THE MOVEMENT AND METABOLISM OF 2,4-DICHLOROPHENOXYACETIC ACID IN ROOT TISSUES OF PISUM SATIVUM L.

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by

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SUMMARY

The movement of 2,4-dichlorophenoxyacetic acid, labelled with ^{14}C in the -COOH or the $-CH_2$ group of the acetic acid side chain, was monitored in sub-apical segments and intact roots of Pisum sativum maintained at 25°C ± 1°C in white light or total darkness.

A strong acropetal polarisation of the movement of radioactivity was detected in root segments, together with a significant basipetal component of transport. Radioactivity applied to the apical end of the segments appeared to be retained mainly in the tissue adjacent to the donor block, whereas an application to the basal end resulted in a rapid distribution towards the receiving block. The polarity was not related to the ratio of the area of cut surface at either end of the segments, indeed the greatest polarity occurred when the ends were of identical area. The polarity was related to orientation of the segments with respect to gravity and exposure of the segments to white light.

Studies of the importance of several components of the experimental system on the movement of radioactivity, from the labelled herbicide, were made and revealed that the system was complex.

Acropetal and basipetal movement of ^{14}C, in the phloem and xylem respectively, was recorded in intact Pisum seedlings roots but depended on the method of herbicide application.

Degradation of the radioactive herbicide in root segments and intact roots was not significant and the ^{14}C label appeared to be confined to the 2,4-D molecule.

ERRATA

(1) Page 137 — legend to Fig. 43b

2,4-D(-1-^{14}C) should read "2,4-D(-2-^{14}C)".

(2) Page 142 — Delete last half-sentence of heading:

".....and the data are expressed.....".

(3) Page 185 — Final sentence should read:

"The fact that the export of radioactivity into the receiving blocks (fig. 36) exhibited saturation effects would be compatible with this theory, but not the claim that concentrations of IAA up to 10.0 µM did not saturate a Zea root segment system.

(4) Page 193 second paragraph — "Alterations in the acropetal replicates.....were not - please insert not at end of line.

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INTRODUCTION

Over the past three decades, scientists and agriculturalists have recognised the compound 2,4-dichlorophenoxyacetic acid (2,4-D) to be an outstanding herbicidal chemical possessing selective phytotoxicity. Generally, dicotyledonous plants are regarded as susceptible whilst monocotyledonous crops are resistant to the herbicidal action. It is not surprising that a compound of such clear commercial potential should be the centre of specialised research aimed at the elucidation of the mechanisms conferring successful weedkilling capabilities. Such an understanding might be invaluable in the formulation of new products.

In commercial practice 2,4-D is normally applied to crops as a foliar spray which demands that the compound must find an adequate method of penetrating the complex lipophyllic cuticle or the stomata. Addition of surfactants to spray formulations has been employed extensively to bring the compound into intimate contact with the leaf surface (Hauser, 1955). For example, the rate of uptake and subsequent toxicity of 2,4-D and 2,4-dichloro-5-iodophenoxyacetic acid in Phaseolus vulgaris, Alternanthera philoxeroides, Prosopis juliflora and wild morning glory seedlings was increased by Tween 20, Carbowax 1500, Dreft and Emulphor-ELA (Mitchell and Linder, 1950; Mitchell and Hamner, 1944; Crafts, 1956b; Earl et al, 1951; Blair and Fuller, 1952). Ennis and Boyd (1946), however, pointed out that carbowax was not effective as a surface active agent on soybean. Adequate penetration also depended upon the formulation of the 2,4-D spray and easiest entry was gained by the ammonium salt, isopropyl esters and emulsifiable esters (Ennis and Boyd, 1946; Hauser, 1955; Crafts, 1956b). The sodium salt and the amine of 2,4-D were absorbed more slowly but the addition of surfactants increased the penetration and reduced the differential (Hauser, 1955). Progressive chlorination of the phenoxyacetic acid molecule also resulted in increased rates of entry in the light and dark (Sargent et al, 1969; Kenney-Wallace and Blackman, 1972), as did increased temperature and high humidity (Pallas, 1960; Clor, 1963).

Sargent (1968) reviewed the data concerning the uptake of 2,4-D into

leaf discs from Phaseolus vulgaris and emphasised the occurrence of an immediate surge of penetration into dark-treated discs excised from seedlings up to ten days old. The rate was pH, temperature and concentration dependent (Sargent, 1968; Bach and Fellig, 1961; Blackman, 1953; Audus, 1948; Orgwell, 1957) and an enhancement under high light intensity was metabolically dependent. Further investigations revealed that the loss of the initial surge in aged discs could be prevented by the addition of 2,4-D, IAA or NAA which might assume the role of endogenous auxin destroyed at the time of excision. A decrease in the level of the natural hormone by the addition of the antiauxin TIBA produced no effect in young or old darkened tissue, but resulted in increased penetration in young illuminated tissue. Since a close correlation existed between stomatal density and the rate of 2,4-D penetration, even in darkness, it was proposed that the guard cells could be major sites of entry (Sargent, 1968). Another correlation was found between the mechanism of uptake of phenoxyacetic acid compounds and the degree of auxin-like activity associated with the particular compound (Saunders et al, 1956a; Saunders et al, 1956b). 'Type 1' uptake depended upon mechanisms which became disorganised after excision of the segments,and 'Type 2' uptake depended upon more stable mechanisms. In general, Type 1 was associated with the uptake of auxin-like compounds such as 2,4-D by Lemna minor Gossypium hirsutum, Triticum vulgare, Sorghum vulgare and Avena sativa, in which the rate was rapid initially but then declined progressively until a net movement of the herbicide back into the donor solution took place (Blackman et al, 1959; Saunders et al, 1956a) When phenoxyacetic acid was tested the phase of egress was followed by a second phase of uptake (Blackman, 1959). In Avena mesocotyl segments the phase of egress could be prevented by pretreatment with buffer or unlabelled 2,4-D solution (Jenner et al, 1968a). Blackman (1964) also discovered that egress occurred mainly in susceptible species and rarely in resistant monocotyledonous plants. He proposed that the lack of egress might indicate the presence of a protection mechanism which restricted the movement of 2,4-D within the plant. In complete contrast, no difference in uptake could be detected between resistant wild cucumber and susceptible cultivated cucumber (Slife et al, 1962), or between resistant sugarcane and susceptible bean plants (Ashton, 1958). Thus the involvement of penetration in the mechanisms of resistance is not fully understood.

The movement of unlabelled and ^{14}C-labelled 2,4-D in intact plants has been the subject of many investigations in which the appearance of

characteristic growth responses or ^{14}C have been used to follow the progress of translocation. There can be little doubt that the movement of the herbicide into the petioles, stems and roots of several species following a foliar application was dependent upon the photosynthetic activity of the plants. Mitchell and Brown (1946) demonstrated that a phytotoxic stimulus was not translocated from sugar-deficient leaves of Phaseolus vulgaris seedlings maintained in darkness. Rice (1948) found that darkness or low intensity light resulted in folding of the leaves but not bending of the stem, whilst Linder (1949) claimed that the stems were still capable of responding to a local application of the herbicide. Leaf-clipping or defoliation of soybean and alfalfa seedlings hampered the movement of the herbicide into the other parts of the plant (Weaver and deRose, 1946). Application of sucrose, fructose or glucose to the treated leaves of darkened Phaseolus or Glycine seedlings resulted in a detectable movement of 2,4-D out of the leaves (Rohrbaugh and Rice, 1949; Weintraub and Brown, 1950; Jaworski et al, 1955; Barrier and Loomis, 1957), Since mannitol, arabinose, nor urea could substitute for sucrose, it was concluded that the effect was metabolic and not osmotic (Hay and Thimann, 1956). Further work by Mitchell et al (1953) revealed that an application of sucrose and boron together produced a synergistic effect on 2,4-D translocation. They concluded that the effect was due to the promotion of sugar movement by boron, but unfortunately they did not check whether boron alone could produce stem curvature. Additional evidence for the movement of 2,4-D with photosynthates came from Weaver and deRose (1946) and Eliason (1965), who demonstrated that the movement through dead stem tissue was possible only in the upward direction. This was supported by Hay and Thimann (1956) who found that removal of the cortex and phloem of Phaseolus stems reduced the amount of herbicide moving into the root system following a foliar application. The rate of movement of unlabelled and labelled herbicide out of Phaseolus leaves was 10-100 cm h^{-1} and 10-12 cm h^{-1} which compared favourably with published rates for phloem translocation (Day, 1952; Little and Blackman, 1963). Earle et al (1951) found that the rate of acropetal and basipetal movement in stems of Alternanthera philoxeroides (alligator weed) was only 4.2-4.3 cm h^{-1}. The rate was independent of herbicide concentration but increased temperature or humidity resulted in enhanced translocation (Rice, 1948; Pallas, 1960; Basler, 1961; Clor etal, 1963). In conclusion, it would appear logical that stem curvatures in response to 2,4-D do not occur in the dark because of the lack of metabolite translocation (Leonard and Crafts, 1956). Jaworski

et al (1955), on the other hand, could not detect curvature after supplying 2,4-D and sugar solution in the dark and suggested that light activation of some other system might be required.

Another factor which greatly influenced the pattern of 2,4-D translocation was the location of active metabolic sinks within the plant. Active root growth of Hordeum, Gossypium, Zebrina and Tradescantia enhanced the movement of the herbicide from the leaves (Crafts and Yamaguchi, 1958; Crafts, 1959) and accumulation occurred in cambium, expanding buds, floral structures, young leaves and other regions of active growth in the Tokay grape and certain monocotyledonous plants (Leonard and Weaver, 1961; Gallup and Gustafson, 1952; Fang and Butts, 1954). Movement of ^{14}C from the treated leaves of Zea, Triticum, Avena, Hordeum, Prosopis, Phaseolus, and Pisum resulted in accumulation in the stem, sheath, hypocotyl or root system (Blair and Fuller, 1952; Peterson, 1958; Fang, 1951; Fang, 1958; Hay and Thimann, 1956). The site of application of the herbicide also influenced the eventual destination, for example, an application to the cotyledons of wild morning glory resulted in translocation to the roots, whilst acropetal and basipetal movement was recorded when the middle leaves were treated. Movement did not occur at all when the young expanding leaves were treated, but this would be expected in view of the metabolic sink theory (Crafts, 1956a; Crafts, 1956b). However, when the cotyledons or primary leaves of Glycine soja were treated bidirectional translocation through the epicotyl resulted in accumulation of radioactivity in the root and shoot tips (Crafts, 1966-1967). In other investigations 2,4-D was supplied to the root system of Zebrina, Hordeum, Phaseolus, Gossypium, Sicyos and Cucumis and most of the herbicide was retained in the roots by loose physical binding or metabolic binding (Yamaguchi, 1964; Slife, et al, 1962; Crafts, 1966-1967). Dhillon and Lucas (1950), on the other hand, reported rapid movement into aerial structures following root applications to tomato, bean, corn and oat seedlings. In most of the species the translocation of the herbicide from roots or shoots was limited by immobilisation on application and during translocation such that the proportion of readily extractable 2,4-D steadily decreased along the path of movement (Crafts, 1956b; Crafts and Yamaguchi, 1958; Leonard, 1958)

Several workers have attempted to correlate the translocatability of 2,4-D in susceptible and resistant species with the degree of phytotoxicity

observed (Butts and Fang, 1961 ; Fang and Butts, 1954; Weintraub et al, 1954 ; Crafts and Yamaguchi, 1960). The translocation in Gossypium, Pisum and Phaseolus seedlings was found to be up to 70 times greater than that in resistant Zea and Saccharum and the concentration of the herbicide was 10 times greater in the apical meristem of Phaseolus than in Saccharum. Further studies by Gallup and Gustafson (1952) revealed that the lack of translocation associated with resistant cereal plants was due to a block in the intercalary meristem of the monocotyledonous plants' leaf.

On entry into the translocation stream the herbicide is attacked by degrading mechanisms which attempt to alter the molecular form of the compound. Reports range from no degradation in Ampelamus albidus (milkweed) to the transformation of 75% of the 2,4-D(-1-^{14}C) into two radioactive metabolites in wild and cultivated cucumbers (Slife et al, 1962; Coble et al, 1970). Weintraub et al (1954) found that the extent of 2,4-D metabolism in overwintering cherry trees was directly dependent upon the length of exposure to the tissue. A foliar application to Phaseolus seedlings resulted in the formation of two unknown radioactive compounds. 'Unknown 1' was formed at the same rate in light and darkness and was readily hydrolysable to release free 2,4-D (Jaworski and Butts, 1952; Jaworski et al, 1955). Incubation of Phaseolus stem segments with 2,4-D produced two major radioactive compounds together with eight minor metabolites, all of which retained the aromatic nucleus. Acid hydrolysis of a compound running at Rf 0.5 in butanol:propionic acid:water released free 2,4-D and indicated that the product might be a glycoside or peptide of the herbicide (Bach, 1961). Further evidence for 2,4-D-protein complexes was provided when hydrolysis of a Phaseolus extract produced free 2,4-D and at least 12 amino acids (Butts and Fang, 1955) and by the extraction of conjugation products from cotton, sorghum, tickbeans and jimsonweed (Canny, 1960; Morgan, 1963; Fites et al, 1964). A water soluble extract formed in the primary leaves of Phaseolus within 6 hours' of treatment was thought to be 2,4-D bearing an extra constituent (Holley, 1952). Furthermore, Thomas et al (1964b) proposed that a shift of chlorine atoms during the formation of 2,5-dichlorophenoxyacetic acid and 2,3-dichlorophenoxyacetic acidfrom 2,4-D might be a prerequisite for the formation of conjugated products. The hydroxyl-chlorine replacement reaction was confirmed in Avena mesocotyl segments and it was suggested that glucose esters or hydroxylation products were formed in the first instance, which were then converted to glycosides (Thomas et al, 1963; Thomas et al, 1964a)

Herbicide degradation has been tackled from a different angle which did not involve the chromatographic analysis of plant extracts, but the collection of radioactive carbon dioxide (Fang et al, 1951; Basler, 1964). Clearly, evolution of ^{14}C from treated plants must indicate that the applied molecules are undergoing degradation. Weintraub et al (1952) demonstrated that $^{14}CO_2$ was lost from the terminal buds, primary leaves and stem explants of Phaseolus treated with carboxyl-, methylene- or ring-labelled 2,4-D. Little radioactive carbon dioxide was lost from plants treated with the latter compound but 2,4-D(-1-^{14}C) yielded a three times greater output than 2,4-D(2-^{14}C). An identical situation was discovered with sorghum, cotton and red currant leaves (Luckwill and Lloyd-Jones, 1960a; Morgan, 1963) and indicated that the acetic acid side chain was being subjected to sequential degradation from the -COOH group end, whilst the aromatic nucleus remained intact. Canny (1960) found that the output from tickbean roots was identical whether the carboxyl or the methylene-labelled compound was used. Several authors have tried to relate the production of the radioactive gas with the formation of detoxification products. Butts and Fang (1955), for example, claimed that 2,4-D-protein complexes were partially detoxified since injection into Phaseolus stems caused a faster rate of $^{14}CO_2$ evolution than when 2,4-D(-1-^{14}C) was used. Luckwill and Lloyd-Jones (1960a, 1960b) demonstrated that resistant red currant and Coxs' apples lost up to 60% of the applied radioactivity whilst susceptible blackcurrant and Bramley apple lost only 2% as carbon dioxide in the same period. Susceptible cotton seedlings released 1% whilst resistant sorghum released only 0.09% of the applied ^{14}C. In contrast, several other resistant species, including corn and bean, gave the same rate of evolution indicating that in these species, at least, decarboxylation was not an important mechanism of detoxification (Luckwill and Lloyd-Jones, 1960b; Weintraub et al, 1954 ; Williams et al, 1960). It is obvious that the evolution of radioactive gas cannot be regarded as a universal symptom of 2,4-D degradation and detoxification.

Having considered the mechanisms of uptake, translocation and metabolism of 2,4-D, attention can now be turned to the effects of the compound whilst it is in the plant. The gross morphological responses of intact plants to herbicidal concentrations of 2,4-D have been well documented. Beal (1944) described effects which were induced or incited at considerable distance from the point of herbicide application as 'Telemorphic! A good example of this was the inhibition of root elongation and swelling adjacent to the root tip following foliar application of the herbicide to

Lathyrus odoratus, Phaseolus vulgaris, Zea mays, Triticum vulgare and Pisum sativum (Beal, 1944; Taylor, 1946; Botrill and Hanson, 1968; Scott and Morris, 1970). Confirmatory evidence was provided by Eliason (1959, 1961, 1963, 1972), who found that similar responses in Populus tremula and Pisum sativum appeared after 12-24 hours and were enhanced by white light. The inhibition of elongation was not permanent and could be removed by washing the root system and Eliason proposed that a leachable, growth inhibitory substance was formed in Pisum roots in response to 2,4-D. Johanson and Muzik (1961) found that the normal resumption of root growth in Triticum vulgare took place 10 days after foliar treatment, but only 4 days were required after a localised treatment of the root system. In general, root swellings were associated with a massive initiation of lateral roots. In addition to root responses, telemorphic effects of 2,4-D can be seen in the aerial structures of many species. Foliar applications of the herbicide resulted in the swelling of apical and nodal regions of several monocotyledonous and dicotyledonous plants (Taylor, 1946; Eames, 1951)and curvature, epinasty and thickening of the stem of Lathyrus odoratus, Gossypium hirsutum and Zea mays (Beal, 1944; Dunlap, 1948; Botrill and Hanson, 1968). Inhibition of hypocotyl and epicotyl elongation in Glycine max was reported by Gifford and Dengler (1966) and Key et al (1966). Studies with Phaseolus have shown that 2,4-D can cause the cessation of growth and swelling of the internodes (Beal, 1945), epinasty of the petiole and leaf curling (Brown, 1946; Watson, 1948; Kelly, 1949), and the appearance of outgrowths on both surfaces of the trifoliate leaves (Felber, 1948). Treatment of the seeds killed the embryo and induced the initiation of root primordia on the cotyledons (Akamine, 1948). The disorganisation of floral, pollen grain and chlorophyll development in bindweed was reported by Tukey (1945) and the loss of chloroplasts from cortical cells of Glycine max by Rojas-Garciduenas and Kommendahl (1958).

The external alterations in plant form in response to 2,4-D are, without doubt, the manifestation of more subtle changes at the cellular level. Within three days of a foliar application Phaseolus seedlings exhibited extensive periclinal and anticlinal cell division in the cambium, endodermis, medullary rays and phloem parenchyma of the hypocotyl. Eventually a wide band of highly meristematic cells was formed from which root initials were differentiated and culminated in the crushing of all tissues external to the inner pericycle (Smith, 1948; Eames, 1950). As Swanson pointed out, however, the epidermis, cortex and pith showed no direct response to 2,4-D treatment. The integrity of the vascular system

was disrupted through a lack of fresh differentiation and by the proliferation of the phloem parenchyma of the companion cells and sieve tubes formed prior to treatment (Swanson, 1945; Eames, 1950). Activation of cell division in Glycine max and Zea mays occurred in close proximity to the vascular cylinder in the aerial structures (Key et al, 1966; Hoshaw and Guard, 1951). Additional evidence of the meristematic response came from Muzic and Cruzado (1958) using Phaseolus internodes and by Grant and Fuller (1971) using Vicia root tips. Furthermore, Watson (1948) reported that distortion of Phaseolus leaves was due to the formation of turgid, thick-walled parenchyma cells, which had no intercellular spaces and replaced the essential chlorophyll-bearing mesophyll cells. Hallam (1970) found that, in the presence of light, 2,4-D promoted the degradation of membranes in epidermal, palisade and mesophyll cells followed by a recession of the cytoplasm from the cell wall. The chloroplasts of the Phaseolus leaves became granular and vacuolated whilst the membranes disintegrated, leaving the internal structure unidentifiable.

It has been shown that 2,4-D treatment invoked specific morphological and histological modifications in a wide range of plant species, but the underlying causes for the responses are to be found at the biochemical level. Reports are numerous and this review merely attempts to indicate the major lines of research. O'Brien et al (1968) and Leffler et al (1971) discovered that 2,4-D enhanced the activity of actinomycin-D-sensitive DNA and RNA polymerase activity of soybean hypocotyl chromatin, but the product RNA possessed a modified structure. Increased levels of DNA and RNA have been detected in stem segments, hypocotyls and microsomes of corn, cucumber and soybean and in the vascular system of Vicia roots (West et al, 1960; Key and Hanson, 1961; Key et al, 1966; Grant and Fuller, 1971). Basler (1961) reported that 2,4-D prevented a loss of microsomal RNA and protein, but increased chloroplast RNA in cultured Gossypium cotyledon tissue. Incorporation studies with adenosine-8-^{14}C, however, revealed that low levels of the herbicide enhanced the degradation of RNA and protein whilst high concentrations prevented the breakdown in corn and cucumber mesocotyl and hypocotyl tissue (Key, 1963; West et al, 1960). The decrease in RNA mainly at the expense of microsomal and t-RNA but not nuclear or mitochondrial RNA (Key, 1963). Osborne (1964), using a ^{14}C incorporation technique, found that 2,4-D retarded a loss of protein and chlorophyll but increased the soluble nitrogen content of detached autumnal Prunus leaves.

She proposed that an auxin balance could limit protein synthesis during senescence. Confirmatory evidence for inhibition of protochlorophyllide and chlorophyll synthesis was provided by Wedding et al.(1954) and Shewry et al.(1971). De novo synthesis of protein in response to 2,4-D was shown in Alaska pea roots by polyacrylamide gel electrophoresis, with the new bands of protein being identical to those in untreated meristematic tissue (Morris, 1966). Changes in the content of aspartic acid, glutamic acid, lysine, valine, methionine and phenylalanine have been detected in Phaseolus seedlings (Sell et al., 1949; Akers and Fang, 1956). The level of thiamine, riboflavine and nicotinic acid decreased in leaves but increased in stems, whilst the level of pantothenic acid in leaves and carotene decreased in the stems of Phaseolus seedlings (Leuke et al., 1949). Enzymes were also affected by herbicide treatment, for instance the activity of pectinmethyl-esterase in bean and artichoke tissue and nitrate reductase activity in corn and cucumber was enhanced (Neely et al., 1950a; Beever et al., 1963; Macey, 1965). Malic dehydrogenase was inhibited whilst there was no effect on -amylase in Phaseolus leaves (Neely et al., 1950b). Further effects on enzymes were revealed when Humphreys and Dugger (1957a) found that 2,4-D switched glucose catabolism from the glycolytic to the pentose phosphate pathway in Pisum, Zea and Avena seedlings. This was verified by Black and Humphreys (1962), who found decreased activity of phosphofructokinase, aldolase and glyceraldehyde phosphate dehydrogenase and increased the activity of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase following the treatment of Zea seedlings with the herbicide. In contrast, Mostafa and Fang (1971) claimed that the pentose phosphate pathway in Zea and Pisum was inhibited whilst glucuronic acid formation in pea roots was stimulated by 2,4-D. Further investigations by Wedding and Black (1962) indicated that 2,4-D could uncouple the energy-storing phosphorylation reaction normally associated with the oxidation of malate, citrate, succinate and NADH in Brassica and Beta mitochondria. There can be little doubt that herbicide application affected enzyme and protein levels in such a way as to stimulate respiratory rates and decrease the levels of carbohydrate reserves (Rasmussan, 1947; Wedding et al., 1954; Smith, 1948)

Having considered the data dealing with the movement of 2,4-D in intact plants of several species, the movement of the herbicide in isolated segments can be assessed. The uptake and movement of 2,4-D(-1-^{14}C) in Phaseolus petiole and petiole/pulvinus segments was basipetally polarised, but uptake into the distal end of the pulvinus segments was smaller than that

into purely petiolar segments (McCready, 1963; Jacobs et al., 1966). Movement of the herbicide through Arachis internode segments was also basipetally polarised and correlated with vascular regeneration following wounding (Thomson, 1968). The polarity in Phaseolus petiole segments persisted against a concentration gradient and could be increased by the inclusion of kinetin or mannitol in the donor and receiving blocks, or decreased by GA. Since GA promoted elongation of the segments but decreased the basipetal movement of 2,4-D, McCready (1967) suggested that the polarity of auxin movement might be dependent upon the elongation of the transporting tissues. However, Harel (1969) revealed that the degree of polarity depended upon the action of white light and McCready et al. (1965) showed that kinetin could stimulate the movement of 2,4-D in aged Phaseolus segments. McCready (1963) noted a very interesting phenomenon, in that ^{14}C in the receiving blocks appeared to move back into the segments after a donation of IAA(-1-^{14}C) or a low concentration of 2,4-D(-1-^{14}C). Nevertheless, no more than 4.3% and 0.7% of the applied IAA and 5.5% and 0.5% of the initial donor supply of 2,4-D reached the receiving blocks of the basipetal and acropetal replicates respectively. The acropetal movement increased disproportionately as the donor concentration increased and as the age of the segment increased, resulting in an apparent reduction in the degree of polarity. An increase in the length of the segments produced an increase in polarity. Calculations of velocity of 2,4-D movement gave values of 0.6-1.6 mm h^{-1} for movement through petiole and petiole/pulvinus segments. Chromatographic analysis revealed that 2,4-D was the only radioactive compound accumulating in receiving blocks applied to Phaseolus petiole segments. One metabolic product was found in tissue extracts and the compound ran to a lower Rf than the major peak of 2,4-D.

Although the movement and metabolism of growth regulating compounds have been well characterised in the shoots of many plant species, little attention has been paid to the mechanisms existing in root tissues. By the end of 1971 the movement of only two compounds had been investigated, namely, IAA and kinetin. Of these, IAA was the most fully studied whilst only one report of kinetin movement was documented. The whole realm of the synthetic regulators, including 2,4-D, remained untouched.

In common with the investigations of aerial structures, the primary objective was the determination of polarity, if any, of auxin movement in roots. Torrey (1950, 1958) and others employed auxin-induced responses such

as lateral root formation and cambial initiation to demonstrate the preferential movement of auxin towards the root apex of several species. Initial investigations using radioactively-labelled IAA presented a confused picture, with reports of a basipetal polarity in Zea (Hertel and Leopold, 1963) and an acropetal polarity in Lens, Vicia and Convolvulus root segments (Pilet, 1964; Yeomans and Audus, 1964; Bonnett and Torrey, 1965). The situation was resolved by reports from independent laboratories showing that IAA moved preferentially toward the root apex in Lens and Phaseolus (Kirk and Jacobs, 1968) and in Zea, Avena, Triticum and Helianthus root segments (Wilkins and Scott, 1968; Scott and Wilkins, 1968). Confirmatory evidence came from studies of Helianthus and Pisum segments (Iversen and Aasheim, 1970; Aasheim and Iversen, 1971; Hillman and Phillips, 1970).

A differential loss of radioactivity from donor blocks during acropetal and basipetal treatments can be used to demonstrate the polarisation of auxin movement towards the root apex. Basal application to Lens and Phaseolus segments for 8 hours (Kirk and Jacobs, 1968) resulted in a loss of up to 40% of the original donor content, but the maximum loss from apical blocks was only 30%. The loss from apical blocks was found to be slightly less than the 50% loss in 6 hours and the 70% loss in 24 hours for Vicia and Pisum respectively (Yeomans and Audus, 1964; Hillman and Phillips, 1970). Pilet (1964) detected a 17% loss of ^{14}C following a basal donation for 4 hours to Lens roots. Several other workers have ignored this part of the experimental system completely-Bonnett and Torrey, 1965; Wilkins and Scott, 1968; Scott and Wilkins, 1968; Wilkins and Cane, 1970; Cane and Wilkins, 1970; Iversen and Aasheim, 1970; Aasheim and Iversen, 1971; Wilkins et al, 1972.

The movement of radioactivity within the root segments has been monitored as the distribution of radioactivity along the root segments with time. Dissection of Zea segments revealed that the radioactivity from IAA(-1-^{14}C) decreased in an approximately logarithmic fashion with increasing distance from the donor block (Wilkins et al., 1972). A similar situation was found in the first three 1.0mm pieces of Lens segments, but the distal pieces had a higher content than would be expected from such a relationship This upward trend at the receiving block end of the system was not apparent after apical donation (Kirk and Jacobs, 1968). In all the species studied, the level of ^{14}C in the half-segment nearest to the donor block was virtually

identical after acropetal or basipetal treatments (Yeomans and Audus, 1964; Bonnett and Torrey, 1965; Kirk and Jacobs, 1968; Hillman and Phillips, 1970; Iversen and Aasheim, 1970; Aasheim and Iversen, 1971). A more detailed examination by Yeomans and Audus (1964) showed that the thin apical slice of a Vicia segment in contact with the donor block was capable of accumulating more radioactivity than the comparable basal slice. However, the converse was true for Lens (Kirk and Jacobs, 1968). Also detectable in Vicia was a greater radioactive content of the half-segment nearest the receiving block after basal than after apical donation. This preferential movement of IAA towards the root apex was present, but to differing extents, in Convolvulus (Bonnett and Torrey, 1965), Lens (Kirk and Jacobs, 1968), Pisum (Hillman and Phillips, 1970), Brassica and Helianthus (Iversen and Aasheim, 1970; Aasheim and Iversen, 1971). The degree of polarity in the latter two species was reduced by increasing the duration of the experiments and was increased in Convolvulus by raising the IAA concentration. Tissue studies have been shown, therefore, to be useful in providing indisputable evidence in polarity studies and it is surprising that some workers have neglected this part of the system completely.

The accumulation of radioactivity in agar receiving blocks placed on the end of the segments distal to the point of donation, has been an almost universal method of determining the polarity of auxin movement. Difficulty in detecting significant radioactivity in the receiving blocks forced some workers to look at the accumulation of ^{14}C in the tissue only (Yeomans and Audus, 1964), whilst Bonnett and Torrey (1964) preferred not to use blocks at all. In general, apically applied blocks accumulated more ^{14}C than those applied basally, indicating a strong polarity of IAA movement towards the root apex. For example, Pilet, using Lens segments, detected a steady increase of radioactivity in both apical and basal blocks throughout an 8 hour experiment and reported an acropetal/basipetal ratio of 2/1. Kirk and Jacobs (1968), using the same stock of plants, found a steady accumulation of ^{14}C with a ratio of 33/1, the strongest polarity yet detected. Other less absolute polarities were demonstrated in Avena, Triticum, Helianthus, two varieties of Zea (Wilkins and Scott,1968) and Brassica and Helianthus (Iversen and Aasheim, 1970). A linear increase in receiving block content of ^{14}C, followed by a decrease in block content after 7 hours was reported to be gravity insensitive (Scott and Wilkins, 1968). The onset of the decline phase was dependent upon temperature (Wilkins and Cane, 1970) and the initial concentration of the donor blocks (Scott and Wilkins, 1968). Similarly,

Hillman and Phillips (1970) showed that a decline phase in Pisum, which they explained as the loss of a volatile product from the system, set in after 12-18 hours. Scott and Wilkins (1968), and Iversen and Aasheim (1970) also reported that the degree of polarity was reduced with increased duration of the experiments. Polarity was insignificant, however, when the data were expressed as a proportion of the ^{14}C supplied originally. Pilet (1964) claimed that 5.8% of the original donor block activity appeared in receiving blocks applied to Lens segments, whilst only 0.3% was detected in the same tissue by Kirk and Jacobs (1968). A maximum of 3.29% in Pisum was reported by Hillman and Phillips (1970). Consideration of the system from this viewpoint places the capacity of the auxin transport system in a true perspective.

Although maximum promotion of root elongation can be induced by 0.1 nMolar solution, much higher concentrations, 0.1μM-1.0 mM have been employed in tracer studies because of the limits of detection. An increase in donor concentration supplied to Zea root segments led to an increased accumulation of ^{14}C in apical but not basal receiving blocks (Scott and Wilkins, 1968). The system was not saturated by concentrations up to 10^{-2} M and this was true for Brassica (Iversen and Aasheim, 1970). However, the capacity of the Convolvulus system was exceeded by 5 μM IAA (Bonnett and Torrey, 1965). This was detectable in receiving block accumulation only, whilst the amount of ^{14}C moving through the tissue was directly proportional to that supplied over the range 0.0239μM-1.0 mM. Increasing the donor concentration also accelerated the onset of the decline phase in receiving block content with Zea (Scott and Wilkins, 1968).

