of bud outgrowth in Phaseolus vulgaris L. Planta, 90, 222-229.

Hillman, S.K., and I.D.J. Phillips, 1970. Transport and metabolism of indol-3yl (Acetic acid-2-^{14}C) in pea roots. J. Exp. Bot. 21, 959-967.

Hoad, G.V., 1967. (+)-Abscisin II, (+ Dormin) in phloem exudate of willow. Life Sci. 6, 1113-1118.

Hoagland, D.R. and W.C. Synder, 1933. Nutrition of strawberry plants under controlled conditions. Proc. Amer. Soc. Hort. Sci. 30, 288.

Hocking, T.J., Hillman, J.R. and M.B. Wilkins, 1972. Movement of Abscisic acid in Phaseolus vulgaris plants. Nature New Biol., London, 235, 124-125.

Housley, S. and W.C. Taylor, 1958. Studies on plant growth hormones VI. The nature of inhibitor β in potato. J. Exp. Bot. 9, 458-471.

Imber, D. and M. Tal, 1970. Phenotypic reversion of Flacca, a wilty mutant of tomato, by abscisic acid. Science, 169, 592-593.

Ingersoll, R.B. and O.E. Smith, 1970. Movement of (R,S)-abscisic acid in the cotton explant. Plant Physiol. 45, 576-578.

Ingersoll, R.B. and O.E. Smith, 1971. Transport of abscisic acid. Plant. Cell Physiol. 12, 301-309.

Irving, R.M., 1968. Study of germination, dormancy and growth of seeds and buds of Acer negundo. Plant Physiol. 43, suppl., 5-49.

Iversen,* T.- H., and T. Aasheim, 1970. IAA transport. Planta, 93, 354-362.

Jones, R.J. and T.A. Mansfield, 1970. Suppression of stomatal opening in leaves treated with abscisic acid. J. Exp. Bot. 21, 714-719.

Jones, R.J. and T.A. Mansfield, 1972. Effects of abscisic acid and its esters of stomatal aperture and the transpiration ratio. Physiol. Plant. 26, 321-327.

Kaldeway, H., 1968. Auxin transport: general remarks concerning the terminology and the methods. In Transport of Plant Hormones, ed. by Y. Vardar, North-Holland, Amsterdam, pp. 1-23.

Kawase, M., 1961. Growth substances related to dormancy in Betula. Proc. Amer. Soc. Hort. Sci. 78, 532-544.

Kawase, M. and J.P. Nitsch, 1958. Inhibitory activity in Betula pubescens. Plant Physiol. 33 xix.

Kirk, S.C. and W.P. Jacobs, 1968. The movement of 3-indoleacetic acid-^{14}C in roots of Lens and Phaseolus. In Biochemistry and Physiology of Plant Growth Substances, eds. F. Wightman and G. Setterfield, Runge Press, Ottawa. pp. 1077-1094.

Koshimuzu,* K., Invi, M., Fukui, H. and T. Mitsui, 1968. Isolation of (+)-abscisyl- β -D-gluco-pyranoside from immature fruit of Lupinus luteus. Agr. Biol. Chem. 32, 789-791.

Lane, F.E. and L.F. Bailey, 1964. Isolation and characterization studies on the β -inhibitor in dormant buds of the silver maple, Acer saccharinum L. Physiol. Plant. 17, 91-99.

Larsen, P., 1955. Growth substances in higher plants. In Modern Methods of Plant Analysis, eds. K. Paech and M.V. Tracey, Vol. 3, Springer-Verlag, Berlin, pp. 565-625.

Lenton, J.R., Bowen, M.R. and P.F. Saunders, 1968. Detection of abscisic acid in the xylem sap of Willow (Salix viminalis L.) by Gas-Liquid Chromatography. Nature, London, 220, 86-87.

Lenton, J.R., Perry, V.M. and P.F. Saunders, 1971. The identification and quantitative analysis of abscisic acid in plant extracts by gas-liquid chromatography. Planta, 96, 271-280.

Lenton, J.R., Perry, V.M. and P.F. Saunders, 1972. Endogenous abscisic acid in relation to photoperiodically induced bud dormancy. Planta, 106, 13-22.

Leopold, A.C., 1963. The polarity of auxin transport. In Meristems and Differentiation, Brookhavan Symposia in Biology No. 16. pp. 218-233.

Lipe, W.N. and J.C. Crane, 1966. Dormancy regulation in peach seeds. Science, 153, 541-542.

Little, C.H.A. and D.C. Eidt, 1968. Effect of abscisic acid on budbreak and transpiration in woody species. Nature, 220, 498-499.

Little, C.H.A. and M.H.M. Goldsmith, 1967. Effect of inversion on growth and movement of indole-3-acetic acid in coleoptiles. Plant Physiol. 42, 1239-1245.