The time required for radioactive molecules to pass from end to end of a root segment of known length has been used in the calculation of the velocity of growth substance movement. Bonnett and Torrey (1965) found an acropetal velocity of 10.4 mm h^{-1} in 12mm Convolvulus segments and 9.1 mm h^{-1} in 13.5 mm segments at 25°C. Wilkins and Cane (1970) discovered that the acropetal velocity in Zea was independent of segment length, but showed a clear optimum at 31°C with a value of 7-8mm h^{-1}. An acropetal velocity of 5mm h^{-1} at 25°C, though slower than the basipetal value, confirmed an earlier estimate of 4-6mm h^{-1} in the same species (Scott and Wilkins, 1968). Slower velocities of 2.2 mm h^{-1} have been reported in root segments of Lens and Phaseolus respectively (Kirk and Jacobs, 1968) and of 2.07 mm h^{-1} in Zea at 15°C (Wilkins et al., 1972).

The polar acropetal flux, that is the quantity of ^{14}C moving towards the root apex of Zea was greatest at 10-15°C and 40-50°C (Wilkins and Cane, 1970), and was dependent upon metabolism (Wilkins and Scott, 1968). The flux was promoted by white, red and blue light (Scott and Wilkins, 1969). Excision of the segments at increasing distances from the root apex reduced both the acropetal and basipetal fluxes of IAA in Zea (Wilkins and Cane, 1970). In general, the basipetal flux in this species was smaller than the acropetal flux.

Demonstrations that the velocity and flux of IAA movement were temperature dependent led several workers to explore the relationship between movement and metabolism. Wilkins and Scott (1968) found basipetal movement and the acropetal polarity of IAA translocation in Zea root segments to be unaltered by anaerobic conditions, although the acropetal flux was reduced by 92%. Prolonged anaerobiosis resulted in a resumption of the acropetal movement, presumeably as the segments adapted to anaerobic metabolic pathways. If both aerobic and anaerobic metabolism were prevented, however, the acropetal polarity was replaced by a basipetal polarity and the ratio of basipetal to acropetal movement was almost identical to that through dead segments (Wilkins and Scott, 1968). A reversal of polarity, also in Zea, was shown at 1-5°C under anaerobic conditions. It was concluded that anaerobic metabolism at this temperature was not sufficient to maintain the acropetal polarisation of IAA (Wilkins and Cane, 1970). Yeomans and Audus (1964) demonstrated that the uptake of auxin could be reduced by the treatment of Vicia segments with 2,4-dinitrophenol, potassium cyanide or anaerobic conditions, all of which are inhibitors of energy-synthesising processes.

Isolated segments are intended to provide an experimental system of less complexity than that encountered in whole-plant experimentation. but it has become apparent that artefacts could be inherent in the technique. For example, Yeomans and Audus (1964), observing the tissue composition at either end of a segment to be different, compared uptake into the ends of contiguous segments excised 4-8mm and 8-12mm behind the root apex of Vicia and found little difference. This was corroborated by evidence from Pisum (Hillman and Phillips, 1970), showing little difference in uptake or movement of IAA in segments excised from three different regions of the root. On the other hand, Zea segments from corresponding regions were capable of moving less ^{14}C on excision from the more basal parts of the roots. Wilkins and

Scott (1968a) and Kirk and Jacobs (1968) pointed out that Zea and Lens were frustums in which the basal surface area was more than double the apical surface area. They suggested that weak acropetal polarities could, perhaps, be explained as a greater diffusion of the auxin through the larger basal cut surface. However, as Avena, Triticum and Helianthus segments were virtually cylinders it was thought that the polar flux could not be due to a surface area effect alone (Wilkins and Scott, 1968a). Since a basipetal polarity of IAA(-1-^{14}C) movement was evident on killing or the suspension of metabolism of Zea root segments, it was felt that the phenomenon could be related to the geometry of the frustum (Wilkins and Scott, 1968b). Diffusion of IAA through agar cylinders of identical dimensions to Vicia segments led Yeomans and Audus to believe that root tissues restricted the free movement of IAA. They concluded that the acropetal polarity was due to 'a positive gradient of accumulation potential as one moves towards the root tip'.

The movement of auxin through shoot segments resulted in immobilisation and/or partial degradation of the IAA molecules (Goldsmith and Thimann, 1962). The radioactivity accumulating in the segments or receiving blocks did not automatically indicate the presence of the exogenously applied compound. Hence, following a donation of IAA(-1-^{14}C), radioactive compounds present in receiving blocks applied to Convolvulus (Bonnett and Torrey, 1965), Lens (Kirk and Jacobs, 1968) and Zea root segments (Scott and Wilkins, 1968; Wilkins and Cane, 1970) were extracted in ethanol or ether and analysed by developing paper chromatograms in 8:1:1 or 9:1:1 (isopropanol : NH_3 : H_2O). Only one radioactive peak was isolated from all the species tested and this ran to the same Rf as authentic samples of pure labelled and unlabelled IAA. Further analyses using Zea roots revealed that 85, 96 and 95% at 5 C, and 72, 79 and 70% at 25 C of the ^{14}C extracted from IAA(-1-^{14}C) stock solution, used donor blocks and receiving blocks respectively, was still confined to the IAA molecule. On the basis of colorimetric and radioactivity determinations Aasheim and Iversen (1971) isolated three main compounds originating from the labelled compound supplied to cabbage segments. These ran to Rf 0-0.1, 0.4-0.6 (IAA), and 0.8-0.9 in 8:1:1 and the amount of identifiable IAA decreased with increasing duration of the experiment. Receiving block extracts were reported to contain only one compound which ran to Rf 0.45-0.60 (IAA), yet the data clearly showed that 23% of the ^{14}C ran to Rf 0-0.1! The translocated compounds in Helianthus were claimed, by

Aasheim and Iversen (1971), to be identical to those in Brassica, but the evidence was not presented. IAA, ring-labelled with tritium, was found to be degraded completely by the same two species, leaving two radioactive compounds running at Rf 0-0.1 and Rf 0.85-1.00. The stock donor solution contained IAA and a compound running at Rf 0.85-1.00, but at another point in the same paper the results were claimed to relate to control donor blocks. Clearly, the extraction proce dures employed in the analysis of donor block content might influence the loss of the auxin, so it is imperative to know whether the data related to extracted material. Hillman and Phillips (1970) studying the metabolism of IAA(-2-^{14}C) in pea roots, discovered three radioactive compounds in tissue and receiving blocks running at Rf 0-0.1, Rf 0.2-0.4 (IAA) and Rf 0.8-0.9. The identities of the two unknowns were suggested as indole-3-acetylaspartic acid and indole-3-aldehyde respectively. All three were growth promotory in a wheat coleoptile bioassay. Used and aged donor blocks contained the same compounds, thus preventing any distinction between spontaneous or metabolic degradation of the auxin. Criticism of the majority of papers relating to degradation studies lies mainly in the limited range of solvent systems in which the chromatograms, thin-layer or paper, were developed.

Decarboxylation studies have provided further evidence of the degradation of exogenously applied auxin. Pilet (1964), for example, supplied IAA to Lens root segments and found that the quantity of ^{14}C appearing in the receiving blocks was doubled if the compound was labelled on the indole nucleus rather than on the acetic acid side chain. He concluded that the IAA oxidases which had been demonstrated in Lens roots previously, were more capable of decarboxylating the side chain than of opening the indole ring. In addition, the position of ^{14}C-labelling can be used to show sequential degradation of the IAA side chain. Donation of IAA(-1-^{14}C) to Pisum (Wilkins and Scott, 1968a) resulted in little accumulation of radioactivity in receiving blocks, but donation of IAA(-2-^{14}C) (Hillman and Phillips, 1970) revealed a distinct acropetally polarised movement. This indicated that endogenous enzyme systems probably acted preferentially on the carboxyl group before attacking the methylene group of the side chain. Iversen and Aasheim (1970) found that enzymatic decarboxylation of IAA(-1-^{14}C) by Helianthus root segments was far greater than non-enzymatic decarboxylation, which occurred in response to impurities and phosphate buffer. Addition of ferulic acid, supposedly a specific

inhibitor of IAA oxidase, to receiving blocks resulted in a greater accumulation of ^{14}C from Brassica root segments. They also demonstrated that $^{14}CO_2$ was lost from receiving blocks on air-drying. Collection of the gas in barium hydroxide solution resulted in a threefold increase in recoverable receiving block radioactivity, which identifies another part of the system from which the applied ^{14}C can be lost without trace.

Kinetin is the only other growth substance with which root transport studies have been carried out. El-Saidi (1971) found that kinetin-8-^{14}C moved preferentially from the apex to the base of intact or decapitated Zea segments. That is, it was basipetally polarised and quite different to the movement of auxin. Devascularisation of the segments, by an undefined technique, led to an abolition of polarity, but the author failed to note that the control segments exhibited little polarity either. Clear interpretation of the chromatographic analyses was hindered by gross overloading of the extracts, but the breakdown of kinetin into adenine and several other labelled compounds was indicated by autoradiograms.

The literature survey presented above was not intended to be a complete review of relevant papers but should serve to inform the reader of the many lines of research and of the current state of knowledge. Much is known about the problems of uptake, translocation and metabolism of 2,4-D in intact plants, not to mention the surfeit of data regarding the characteristic morphological and histological responses invoked by the herbicide. Even more is to be learned on the applied agricultural front, with many publications on 2,4-D specificity, toxicity, formulations, application rates etc. Although work on intact plants is still carried out with great success, many Plant Physiologists regard the intact plant as a complex experimental system, especially when grown under natural environmental conditions. It is not surprising, therefore, that considerable effort has been directed towards the simpler isolated segment technique. For example, the study of IAA movement in shoot and root segments of several species revealed that the overall direction of movement of auxin was probably from the shoot apex to the root apex. This closely paralleled the true situation found in intact plants. It was apparent, however, that whilst the movement of 2,4-D in shoot segments had been adequately documented, no detailed, critical examination of the herbicide in root segments had been undertaken. Consequently, an investigation of such a system was considered

invaluable in:

(a) Supplementing the existing knowledge and understanding of the physiological characteristics of the herbicide.

(b) Increasing the available information on plant growth regulator movement in root segments.

(c) Providing an academic study of the isolated segment technique using a relatively stable compound of the auxin type.

It was hoped that the data from this investigation might provide an insight into the relevance of segment systems to whole plant physiology through a direct comparison of results obtained from both techniques.

MATERIALS AND METHODS

MATERIALS AND METHODS

Plant material

Seeds of Pisum sativum L., variety Alaska, supplied by Charles Sharpe and Company, Sleaford and by Alex S. Mair, Glasgow, were stored dry at 2-3°C. Non-viable debris was removed from the samples by two washings prior to an eight-hour imbibition period in cold, flowing tap water. Germination took place between sheets of moistened filter paper in closed polythene boxes at 29 ±1°C in total darkness in a growth room. 72 hours later, seedlings with straight radicles 2-3cm long were selected either for immediate use in root segment experiments, or for growing-on into young seedlings. The latter were transferred to moistened filter paper in clean boxes to allow space for continued growth of the radicle and plumule for a further 72 hours. Selection was carried out in physiologically-safe green light (510-550nm)

2,4-dichlorophenoxyacetic acid (2,4-D)

Movement of the herbicide in Pisum root tissue was monitored using radioactive isotopic tracer techniques. Radioactive 2,4-D was obtained from the Radiochemical Centre, Amersham, England, with ^{14}C labelling either in the carboxyl or the methylene group of the acetic acid side chain. In other words, as 2,4-D(-1-^{14}C) or as 2,4-D(-2-^{14}C) with specific activities of 153 μCi/mg and 131 μCi/mg respectively. Manufacturers' analysis indicated a purity exceeding 99%.

The labelled compounds were supplied as dry benzene solutions in glass ampoules sealed under nitrogen. Benzene was removed in a stream of nitrogen and the herbicide remained as a white residue. A stock solution of the soluble sodium salt of 2,4-D(-1-^{14}C) was prepared by the addition of 5.9mls. of sodium hydroxide solution (NaOH), containing 0.01 mgms/ml. of the alkali, to neutralise the acid. Further dilution of the stock with 4.1mls. distilled water produced a stock concentration of 1.4×10^{-4} molar. The methylene labelled stock was prepared in a similar manner. In this case, 6.9mls. of NaOH solution, containing 0.01 mgms/ml. of alkali, followed by the addition of 3.1mls. distilled water gave a stock concentration of 1.7×10^{-4}molar. The stock solutions were deep-frozen until required for dilution to working concentrations in the range 10^{-7}-10^{-5} molar (0.1 - 10.0 μM).

Physiological responses of Pisum roots to the herbicide were investigated using pure, unlabelled 2,4-D (Sigma Chemicals). Solutions of the sodium salt were prepared in the way described for the radioactive solutions, with neutralisation of the acid being the initial step. A range of concentrations from 10^{-14}-10^{-4} molar was employed (0.01 pM - 0.1 mM).

Preparation of Agar Blocks

In the majority of experiments in which the movement of 2,4-D was followed, the radioactive compound was incorporated into agar blocks and supplied to the roots. This provided a readily available source of the herbicide in an easily locatable form not subject to rapid dessication.

A 3% stock of Difco-Bact agar or Ionagar No.2 was dispensed into suitable aliquots and sterilised at 15lbs/in^2 for 15 minutes. The stock was stored at 2-3°C until required. Receiving and donor blocks were prepared by diluting molten 3% agar with an equal volume of distilled water or herbicide solution respectively, giving a final gel strength of 1.5%. This was then cast either into brass moulds (8 x 8 x 2 mm.) or into constant-bore glass tubing (8mm. diameter, cut into 2mm. thick discs). Concentration of the radioactive donor blocks was within the range 0.1 - 10.0 μ molar 2,4-D. Blocks were prepared on the day before the experiment commenced and were stored in damp chambers at 2-3°C. In several experiments, the application of radioactive donor blocks was either preceeded or followed by a donation of unlabelled 2,4-D. Donor blocks for these experiments were prepared using the appropriate concentration of labelled or unlabelled 2,4-D.

Identification of Radioactive Compounds

To determine whether or not the ^{14}C molecule remained confined to the herbicide structure, ethanolic extracts of the radioactive compounds in the tissue and receiving blocks were analysed by descending paper chromatography. At the end of transport periods, segments were extracted for 24 hours in 2mls. of 95% ethanol at 2°C in darkness. Tissue was then transferred to 2mls. ethanol/acetone mixture (6:4 v/v) and extracted for a further 24 hours. Extracts were bulked, reduced to dryness under vacuum and finally taken up in 1ml. absolute ethanol. Radioactivity in donor and receiving blocks was extracted in the same way. An alternative method of extraction was employed on some occasions, in which the extracting solvent was methanol and not ethanol.

0.1ml. of each extract was spotted on the origin of a pre-eluted paper chromatogram, Whatmans No.1, together with marker spots of pure 2,4-D and stock radioactive 2,4-D. Three solvent systems were used, namely isopropanol:ammonia:water (10:1:1), n-butanol:acetic acid:water (5:1:2.2) and n-butanol:acetone:water (5:2:3). Chromatograms were equilibrated and developed in a vapour-filled Shandon chromatography tank, after which they were dried and cut into at least 10 equal pieces. Activity in each Rf or part-Rf region was eluted in 1.5mls. 95% ethanol for a minimum of 24 hours at 2°C in a scintillation vial, which was then processed for scintillation counting.

Duplicate paper chromatograms were clipped to Ilford "Ilfex" X-ray plates in total darkness and stored inside protective envelopes for four months. Films were developed in Ilford "Phenisol" Developer, diluted 1:4 with distilled water, and fixed for 30 minutes in May and Baker "Amfix" high speed fixer, diluted 1:3 and containing 1:40 parts Kodak "HX-40" X-ray liquid hardener. Films were washed in flowing tap water for 4 hours, rinsed in distilled water and finally dried in air. Film processing was carried out in complete darkness.

Detection of Radioactivity

Radioactivity in root tissue, donor and receiving blocks was extracted in 1.5mls. of 95% ethanol for at least 30 hours at 2-3°C. A control experiment showed that no further extraction of ^{14}C took place after this time (table 1). Extracts were dried under vacuum and to each vial was added 10mls. of scintillation fluid, containing 4gms. of 2,5-diphenyloxazole in each litre of toluene. Three liquid scintillation counting systems were employed to determine the ^{14}C content of the samples during the course of the experimental programme. These were:

(a) Nuclear Chicago "Unilux 3"

(b) Tracer Lab "Corumatic 200"

(c) Packard Liquid Scintillation Counter with "Absolute Activity Analyser"

Each system was calibrated according to the manufacturers recomendations and quench correction curves were prepared for use in conjunction with external standardisation ratios. The data presented in this thesis have been corrected for machine efficiency, quenching and background radiation either manually, using an Olivetti 101 desk-top computer, or automatically by an in-line computer incorporated in the Packard system.

TABLE 1

Effect of time on the efficiency of extraction of ^{14}C, at 2-3°C, from Pisum root segments and receiving blocks applied to the segments for 24 hours at 25°C.

Extraction time (h)	Radioactivity in tissue (Dpm)	Radioactivity in receivers (Dpm)
5.0	2058.9	93.3
10.0	2155.8	83.8
20.0	2314.8	73.3
30.0	2247.6	114.5
45.0	2185.9	81.9
55.0	2200.9	102.8
150.0	2286.5	101.7

The data are the mean of four replicates each containing four root segments.

Movement of 2,4-D through root segments of Pisum sativum

The movement and uptake of 1.0μ molar 2,4-D(-1-^{14}) and 2,4-D(-2-^{14}C) were studied at two and five-hourly intervals over a period of 60 hours. Replicates were not set up in chronological order, but over an eight hour period such that the transport periods ended at relatively convenient times.

A double-bladed cutter was used to excise 10mm. long subapical segments 2mm. behind the root tip of three day old pea seedlings. Four independent replicates, each consisting of a donor block beneath and a receiving block above four segments, were held vertically in a perspex holder (fig.1). Acropetal replicates had the bases of the segments in contact with the donor block, whereas basipetal replicates had the tips in contact (fig.2). A high relative humidity was maintained throughout the experiment by enclosing the holders in boxes lined with damp filter paper. All experimental manipulations were carried out under green light (510-530nm.) and transport periods run in total darkness at 25°+ 1° C in a temperature controlled growth room. At the end of each experimental period, the segments were cut into 2mm. pieces from the donor block end, using a multibladed cutter, and the level of radioactivity was estimated by scintillation counting.

Movement of 2,4-D through the roots of intact Pisum seedlings

1.0μ molar 2,4-D(-1-^{14}C) was supplied to the root system of three day old Pisum seedlings by two methods, namely agar blocks and aqueous solution. In the first method, circular radioactive donor blocks encircled the root either at the tip or 5mm. away from the cotyledons. In the second, the radicles were inserted through small holes drilled in the lids of plastic petri dishes such that approximately 5mm. of the root tip was in contact with the 5mls. of donor solution. Following experimental periods of five to sixty hours, the level of radioactivity in each one third portion of the root (A,B and C as illustrated in fig.1), the cotyledons and the shoots was determined by liquid scintillation counting.

Dependence of 2,4-D movement on metabolism

The aim of this series of experiments was to observe the effect of reducing or abolishing metabolism on the movement of 1.0μ molar 2,4-D(-1-^{14}C) through 10mm. long Pisum root segments.

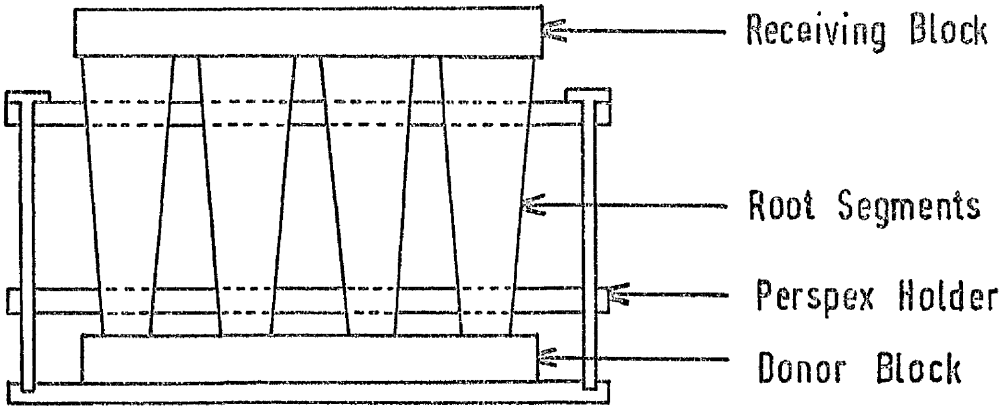
FIGURE 1

(A) Perspex holder used to maintain root segments in a vertical orientation during transport experiments.

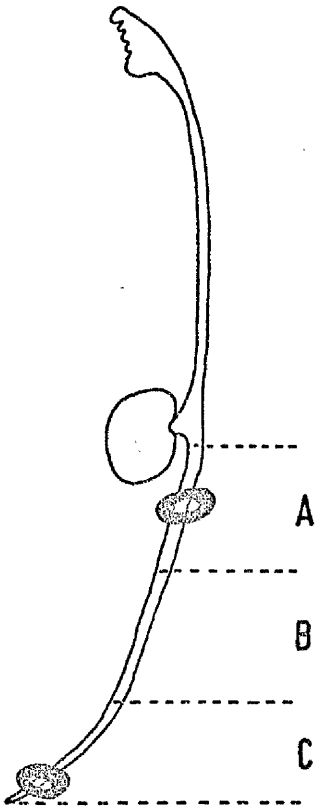
(B) Seedling showing the two sites of donation by agar discs (a) at the root apex (b) 5mm. from the cotyledons, and the designation of each one-third portion of the root (A,B and C)

(C) Seedling showing method of donation to the root apex.

(A)


Receiving Block
Root Segments
Perspex Holder
Donor Block

(B)


A
B
C

(C)

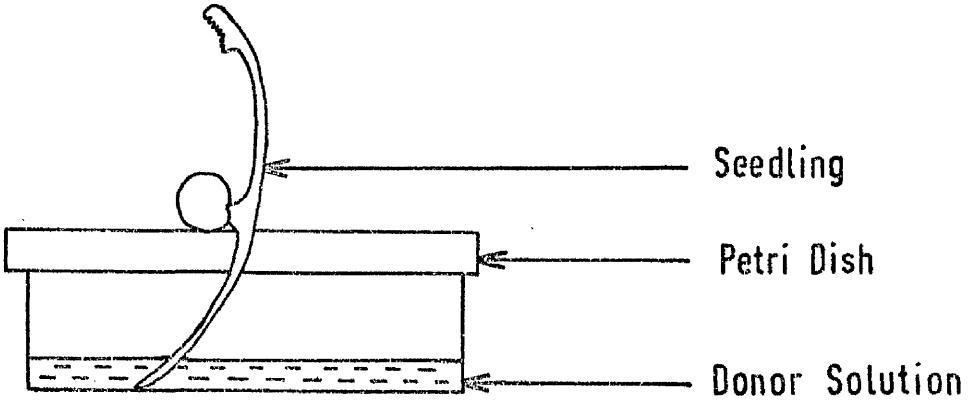
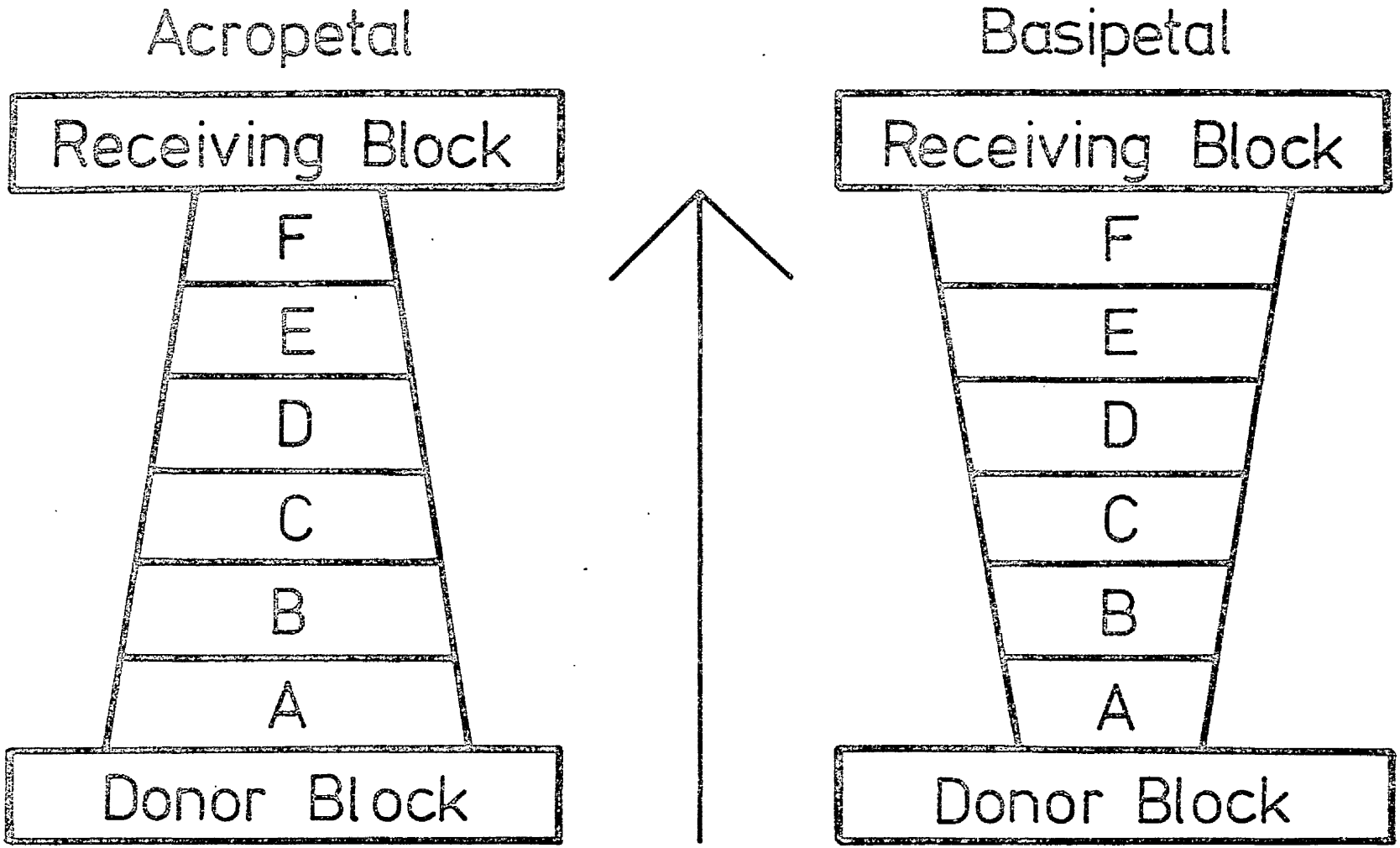

Seedling
Petri Dish
Donor Solution

FIGURE 2

Diagramatic representation of acropetal and basipetal replicates showing designation of each successive 2mm. piece of root segment as prepared for scintillation counting.


Acropetal
Receiving Block
F
E
D
C
B
A
Donor Block
Basipetal
Receiving Block
F
E
D
C
B
A
Donor Block

Segments were soaked either in distilled water or in 2.0mM. sodium fluoride solution for 2.5 hours, prior to setting up as acropetal or basipetal replicates. These were transferred to a nitrogen-filled vacuum desicator lined with damp filter paper. Anaerobic conditions were achieved by evacuation to 59mm. of mercury followed by release in pure, humidified nitrogen. The process was repeated six times to ensure the complete absence of oxygen. Air controls were also subjected to the evacuation treatment. The system employed is represented diagramatically in figure 3. Further work was carried out in which the necessity for soaking the segments was avoided by supplying the 2.0mM. sodium fluoride in the donor and/or the receiving block. Both techniques were carried out under the conditions previously described for transport experiments and the segments were processed for scintillation counting.

Degradation of 2,4-D

Metabolic breakdown of the herbicide in Pisum sativum was investigated by monitoring the evolution of radioactive carbon dioxide following the application of donor blocks containing 1.0μ molar 2,4-D(-1-^{14}C) or 2,4-D(-2-^{14}C).

The apparatus, illustrated in figure 4, consisted of two chambers containing the experimental material and through which humidified air was sucked by a vacuum pump. The air then passed through a series of two small bubblers each containing 7.5mls. of a CO_2 trapping-agent consisting of ethanolamine:ethylene glycol monomethyl ether:toluene (1:8:10 v/v). The trapping-agent was changed every two hours throughout the experiment and was drained directly into scintillation vials, to which were added 10mls. scintillation fluid prior to counting.

The experiment was carried out in physiologically inactive green light at 25° C.

Growth tests

The growth rate of 10mm. long Pisum root segments excised 2mm. behind apex of three day old seedlings was determined in 2,4-D solutions over the range 0.1 mM - 0.01 pM. A control was carried out using distilled water. Four replicates of ten segments were floated in 10mls. of herbicide

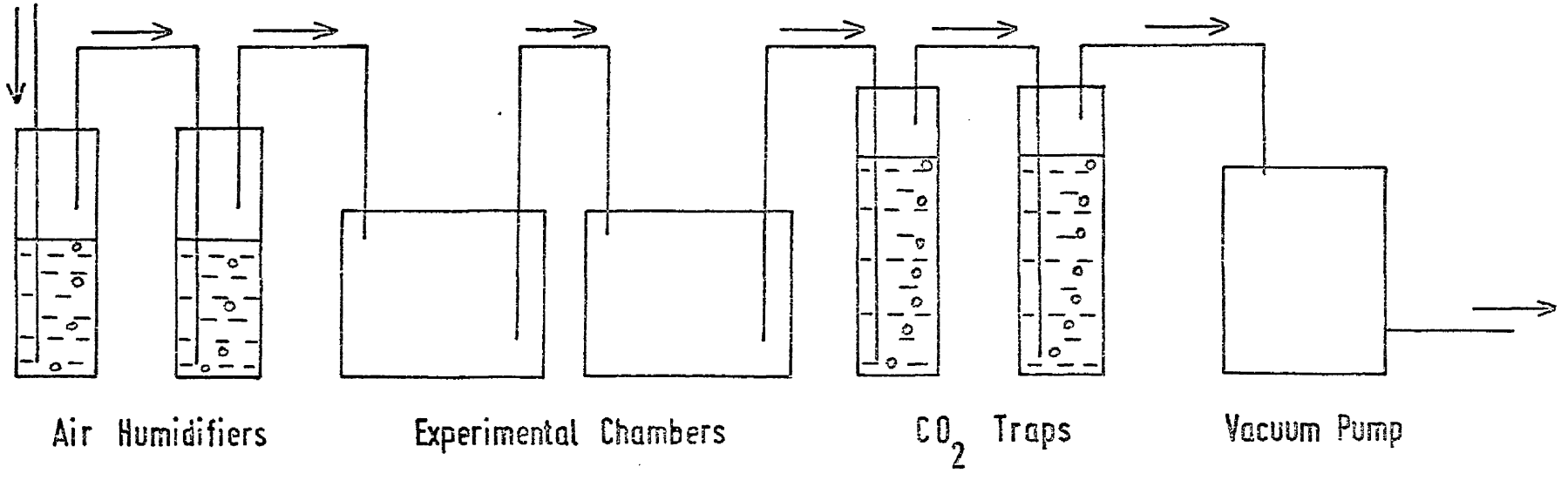
FIGURE 3

Experimental system for achieving an anaerobic environment in investigations into the dependence of herbicide translocation on the metabolic activity of the root segments.

Nitrogen Source
1
2
3
Desiccator
Manometer
Vacuum Pump
1 and 2 Pressure and Humidity Control
3 Safety Valve
Valves in gas flow system

FIGURE 4

Experimental system for trapping radioactivity evolved from the root segments during the translocation of 2,4-D(-1-^{14}C) and 2,4-D(-2-^{14}C).


Air Humidifiers
Experimental Chambers
CO_2 Traps
Vacuum Pump

solution or water in individual plastic petri dishes. Growth was permitted for a predetermined period in a growth room at 25°C in total darkness. Segments were wiped dry before shadowgraphs were taken at a magnification of five times. Ilford "IB4 IP" photographic paper was exposed for six seconds and was developed in Ilford contrast FF developer, diluted 1:4 with distilled water, until the image of the segments was contrasted clearly. They were then rinsed in tap water before being transferred to a 1:4 dilution of Kodafix solution for ten minutes. A final rinse in tap water was given prior to glazing.

The length of each segment was measured along the median line using a flexible millimetre scale.

Measurements of the length of the radicles of intact seedlings were taken at the conclusion of appropriate experiments. In other instances, agar discs were encircled around the radicle and measurements taken at intervals.

Statistical analysis

The experiments presented in this thesis were designed to demonstrate the presence or absence of clearly defined trends in the translocation of 2,4-D in the roots of Pisum sativum. In general, the work has been concerned with comparisons of acropetal and basipetal translocation and the effect of certain treatments. Adequate replication within each experiment was limited by the time-consuming nature of the technique, but since conditions in the growth rooms were constant, data from identical experiments carried out on different occasions were pooled for statistical analysis. Details of the number of occasions and replication are quoted at the beginning of each section of results. The only analysis undertaken was Students' T-test for the comparison of two sets of means. Significance levels are given as star-ratings:

*	= 5% level
**	= 1% level
***	= 0.1% level

Degrees of freedom are presented with each table of data.

On other occasions, the significance of fluctuations from the "general trend" was analysed using techniques advised by the Statistics Unit, Wye College, University of London. The normally accepted version of orthogonal polynomial regression analysis was favoured but was found to be unacceptable since the first observation for most of the experiments passed through the origin. With this constraint on the data the analysis was modified as follows:

To fit a regression line $y = \alpha + \beta x + \gamma x^2 + \varepsilon$ (eg. parabola) to a set of data use the estimated equation:

$$y = a + bx + cx^2 + e$$

Minimising Σe^2, this gives:

$$\Sigma y = an + b\Sigma x + c\Sigma x^2$$
$$\Sigma xy = a\Sigma x + b\Sigma x^2 + c\Sigma x^3$$
$$\Sigma x^2 y = a\Sigma x^2 + b\Sigma x^3 + c\Sigma x^4$$

where n = number of points

a,b & c = estimated regression coefficients

But in the case where the first point is at the origin:

$$y = \beta x + \gamma x^2 + \varepsilon$$

-which is estimated by:

$$y = bX + cX^2 + e$$

Least squares regression yields the equations:

$$\Sigma xy = b\Sigma x^2 + c\Sigma x^3$$
$$\Sigma x^2 y = b\Sigma x^3 + c\Sigma x^4$$

In order to determine the least significant value by which the fluctuations must be different from the fitted regression line, particular values of x were substituted into the following equations:

(a) LINEAR

$$\text{LSD} = \text{t value on 11DF} \times \sqrt{s^2\left(\frac{X^2}{\Sigma X^2} + 1\right)}$$

(b) QUADRATIC

$$\text{LSD} = \text{t value on 10 DF} \times \sqrt{s^2\left[1 + \frac{1}{\Delta}\left\{X^2\Sigma X^4 + X^4\Sigma X^2 - 2X^3\Sigma X^3\right\}\right]}$$

-where LSD = least significant difference

DF = degrees of freedom

s^2 = residual mean square

$\Delta = X^2\Sigma X^4 - (\Sigma X^3)^2$

Where presented ~~Δ~~ presented by vertical lines on figures.

PHYTOTOXICITY OF 2,4-D

In order to determine the relative phytotoxicity of the herbicide concentrations proposed for the tracer studies, the effect of a range of concentrations of unlabelled 2,4-D were evaluated by two straight-growth bioassays. The first involved the floatation of 10mm long Pisum root segments in 2,4-D solutions for 24 hours, whilst the second had herbicide supplied in agar blocks either to the apical or the basal end of the segments. Both bioassays were set up in green light and run in total darkness at 25°C. Shadowgraphs of the segments were taken at five-times magnification and the image length measured and computed to give actual length. Each point on the graphs is the mean of forty observations.

Floatation of the segments in 2,4-D solutions ranging from 0.01 pM to 0.1 mM, produced a growth response which was consistently smaller than that observed in the water control (fig.5). At 0.01 pM and 01 mM, for example, the segments were 0.6 and 1.2mm shorter than the control and were significant at the 0.1% level of probability. Maximum elongation was recorded at 0.1 nM and 10.0 nM 2,4-D, but the lengths were not significantly different from the controls. Paired t-test analyses gave t-values of 3.943, 4.625, 0.321 and 0.462 in each of these cases respectively.

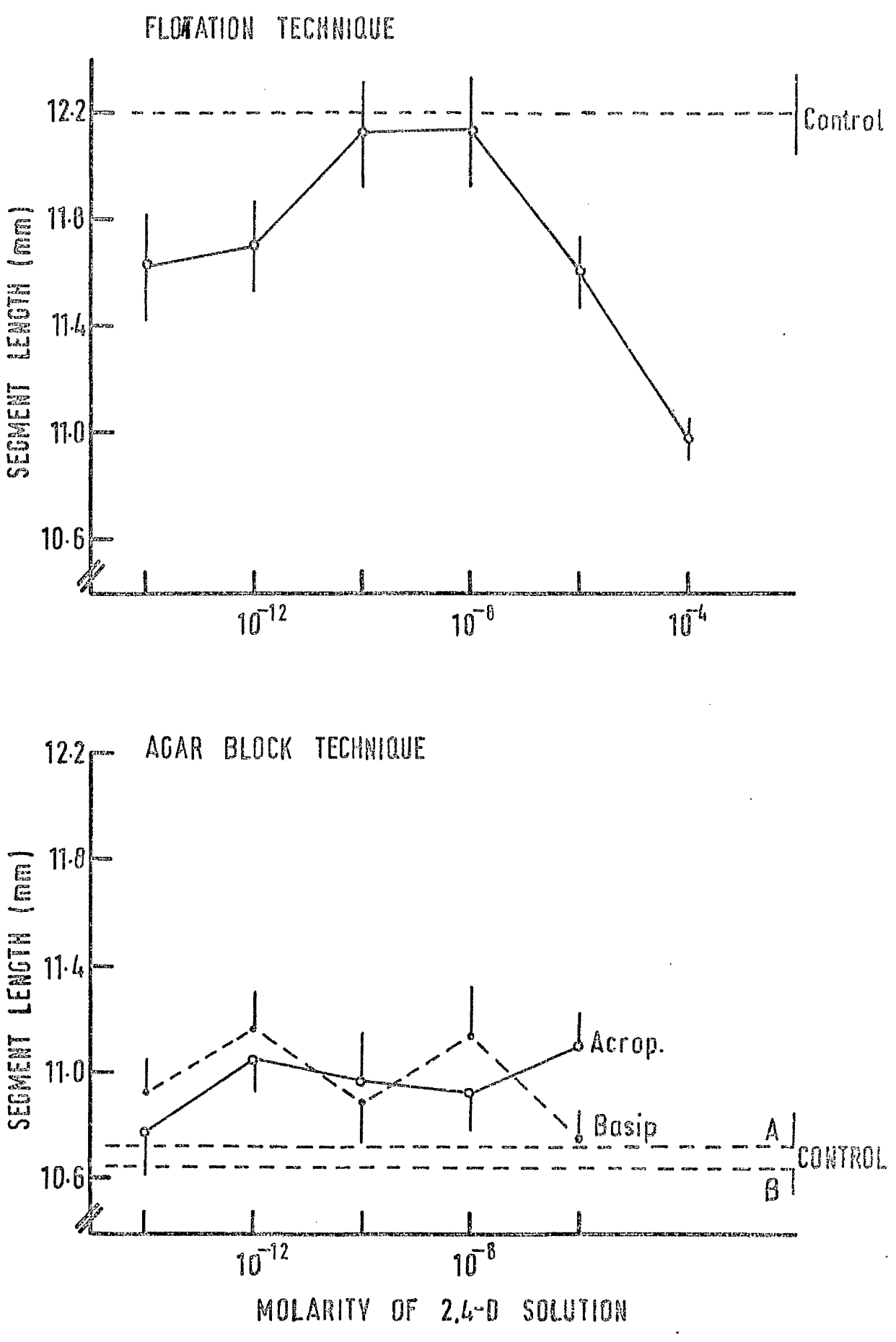
Application of the herbicide in agar blocks resulted in the promotion of elongation, such that the segments were longer than the controls. Little significance could be attached to the variations in length detected after donation of the compound to either end of the segments and no clearly defined concentrations for optimum elongation or phytotoxicity could be defined.

Thus, on the basis of these two bioassays, the use of 1.0 µM 2,4-D in tracer experiments can be considered to be a useful compromise between phytotoxicity and an adequate specific activity of the radioactive compound.

FIGURE 5

(a) Length of Pisum root segments, initially 10mm long, after floatation in a range of 2,4-D concentrations for 24 hours at 25°C in darkness;

(b) Length of Pisum root segments, initially 10mm long, after supplying agar blocks containing 2,4-D to either the apical or basal ends for 24 hours at 25°C in darkness.


FLOTATION TECHNIQUE
12·2
11·8
11·4
11·0
10·6
SEGMENT LENGTH (mm)
Control
10⁻¹²
10⁻⁸
10⁻⁴
AGAR BLOCK TECHNIQUE
12·2
11·8
11·4
11·0
10·6
SEGMENT LENGTH (mm)
Acrop.
Basip
A
B
CONTROL
10⁻¹²
10⁻⁸
MOLARITY OF 2,4-D SOLUTION

THE MOVEMENT OF 2,4-D(-1-^{14}C) AND 2,4-D(-2-^{14}C) IN PISUM ROOT SEGMENTS.

The primary objective of the investigations reported in this thesis was to establish whether or not 2,4-D could move through sub-apical root segments of Pisum sativum in a polar or apolar manner.

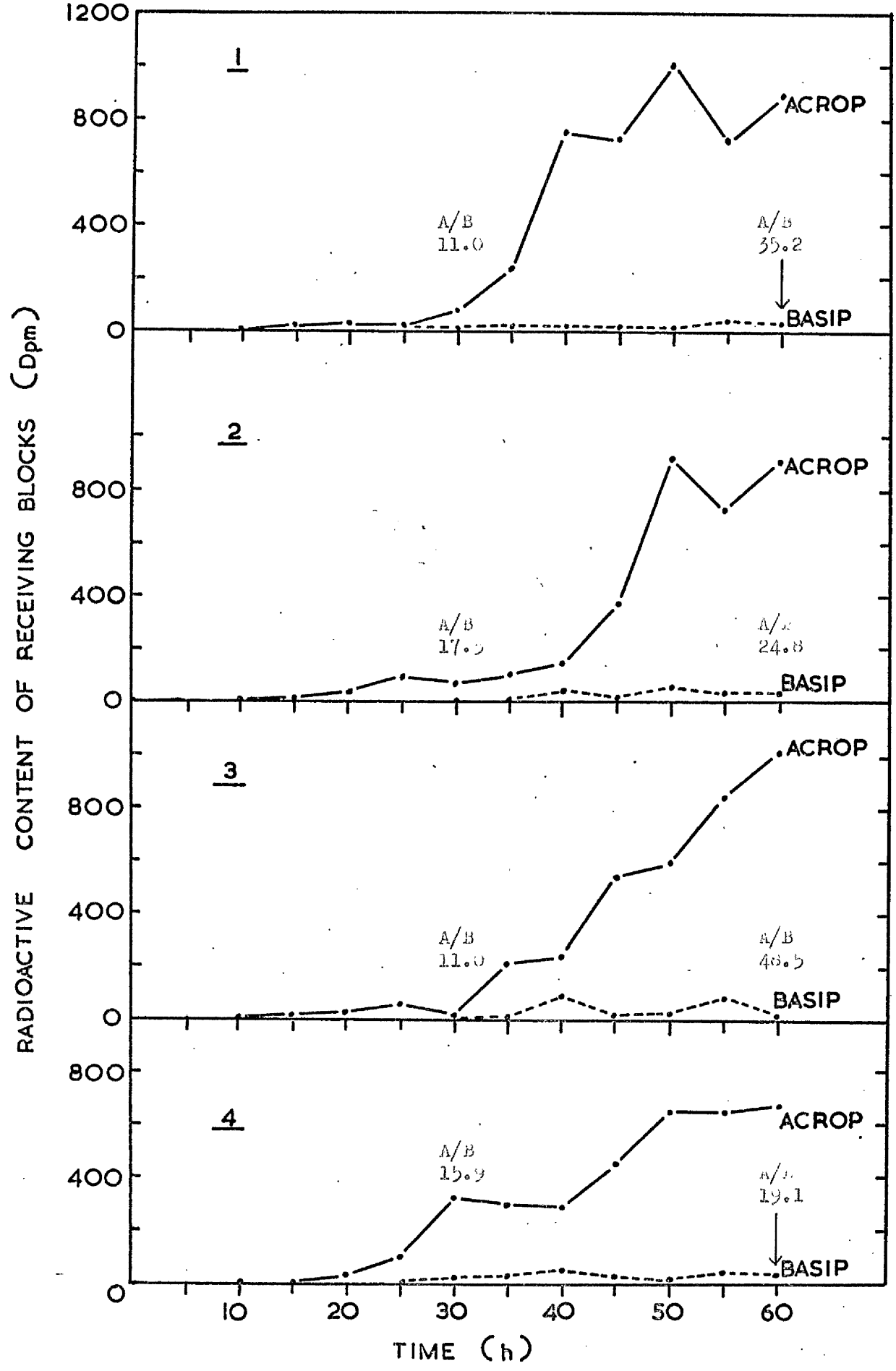
Donor blocks of 1.0 µM 2,4-D(-1-^{14}C) or 2,4-D(-2-^{14}C) were supplied to either the apical or the basal ends of 10mm long Pisum root segments, for the determination of basipetal and acropetal translocation of the herbicide respectively. Plain agar receiving blocks were applied to the opposite end of the segments. Twenty-four independent sets of four root segments, each with communal agar blocks, were set up in order that herbicide translocation could be assessed at 5-hourly intervals from 0-60 hours, using a destructive sampling technique. Green light was used during experimental manipulations but the transport period was in total darkness in a light-proof cupboard at 25° ± 1°C. The experiment was repeated on several occasions and the data from experiments carried out on four different days are presented for each labelled compound.

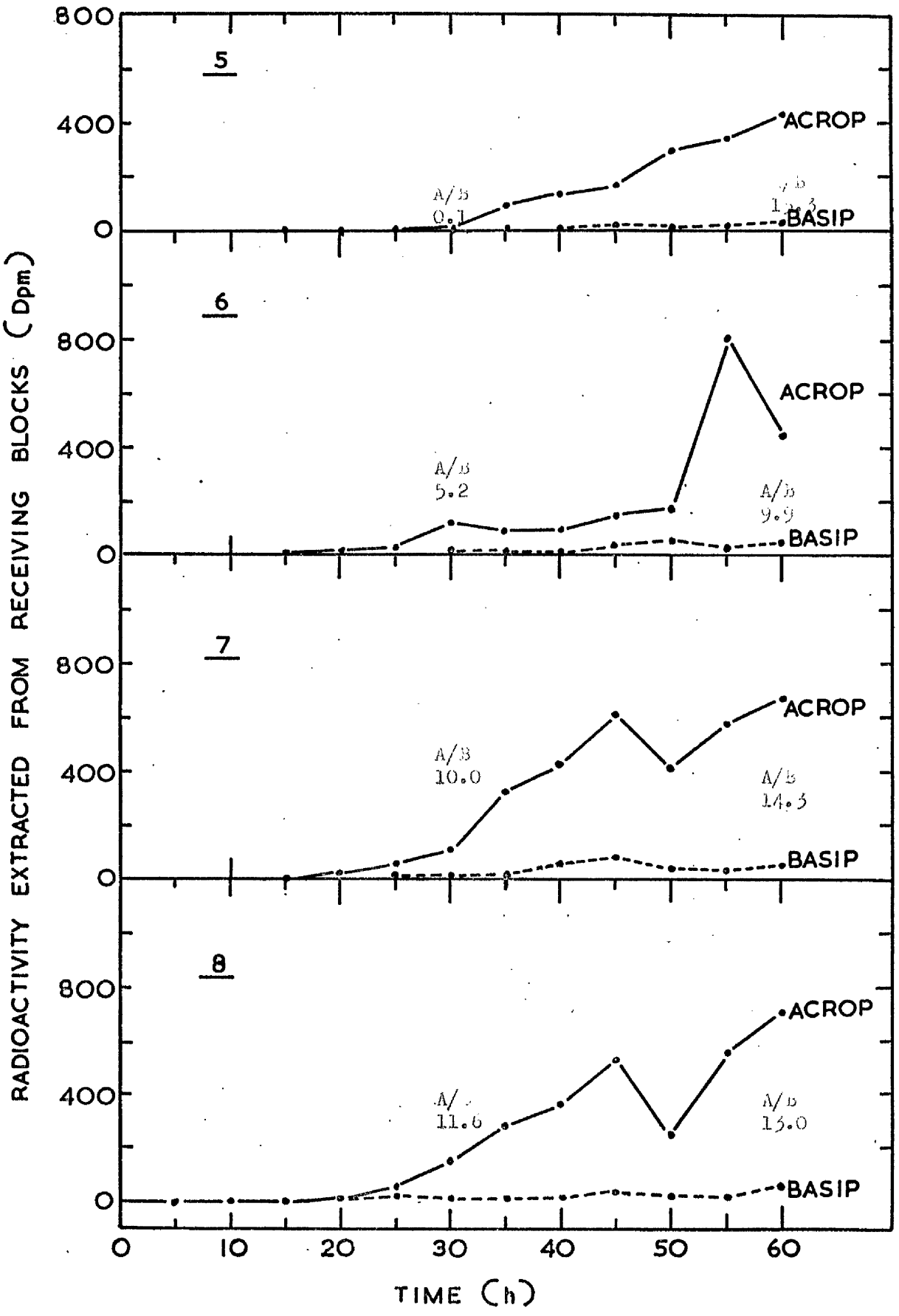
The ^{14}C content of receiving blocks applied to the apical or basal ends of Pisum root segments supplied with the -COOH or -CH_2 labelled compound is presented in figure 6. At each sampling interval one donor and one receiving block were applied to four replicate segments. After an initial lag-phase of 10-15 hours, ^{14}C could be extracted from apical and basal receiving blocks, indicating a velocity of 0.7-1.0mm h^{-1}. A subsequent rapid increase in apical block content was accompanied by a much smaller increase in the level of radioactivity in the basal blocks. In fact, the content of the apical blocks following a 30 hour donation of 2,4-D(-1-^{14}C) or 2,4-D(-2-^{14}C) was, on average, 13.8 and 6.7 times greater than that of the basal blocks. Differences of 31.9 and 12.6 fold respectively after a 60 hour transport period indicated a preferential movement of ^{14}C into the receiving blocks of the acropetal treatments.

Total radioactivity extracted from the root segmentsand receiving blocks in the eight independent experiments is presented in figure 7. for the -COOH and the -CH_2 labelled compound. Over the initial 10-15 hours the levels of ^{14}C in the acropetal and basipetal treatments were virtually identical. After a transport period of 30 hours the radioactivity in the former exceeded that in the latter

FIGURE 6

Radioactivity extracted from agar receiving blocks applied to the apical or basal ends of Pisum root segments supplied with 1.0 μM 2,4-D(-1-^{14}C) (expts. 1-4) or 2,4-D(-2-^{14}C) (expts.5-8) for 0-60 hours at 25°C in darkness. The experiment was repeated on eight different occasions and the data show the total accumulation from sets of four root segments.


1200
800
400
0
1
ACROP
A/B
11.0
A/B
35.2
BASIP
2
ACROP
A/B
17.2
A/B
24.8
BASIP
3
ACROP
A/B
11.0
A/B
48.5
BASIP
4
ACROP
A/B
15.9
A/B
19.1
BASIP
10
20
30
40
50
60
TIME (h)
RADIOACTIVE CONTENT OF RECEIVING BLOCKS (Dpm)


800
400
0
5
ACROP
A/b
0.1
BASIP
6
ACROP
A/b
5.2
A/b
9.9
BASIP
7
ACROP
A/B
10.0
A/b
14.3
BASIP
8
ACROP
A/
11.6
A/b
13.0
BASIP
0
10
20
30
40
50
60
TIME (h)
RADIOACTIVITY EXTRACTED FROM RECEIVING BLOCKS (Dpm)

by an average of 1.7 times with the compound labelled in either position. By the end of the experiments the level of ^{14}C in the acropetal treatments was an average of 4.9 and 3.5 times greater than that in the basipetal treatments following donations of 2,4-D(-1-^{14}C) and 2,4-D(-2-^{14}C) respectively.

Peaks of high ^{14}C content in the acropetal treatments were detected after 10-15, 35 and , except in experiments 5 and 6, after 50-55 hours. Additional peaks were recorded after 20-25 hours in experiments 3,5 and 6 and after 45 hours in experiments 6 and 7. Statistical significance of these peaks was not tested.

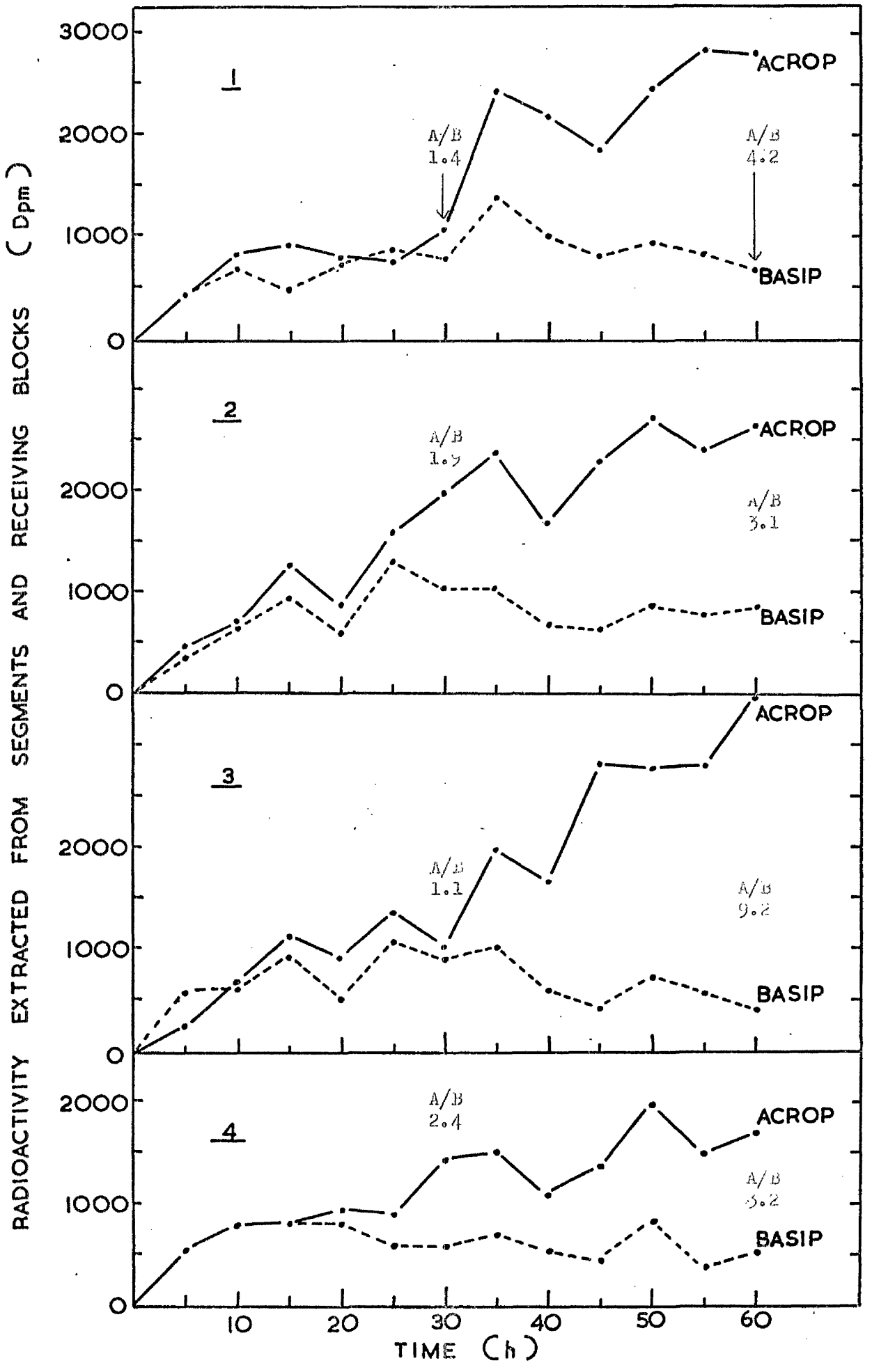
Donor blocks containing higher or lower than normal levels of radioactivity would be a source of error likely to result in fluctuations of extractable ^{14}C from the experimental system. A comparison of the level of radioactivity in the segments and receiving blocks and the amount remaining in basally applied donor blocks with time (fig.8) revealed that peaking in extractable ^{14}C corresponded to a high level of activity in the donor block only at the 20 hour point in experiment 8. In many cases, increased donor block activity appeared to be counterbalanced by decreases in the remainder of the experimental system. The relationship in the basipetal treatments was not so clearly defined but, allowing for the scale differences in figure 9, the same basic trends were present.

On average, the radioactivity in donor blocks applied to the basal end of Pisum root segments for 15 hours amounted to 91-95% of that present in the apical blocks, indicating little difference in uptake of ^{14}C by the two ends of the segments (table 2). Differential uptake increased steadily throughout the experiment, however, so that after 60 hours the basal block content was only 57% and 65% of that in the apically applied blocks of 2,4-D(-1-^{14}C) and 2,4-D(-2-^{14}C) respectively. Clearly, the ^{14}C loss from basally applied blocks exceeded that from apically applied blocks.

Figure 10 shows the distribution of radioactivity along the root segments with time. These data from experiment 1 are typical of the pattern detected on cutting successive 2mm pieces of the four segments at each sampling interval. Donation to the apical ends of the segments resulted in less movement of the radioactive molecules along

FIGURE 7

Radioactivity extracted from receiving blocks and corresponding sets of four root segments supplied with 1.0 μM 2,4-D(-1-^{14}C) (expts. 1-4) or 2,4-D(-2-^{14}C) (expts. 5-8) from 0-60 hours at 25°C in darkness. The experiments were carried out on eight different days and each acropetal and basipetal treatment was set up independently and run in a temperature controlled darkroom.


RADIOACTIVITY EXTRACTED FROM SEGMENTS AND RECEIVING BLOCKS (Dpm)
3000
2000
1000
0
1
A/B 1.4
A/B 4.2
ACROP
BASIP
2
A/B 1.9
A/B 3.1
ACROP
BASIP
3
A/B 1.1
A/B 9.2
ACROP
BASIP
4
A/B 2.4
A/B 5.2
ACROP
BASIP
10
20
30
40
50
60
TIME (h)

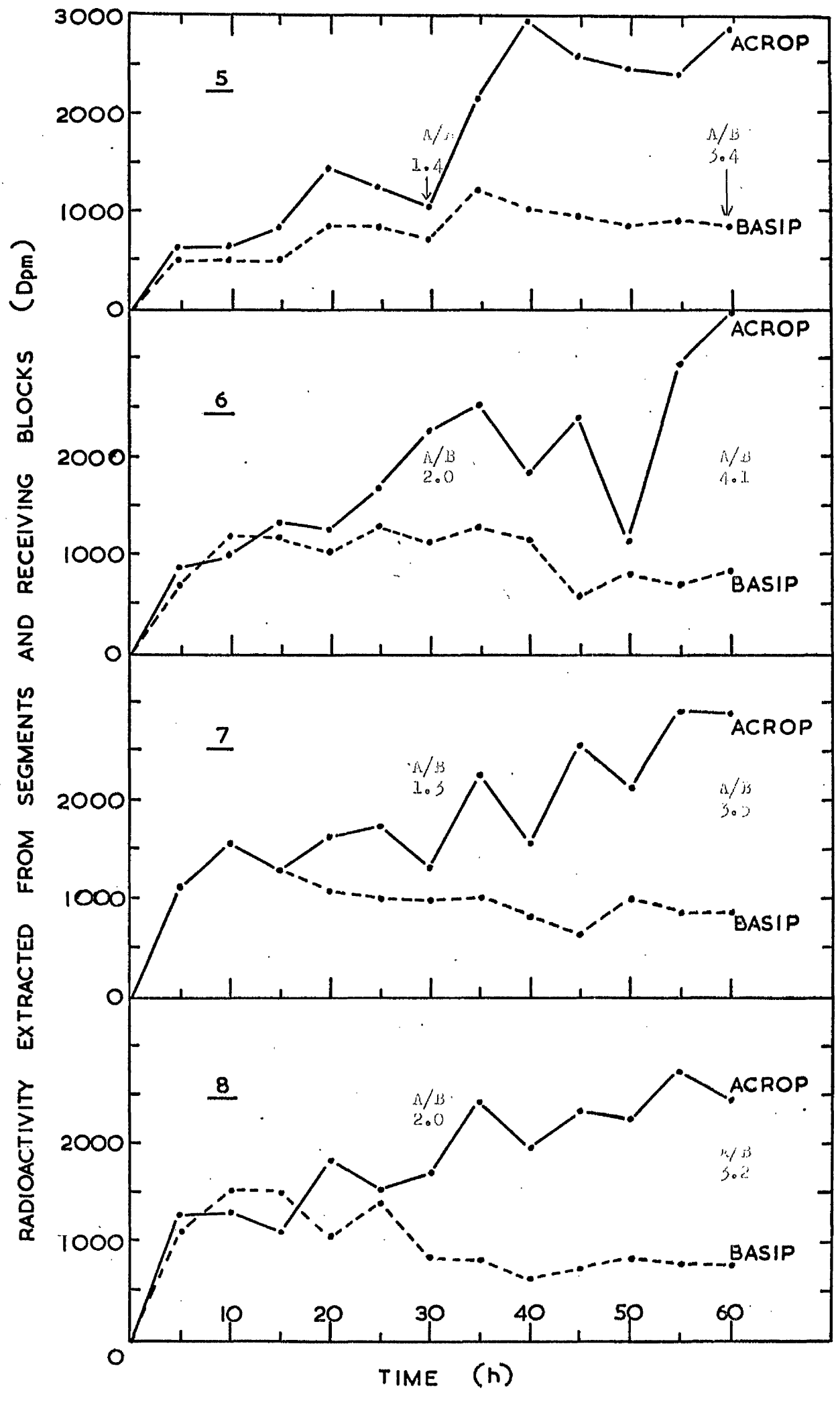
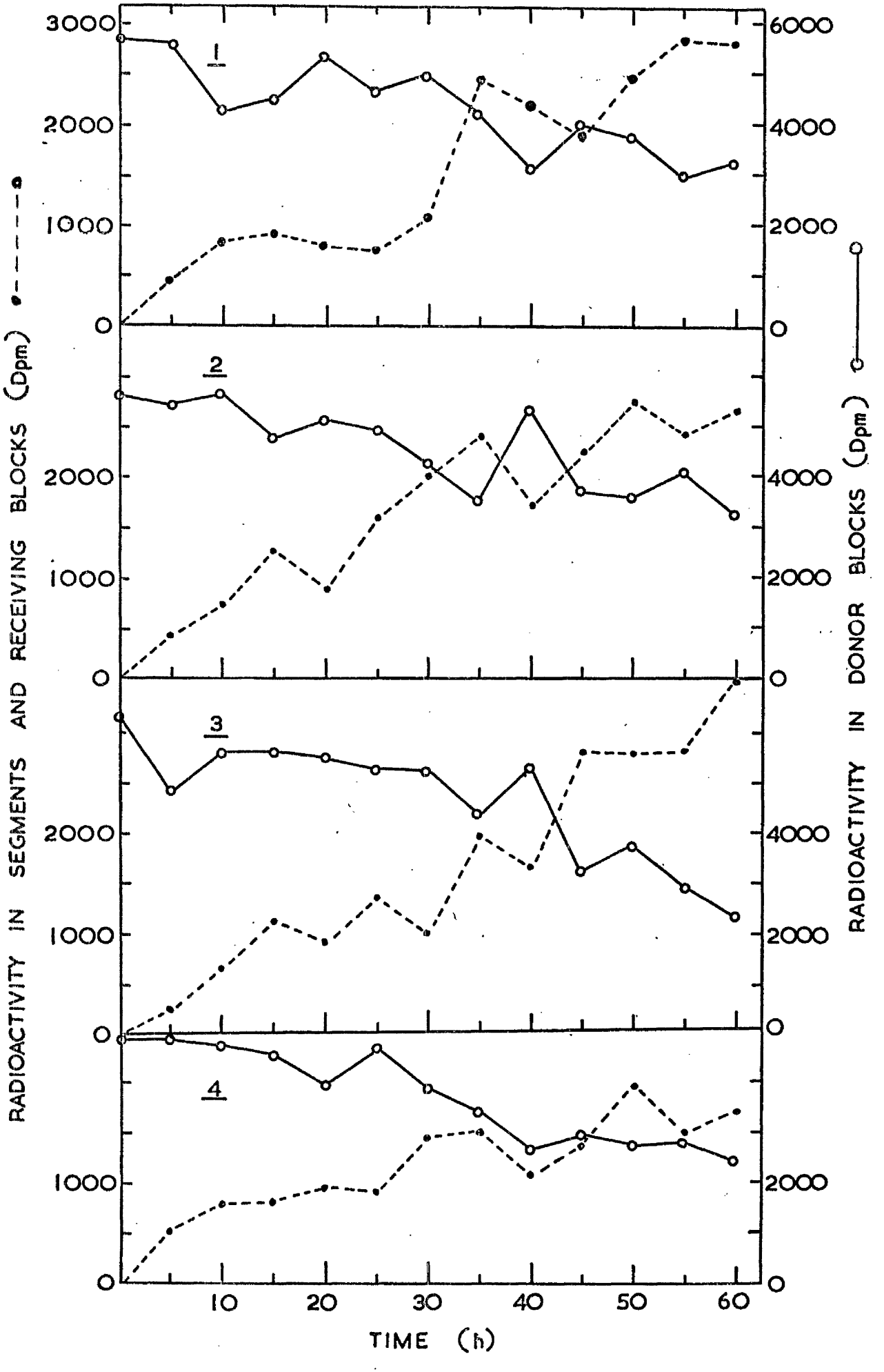

RADIOACTIVITY EXTRACTED FROM SEGMENTS AND RECEIVING BLOCKS (Dpm)
3000
2000
1000
0
5
ACROP
A/B 1.4
A/B 3.4
BASIP
6
ACROP
A/B 2.0
A/B 4.1
BASIP
7
ACROP
A/B 1.3
A/B 3.0
BASIP
8
ACROP
A/B 2.0
A/B 3.2
BASIP
10
20
30
40
50
60
TIME (h)

FIGURE 8

Radioactivity extracted from 1.0 μM donor blocks of 2,4-D(-1-^{14}C) (expts.1-4) or 2,4-D(-2-^{14}C) (expts.5-8) supplied to the basal ends of Pisum root segments, compared with ^{14}C extracted from the root segments and receiving blocks. The experiments were carried out on eight different occasions at 25°C in darkness and each donor block supplied four segments.