Little, E.C.S. and G.E. Blackman, 1963. The movement of growth regulators in plants, III. Comparative studies of transport in Phaseolus vulgaris. New. Phytol., 62, 173-197.

Long, J. and E. Basler, 1973. Some factors regulating auxin translocation in intact bean seedlings. Plant Physiol., 51, 128-135.

MacMillan, J. and R.J. Pryce, 1968. Phaseic acid, a putative relative of abscisic acid from seed of Phaseolus multiflorus. Chem. Commun. 3, 124-126.

McCready, C.C., 1963. Movement of growth regulators in plants. I. Polar transport of 2,4-dichloro phenoxyacetic acid in segments from the petioles of Phaseolus vulgaris. New Phytol. 62, 360-366.

McCready, C.C., 1968. The polarity of auxin movement in segments excised from petioles of Phaseolus vulgaris L. In Biochemistry and Physiology of Plant Growth substances, Proc. 6th Intern. Conf. Plant Growth Substances, eds. F. Wightman and G. Setterfield, Rung Press, Ottawa, 1005-10024.

McCready, C.C. and W.P. Jacobs, 1963. Movement of growth regulators in plants. II Polar transport of radioactivity from indoleacetic acid-(^{14}C) and 2,4-dichlorophenoxyacetic acid-(^{14}C) in petioles of Phaseolus vulgaris. New Phytologist, 62, 19-34.

McWha, J.A., and J.R. Hillman, 1973. Uptake and metabolism of 2-(^{14}C) Abscisic acid in Lettuce fruits var. Great Lakes. Planta, 110, 345-351.

McWha, J.A., Philipson, J.J., Hillman, J.R. and M.B. Wilkins, 1973. Molecular requirements for abscisic acid activity in two bioassay systems. Planta, 109, 327-336.

Milborrow, B.V., 1968. Identification and measurement of (+)-abscisic acid in plants. In Biochemistry and Physiology of Plant Growth Substances, eds. F. Wightman and G. Setterfield, Runge Press, Ottawa, pp. 1531-1545.

Milborrow, B.V., 1969a. Identification of "Metabolite C" from abscisic acid and a new structure for phaseic acid. Chem. Comm. 966-967.

Milborrow, B.V., 1969b. The occurrence and function of abscisic acid in plants. Sci. Progr. 57, 533-558.

Milborrow, B.V., 1970. The metabolism of abscisic acid. J. Exp. Bot. 21, 17-29.

Milborrow, B.V., 1972. Studies on the Biosynthesis and Degredation of abscisic acid. In Plant Growth Substances 1970, ed. D.J. Carr, Springer-Verlag, pp. 281-290.

Mitchell, J.W. and J.W. Brown, 1946. Movement of 2,4-D stimulus and its relation to the translocation of organic food materials in plants. Bot. Gaz. 107, 393-407.

Mittleheuser, C.J. and R.F.M. van Stevenick, 1969. Stomatal closure and inhibition of transpiration induced by (RS)-abscisic acid. Nature, 221, 281-282.

Mizrahi, Y., Blumenfeld, A. and A.E. Richmond, 1970. Abscisic acid and transpiration in leaves in relation to osmotic root stress. Plant Physiol. 46, 169-171.

Mizrahi, Y. and A.E. Richmond, 1972. Abscisic acid in relation to mineral deprivation. Plant Physiol. 50, 667-670.

Molisch, H., 1922. Pflanzenphysiolie als Theorie der Gartnerei. 5th Ed. Jena.

Mullins, M.G., 1970. Transport of ^{14}C-assimilates in seedlings of Phaseolus vulgaris L. in relation to vascular anatomy. Ann. Bot., 34, 889-896.

Naqui, S.M. and S.A. Gordon, 1966. Auxin transport in Zea mays L. coleoptiles. I. Influence of gravity on the transport of indoleacetic acid-2-^{14}C. Plant Physiol. 41, 1113-1118.

Nelson, C.D., Perkins, M.J. and P.R. Gorham, 1958. Note on a rapid translocation of photosynthetically assimilated ^{14}C out of the primary leaf of a young soybean plant. Can. J. Biochem. Physiol. 36, 1277-1279.

Nitsch, J.P., 1957. Growth responses of woody plants to photoperiodic stimuli. Proc. Amer. Soc. Hort. Sci. 70, 512-525.

Nitsch, J.P., 1963. The mediation of climatic effects through endogenous regulating substances. In Environmental Control of Plant Growth, ed. L.T. Evans, Academic Press, New York.

Okhuma, K., Lyon, J.L., Addicott, F.T. and O.E. Smith, 1963. Abscisin II, and abscission-accelerating substance from young cotton fruit. Science, 142, 1592.