RADIOACTIVITY IN SEGMENTS AND RECEIVING BLOCKS (Dpm)
RADIOACTIVITY IN DONOR BLOCKS (Dpm)
TIME (h)
1
2
3
4
3000
2000
1000
0
6000
4000
2000
10
20
30
40
50
60

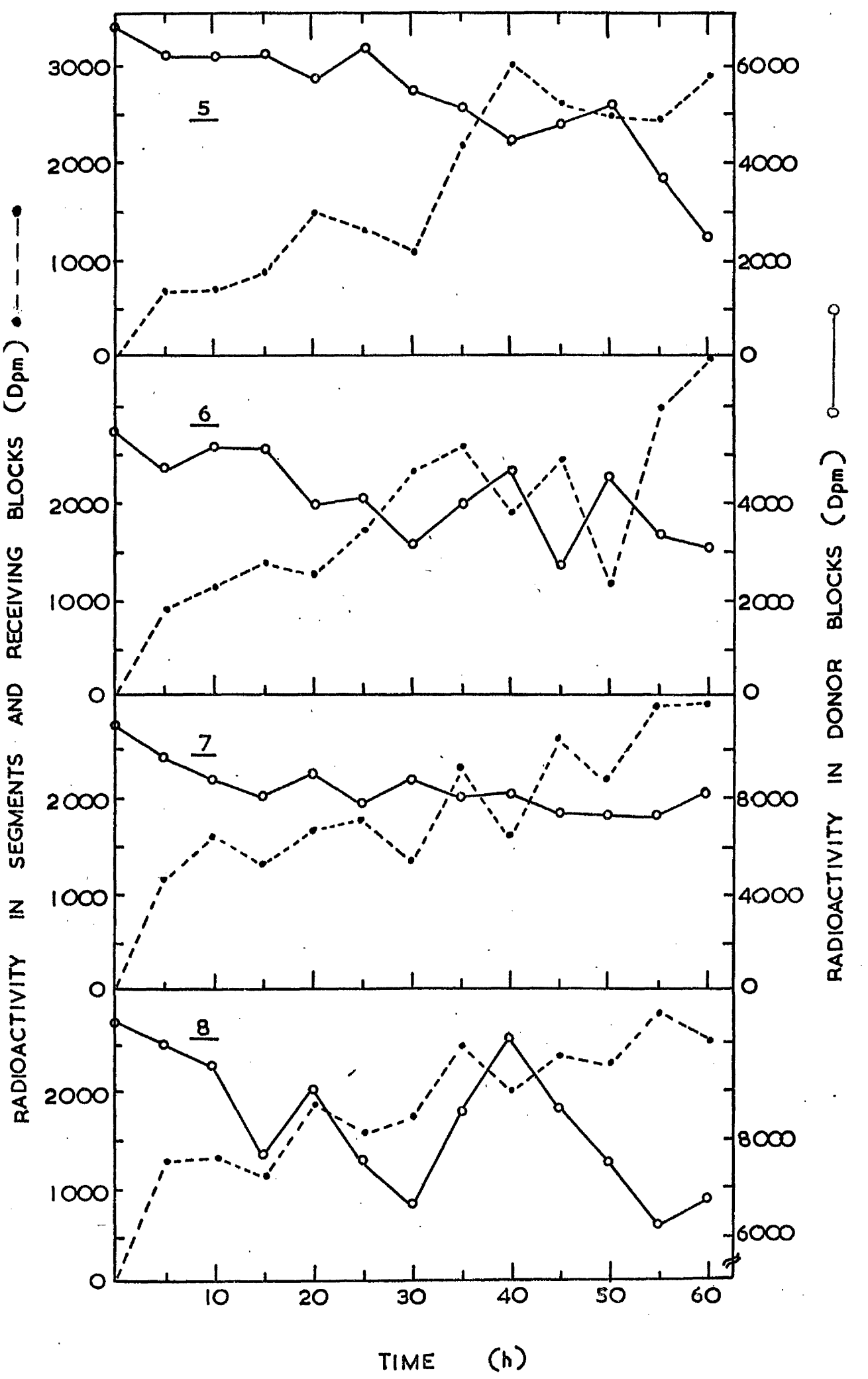
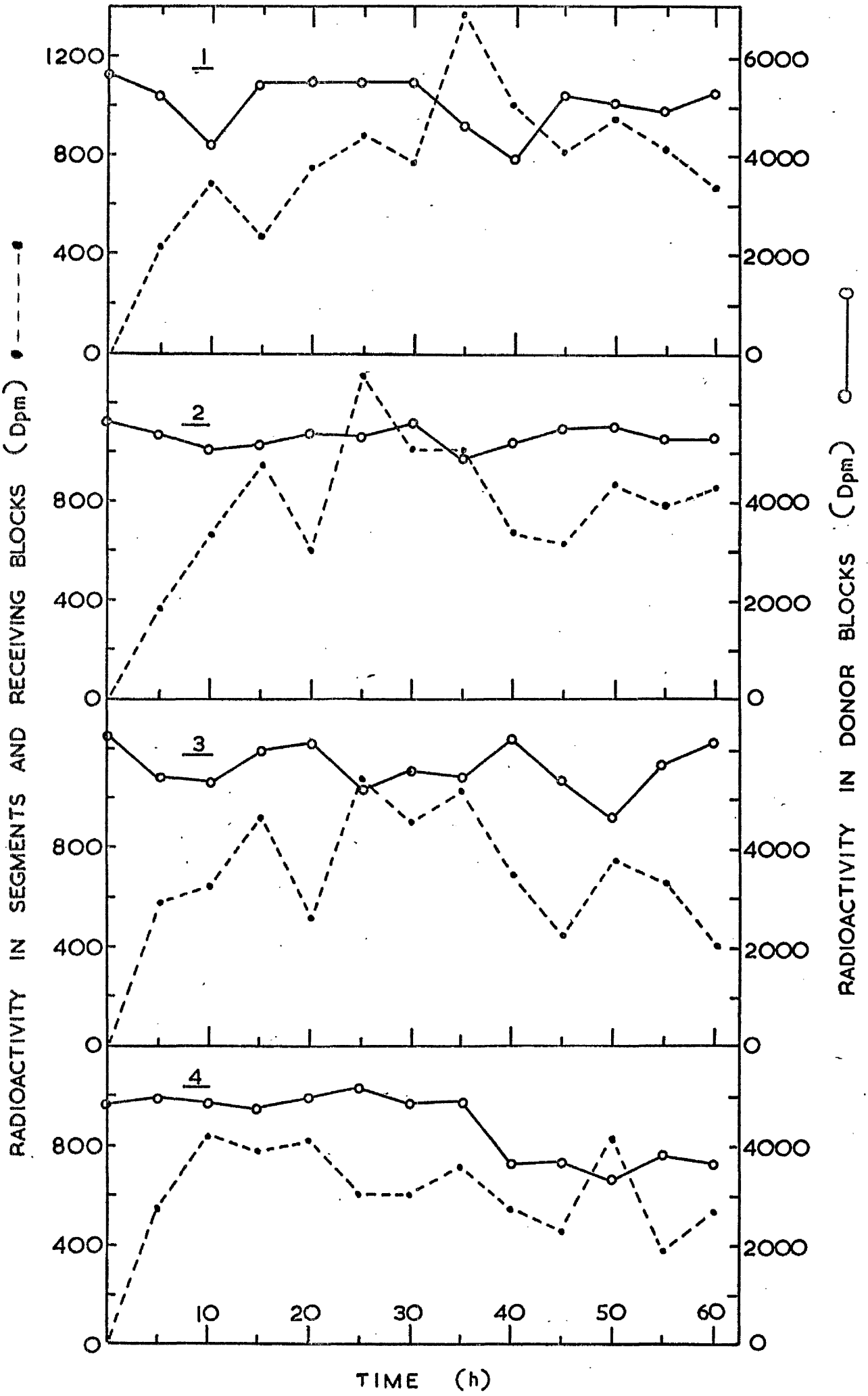

RADIOACTIVITY IN SEGMENTS AND RECEIVING BLOCKS (Dpm) •— — —•
RADIOACTIVITY IN DONOR BLOCKS (Dpm) o——o
5
6
7
8
3000
2000
1000
0
6000
4000
2000
0
2000
1000
0
4000
2000
0
2000
1000
0
8000
4000
0
2000
1000
0
8000
6000
10
20
30
40
50
60
TIME (h)

FIGURE 9

Radioactivity extracted from 1.0 μM donor blocks of 2,4-D(-1-^{14}C) (expts.1-4) or 2,4-D(-2-^{14}C) (expts.5-8) supplied to the apical ends of Pisum root segments, compared with ^{14}C extracted from segments and receiving blocks. The experiments were carried out on eight different occasions at 25°C in darkness and each donor block supplied four root segments.


1
2
3
4
1200
800
400
0
6000
4000
2000
0
10
20
30
40
50
60
TIME (h)
RADIOACTIVITY IN SEGMENTS AND RECEIVING BLOCKS (Dpm) •- - - -•
RADIOACTIVITY IN DONOR BLOCKS (Dpm) o——o

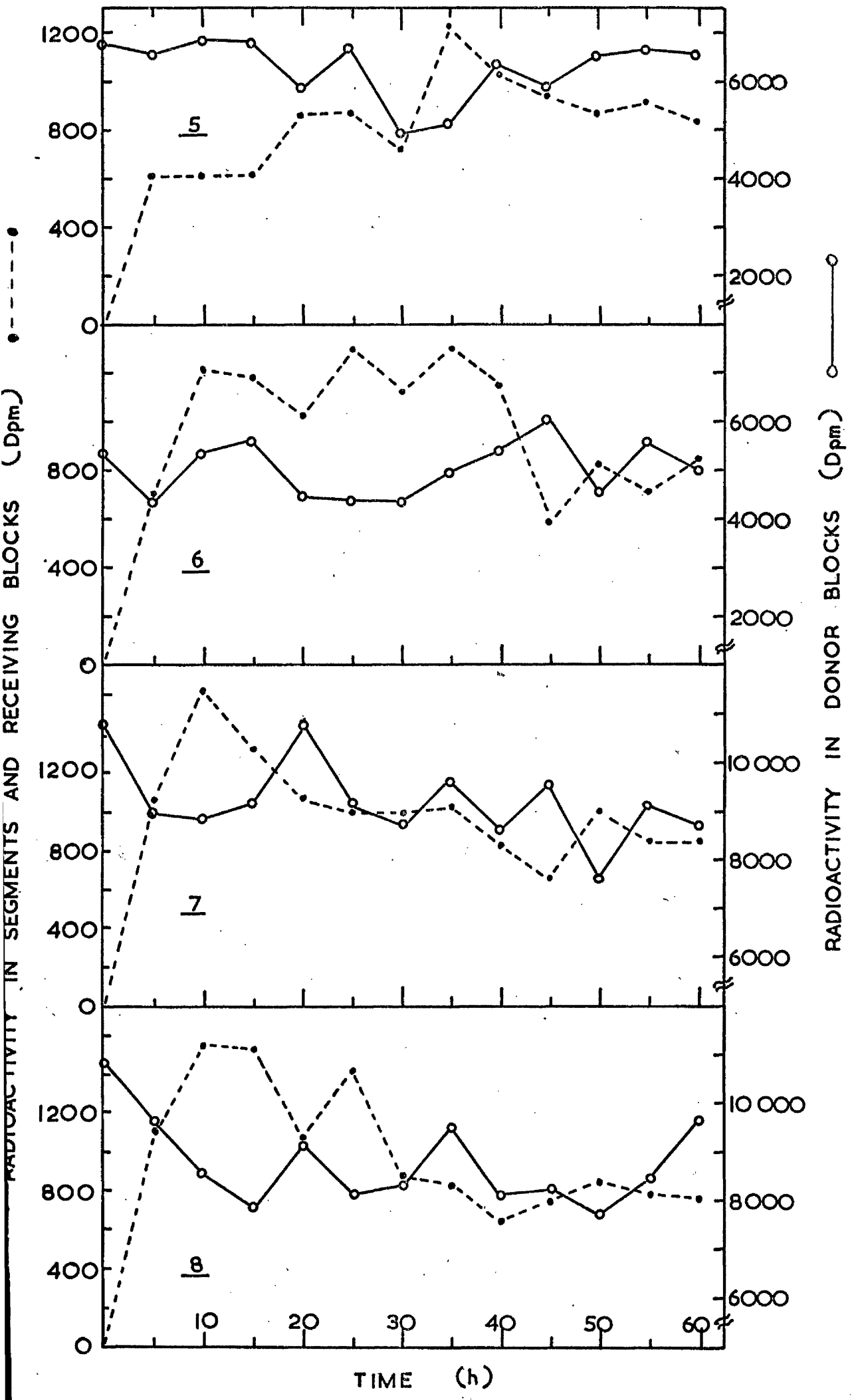

RADIOACTIVITY IN SEGMENTS AND RECEIVING BLOCKS (Dpm)
RADIOACTIVITY IN DONOR BLOCKS (Dpm)
TIME (h)
5
6
7
8
1200
800
400
0
6000
4000
2000
10 000
8000
10
20
30
40
50
60

TABLE 2

Ratio of ^{14}C content in basal/apical donor blocks of 1.0 µM 2,4-D(-1-^{14}C) or 2,4-D(-2-^{14}C) applied to Pisum root segments at 25°C in darkness (refer to figures 8 and 9 for absolute count levels).

Expt. No.	Acrop/Basip x 100			
	15h	30h	45h	60h
1	82.3	88.9	97.4	60.9
2	92.8	74.4	67.3	61.0
3	95.1	80.5	81.6	67.0
4	94.7	93.3	60.9	38.5
Mean:	91.2	84.3	76.8	56.8
5	91.3	103.2	82.2	37.2
6	104.2	71.1	63.8	60.5
7	86.9	99.9	77.3	93.1
8	96.1	79.7	79.4	69.7
Mean:	94.6	88.5	75.7	65.1

Experiments 1-4 = donation with 2,4-D(-1-^{14}C) .
Experiments 5-8 = donation with 2,4-D(-2-^{14}C).

the segment than when the herbicide was supplied to the base. For example, the content of section B was 530 dpm and 140 dpm in the acropetal and basipetal treatments whilst section F contained 140 and 10 dpm respectively after a transport period of 60 hours. Thus the acropetal polarity of ^{14}C movement detected in the ^{14}C content of receiving blocks (fig.6) was reflected by a restricted movement of radioactivity towards the receiving block in the basipetal treatments.

Fluctuations in the level of ^{14}C present in the 2mm of segment in contact with the donor block (A) were recorded after 10, 35 and 55 hours with basal donation and after 10, 25, 35 and 50 hours with apical donation. The peaks of ^{14}C content could be detected in sections B-E of the acropetal replicates. Statistical significance of these peaks was not ascertained.

Expression of the data as % of the total uptake of radioactivity revealed that 24% and 50% of the ^{14}C was retained in the 2mm of segment adjacent to the donor block in the acropetal and basipetal replicates respectively after 60 hours. The values for the corresponding region adjacent to the receiving block were 3-4% and 1-2% respectively (fig.11). It was apparent from this treatment of the results that a steady-state was established in which each 2mm piece of the segment contained a definite proportion of the total uptake after 25-30 hours. Following a basal donation, for example, the equilibrium distribution was approximately 25, 15, 11, 8, 7 and 4% from the donor to the receiving block end of the segments. Comparable data after an apical donation were 55, 22, 10, 6, 3 and 1%. A preferential movement of radioactivity towards the root apex was detectable, therefore, in the proportional distribution of ^{14}C along the root segments.

A semi-logarithmic plot of the distribution data showed that an exponential decrease in ^{14}C-content occurred from one end of the segment to the other (fig.12). But as time progressed, only the central part of the segments exhibited the linear relationship, which might indicate the involvement of an unidentified process at the ends of the tissue.

Figure 10 revealed that the 2mm of the root segments in contact with the donor block exhibited the most erratic fluctuations in ^{14}C content. The data presented in figure 13 illustrate that the highest levels of radioactivity occurred after 10-15, 35-40 and 45-55h on each occasion except experiments 5 and 6. An additional peak was recorded after

FIGURE 10

Distribution of radioactivity in successive 2mm sections of Pisum root segments supplied with 1.0 μM 2,4-D(-1-^{14}C) at the apical or basal ends at 25°C in darkness. The donor block end of the segment is denoted by A and the receiving block end by F. Each point is the total radioactivity extracted from corresponding sections of four root segments supplied by a communal donor block.

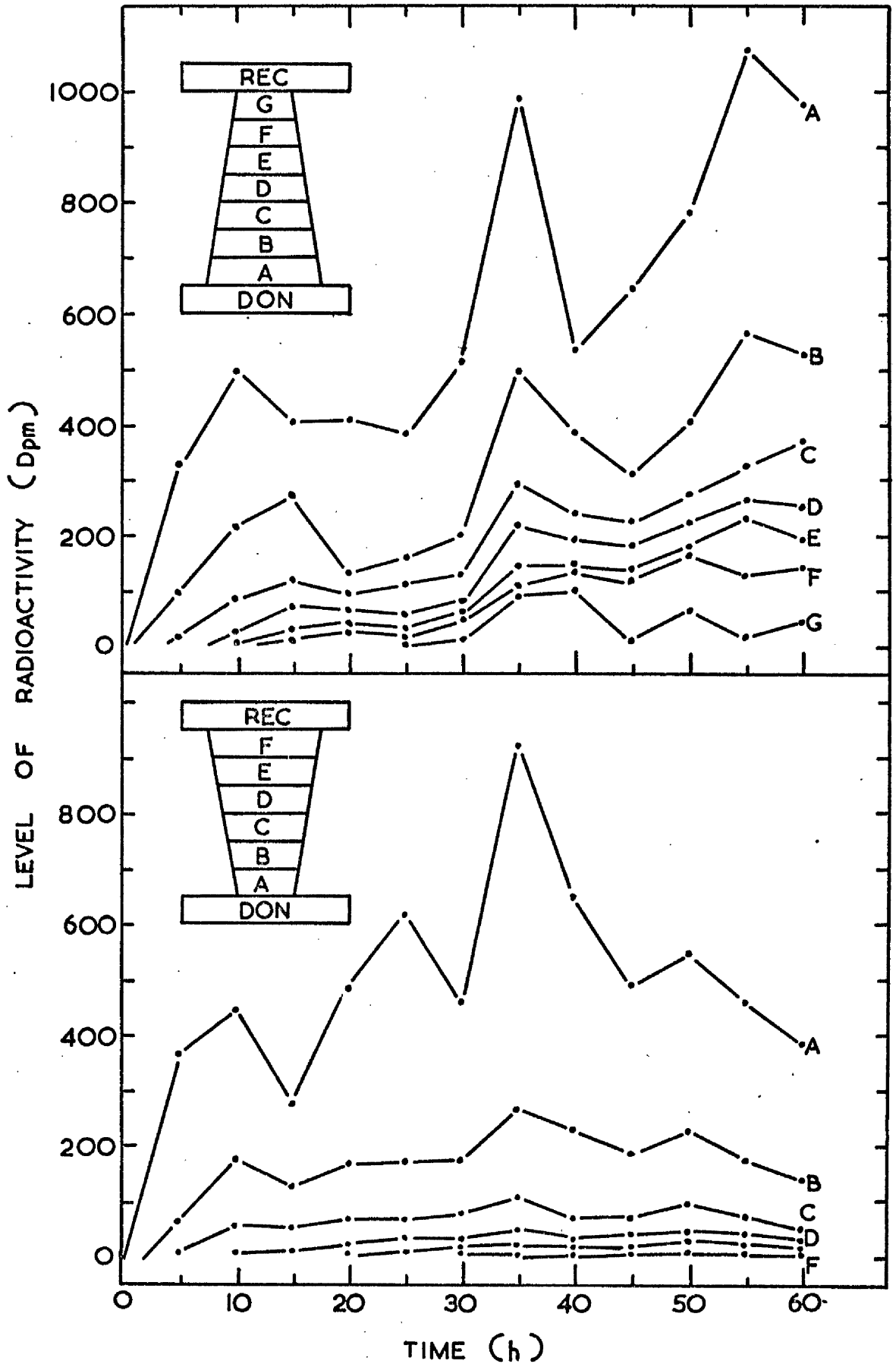
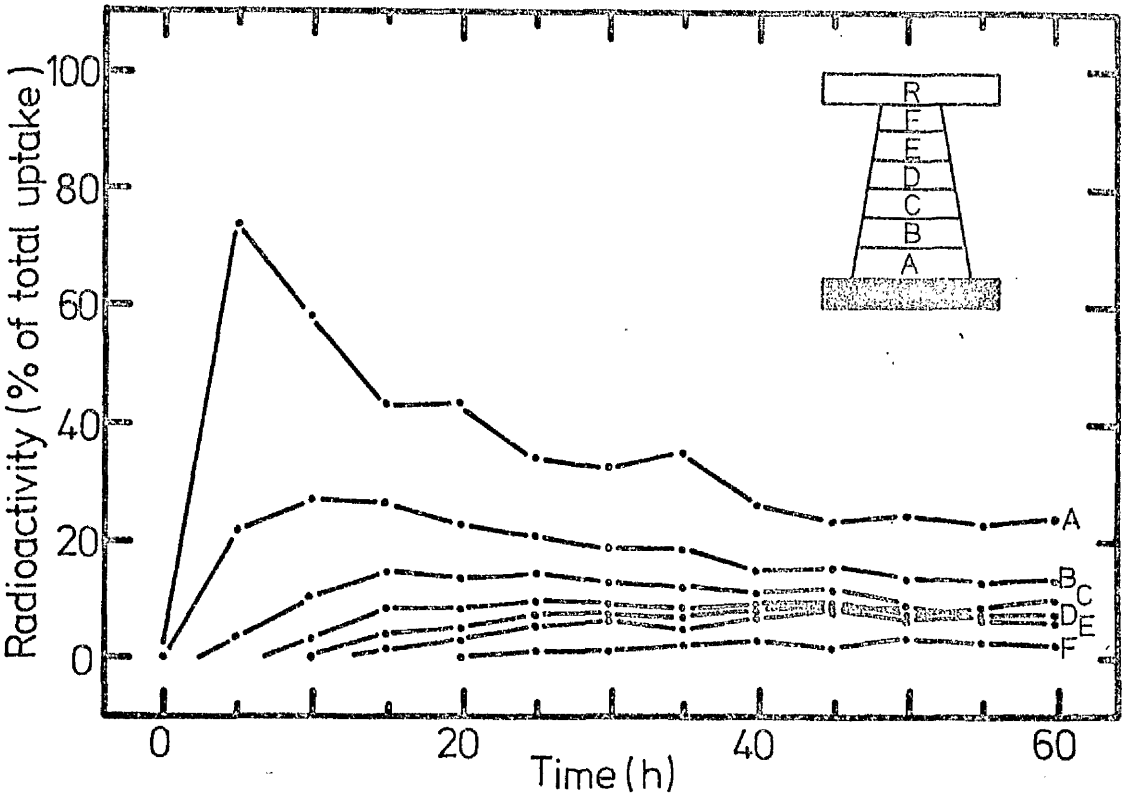

REC
G
F
E
D
C
B
A
DON
1000
800
600
400
200
0
A
B
C
D
E
F
G
REC
F
E
D
C
B
A
DON
800
600
400
200
0
A
B
C
D
F
0
10
20
30
40
50
60
TIME (h)
LEVEL OF RADIOACTIVITY (Dpm)

FIGURE 11

Distribution of radioactivity in successive 2mm sections of Pisum root segments supplied with 2,4-D(-1-^{14}C) at the apical or basal ends at 25^{o}C in darkness. The donor block end is denoted by A and the receiving block end by F. Each point is the radioactivity, expressed as % of the total extracted from four root segments and communal receiving block at each sampling interval.


Radioactivity (% of total uptake)
100
80
60
40
20
0
0
20
40
60
Time (h)
R
F
E
D
C
B
A
A
B
C
D
E
F

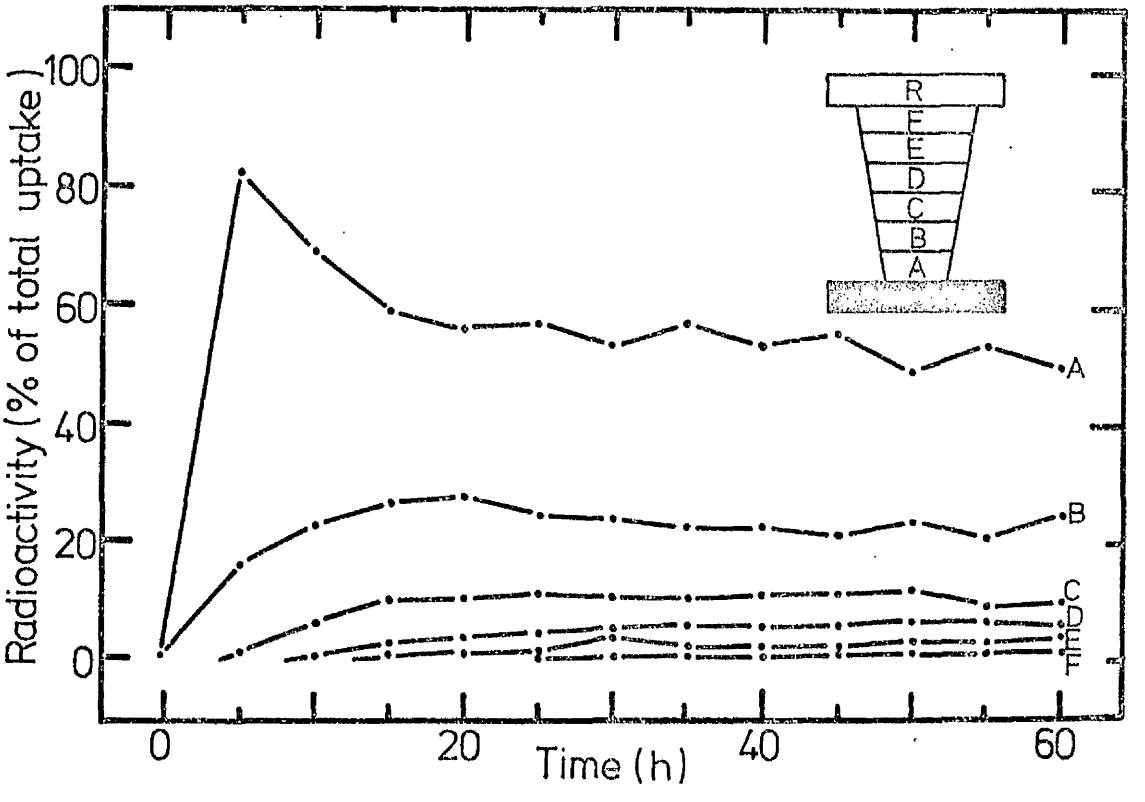
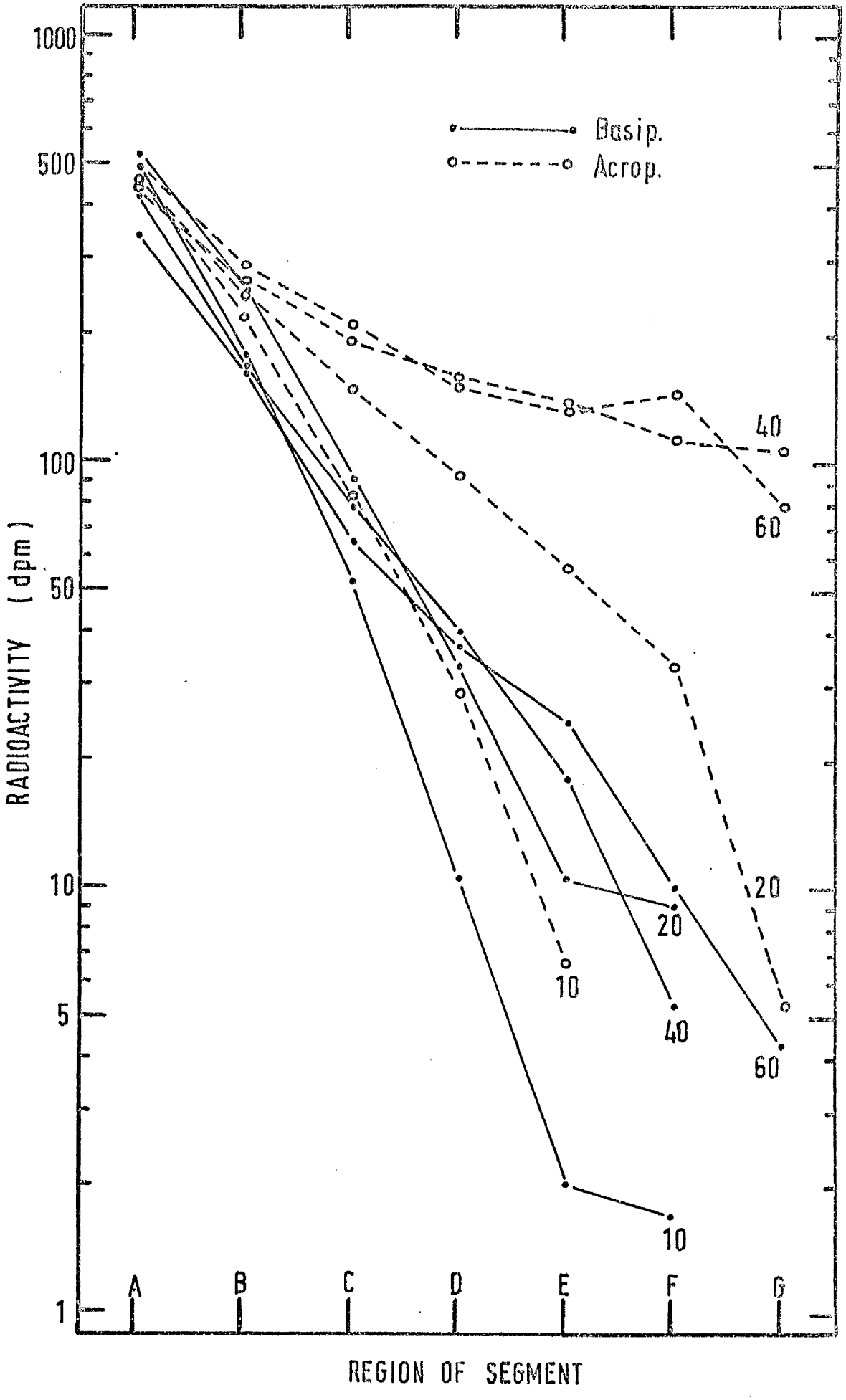

Radioactivity (% of total uptake)
100
80
60
40
20
0
0
20
40
60
Time (h)
R
E
E
D
C
B
A
A
B
C
D
E
F

FIGURE 12

Distribution of radioactivity along Pisum root segments after 10, 20, 40 and 60 hours, presented on a semi-logarithmic scale. The data are the mean of four experiments carried out on different days at 25°C in darkness using a donor concentration of 1.0 μM 2,4-D(-1-^{14}C).


1000
500
100
50
10
5
1
RADIOACTIVITY (dpm)
Basip.
Acrop.
8
8
8
40
60
20
10
20
40
60
10
A
B
C
D
E
F
G
REGION OF SEGMENT

20-25 hours in experiments 1, 5 and 8. Peaking of acropetal and basipetal treatments within an individual experiment were closely synchronous. For example, peaks after 10-15 hours were present on each occasion in both acropetal and basipetal treatments. This was true at the 35 hour point with the exception of the basipetal treatments of experiments 7 and 8.

Monitoring the ^{14}C content of the donor supply revealed that more radioactivity was lost from the blocks applied to the basal than to the apical ends of the segments (table 3). After a transport period of 30 hours, up to 3.3 times more ^{14}C was lost from the basal than from the apical blocks, whilst the difference was 4-5 fold by the end of the experiment. This was equivalent to a loss of 49.4% and 42.6% of the initial 2,4-D(-1-^{14}C) and 2,4-D(-2-^{14}C) donor supply to the acropetal treatments and 10% from the basipetal treatments after a 60 hour experiment.

The radioactivity in the root segments and receiving blocks accounted for 19-23% and 11-13% of the total ^{14}C present in the acropetal and basipetal systems respectively after 30 hours. By the end of the experiments the corresponding values were 40-48% and 10-11% (table4). The polarity ratios (acrop/basip) exhibited the same trend as those for ^{14}C content of receiving blocks and ^{14}C-loss from donor blocks in that the levels of radioactivity in the acropetal treatments were consistently greater than those in the basipetal treatments. Little difference between the -COOH and $-CH_2$ labelled compound could be detected.

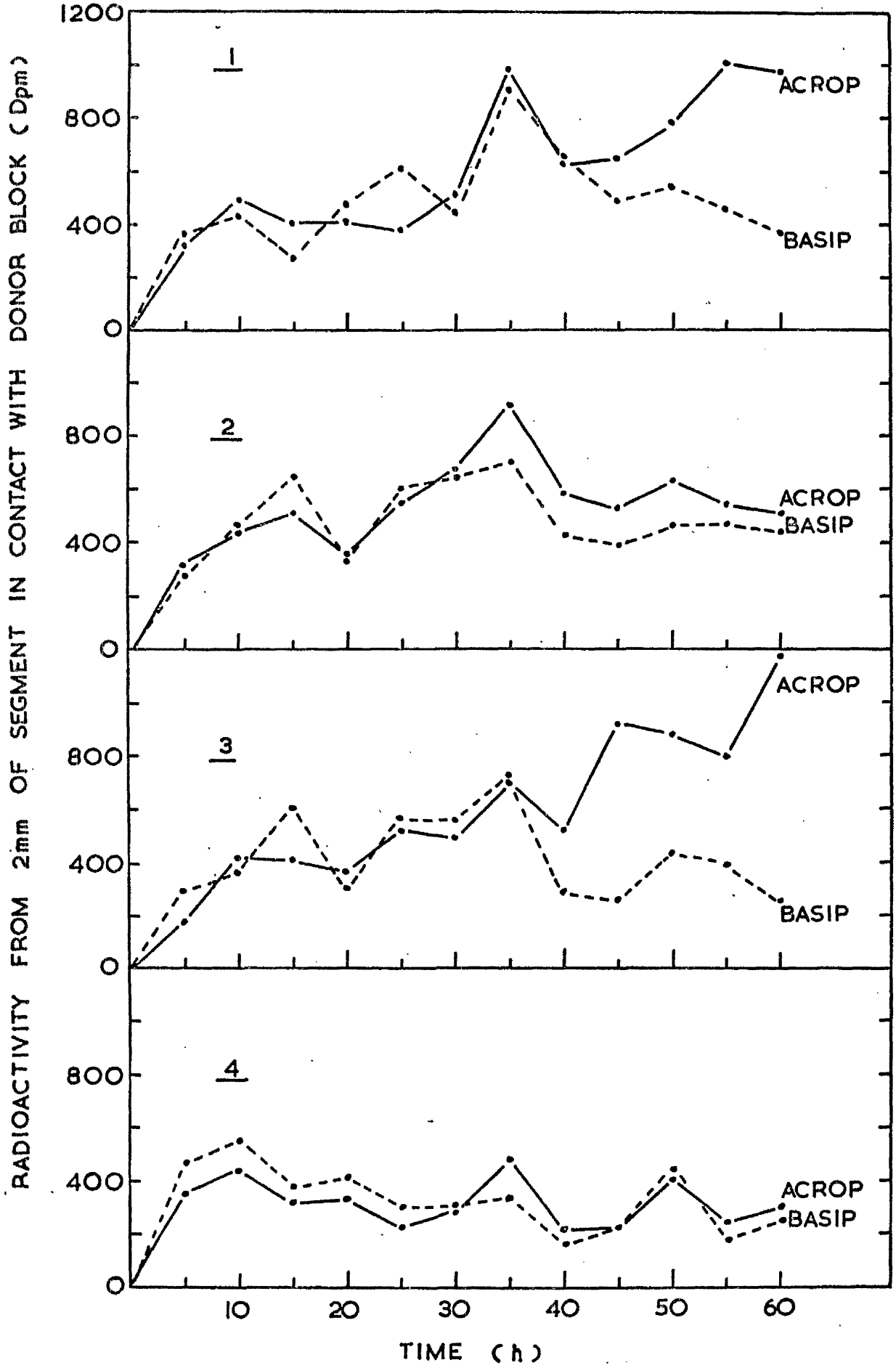
It is clear from these data that radioactivity, applied as 2,4-D(-1-^{14}C) or 2,4-D(-2-^{14}C) moved preferentially towards the root tip ie. exhibited an acropetal polarity.

FIGURE 13

Radioactivity extracted from apical or basal 2mm pieces of Pisum root segments in contact with 1.0 μM donor blocks of 2,4-D(-1-^{14}C) or 2,4-D(-2-^{14}C) at 25^{o}C in darkness. The eight experiments were carried out on different days and the data are the total radioactivity present in the four segments supplied by a communal donor block at each sampling interval.

Experiments 1-4 ..... 1.0 μM 2,4-D(-1-^{14}C)

Experiments 5-8 ..... 1.0 μM 2,4-D(-2-^{14}C)


RADIOACTIVITY FROM 2mm OF SEGMENT IN CONTACT WITH DONOR BLOCK (Dpm)
1200
800
400
0
1
ACROP
BASIP
2
ACROP
BASIP
3
ACROP
BASIP
4
ACROP
BASIP
10
20
30
40
50
60
TIME (h)

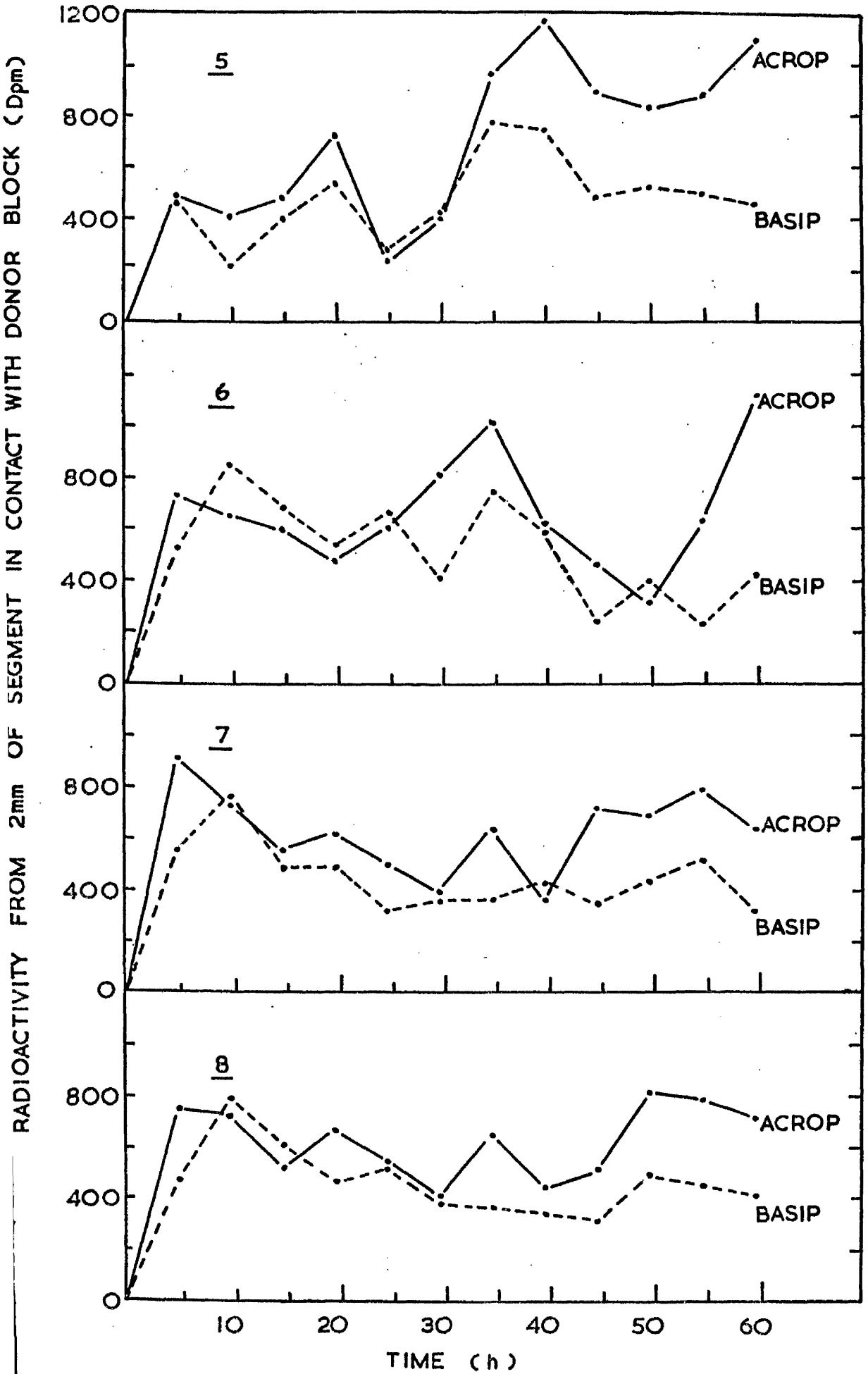

RADIOACTIVITY FROM 2mm OF SEGMENT IN CONTACT WITH DONOR BLOCK (Dpm)
1200
800
400
0
5
ACROP
BASIP
6
ACROP
BASIP
7
ACROP
BASIP
8
ACROP
BASIP
10
20
30
40
50
60
TIME (h)

TABLE 3

The loss of radioactivity from 1.0 μM donor blocks of 2,4-D(-1-^{14}C) or 2,4-D(-2-^{14}C) supplied to Pisum root segments.

Experiments 1-4 ..... 2,4-D(-1-^{14}C)
Experiments 5-8 ..... 2,4-D(-2-^{14}C)

Expt. No.	% loss of ^{14}C from donor blocks			
	30 hours		60 hours	
	Acrop	Basip	Acrop	Basip
1	13.2	2.4	43.0	6.2
2	25.1	6.4	43.0	6.4
3	17.5	11.5	62.3	2.2
4	18.9	2.5	49.4	24.4
Mean	18.7	5.7	49.4	9.8
Acrop/basip	3.3		5.0	
5	20.5	27.2	64.7	5.2
6	42.9	19.7	43.3	6.2
7	19.0	18.9	24.6	19.1
8	38.3	22.5	37.7	10.7
Mean	30.1	22.1	42.6	10.3
Acrop/basip	1.4		4.1	

TABLE 4

Radioactive content of root segments and receiving blocks, expressed as % of that in the segments, receiving and donor blocks.

Experiments 1-4 ..... 1.0 μM 2,4-D(-1-^{14}C)

Experiments 5-8 ..... 1.0 μM 2,4-D(-2-^{14}C)

Expt. No.	^{14}C in tissue + rec. as % of total in system			
	30 hours		60 hours	
	Acrop	Basip	Acrop	Basip
1	17.4	12.2	46.4	11.2
2	32.0	15.6	45.0	14.0
3	16.3	14.0	60.7	6.1
4	27.0	11.1	41.2	12.9
Mean	23.0	13.2	48.3	11.0
Acrop/basip	1.7		4.4	
5	13.3	9.6	54.0	11.5
6	29.7	17.2	53.4	14.4
7	13.1	10.3	26.3	8.7
8	20.6	9.4	26.7	7.3
Mean	19.2	11.6	40.1	10.5
Acrop/basip	1.6		3.8	

Fluctuations in transport data

Throughout the course of the experimental programme reported in this thesis it became increasingly clear that the process(es) involved in the movement of 2,4-D through root segments did not proceed at constant rates. In fact, the possibility that a reversal of certain processes might occur could not be discounted. A series of experiments was designed in an attempt to clarify the situation.

(i) Effect of time of setting-up

Since experiments in which destructive sampling was carried out were set-up at staggered intervals over the period 09.00-18.00 hours for ease of harvesting, it was possible that the fluctuations could be linked to regular fluctuations of an environmental parameter. In addition, the initial age of the segments could vary by up to 9 hours because all segments for one experiment were excised from the same batch of germinated seeds.

Identical acropetal and basipetal replicates were set-up at two-hourly intervals throughout the day and were kept in contact with 1.0 μM 2,4-D(-1-^{14}C) for 24 hours at 25°C in darkness. The data are presented in table 5 , together with the results of a statistical analysis in table 6

The time of setting-up had little effect on the total uptake or the ^{14}C level in the segments of the basipetal replicates. Accumulation in the associated receiving blocks, however, fell steadily so that a 62% difference between the first and the last set of replicates was recorded. The total uptake and segment content of radioactivity of the acropetal replicates were significantly different from the 09.00 hour point on two occasions. Accumulation of radioactivity in the receiving blocks of the acropetal replicates was reasonably stable.

In conclusion, the time of setting-up did not appear to have an effect on the movement of 2,4-D in the acropetal replicates, but did appear to be a significant factor in the basipetal replicates.

TABLE 5

The effect of time of setting-up on the uptake and translocation of 2,4-D(- 1-^{14}C) supplied to Pisum root segments at 25°C in darkness. The data are based on a total of eight independent replicates.

Time (h)	Sample	RADIOACTIVITY (dpm) Acropetal	Basipetal
09.00	Receiver	120.2 ± 12.9	39.3 ± 3.4
	Tissue	2117.9 ± 153.8	1189.0 ± 104.1
	Uptake	2238.1 ± 146.3	1228.3 ± 103.1
11.00	Receiver	118.4 ± 8.7	33.2 ± 4.5
	Tissue	1709.1 ± 125.5	1229.1 ± 79.8
	Uptake	1827.5 ± 131.0	1252.3 ± 67.6
13.00	Receiver	98.5 ± 9.9	28.1 ± 5.1
	Tissue	1910.4 ± 234.6	1018.3 ± 84.5
	Uptake	2008.9 ± 235.1	1046.4 ± 79.9
15.00	Receiver	108.9 ± 10.9	19.0 ± 4.4
	Tissue	1667.9 ± 112.4	1066.2 ± 92.3
	Uptake	1776.8 ± 109.5	1085.2 ± 90.1
17.00	Receiver	90.1 ± 6.7	23.8 ± 4.6
	Tissue	1888.9 ± 143.0	1141.9 ± 43.4
	Uptake	1979.0 ± 147.2	1165.2 ± 44.7
19.00	Receiver	79.7 ± 8.9	15.7 ± 1.9
	Tissue	1933.8 ± 93.2	1230.4 ± 89.0
	Uptake	2013.5 ± 87.0	1246.1 ± 89.2
21.00	Receiver	98.8 ± 24.4	14.8 ± 3.1
	Tissue	1884.6 ± 219.9	1104.7 ± 71.5
	Uptake	1983.4 ± 226.0	1119.5 ± 72.6

TABLE 6

Statistical analysis of data presented in table 5.

Paired t-test: $t_{0.05} = 2.365$ $t_{0.01} = 3.499$ $t_{0.001} = 5.405$ (7 DF)

Times compared (h)	Sample	t - values	
		Acrop	Basip
09.00-11.00	Receiver	0.12	1.91
	Tissue	2.15	1.33
	Uptake	2.18	1.45
	Donor	0.17	-1.48
09.00-13.00	Receiver	1.39	1.91
	Tissue	0.77	1.33
	Uptake	0.86	1.45
	Donor	0.74	-1.06
09.00-15.00	Receiver	0.69	3.82 **
	Tissue	2.46 *	0.92
	Uptake	2.63 *	1.09
	Donor	0.09	-2.01
09.00-17.00	Receiver	2.12	2.80 *
	Tissue	1.13	0.43
	Uptake	1.30	0.58
	Donor	1.93	-0.63
09.00-19.00	Receiver	2.70 *	6.29 ***
	Tissue	1.06	-0.31
	Uptake	1.37	-0.13
	Donor	1.39	-1.50
09.00-21.00	Receiver	0.80	5.55 ***
	Tissue	0.90	0.69
	Uptake	0.99	0.90
	Donor	0.12	-1.12

(ii) The use of shorter sampling intervals

The radioactive content of the donor, tissue and receiving block was estimated by the destructive sampling technique employed previously but using 2-hourly, instead of 5- hourly sampling intervals. The necessity for an independent set of roots at each sampling governed the possible number of replicates. Consequently, 1.0 μM 2,4-D(-1-^{14}C) was supplied to only two acropetal and two basipetal replicates, each containing four root segments. The experiment was carried out on two occasions and the mean data from each experiment are presented separately.

During the initial 10 hour period of experiments A and B approximately 1700 - 2000 dpm entered the root segments (figure 14). Rapid fluctuations in the level of ^{14}C over the subsequent 10 hours of experiment A and 20 hours of experiment B were not significantly different from the fitted quadratic curves (table 7). Further peaks and declines in the level of radioactivity in the segments were noted at intervals during the remainder of the experiment. Polynomial regression analysis revealed that the trough at 38 hours and the peak at 50 hours in experiment A were significantly different from the fitted curve. Only the peaks recorded after 54 and 58 hours in experiment B proved to be significant. Nevertheless, there was a striking resemblance in the pattern of peaking in the two experiments. It is tentatively suggested that the peaks 1, 2, 3 and 4 might be essentially similar except that a 10 hour delay in experiment B might have occurred.

Export of radioactivity into the receiving blocks was also characterised by periods in which the detectable ^{14}C increased rapidly whilst at other times a loss of ^{14}C from the blocks appeared to take place. For example, maxima in experiment A were noted after 30, 40, 50 and 58 hours and after 28, 38, 48 and 58 hours in experiment B. The situation was less clearly defined in the receiving blocks applied to the basipetal replicates (figure15).

FIGURE 14

Total radioactive content of Pisum root segments supplied with 1.0 μM 2,4-D(-1-^{14}C) either at the apical or the basal end of the cut tissue. Mean data from two experiments (A & B) are presented and both were carried out at 25°C in darkness.

° ——— ° basal donation

• - - - - - • apical donation

x ——— x fitted regression line

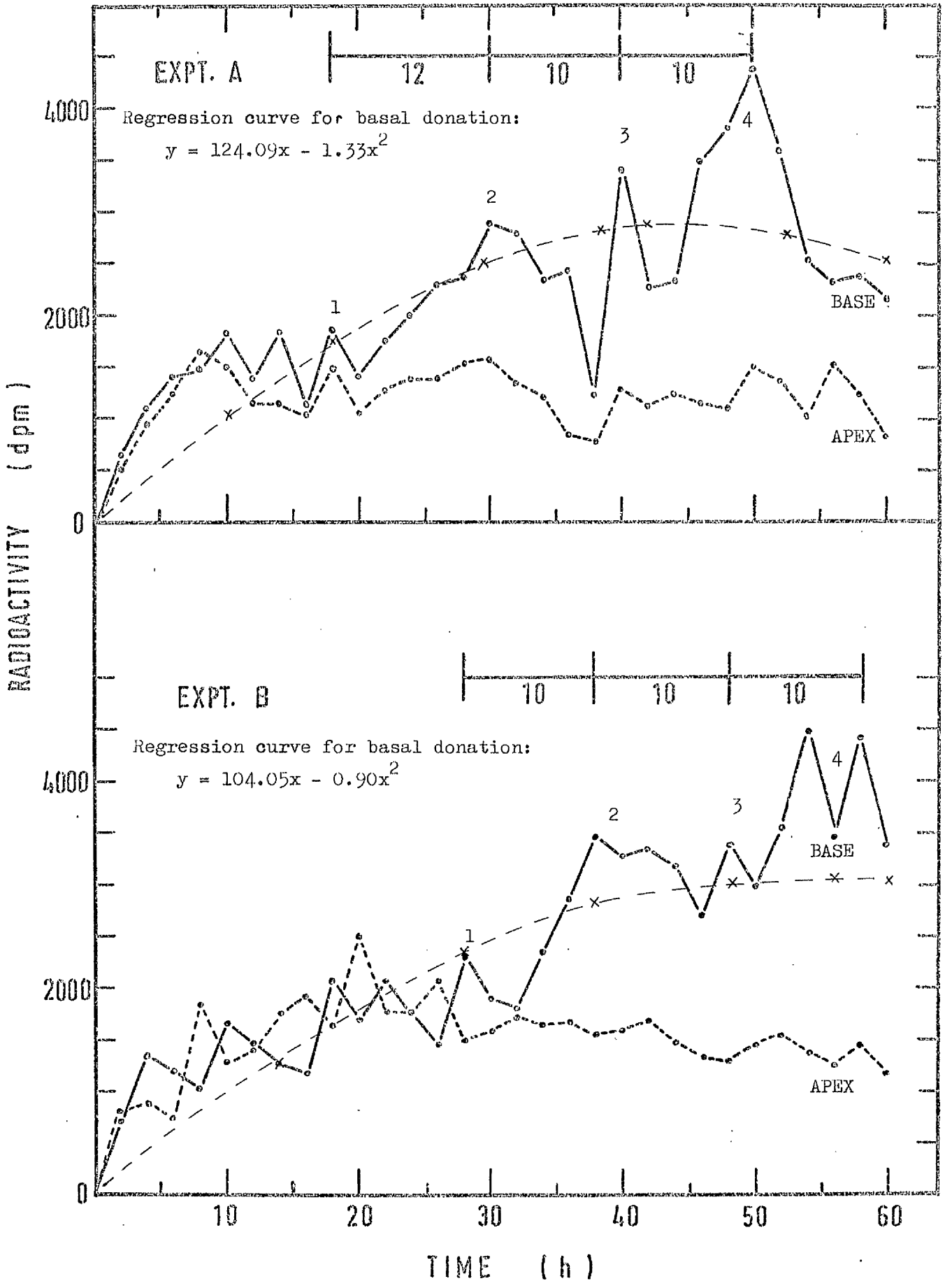

EXPT. A
12
10
10
Regression curve for basal donation:
y = 124.09x - 1.33x²
1
2
3
4
BASE
APEX
EXPT. B
10
10
10
Regression curve for basal donation:
y = 104.05x - 0.90x²
1
2
3
4
BASE
APEX
4000
2000
0
RADIOACTIVITY (dpm)
10
20
30
40
50
60
TIME (h)

FIGURE 15

The content of radioactivity in receiving blocks applied to the apical or the basal end of Pisum root segments supplied with 1.0 µM 2,4-D(-1-^{14}C) at 25°C in darkness. The data are the mean from two experiments (A & B) each of which consisted of four replicates.

° ——— ° basal donation

• - - - - • apical donation

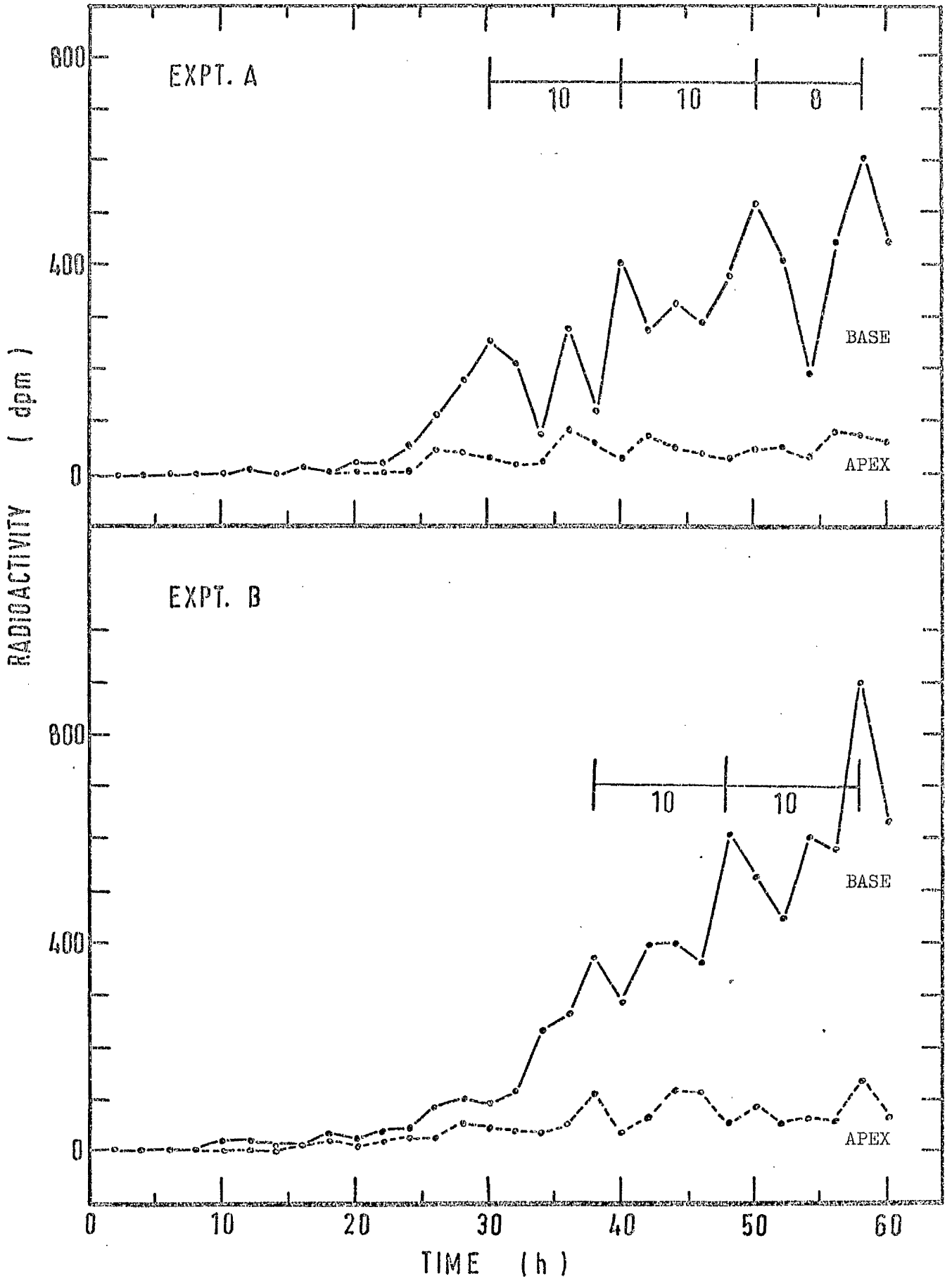

EXPT. A
10
10
0
800
400
0
BASE
APEX
EXPT. B
10
10
800
400
0
BASE
APEX
0
10
20
30
40
50
60
TIME (h)
RADIOACTIVITY (dpm)

TABLE 7

Polynomial regression analysis

(1) Experiment A

Source	DF	SS	MS	F
Linear	1	0.14415520^9	0.14415520^9	361.69
Quadratic	1	9011411.95	9011411.95	22.61
Residual	28	11159810.94	398564.68	
Total	30	0.16432643^9		

$y = 124.09x - 1.33x^2$

Time (h)	LSD from fitted line	Calculated y	Observed y	Difference	Sig.
6	1425.4	696.7	1410.9	714.2	-
38	1495.2	2798.9	1236.7	1553.2	*
50	1514.9	2870.9	4386.0	1515.1	*

Experiment B

Source	DF	SS	MS	F
Linear	1	0.14409657^9	0.14409657^9	781.02
Quadratic	1	4097523.01	4097523.01	22.21
Residual	28	5165951.23	184498.26	
Total	30	0.15336005^9		

$y = 104.05x - 0.90x^2$

Time (h)	LSD from fitted line	Calculated y	Observed y	Difference	Sig.
6	969.8	591.9	1194.5	602.6	-
38	1019.8	2655.4	3094.8	439.4	-
54	1065.7	2996.0	4500.0	1504.0	*
58	1124.2	3010.0	4410.0	1400.0	*

$F_{1, 28}$ 4.20 * 5%
7.64 ** 1%
*** 0.1%

DF = degrees of freedom
SS = sum of squares
MS = mean square
F = variance ratio

LSD = least significant difference
Sig = indication of a significant difference from the fitted line.

(iii) The use of non-destructive sampling techniques

A non-destructive sampling technique was employed in order that the innate variability associated with the use of several sets of root segments, in the destructive sampling experiments, could be reduced by using only one set of roots. The movement of 2,4-D was studied in terms of ^{14}C-loss from donor blocks and the ^{14}C-content of receiving blocks applied to one set of Pisum root segments.

1.0 μM 2,4-D(-1-^{14}C) was supplied to the basal and apical ends of root segments in acropetal and basipetal replicates. In each case, four replicates were set up in which the donor blocks were replaced at 2-hourly intervals, and another four in which the receiving blocks were replaced at similar intervals. This experiment was run (simultaneously with experiment B in section ii) at 25°C in total darkness.

Figure 16 presents the loss of ^{14}C from donor blocks applied to the basal end of the segments for successive periods of 2 hours. Periods of greatest ^{14}C loss were 7-9,17-19, 25-27, 31-33, 39 and 55-57 hours, with those underlined being periods of very high loss. Greatest loss in the basipetal replicates occurred after 9, 15, 23, 31-33, 37, 53, 57 and 62 hours.

Table 8 reveals that the level of extractable radioactivity in the donor blocks apparently increased at intervals throughout the experiment. For example, an additional 1932 and 2862 dpm were detected in basal donor blocks after the 35 and 49 hour points of the experiment.

Figure 17 shows the accumulation of radioactivity in receiving blocks during each 2-hour period of the experiment. The acropetal replicates had high rates of accumulation after 29, 45 and 73 hours, whilst the peaks in the basipetal replicates occurred after 15,23,33,43, 63 and 73 hours.

The results of this experiment revealed that, in general, the periods of high loss of ^{14}C from the donor blocks could be related to periods of greater accumulation of ^{14}C in the receiving blocks 14-16 hours later. This time interval would be acceptable since it is the approximate time taken for ^{14}C to pass from one end of the segment to the other.