Okhuma, K., Addicott, F.T., Smith, O.E. and W.E. Thilssen, 1965. The structure of abscisin II. Tetrahedron Letters, 29, 2529.

Overbeck, J. van, Loeffler, J.E. and M.I.R. Mason, 1967. Dormin (Abscisin II), Inhibitor of plant DNA synthesis? Science, 156, 1497-1499.

Phillips, I.D.J. and P.F. Wareing, 1958. Studies in Dormancy of Sycamore. I. Seasonal changes in the growth-substance content of the shoot. J. Exp. Bot. 9, 350-364.

Phillips, I.D.J. and P.F. Wareing, 1959. Studies in Dormancy of Sycamore. II. The effect of daylength on the natural growth-inhibitor content of the shoot. J. Exp. Bot. 10, 504-514.

Pieniazek,* J. and R. Rudnicki, 1967. The presence of Abscisin II in apple leaves and apple juice. Bull. Acad. Pol. Sci., Ser. Sci. Biol. 15, 251-254.

Pieniazek,* J. and R. Rudnicki, 1969. The inhibitory effect of endogenous abscisic acid on after-ripening of apple seeds in the fruit. Bull. Acad. Pol. Sci., Ser. Sci. Biol. 17, 707-711.

Pilet, P.E., 1964. Auxin transport in roots. Lens culinaris. Nature, 204, 561-562.

Pilet, P.E., 1965. Polar transport of radioactivity from ^{14}C-labelled--indolylacetic acid in stems of Lens culinaris. Physiol. Plant. 18, 687-702.

Pilet, P.E., 1968. In vitro and in vivo auxin and cytokinin translocation. In Biochemistry and Physiology of Plant Growth Substances, eds. F. Wightman and G. Setterfield, Runge Press, Ottawa, pp. 993-1004.

Rehm, M.M. and M.G. Cline, 1973. Rapid growth inhibition of Avena coleoptile segments by abscisic acid. Plant Physiol. 51, 93-96.

Robinson, P.M., 1962. PhD thesis. Univ. of Wales.

Robinson, P.M. and P.F. Wareing, 1964. Chemical nature and biological properties of the inhibitor varying with photoperiod in sycamore (Acer pseudoplatanus). Physiol. Plant. 17, 314-323.

Rothwell, K. and R.L. Wain, 1964. Studies on a growth inhibitor in yellow lupin (Lupinus luteus L.). In Regulateurs Naturels de la Croissance Vegetale, ed. J.P. Nitsch, Gif-sur-Yvette, 1963; Editions du Centre National de la Recherche Scientifique, Paris. pp. 457-474.

Sankhla, N., and D. Sankhla, 1968. Interaction between growth regulators and (±)-abscisin II in seed germination. 2. Pfanzenphysiol. 58, 402-409.

Sargent, J.A. and G.E. Blackman, 1962. Studies on foliar penetration. I. Factors controlling the entry of 2,4-dichlorophenoxyacetic acid. J. Exp. Bot. 13, 348-368.

Scott, T.K. and M.B. Wilkins, 1968. Auxin transport in roots. II. Polar flux of IAA in Zea roots Planta, 83, 323-334.

Scott, T.K. and M.B. Wilkins, 1969. Auxin transport in roots. Effect of light on IAA transport and geotropic responsiveness in Zea roots. Planta, 87, 249-258.

Shein, T. and D.I. Jackson, 1971. Hormone interaction in apical dominance in Phaseolus vulgaris L. Ann. Bot. 35, 555-564.

Skoog, F., 1938. Absorption and translocation of auxin. Am. J. Bot. 25, 361-372.

Slife, F.W., Key, J.L., Yamaguchi, S. and A.S. Crafts, 1962. Penetration, translocation and metabolism of 2,4-D and 2,4,5-T in wild and cultivated cucumber plants. Weeds, 10, 29-35.

Smith, O.E., Lyon, J.L., Addicott, F.T., Johnson, R.E., 1968. Abscission physiology of abscisic acid. In Biochemistry and Physiology of Plant Growth Substances, eds. F. Wightman and G. Setterfield, Runge Press, Ottawa, pp. 1547-60.

Sondheimer, E. and E.C. Galson, 1966. Effects of abscisin II and other plant growth substances on germination of seeds with stratification requirements. Plant Physiol. 41, 1397-1398.

Sondheimer, E. and D.C. Walton 1970. Structure-Activity correlations with compounds related to abscisic acid. Plant Physiol. 45, 244-248.

Steveninck, R.F.M., van, 1958. Straight growth inhibitors in Glucose. Nature, 182, 950-951.

Steveninck, R.F.M., van, 1959. Abscission-accelerators in Lupins (Lupinus luteus L.). Nature, 183, 1246-1248.