FIGURE 16

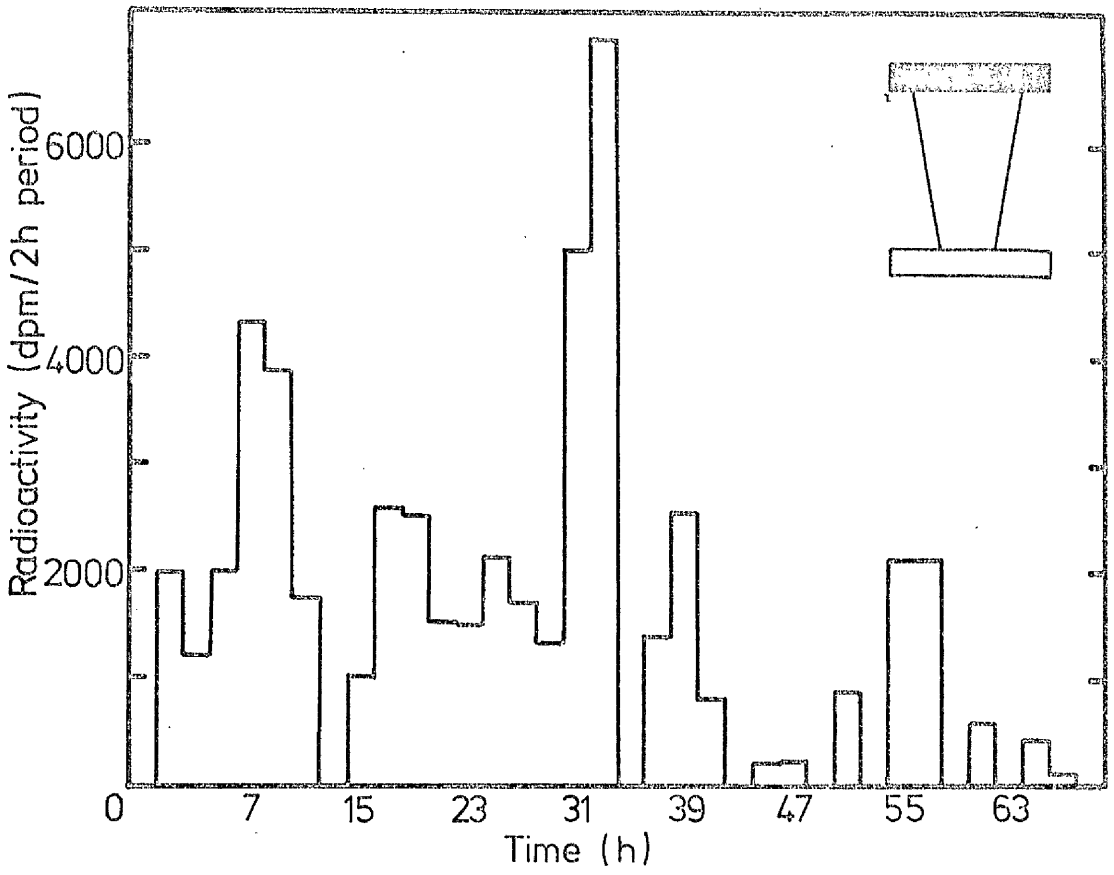
The loss of radioactivity from donor blocks of 1.0 μM 2,4-D(-1-^{14}C) supplied to the apical or the basal end of Pisum root segments at 25°C in darkness. The data are the mean of four replicates at each 2-hour harvest and are presented as the loss of radioactivity during each successive 2-hour interval.

TABLE 8

Increased levels of radioactivity were recorded in the donor blocks at several intervals during the experiment:

Time (h)	Increase in donor block counts (dpm/2h period)	
	ACROPETAL	BASIPETAL
13	4780.1	1392.9
17	-	1513.3
35	1932.3	184.9
43	866.7	1340.3
45	-	36.7
47	-	525.0
49	2862.0	525.0
53	1652.1	-
55	-	802.1
59	3095.4	543.7
63	388.3	-

Donor Blocks



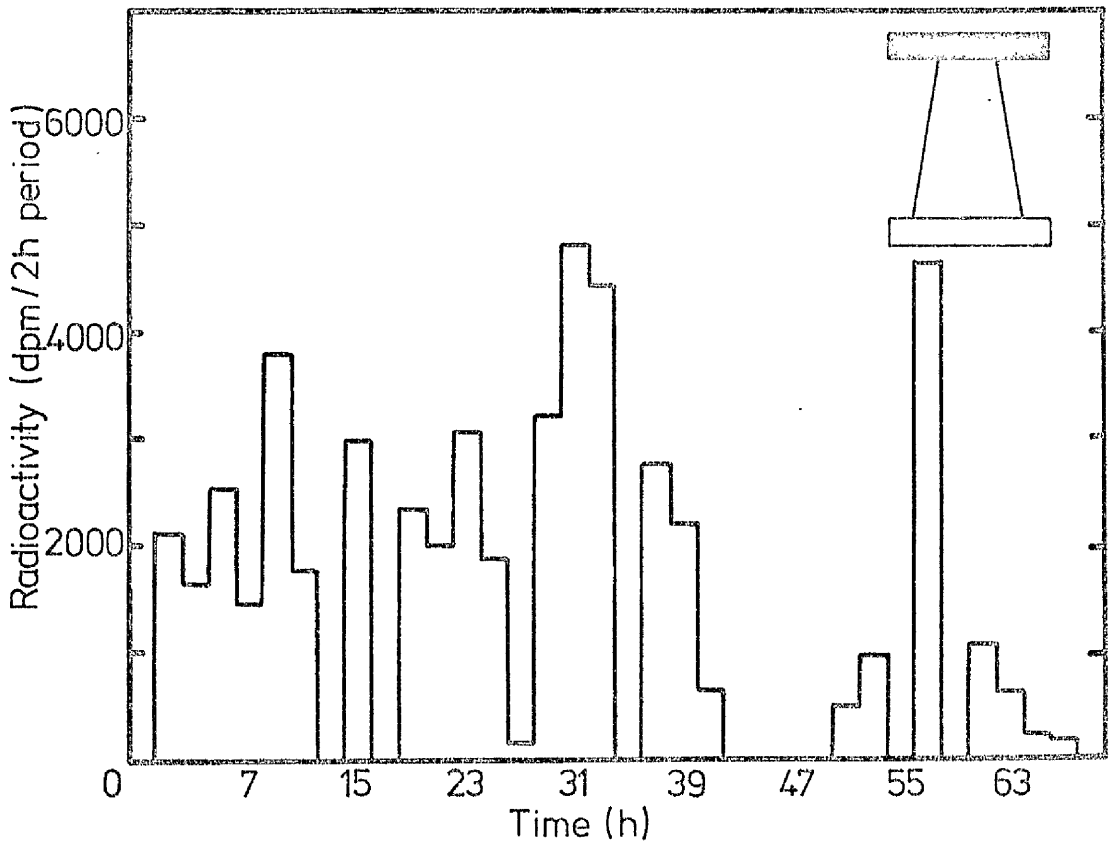
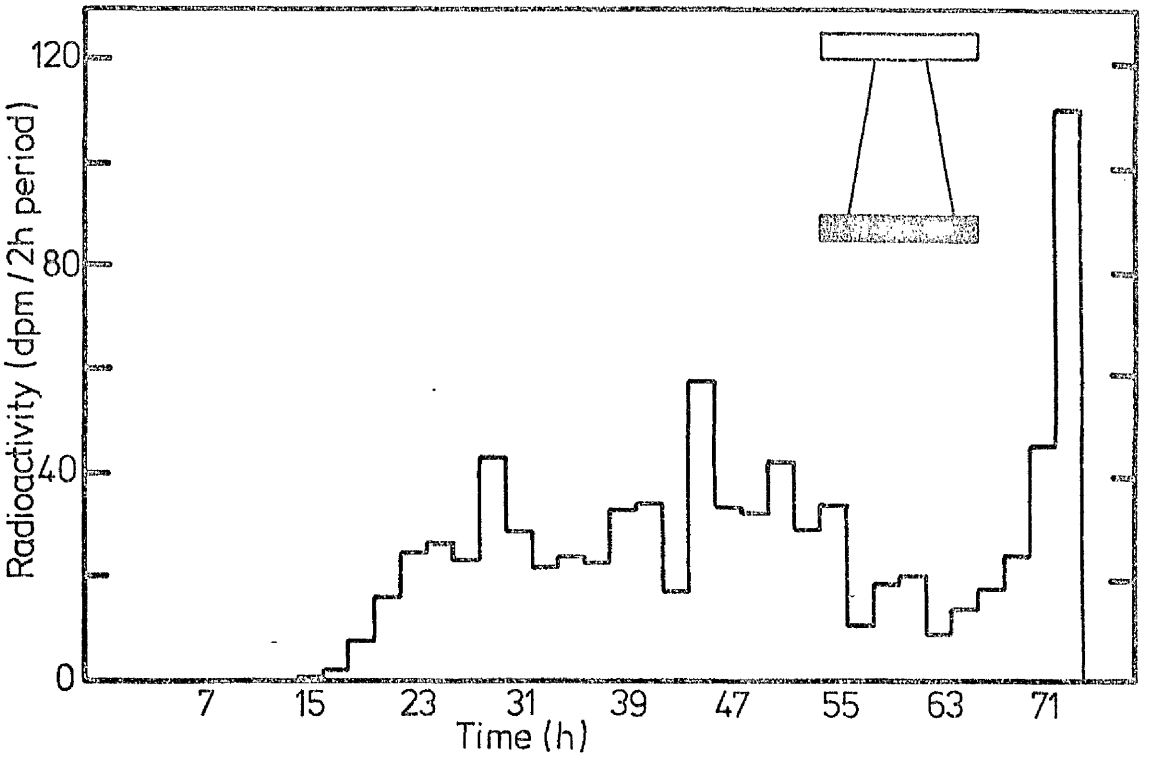
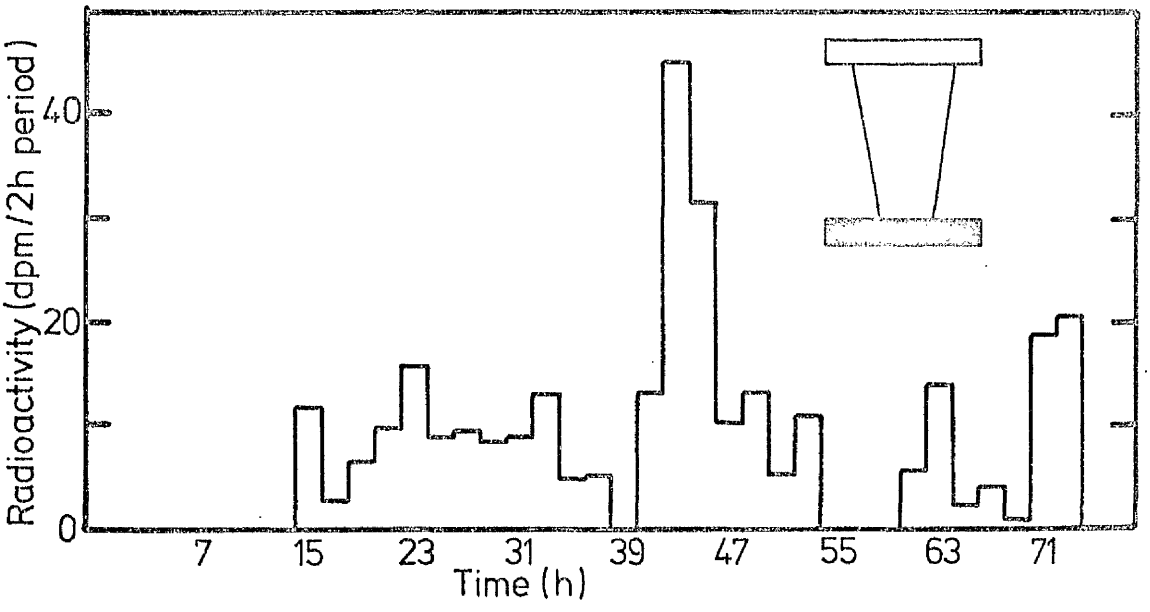


FIGURE 17

Radioactivity extracted from receiving blocks applied to the apical or basal end of Pisum root segments at 25°C in darkness. The data are the mean of four replicates at each harvest and are presented as the increase in ^{14}C content during each successive 2-hour period.

Receiving Blocks





IMMOBILISATION OF 2,4-D

Investigations to determine the extent of immobilisation of the radioactive herbicide within the root segments were carried out using the normal segment technique.

(A) Control

Donor blocks of 1.0 μM 2,4-D(-1-^{14}C) were supplied to the apical or basal ends of 10mm long Pisum root segments for assessment of basipetal and acropetal transport.

(B) Plain agar treatment

Pisum root segments were supplied with 2,4-D(-1-^{14}C) in the manner described for the control experiment, but after 25 hours the donor block was replaced by a plain agar block. This prodedure was adopted since figure 11 indicated that the level of ^{14}C in successive 2mm pieces of the segment came to a 'steady state' after approximately 25 hours.

(C) Unlabelled (cold) 2,4-D treatment

Procedure was identical to that for the plain agar treatment (B) except that the donor blocks were replaced with blocks containing 1.0 μM unlabelled 2,4-D after 25 hours.

Levels of radioactivity in the segments, donor blocks, receiving blocks and replacement blocks were determined by liquid scintillation counting of ethanol extracts. Experimental manipulations were carried out in green light and transport periods run in humidified chambers placed in a dark cupboard. The temperature was 25° ± 1°C and thermograph records revealed that fluctuations were minimal.

Each point on the graphs represents the mean value from eight independent replicate samples, each consisting of four root segments with communal donor and receiving blocks. The associated standard errors are presented as vertical lines on the figures.

Data for the control experiment (figure 18) illustrate that an acropetal polarity of ^{14}C movement could be defined as a restricted distribution of radioactivity along the root segments and an absence of a continued increase in ^{14}C content of the segments following apical donation of the compound. Standard errors for these data are presented

in table 10. These points will not be considered further since the data in figure 10, bearing a striking similarity, have been covered adequately.

A polynomial regression analysis revealed that the levels of ^{14}C in the 2mm of segment (A) adjacent to the donor block and the subsequent 2mm piece (B) of the acropetal replicates possessed a significant (*) quadratic relationship with the x-axis. Further analysis indicated that points of greatest deviation from the fitted curve to section (A) of the acropetal replicates ie. after 10, 35, 55 hours were not significantly different ffom the curve. Fluctuations in section (B) after 30, 35 and 55 hours were not significantly different from the fitted regression curves. The time-course of ^{14}C content in section (F) proved to be not significantly different from the fitted linear regression (table 16).

Fluctuations were not detectable in the basipetal replicates.

Replacement of basal donor blocks by plain agar blocks after 25 hours (figure 20) resulted in a 60% decrease in ^{14}C content of the 2mm of segment in contact with the donor block, within 5 hours. Replacement by blocks of unlabelled 2,4-D produced an 85 % decrease of ^{14}C in the 2mm of segment adjacent to the donor block (figure 19). Radioactivity in this part of the segment decreased so that by the end of the experiment the content, after applying plain blocks, was only 8% of that found after 25 hours and was only 17% when unlabelled 2,4-D was used.

It should be noted that following the removal of the donor blocks the ^{14}C contentof each successive 2mm piece of segment became virtually identical. The values were 40-80 dpm and approximately 100 dpm for the plain and unlabelled 2,4-D blocks respectively. This was not true for the basipetal replicates, however, where the initial decreases in the ^{14}C content of each part of the system, in response to the removal of the donor block, were followed by the segment reaching an equilibrium in which each 2mm piece contained a definite proportion of the total radioactive uptake. After 50 hours, for example, the levels were 16, 8, 4, 2, 1 and 0.6% with plain agar blocks and 11, 5, 4, 3, 3 and 2.5% with unlabelled 2,4-D blocks. It would appear that the major portion of the ^{14}C either remained in the replacement blocks or in the receiving blocks.

The radioactivity in the agar blocks which replaced the radioactive donor blocks was monitored at 5-hourly intervals following the change. Data and the results of a statistical analysis are presented in figures 21 and 22 and table 13. Within 5 hours, the plain agar blocks applied to the basal end of the acropetal replicates contained 385 dpm which accounted for 16% of the radioactivity in the system. A further increment of 90 dpm was detected during the following 5 hours, but no significant change in ^{14}C content could be detected over the subsequent 10 hour period. A decrease of 179 dpm during the 45-50 hour period, however, was significant at the 5% level of probability. The export of radioactivity into plain agar blocks applied to the apical ends of the basipetal replicates accounted for 74% of the total radioactivity in the system 10 hours after the change-over, but no further change occurred during the experiment. These data closely fitted a calculated cubic regression curve (dotted line in figures 21 and 22) at the 5% level of probability (table 16).

Export of radioactivity into unlabelled 2,4-D blocks applied to the basal end of the acropetal replicates during the initial 10-15 hours of the replacement period accounted for 31% of the entire radioactivity in the system. The subsequent decline in the ^{14}C level lasted for 5 hours longer than in the corresponding plain agar treatment, but an upward trend (* sign.) was apparent at the 60 hour point. 52% of the radioactivity in the segments of the basipetal replicates supplied with unlabelled 2,4-D blocks was found in the replacement donor blocks 20 hours after the change-over. Further increase did not occur for 15 hours and the quiescent phase corresponded to the trough in the ^{14}C content of the unlabelled 2,4-D blocks applied to the acropetal replicates. Statistical analysis is presented in table 16.

Accumulation of ^{14}C in the plain agar and the unlabelled 2,4-D blocks applied to the basipetal replicates was significantly greater than the accumulation in the acropetal replicates on a number of occasions after the 35 hour point (table 13).

Accumulation of radioactivity in the receiving blocks applied to the control segments, which were given a continuous donation of the radioactive herbicide throughout the experiment, followed the pattern established for the acropetal polarisation of ^{14}C movement. After 60

hours the level of radioactivity in the blocks applied to the apical end of the segments was 23 times greater than that in the blocks applied to the opposite end (figure 23). Orthogonal polynomial regression analysis revealed that the data for the ^{14}C content of the acropetal and basipetal controls was not significantly different from the fitted curves (table 16).

Further consideration of figure 23 reveals that the withdrawal of the radioactive donor blocks after 25 hours had little effect on the export of ^{14}C into the receiving blocks of the acropetal replicates until 30 hours later. Accordingly, the ^{14}C in the controls at the 55 and 60 hour points was up to 73% greater than that in the receiving blocks applied to the plain agar or the unlabelled 2,4-D treatments. These differences proved to be highly significant in t-test analyses (table 14), however, the accumulation following the two treatments was identical. Accumulation of radioactivity in the receiving blocks applied to the basipetal replicates was virtually identical in the control and plain agar treatments.

Replacement of the donor blocks by blocks of unlabelled 2,4-D resulted in a rapid increase in export of radioactivity into the basal receiving blocks. In fact, within 5 hours the level in the control was only 19% of that in the treated replicates. The level of enhancement decreased steadily over the following 15 hours prior to a renewed upsurge. Only the 55 hour reading, however, was greater than the control, but at the 35, 40, 45 and 55 hour readings the level of ^{14}C following the unlabelled 2,4-D treatment was greater than after the plain agar treatment.

The total ^{14}C content of the segments and receiving blocks (figure 24, and table 15) revealed a greater uptake by the acropetal than by the basipetal replicates of the control.

A continuous donation of the radioactive herbicide was necessary for the full potential of acropetal uptake into the segments and receiving blocks to be acquired (figure 24 and table 15). Application of non-radioactive blocks resulted in an immediate cessation of uptake, as might be expected. 33% of the radioactivity taken-up during the initial 25 hours was lost by the end of the experiment when plain agar blocks were used, but no loss occurred when unlabelled 2,4-D blocks were used. The

loss might be explained in terms of experimental error but the data for the total content of radioactivity in the system (donor, segment, and receiving block) showed little signs of radioactive-loss during the experiment. For example, the initial donor supply was 15432 dpm and the experimental system of the acropetal treatments still contained 14772 dpm after 60 hours ie. a 4.3% loss. It is likely, therefore, that the 33% decrease in the level of uptake could be accounted for by an increase in donor block radioactivity. The data for the basipetal replicates presented a more difficult situation in that the withdrawal of the donor blocks led to significant increases in the level of radioactivity detectable in the system at the end of the experiment, in the case of the change over to unlabelled 2,4-D blocks. At this time, the uptake of ^{14}C by the control was only 54% of that by the unlabelled 2,4-D treatment.

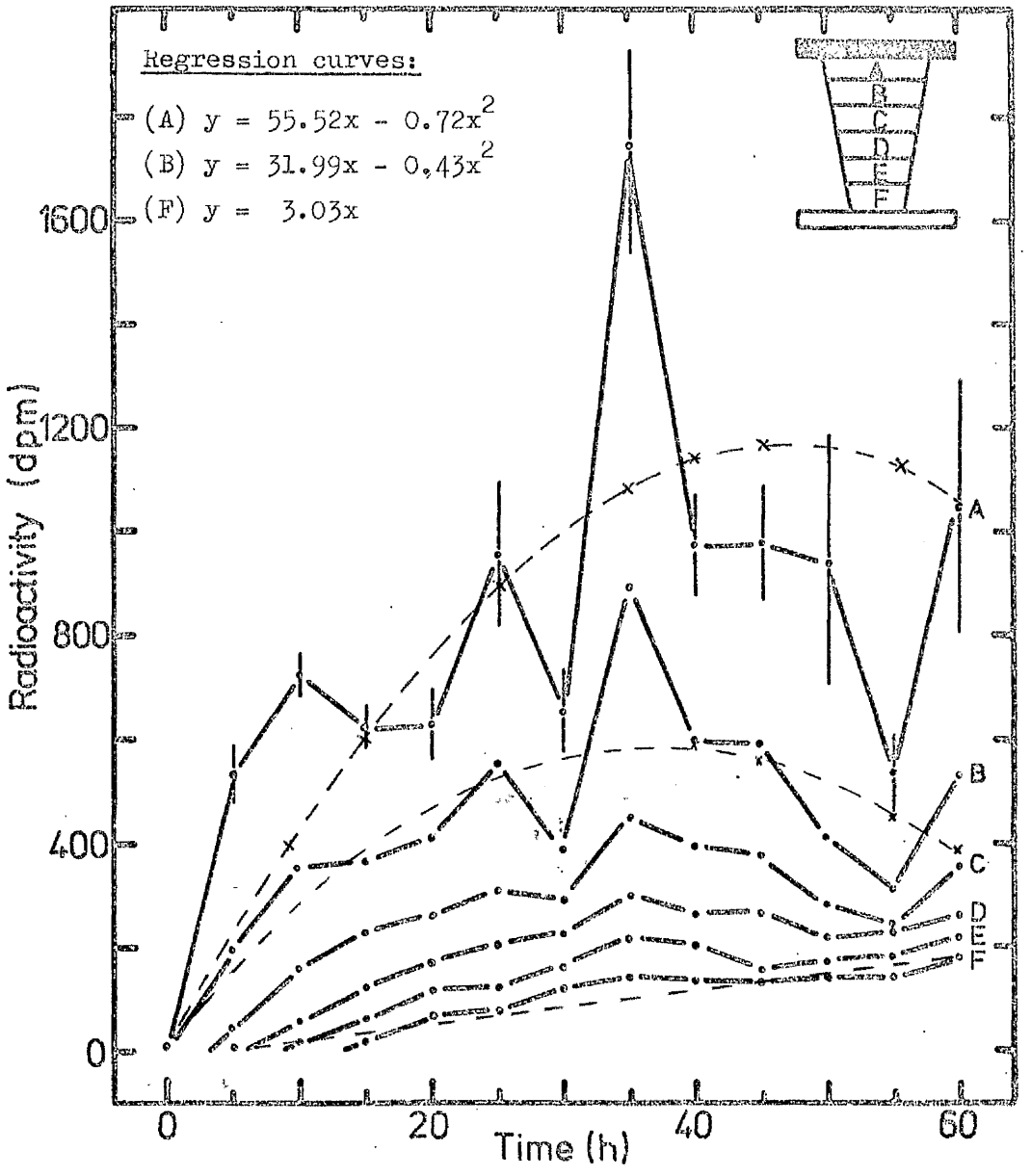
Methanol extracts of the receiving blocks, the replacement blocks and the root segments at the end of the experiment revealed that the only radioactive compounds present ran to Rf values of 0.83-0.85, 0.91-0.92 and 0.72-0.74 in iso-propanol:ammonia:wat er (8:1:1), n-butanol: acetic acid:water (5:1:2.2) and n-butanol:acetone: water (5:2:3) respectively. The stock solution ran to Rf 0.85, 0.90, and 0.70 in the same solvent systems. It would appear unlikely, therefore, that the immobilised radioactivity within the segments was a metabolite of the herbicide.

Clearly, immobilisation of radioactivity within the Pisum root segments was not a factor likely to have a great influence on the characteristics of 2,4-D transport.

FIGURE 18

The distribution of radioactivity along Pisum root segments supplied with 1.0 μM 2,4-D(-1-^{14}C) to the apical or basal cut surfaces at 25^{o}C in darkness. The experiment was carried out on two occasions with a total of eight replicates for each point on the graph and the data are average values.

Dotted lines are fitted regression curves.



Regression curves: (A) $y = 100.98x - 3.42x^2 + 0.03x^3$ (F) $y = 0.29x$

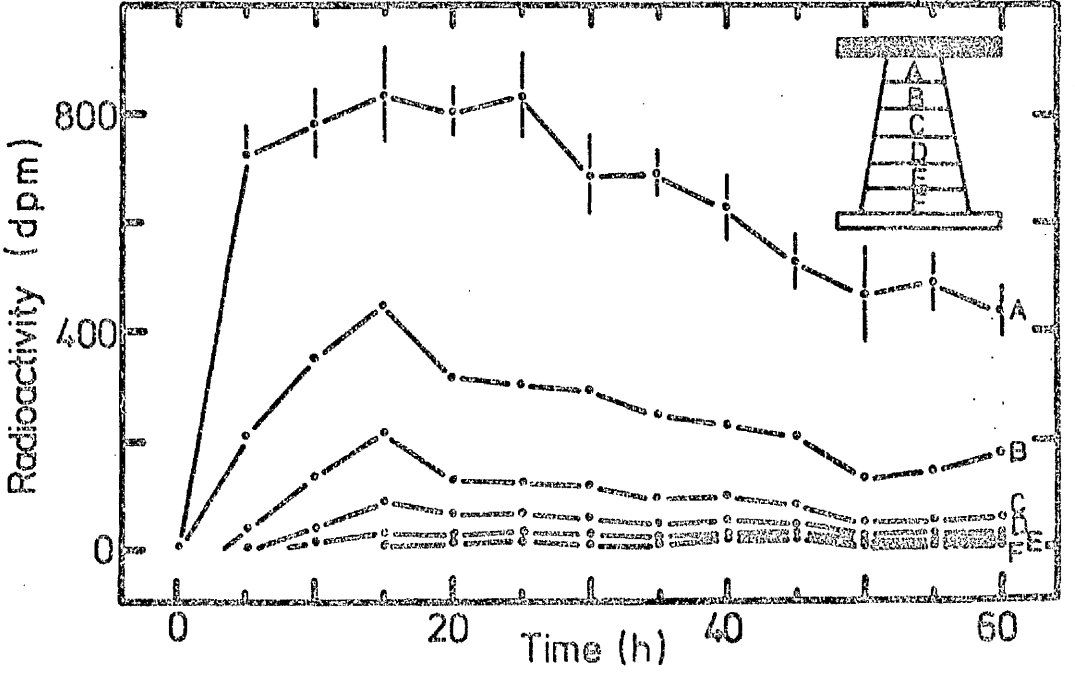


TABLE 10

The standard errors associated with the data presented in figure 18.

Time (h)	Treatment	Standard error of ^{14}C detected along segments (dpm)					
		A	B	C	D	E	F
5	Acrop	0.6	0.3	1.0	4.4	18.7	42.3
	Basip	42.5	17.0	7.2	1.1	1.3	1.0
10	Acrop	3.0	2.3	6.4	14.9	29.7	35.0
	Basip	61.1	32.3	15.2	6.6	6.2	3.0
15	Acrop	2.3	3.0	9.2	12.7	21.8	38.3
	Basip	77.5	47.9	21.8	5.7	4.9	1.7
20	Acrop	25.2	14.1	16.6	25.6	54.5	79.3
	Basip	42.8	20.9	4.6	4.9	4.0	2.9
25	Acrop	10.3	13.6	23.4	38.4	101.7	139.8
	Basip	79.3	37.4	13.6	8.0	5.0	3.1
30	Acrop	13.6	14.7	16.2	22.1	27.5	76.0
	Basip	69.6	28.5	16.4	11.0	5.3	0.9
35	Acrop	16.2	15.7	27.3	37.3	136.3	191.6
	Basip	40.1	49.4	10.6	8.2	3.3	2.4
40	Acrop	23.0	10.7	13.4	35.3	64.6	100.3
	Basip	61.4	21.6	8.0	2.0	6.2	5.4
45	Acrop	14.4	22.7	17.2	29.5	67.2	117.6
	Basip	42.8	33.2	15.9	6.8	2.9	3.9
50	Acrop	25.7	23.7	40.3	54.1	92.6	259.9
	Basip	81.6	13.0	3.3	2.1	0.8	1.3
55	Acrop	19.6	19.7	25.3	24.6	29.0	76.1
	Basip	43.3	8.8	4.6	2.3	1.8	1.4
60	Acrop	41.6	26.7	33.9	45.5	106.6	267.8
	Basip	44.6	30.3	4.4	2.5	3.4	4.5

FIGURE 19

The distribution of radioactivity along Pisum root segments following the replacement of the apical or basal donor blocks of 1.0 µM 2,4-D(-1-^{14}C) by blocks of 1.0 µM unlabelled 2,4-D. The data are the mean of eight replicates carried out on two occasions at 25^{o}C in darkness.

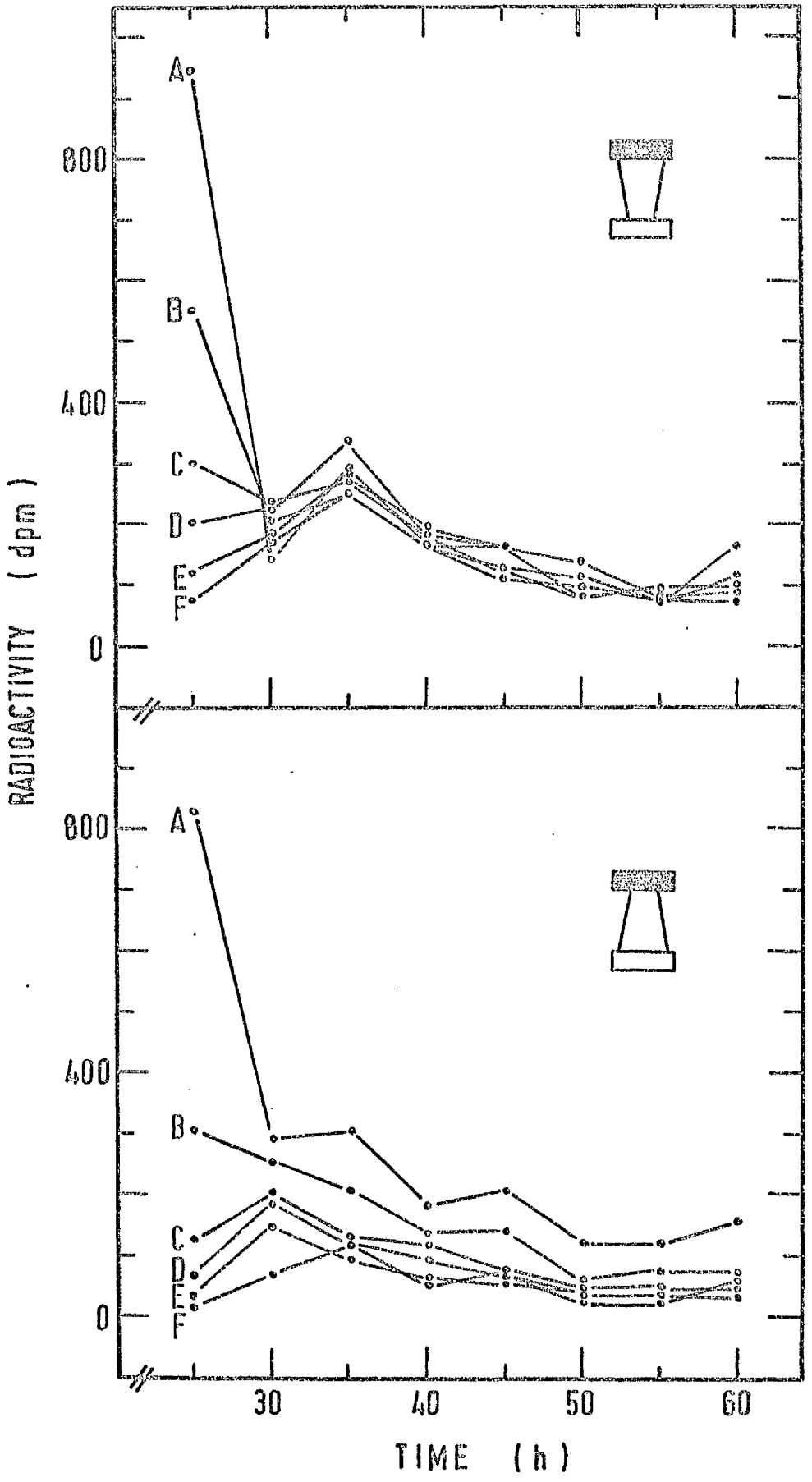

RADIOACTIVITY (dpm)
800
400
0
A
B
C
D
E
F
800
400
0
A
B
C
D
E
F
30
40
50
60
TIME (h)

FIGURE 20

The distribution of radioactivity along Pisum root segments following the replacement of the apical or basal donor blocks of 1.0 μM 2,4-D(-1-^{14}C) by plain agar blocks. Data are the mean of eight replicates carried out on two occasions at 25^{o}C in darkness.

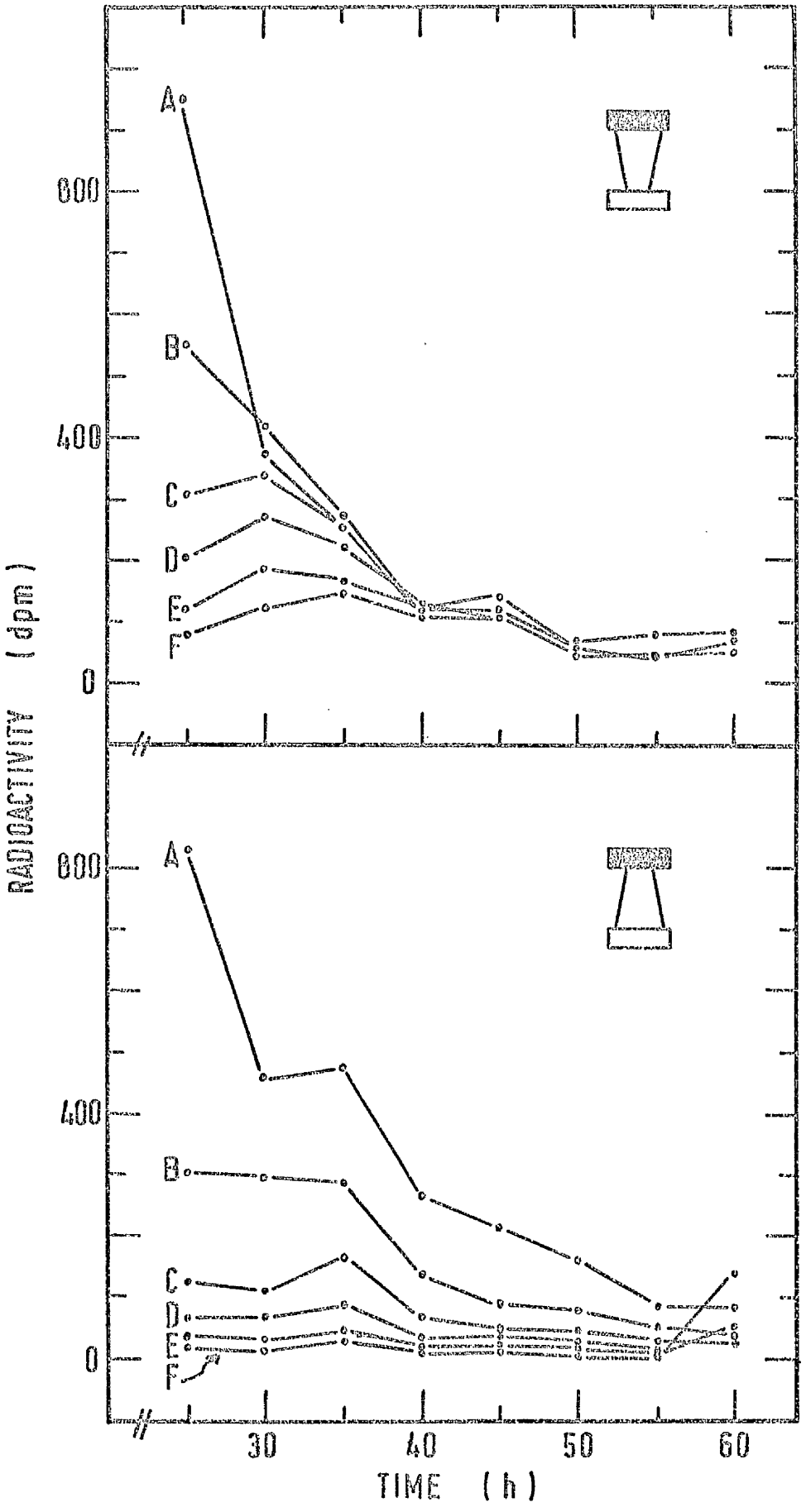

800
400
0
A
B
C
D
E
F
800
400
0
A
B
C
D
E
F
30
40
50
60
RADIOACTIVITY (dpm)
TIME (h)

TABLE 11

The standard errors associated with the data presented in figure 19.

Time (h)	Treatment	Standard error of ^{14}C detected along segment (dpm)					
		A	B	C	D	E	F
30	Acrop	16.6	10.7	14.2	17.6	52.4	84.0
	Basip	78.3	49.5	19.1	8.0	2.8	2.7
35	Acrop	27.3	19.5	18.6	26.5	41.4	41.5
	Basip	30.4	17.1	6.8	2.7	1.5	3.1
40	Acrop	9.0	15.2	11.8	13.6	14.3	15.6
	Basip	70.3	23.3	9.2	3.6	1.5	4.6
45	Acrop	9.1	15.4	17.2	18.9	26.6	31.9
	Basip	60.4	14.5	4.7	2.0	2.2	3.5
50	Acrop	12.1	6.9	5.8	6.3	4.8	8.9
	Basip	51.0	16.4	5.6	3.6	2.3	1.5
55	Acrop	7.1	4.6	3.5	4.5	4.3	6.9
	Basip	21.4	6.2	2.9	2.6	2.5	1.2
60	Acrop	14.0	7.3	5.8	5.1	5.2	8.5
	Basip	37.9	10.3	3.8	5.3	23.0	79.3

TABLE 12

The standard errors associated with the data presented in figure 20.

Time (h)	Treatments	Standard error of ^{14}C along segment (dpm)					
		A	B	C	D	E	F
30	Acrop	29.8	5.7	11.8	16.3	11.5	9.2
	Basip	43.1	10.3	54.3	59.4	63.4	41.8
35	Acrop	43.6	34.2	38.0	38.5	26.6	18.1
	Basip	33.9	16.8	15.7	28.3	25.8	51.2
40	Acrop	20.8	26.6	24.0	23.8	25.4	20.6
	Basip	31.1	10.8	18.5	26.4	17.0	22.9
45	Acrop	13.1	22.4	18.7	19.8	22.2	29.5
	Basip	43.0	23.8	9.7	12.1	17.4	19.3
50	Acrop	24.7	21.3	21.0	17.2	23.2	15.8
	Basip	29.8	7.7	7.5	9.8	8.6	9.4
55	Acrop	10.2	3.5	2.3	5.5	6.7	7.7
	Basip	20.2	6.6	6.5	9.1	8.5	7.3
60	Acrop	8.6	5.8	4.7	8.2	12.0	19.5
	Basip	22.5	10.9	6.4	6.9	19.6	9.0

FIGURE 21

Radioactivity extracted from plain agar blocks which replaced donor blocks of 1.0 μM 2,4-D(-1-^{14}C) at the apical or basal end of Pisum root segments. The data are the mean of eight replicates and the experiments were carried out at 25°C in darkness.

Dotted lines indicate fitted regression curves.

Regression curves:

Acropetal $y = 94.93x - 5.61x^2 + 0.09x^3$

Basipetal $y = 97.82x - 4.71x^2 + 0.07x^3$

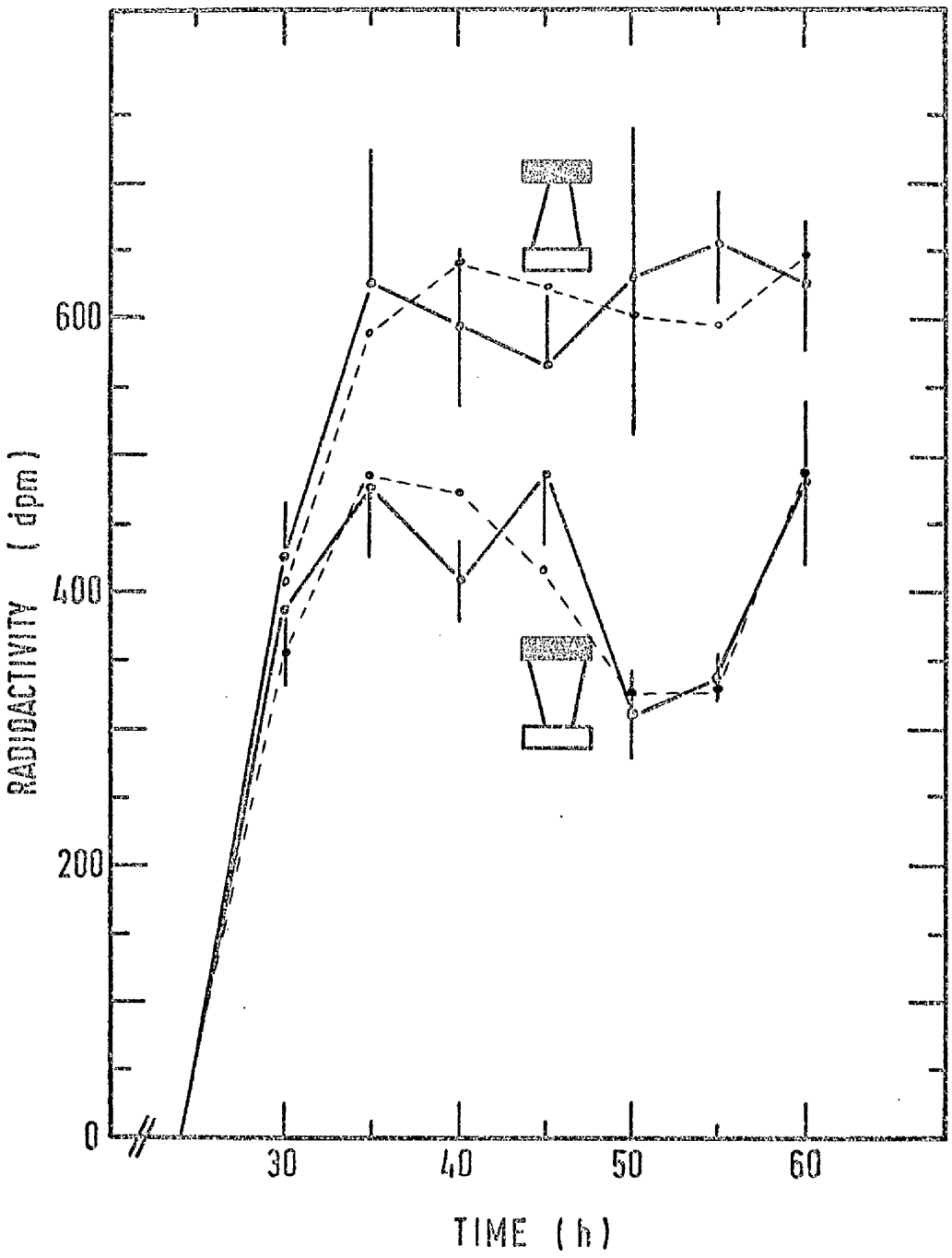


FIGURE 22

Radioactivity extracted from blocks of unlabelled 2,4-D which were used to replace donor blocks of 1.0 μM 2,4-D(-1-^{14}C) at the apical or the basal end of Pisum root segments. The data are the mean of eight replicates and the experiment was carried out at 25°C in darkness.

Dotted lines indicate fitted regression lines.

Regression curves:

Acropetal $y = 84.99x - 5.07x^2 + 0.09x^3$

Basipetal $y = 92.02x - 4.30x^2 + 0.07x^3$

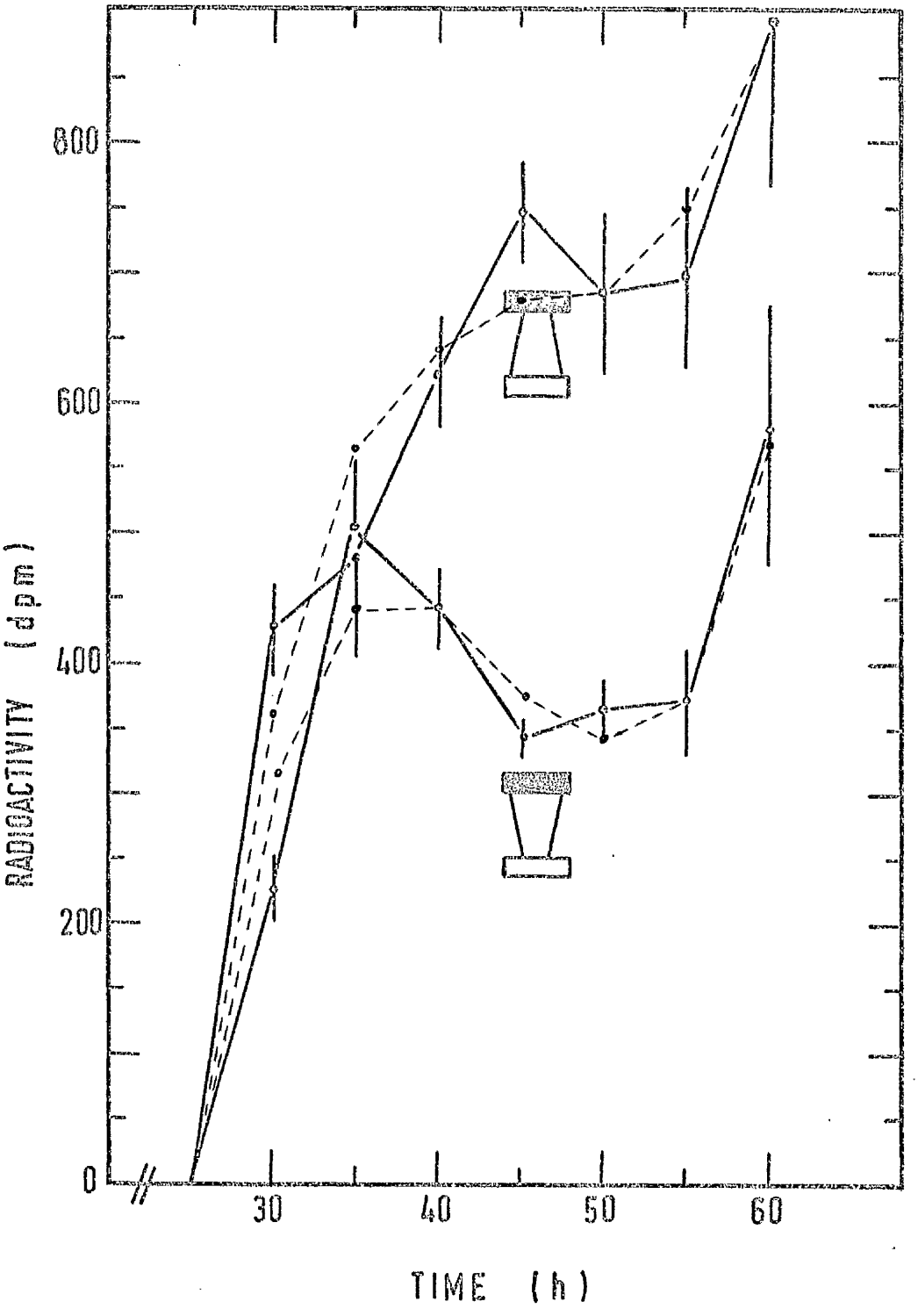


TABLE 13

Paired t-test analysis of ^{14}C content of agar blocks used to replace donor blocks of 2,4-D(-1-^{14}C) after 25 hours (figures 21 and 22). There were eight replicate samples for each treatment.

t with seven degrees of freedom:
* = 2.365 ** = 3.499 *** = 5.405

Time (h)	Comparison of acrop-basip -plain agar blocks		Comparison of acrop-basip -cold 2,4-D blocks	
	t-value	S.E of diff.	t-value	S.E. of diff.
30	-2.833*	1005.6	-2.725*	1715.4
35	-3.766**	2175.9	5.422^{-2}	482.5
40	-5.053**	1974.6	-8.561***	941.8
60	-7.000***	1206.9	-4.162**	3142.7

FIGURE 23

Radioactivity extracted from receiving blocks applied to the apical or basal end of Pisum root segments supplied with 1.0 μM 2,4-D(-1-^{14}C) for 60 hours. In some cases the donor block was removed after 25 hours and replaced by plain agar or blocks containing 1.0 μM unlabelled 2,4-D. Each point on the graph is the mean of eight replicates, each consisting of four root segments supplied with communal donor and receiving blocks.

(A) Control with continuous donation of the radioactive herbicide for 60 hours.

(B) Donation of radioactive herbicide for 25 hours followed by replacement with plain agar blocks.

(C) Donation of radioactive herbicide for 25 hours followed by replacement with unlabelled 2,4-D blocks.

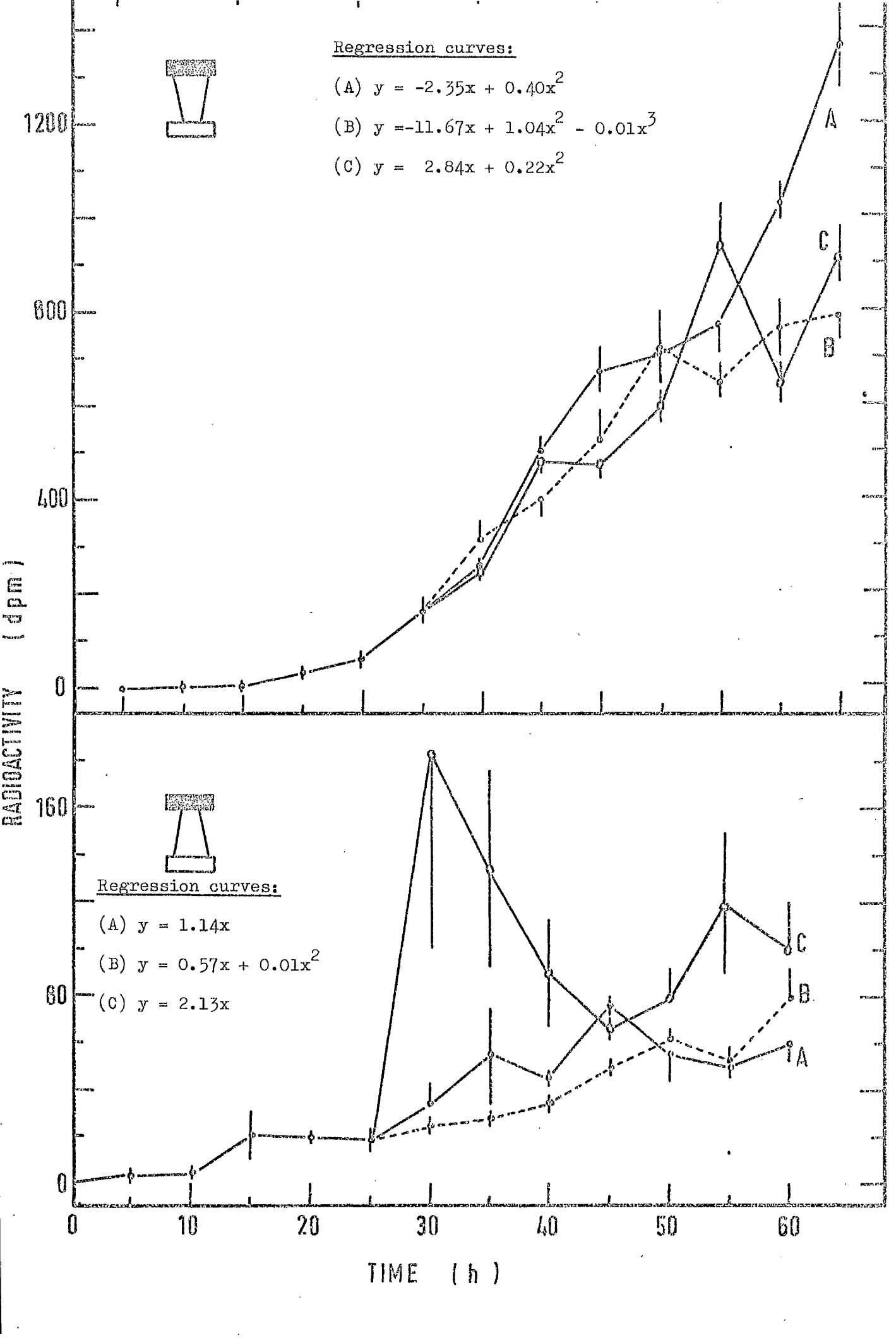

Regression curves:
(A) y = -2.35x + 0.40x²
(B) y = -11.67x + 1.04x² - 0.01x³
(C) y = 2.84x + 0.22x²
A
B
C
1200
800
400
0
RADIOACTIVITY (dpm)
160
60
0
Regression curves:
(A) y = 1.14x
(B) y = 0.57x + 0.01x²
(C) y = 2.13x
A
B
C
0
10
20
30
40
50
60
TIME (h)

TABLE 14

Paired t-test of the data presented in figure 23.

$t_{0.05} = 2.365$ $t_{0.01} = 3.499$ $t_{0.001} = 5.405$ (7 DF)

Time (h)	Treatment	t - values of sample means compared CONTROL- plain agar	CONTROL- 2,4-D	plain agar- 2,4-D
30	Acropetal	-1.54	0.51	1.80
	Basipetal	0.96	-1.88	-2.00
35	Acropetal	3.20*	0.54	-1.76
	Basipetal	1.38	-1.73	-2.62*
40	Acropetal	1.98	3.54**	0.85
	Basipetal	2.55*	-1.99	-2.47*
45	Acropetal	-0.09	1.51	1.70
	Basipetal	3.92**	1.27	-3.01*
50	Acropetal	1.62	-1.63	-3.51**
	Basipetal	-0.64	-1.49	-1.30
55	Acropetal	3.76**	6.30***	1.86
	Basipetal	-0.08	-2.78*	-2.71*
60	Acropetal	5.35**	4.23**	-1.57
	Basipetal	-0.86	-1.87	-0.68

Figure 24

Radioactivity extracted from receiving blocks and Pisum root segments after supplying 2,4-D(-1-^{14}C) to the apical or basal cut surface at 25^{o}C in darkness.

(A) Control with continuous donation of the radioactive herbicide for 60 hours.

(B) Donation of radioactive herbicide for 25 hours followed by replacement with plain agar blocks.

(C) Donation of the radioactive herbicide 25 hours followed by replacement with unlabelled 2,4-D blocks.

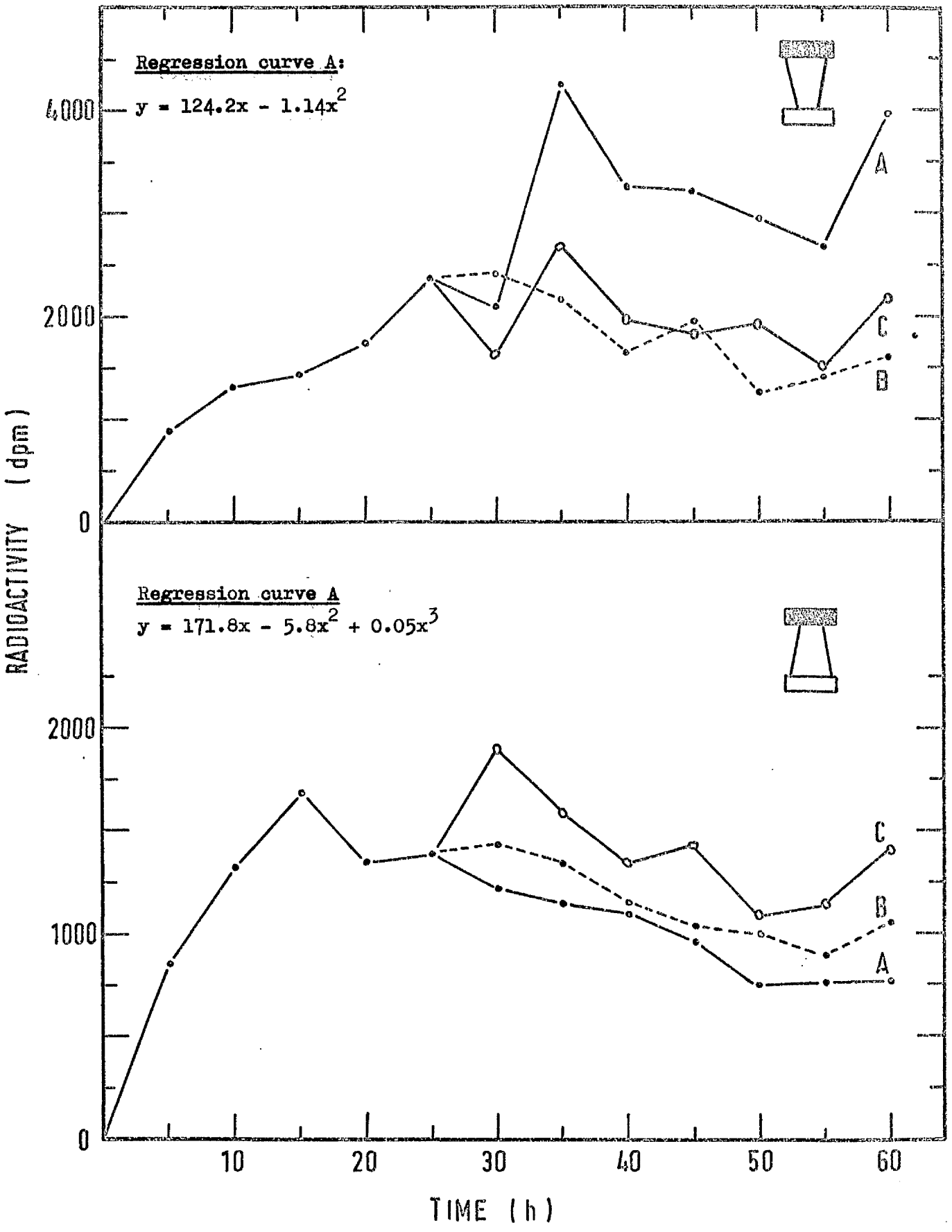

Regression curve A:
y = 124.2x - 1.14x²
4000
2000
0
A
C
B
Regression curve A
y = 171.8x - 5.8x² + 0.05x³
2000
1000
0
C
B
A
10
20
30
40
50
60
RADIOACTIVITY (dpm)
TIME (h)

TABLE 15

Paired t-test for 60 hour data relating to ^{14}C content of root segments and receiving blocks after a continuous donation of 1.0 µM 2,4-D(-1-^{14}C) or after a change to cold 2,4-D or plain agar blocks after 25 hours.

n = 8

Samples compared	t-value	SE of diff. between means
Acrop control-basip control	12.707***	9452.0
" " -acrop cold 2,4-D	9.953***	7419.2
Basip control-basip " "	-5.216**	4476.5
Acrop control-acrop plain agar	9.558***	10746.1
Basip " -basip " "	1.000	2.6^{-8}
Acrop cold 2,4-D-acrop plain agar	6.317***	4568.5
Basip " " -basip " "	3.038*	4386.6

TABLE 16

Results of polynomial regression analyses:

(a) Figure 18 - 2mm of tissue (A) adjacent to donor block of acropetal replicates.

Source	DF	SS	MS	F
linear	1	7837633.84	7837633.84	79.48
quadratic	1	1209632.11	1209632.11	12.27
residual	10	986072.41	98607.24	
total	12	10033338.37		

$y = 55.52x - 0.072x^2$

Time (h)	LSD from fitted line	calc. y	observ. y	diff.	sig.
10	~~720.3~~	483.5	717.5	234.0	-
35	750.3	1064.8	1737.2	672.4	-
55	788.4	884.5	536.2	348.3	-

(b) Figure 18 - second 2mm of tissue (B) of acropetal replicates.

Source	DF	SS	MS	F
linear	1	2319847.58	2319847.58	119.69
quadratic	1	431267.77	431267.77	22.25
residual	10	193816.99	19381.69	
total	12	2944932.34		

$y = 31.99x - 0.43x^2$

Time (h)	LSD from fitted line	calc. y	observed y	diff.	sig.
30	334.1	574.3	385.9	188.3	-
35	332.6	595.0	885.7	290.7	-
55	349.5	464.0	308.1	155.9	-

(c) Figure 18 - 2mm of tissue in contact with receiving block (F) of acropetal replicates.

Source	DF	SS	MS	F
Linear	1	149529.18	149529.18	351.3
Residual	11	4681.87	425.62	
Total	12	154211.05		

$y = 3.03x$

Time (h)	LSD from fitted line	calc. y	observed y	diff.	sig.
25	46.27	75.8	75.6	0.2	-
45	48.15	136.5	129.1	7.4	-
60	50.20	182.0	189.6	7.6	-

(d) Figure 18 - 2mm of tissue (A) adjacent to donor blocks of basipetal replicates.

Source	DF	SS	MS	F
Linear	1	3257847.85	3257847.85	210.20
Quadratic	1	1568430.71	1568430.71	101.19
Cubic	1	446861.57	446861.57	28.83
Residual	9	139491.59	15499.06	
Total	12	5412631.74		

$y = 100.98x - 3.42x^2 + 0.03x^3$

No analysis available to test LSD from regression curve.

(e) Figure 18 - 2mm of tissue (F) in contact with receiving block of basipetal replicates.

Source	DF	SS	MS	F
Linear	1	1349.28	1349.28	10.05
Residual	11	1476.30	134.21	
Total	12	2825.58		

$y = 0.288x$

Time (h)	LSD from fitted line	calc. y	observed y	diff.	sig.
10	25.58	0.28	5.00	4.72	-
30	26.19	8.64	4.30	4.34	-
60	28.18	17.28	9.00	8.28	-

(f) Figure 21 - plain agar replacement blocks - ACROPETAL

Source	DF	SS	MS	F
Linear	1	934549.96	934549.96	267.63
Quadratic	1	164949.42	164949.42	47.24
Cubic	1	103935.71	103935.71	29.76
Residual	4	13967.93	3491.98	
Total	7	1217403.02		

$y = 94.93x - 5.61x^2 + 0.09x^3$

No analysis available to test LSD from fitted regression curve.

(g) Figure 21 - plain agar replacement blocks - BASIPETAL

Source	DF	SS	MS	F
Linear	1	2107961.46	2107961.46	482.55
Quadratic	1	276258.81	276258.81	63.24
Cubic	1	57538.85	57538.85	13.17
Residual	4	17473.57	4368.39	
Total	7	2459232.71		

$y = 97.82x - 4.71x^2 + 0.07x^3$

No analysis available to test LSD from fitted curve.

(h) Figure 22 - unlabelled 2,4-D replacement blocks - ACROPETAL

SOURCE	DF	SS	MS	F
Linear	1	1039468.71	1039468.71	279.33
Quadratic	1	82873.30	82873.30	22.27
Cubic	1	92244.17	92244.17	24.79
Residual	4	14885.05	3721.26	
Total	7	1229471.23		

$y = 84.99x - 5.07x^2 + 0.09x^3$

No analysis to test LSD from fitted curve.

(i) Figure 22 - unlabelled 2,4-D replacement blocks - BASIPETAL

Source	DF	SS	MS	F
Linear	1	2935096.01	2935096.01	593.24
Quadratic	1	145757.37	145757.37	29.46
Cubic	1	55498.54	55498.54	11.21
Residual	4	19790.11	4947.53	
Total	7	3156142.03		

$y = 92.02x - 4.30x^2 + 0.07x^3$

No analysis available to test LSD from fitted curve.

(j) Figure 23 - control receiving blocks - ACROPETAL (A)

Source	DF	SS	MS	F
Linear	1	4493201.53	4493201.53	902.73
Quadratic	1	386947.93	386947.93	77.74
Residual	10	49773.28	4977.33	
Total	12	4929922.75		

$y = -2.35x + 0.40x^2$

Time (h)	LSD from fitted line	calc. y	observed y	diff.	sig.
40	167.0	554.8	678.9	124.1	-
60	191.5	1318.9	1378.9	60.0	-

(k) Figure 23 - control receiving blocks - BASIPETAL (A)

Source	DF	SS	MS	F
Linear	1	21176.68	21176.68	178.37
Residual	11	1305.94	118.72	
Total	12	22482.62		

$y = 1.14x$

Time (h)	LSD from fitted curve	calc. y	observed y	diff.	sig.
35	24.9	39.9	55.8	15.9	-
45	25.4	51.4	76.3	24.9	-

(l) Figure 24 - ACROPETAL CONTROL - radioactivity in segments + receivers.

Source	DF	SS	MS	F
Linear	1	81343857.41	81343857.41	243.96
Quadratic	1	3073409.44	3073409.44	9.22
Residual	10	3334371.66	333437.17	
Total	12	87751638.52		

$y = 124.24x - 1.14x^2$

Time (h)	LSD from fitted line	calc. y	observed y	diff.	sig.
35	1377.8	2948.4	4231.1	1282.7	-
60	1567.9	3339.8	3974.4	634.6	-

(m) Figure 24 - BASIPETAL CONTROL - radioactivity in segments and receivers

Source	DF	SS	MS	F
Linear	1	9508375.40	9508375.40	411.74
Quadratic	1	4744429.38	4744429.38	205.45
Cubic	1	1246279.87	1246279·87	54.00
Residual	9	207839.70	23093.30	
Total	12	15706924.35		

$y = 171.82x - 5.77x^2 + 0.05x^3$

No analysis available to test LSD from fitted curve.