Tamura,* S. and M. Nagao, 1970. Synthesis and biological activities of new plant growth inhibitors structurally related to abscisic acid. Part I. Agr. Biol. Chem. 34, 1393-1401.

Tucker, D.J. and T.A. Mansfield, 1972. Effects of light quality on apical dominance in Xanthium strumarium and the associated changes in endogenous levels of abscisic acid and cytokinins. Planta, 102, 140-151.

Varga, M., 1957. Paperchromatographic Examination of inhibiting substances in fleshy fruits. Naturwissenschaften 44, 398-399.

Veen, R. van der, 1968. Plant hormones and flowering in coffee. Acta Bot. Neerl. 17, 373-376.

Wareing, P.F., 1954. Growth studies in Woody Species VI. The Locus of Photoperiodic Perception in relation to dormancy. Physiol. Plant. 7, 261-277.

Wareing, P.F., 1956. Photoperiodism in Woody Plants. Ann. Rev. Plant Phys. 7, 191-214.

Wareing, P.F. 1965. Endogenous inhibitors in seed germination and dormancy. Encyl. Plant Physiol. 15, 909-924.

Wareing, P.F., 1969. Germination and Dormancy, in Physiology of Plant Growth and Development, ed. M.B. Wilkins, McGraw-Hill, London, pp. 605-644.

Wareing P.F. and H.M.M. El-Antably, 1970. The possible role of endogenous inhibitors in the control of flowering. In Cellular and Molecular Aspects of Floral Induction, ed. G. Bernier, Longman, London, pp. 285-303.

Wareing, P.F., Good, J. and J. Manuel, 1968. Some possible physiological roles of abscisic acid. In Biochemistry and Physiology of Plant Growth Substances, eds. F. Wightman and G.Settlefield, Runge Press, Ottawa, pp. 1561-1579.

Wareing, P.F. and I.D.J. Phillips, 1970. The Control of Growth and Differentiation in Plants. Pergamon, Oxford, pp. 223-254.

Wareing, P.F. and P.F. Saunders, 1971. Hormones and Dormancy. Ann. Rev. Plant Phys. 22, 261-288.

Walton, D.C. and E. Sondheimer, 1972. Metabolism of 2-^{14}C-(±)- abscisic acid in excised bean axes. Plant Physiol. 40, 285-289.

Warner, H.L. and A.C. Leopold, 1969. Timing of Plant Hormone responses in etiolated pea seedlings. Plant Physiol. 44, Suppl. 28.

Weij, H.G. van der, 1932. Der Mechanismus des Wuchsstoff transportes. Rec. Trav. Botan. Neerl. 29, 380-496.

Went, F.W. and R. White, 1939. Experiments on the transport of auxin. Bot. Gaz. 100, 465-484.

Wilkins, M.B. and A.R. Cane, 1970. Auxin transport in roots. V. Effects of temperature on the movement of IAA in Zea roots. J. Exp. Bot. 21, 195-211.

Wilkins, M.B. and M. Martin, 1967. The dependence of basipetal polar transport of auxin upon aerobic metabolism. Plant Physiol. 42, 831-839.

Wilkins, M.B., Cane, A.R. and I. McCorquodale, 1972. Auxin transport in roots. VII. Uptake and movement of radioactivity from IAA-^{14}C by Zea roots. Planta, 105, 93-113.

Wilkins, M.B. and T.K. Scott, 1968a. Auxin transport in roots. Nature, 219, 1388-1389.

Wilkins, M.B. and T.K. Scott, 1968b. Auxin transport in roots. III. Dependence of the polar flux of IAA in Zea roots upon metabolism. Planta, 82, 335-346.

Winter, A., 1967. The promotion of the immobilization of auxin in Avena coleoptiles by triiodobenzoic acid. Physiol Plant. 20, 330-336.

Wright, S.T.C. and R.W.P. Hiron, 1969. (+)-Abscisic acid, the growth inhibitor induced in detached wheat leaves by a period of wilting. Nature, 224, 719-720.

Yeomans, L.M. and L.J. Audus, 1964. Auxin transport in roots: Vicia faba. Nature, 204, 559-561.

Zeevart, J.A.D., 1971a. Effects of photoperiod on growth rate and endogenous gibberellins in the long day rosette plant spinach. Plant Physiol. 47, 821-827.

Zeevart, J.A.D., 1971b. (+)-Abscisic acid content of spinach in relation to photoperiod and water stress. Plant Physiol. 48, 86-90.

Zenk,* M.H., 1970. Phytohormones und Genaktivitat. Ber. Dtsch. Bot. Ges. Bd. 83, 325-344.

Zimmerman, M., 1969. Translocation of Nutrients. In Physiology of Plant Growth and Development, ed. M.B. Wilkins, McGraw-Hill, London, pp. 383-417.

* Cited but not read in detail.