$F_{1,10}$ 4.95 (5% *) 10.04 (1% **) 21.04 (0.1% ***)

DF = degrees of freedom
SS = sum of squares
MS = mean square
F = variance ratio
LSD = least significant difference
calc = calculated
diff = difference
sig = indication of significance from fitted line.

In order to differentiate between two possible mechanisms of translocation ie. diffusion or an energy-requiring process, several investigations were initiated to define the importance of an adequate supply of energy from the roots.

(1) Effect of temperature on 2,4-D movement

The uptake and movement of ^{14}C from 1.0 µM donor blocks of 2,4-D(-1-^{14}C) through subapical segments of Pisum roots was studied at 1°, 5°, 15°, 25°, 35° and 40°C. Three-day-old seedlings were equilibrated at the appropriate temperature for 30 minutes prior to the beginning of the experiments. All experimental manipulations were in green light and the transport periods run in complete darkness. Each sample consisted of four root segments supplied with a communal donor and receiving block and the data are the mean of three experiments carried out on different days.

Uptake of the herbicide ie. the radioactive content of the root segments and receiving blocks, as estimated by scintillation counting of ethanolic extracts, was monitored at five-hourly intervals for 60 hours. The data for each temperature are presented in figure 25. The level of uptake by the acropetal treatments increased throughout the experiments at 25° and 35°C except for decreases after 40 hours and 35-45 hours respectively. At 1°, 5° and 15°C, a phase of uptake over the initial 10-15 hours was followed by little further increase. Increasing the temperature from 1°-5°C had little effect on the uptake of ^{14}C by the basal ends of the segments. A further increase from 5°-15°C resulted in a 21-42% increase in uptake during the experiment, but the 1°C uptake exceeded that at15° by 91 dpm at the 35 hour sampling period. Raising the temperature from 15°-25°C produced a 182% and 231% increase in uptake after transport periods of 30 and 60 hours respectively. The corresponding increases over the range 25°-35°C were 103% and 13%, but the latter value was probably distorted by the fluctuating nature of the data.

The main period of uptake into the apical ends of the segments could be detected over the initial 30 hours of the experiment depending on temperature. The effect of a stepwise elevation of temperature over the range 5^{o}-35^{o}C was to increase the uptake by 1.89, 1.39 and 1.35 times at each step , after a transport period of 20 hours. Increasing the temperature from 5^{o}-15^{o} and 15^{o}-25^{o}C increased the uptake by factors of only 1.08 and 1.06, following a 60 hour period. An increase from 25^{o}-35^{o}C resulted in a slight decrease over the same period.

Uptake at 40^{o}C reached a maximum of 1300 dpm after 15 hours in theacropetal treatments but declined to 904 dpm after 30 hours (figure 25). The basipetal replicates exhibited a maximum after 5 hours before decreasing to 400 dpm after 30 hours.

It is apparent from figure 25 that the ^{14}C content of the segments and receiving blocks, following a 60 hour basal or apical donation of the herbicide, was virtually identical at 1^{o}, 5^{o} and 15^{o}C. However, the acropetal/basipetal ratio at 25^{o} and 35^{o}C were 3.2 and 4.8, indicating that the uptake into the basal end of the segments exceeded that into the apical ends and the difference increased with increasing temperature.

The time-course for the accumulation of radioactivity in receiving blocks applied to the end of the segments opposite to the donor blocks of 1.0 µM 2,4-D(-1-^{14}C) is shown in figure 26. No detectable radioactivity could be found in the receiving blocks of the acropetal or basipetal replicates after a 60 hour experiment at 1^{o} or 5^{o}C. At 15^{o}C, export into apical blocks never exceeded 90 dpm or 30 dpm into basal blocks, which represented an acropetal/basipetal ratio of 3.0. A massive increase in the expression of the acropetal polarity was recorded at 25^{o}C when the ratio was 26.4 and a similar ratio at 35^{o}C represented an accumulation of over 1400 dpm in the acropetal replicates and 55 dpm in the basipetal replicates after an experimental period of 60 hours. The ratio of 2.6 calculated from the receiving block data for 40^{o}C must be viewed with caution until the precise effect of segment death in relation to translocation has been quantitised.

An assessment of the velocity of ^{14}C movement through the segments was based upon the regression lines (see table 17) fitted to the receiving block data presented in figure 26. Knowledge of segment length and the timetaken for the initial detection of radioactivity in the receiving blocks enabled the velocity to be calculated (figure 27A). A maximum velocity of 2.0 mm h^{-1} was recorded for the ^{14}C moving towards the root tip at 40°C, but this must be regarded with caution since root segments became flaccid at this temperature. Similarly, the velocity of 1.66 mm h^{-1} in the basipetal replicates must be suspect. The maximum velocity of radioactivity away from the root tip occurred when the temperature was 25°C but declined with a 10°C increase in temperature. Transport at 35°C resulted in a velocity of 0.8 mm h^{-1} in C^{14} moving towards the root tip.

The flux of radioactivity ie. the quantity moving/unit time, was calculated from the gradient of the fitted regression lines in figure 26. In general, the flux increased as the temperature increased to give a maximum of 25 dpm h^{-1} at 35°C in the acropetal replicates and 4 dpm h^{-1} at 40°C in the basipetal replicates (figure 27B).

The loss of ^{14}C from basally applied donor blocks was studied at each temperature (figure 28) and was found to be 5.1, 4.1, 9.9, 23.6, 65.2 and 45.6% of the original ^{14}C content after transport period of 30 hours at 1°, 5°, 15°, 25°, 35° or 40°C. Over the range from 1°-35°C the losses were 10.5, 17.1, 20.2, 48.2 and 62.2% respectively after transport periods of 60 hours in which the donor blocks were applied to the basal ends of the segments. Following apical donation for 30 hours the losses, over the range 1°-40°C, were 2.2, 0, 21.1, 8.5, 18.1 and 25.3% of the original donor supply. After 60 hours the losses were 11.1, 4.2, 19.7, 17.1 and22.3% over the range 1°-35°C. Thus the ^{14}C loss from the donor blocks applied to the basal ends of the Pisum root segments exceeded that from the apical blocks by 4 times at 5°C and by 2.8 times at 25° and 35°C, whilst the losses at 1° and 15°C were almost the same.

It is clear that the loss of radioactivity from donor blocks, the radioactive content of receiving blocks plus segments and the ^{14}C content of the receiving blocks of the basipetal replicates, at temperatures of 1°-35°C, were closely similar to the 1°-15°C dataof the acropetal replicates. The acropetal replicates exhibited a greater response to temperature increase over the range 15-35°C than basipetal replicates.

FIGURE 25

Time-course of the uptake of radioactivity through the apical and basal ends of Pisum root segments supplied with 1.0 μM 2,4-D(-1-^{14}C) over a range of temperatures from 1° to 40°C. Data are the mean of three experiments carried out on different occasions.

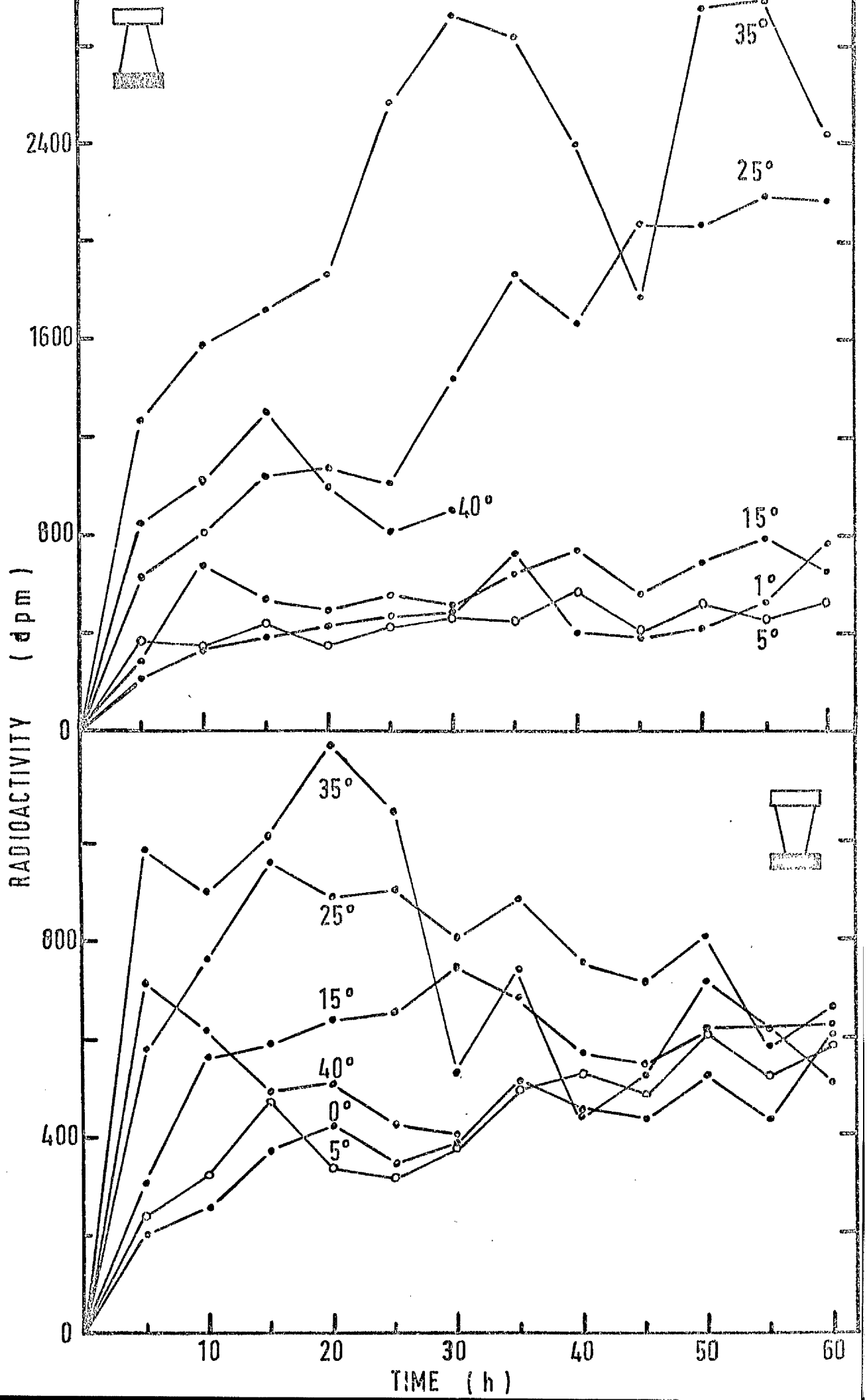

35°
25°
40°
15°
1°
5°
2400
1600
800
0
35°
25°
15°
40°
0°
5°
800
400
0
RADIOACTIVITY (dpm)
10
20
30
40
50
60
TIME (h)

FIGURE 26

Time-course for the ^{14}C content of receiving blocks applied to the apical or basal ends of Pisum root segments supplied with 1.0 µM 2,4-D(-1-^{14}C), over a range of temperatures from 1^{o}-40^{o}C. The data are the mean of three experiments carried out on different days, on each of which every point was the total ^{14}C collected from four replicate segments by a communal receiving block.

Dotted lines indicate fitted regression lines.

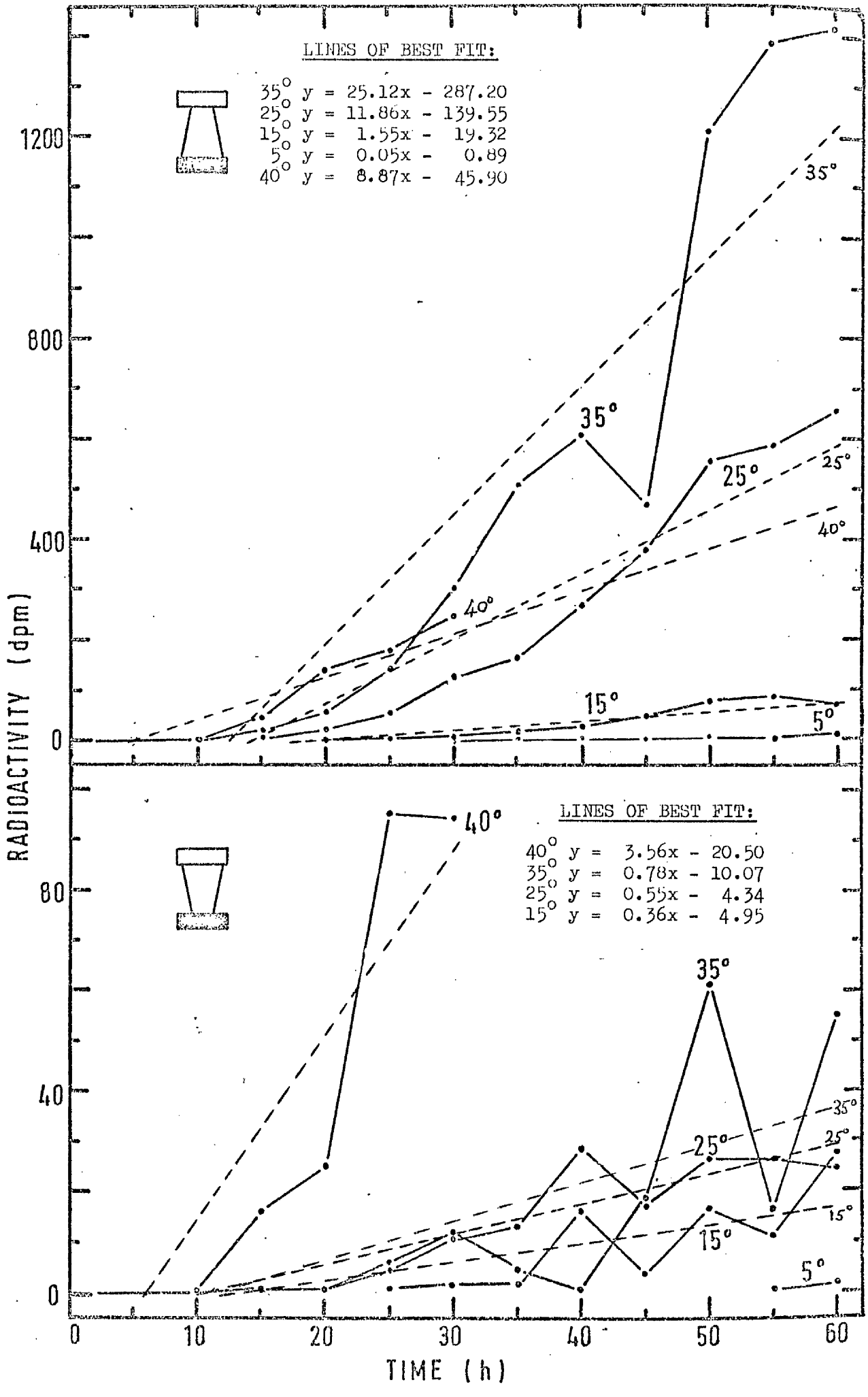
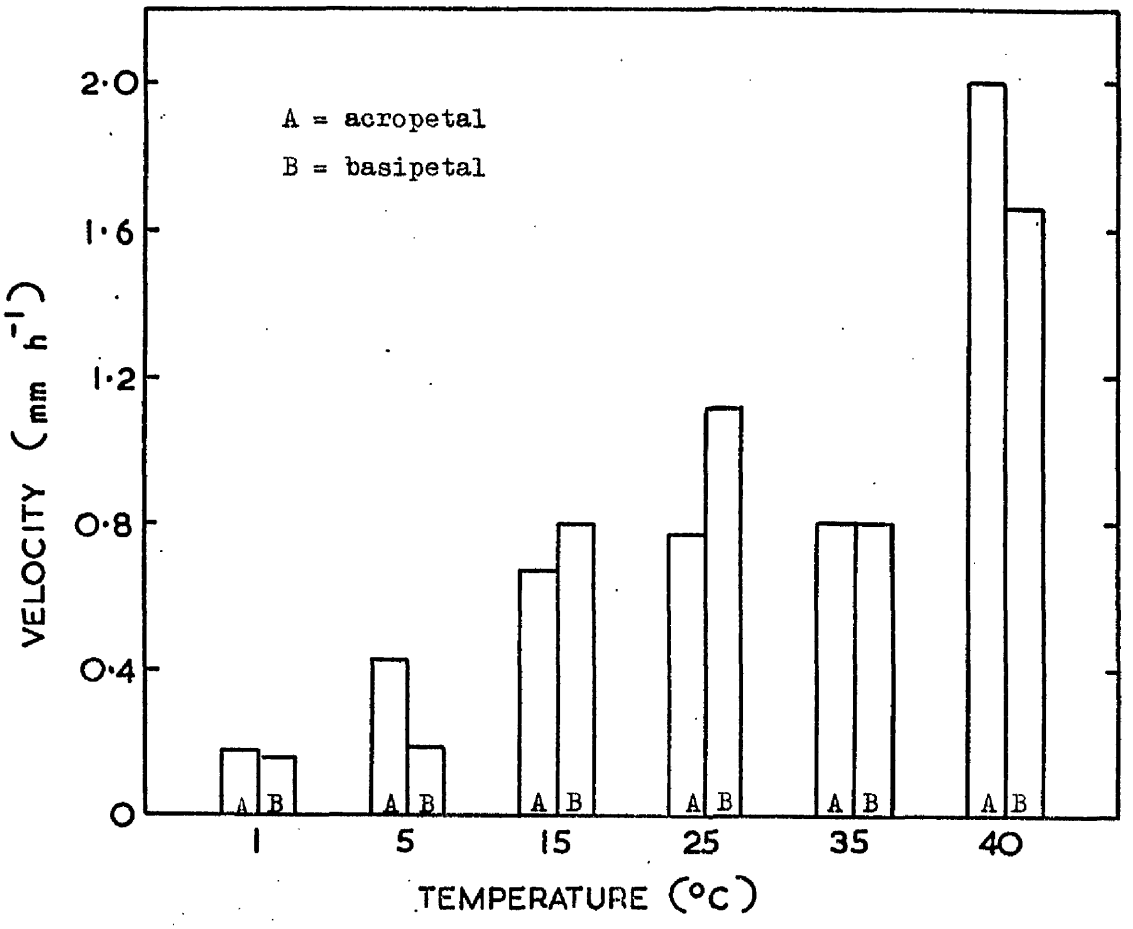

LINES OF BEST FIT:
35° y = 25.12x - 287.20
25° y = 11.86x - 139.55
15° y = 1.55x - 19.32
5° y = 0.05x - 0.89
40° y = 8.87x - 45.90
1200
800
400
0
35°
25°
40°
15°
5°
LINES OF BEST FIT:
40° y = 3.56x - 20.50
35° y = 0.78x - 10.07
25° y = 0.55x - 4.34
15° y = 0.36x - 4.95
80
40
0
0
10
20
30
40
50
60
RADIOACTIVITY (dpm)
TIME (h)

FIGURE 27

(A) The velocity of the radioactivity passing through Pisum root segments supplied with an apical or basal donation of 1.0 μM 2,4-D(-1-^{14}C) over a range of temperatures from 1° to 40°C. Data are from the mean of three experiments carried out on different days with each sample consisting of four root segments supplied with a communal donor and receiving block.

(B) The flux of radioactivity passing through Pisum root segments following the donation of 1.0 μM 2,4-D(-1-^{14}C) to the apical or basal ends, over a range of temperatures from 1° to 40°C. The data are taken from the mean of three experiments (as above).


A = acropetal
B = basipetal
VELOCITY (mm h⁻¹)
2·0
1·6
1·2
0·8
0·4
0
A B
1
5
15
25
35
40
TEMPERATURE (°C)

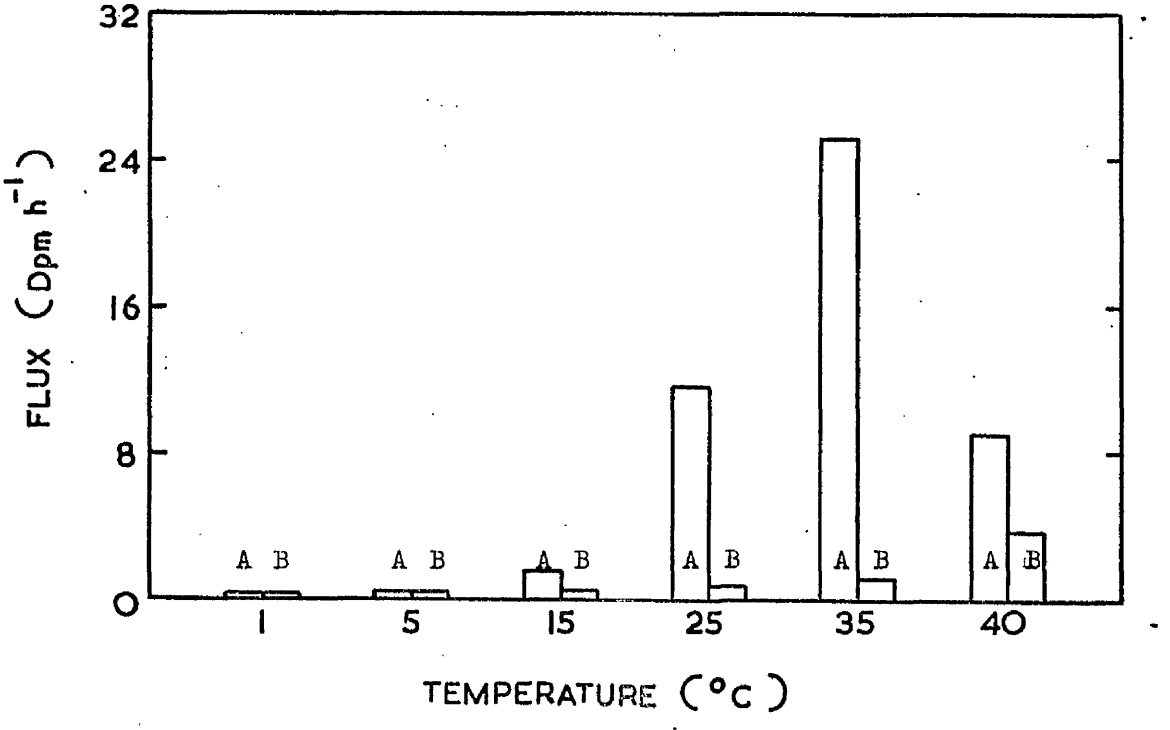

FLUX (Dpm h⁻¹)
32
24
16
8
0
A B
1
5
15
25
35
40
TEMPERATURE (°C)

FIGURE 26

Time-course for the ^{14}C content of donor blocks applied to the apical or basal ends of Pisum root segments over a range of temperatures from 1°-$40^{\circ}C$. The data are the mean of three experiments carried out on different occasions, on each of which every point was the result of ^{14}C uptake by sets of four replicate segments.

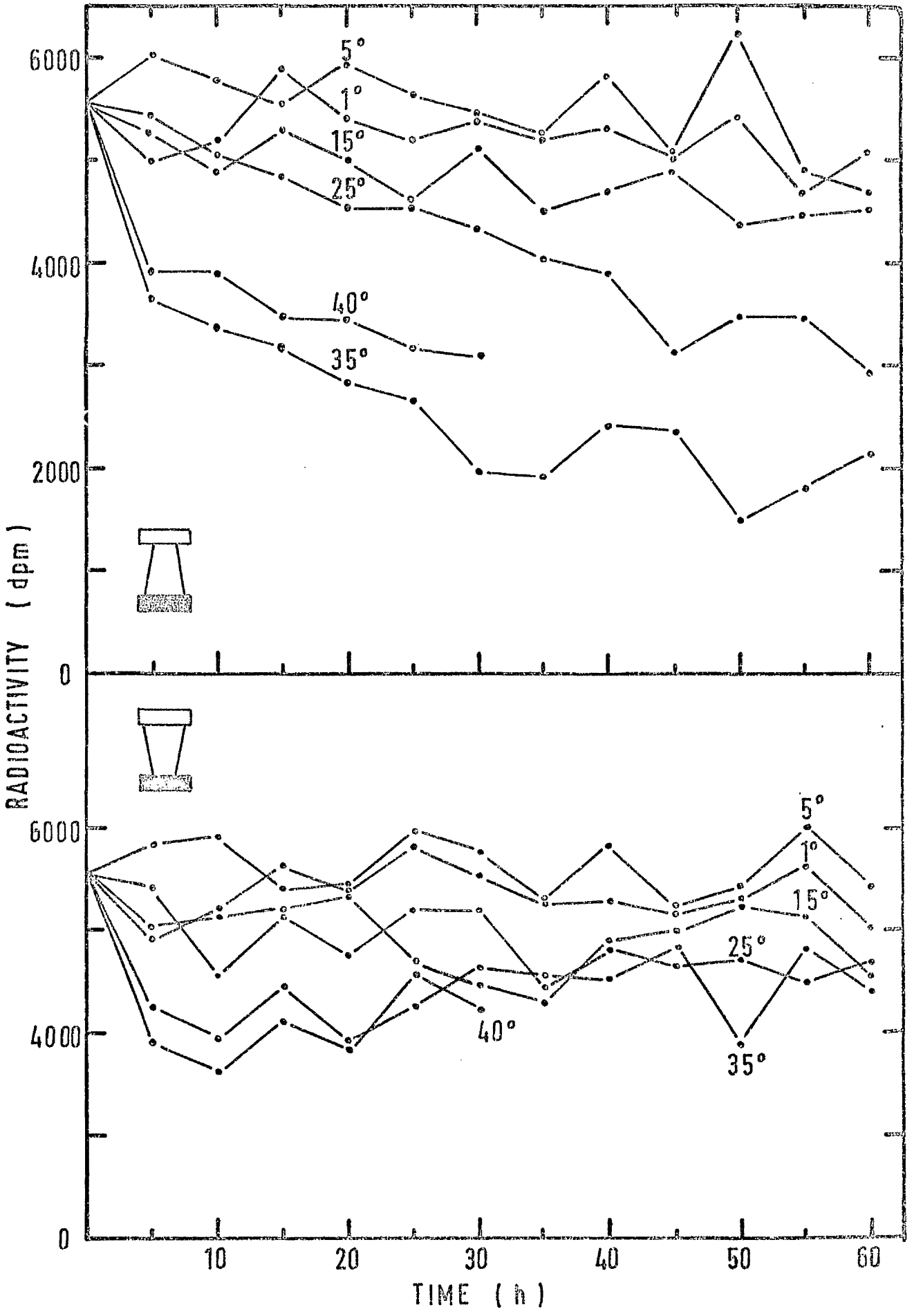

6000
4000
2000
0
RADIOACTIVITY (dpm)
5°
1°
15°
25°
40°
35°
6000
4000
0
5°
1°
15°
25°
40°
35°
10
20
30
40
50
60
TIME (h)

TABLE 17

Linear regressions of data presented in figure 26, for the ^{14}C content of receiving blocks at 5^{o}, 15^{o}, 25^{o}, 35^{o} and $40^{o}C$.

(a) ACROPETAL $5^{o}C$

Source	DF	SS	MS	F
Linear	1	11.42	11.42	3.74
Residual	11	33.58	3.05	
Total	12	45.01		

SE of slope on 11 DF = 2.59^{-2} $F_{0.001} = 19.69$

$\bar{x} = 30$ $\bar{y} = 0.61$

Intercept of regression line on x-axis = 23h, therefore, velocity = 0.43mm h^{-1}

b = 0.05, therefore flux = 0.05 dpm h^{-1}.

(b) ACROPETAL $15^{o}C$

Source	DF	SS	MS	F
Linear	1	10917.43	10917.43	46.07
Residual	11	2606.88	236.99	
Total	12	13524.31		

SE of slope on 11 DF = 0.23 $F_{0,001} = 19.69$

$\bar{x} = 30$ $\bar{y} = 27.15$

Intercept of regression line on x-axis = 15 hours, therefore, velocity = 0.67 mm h^{-1}

b = 1.55, therefore, flux = 1.55 dpm h^{-1}

(c) ACROPETAL 25°C

Source	DF	SS	MS	F
Linear	1	640238.40	640238.40	77.36
Residual	11	91034.96	8275.90	
Total	12	731273.36		

SE of slope on 11 DF = 1.35 $F_{0.001} = 19.69$

$\bar{x} = 30$ $\bar{y} = 216.31$

Intercept of regression line on x-axis = 13 hours, therefore, velocity = 0.77 mm h^{-1}

b = 11.86, therefore, flux = 11.86 dpm h^{-1}

(d) ACROPETAL 35°C

Source	DF	SS	MS	F
Linear	1	2871115.52	2871115.52	59.78
Residual	11	528269.44	48024.49	
Total	12	3399384.96		

SE of slope = 3.25 $F_{0.001} = 19.69$

$\bar{x} = 30$ $\bar{y} = 466.4$

Intercept of regression line on x-axis = 12 hours, therefore, velocity = 0.80 mm h^{-1}

b = 25.12, therefore, flux = 25.12 dpm h^{-1}

(e) ACROPETAL 40°C

Source	DF	SS	MS	F
Linear	1	55082.70	55082.70	40.72
Residual	5	6762.76	1352.55	
Total	6	61845.46		

SE of slope on 5 DF = 1.39 $F_{0.001} = 47.18$

$\bar{x} = 15$ $\bar{y} = 87.2$

Intercept of regression line on x-axis = 5 hours, therefore,

velocity = 2.0 mm h^{-1}

b = 8.87, therefore, flux = 8.87 dpm h^{-1}

(f) BASIPETAL 15°C

Source	DF	SS	MS	F
Linear	1	604.90	604.90	18.12
Residual	11	367.22	33.38	
Total	12	972.12		

SE of slope on 11 DF = 8.56^{-2} $F_{0.001} = 19.69$

$\bar{x} = 30$ $\bar{y} = 5.98$

Intercept of regression line on x-axis = 12.54, therefore,

velocity = 0.80 mm h^{-1}

b = 0.36, therefore, flux = 0.36 dpm h^{-1}

(g) BASIPETAL 25°C

Source	DF	SS	MS	F
Linear	1	1358.28	1358.28	61.04
Residual	11	244.79	22.25	
Total	12	1603.07		

SE of slope on 11 DF = 6.99^{-2} $F_{0.001} = 19.69$

x = 30 y = 12.05

Intercept of regression line on x-axis = 9 hours, therefore velocity = 1.11 mm h^{-1}

b = 0.55, therefore, flux = 0.55 dpm h^{-1}

(H) Basipetal 35°C

Source	DF	SS	MS	F
Linear	1	2757.31	2757.31	12.17
Residual	11	2491.83	226.53	
Total	12	5249.14		

SE of slope on 11 DF = 0.22 $F_{0.001} = 19.69$

x = 30 y = 13.28

Intercept of regression line on x-axis = 12.5 hours, therefore, velocity = 0.80 mm h^{-1}

b = 0.78, therefore, flux = 0.78 dpm h^{-1}

(i) BASIPETAL 40°C

Source	DF	SS	MS	F
Linear	1	8889.33	8889.33	18.79
Residual	5	2365.77	473.15	
Total	6	11255.10		

SE of slope on 5 DF = 0.82 $F_{0.001} = 47.18$

$\bar{x} = 15$ $\bar{y} = 32.96$

Intercept of regression line on x-axis = 6 hours, therefore, velocity = 1.66 mm h^{-1}

b = 3.56, therefore, flux = 3.56 dpm h^{-1}

(ii) Effect of anaerobic conditions and a metabolic inhibitor on 2,4-D transport.

In order to differentiate between an active or a passive movement of 2,4-D through Pisum root segments, the effect of reducing the efficiency of energy-yielding processes was investigated.

Experiments were carried out either in an anaerobic environment of pure, humidified, oxygen-free, nitrogen gas (page 24 and page 28), to prevent aerobic metabolism of the segments, or in air as a control. Sodium fluoride (NaF), a metabolic inhibitor, was incorporated into 1.0 µM donor blocks of 2,4-D(-1-^{14}C) and/or plain agar receiving blocks to provide a working concentration of 2mM NaF. The data for each sampling interval, of 5, 20 and 30 hours, were analysed using a one-way analysis of variance. Experiments were carried out twice on different occasions using two replicate samples for each treatment. Each sample consisted of four root segments supplied with communal donor and receiving blocks. The treatments analysed were:

(1) Air control
(2) Air + NaF applied in receiving block
(3) Air + " " " donor block
(4) Air + " " " donor and receiving block
(5) N_2 control
(6) N_2 + NaF applied in receiving block
(7) N_2 + " " " donor block
(8) N_2 + " " " donor and receiving block

Experiments were carried out at 25°C in darkness. Details of the analysis of variance are presented in table 18.

Histograms representing the levels of radioactivity in the receiving blocks after the donation of 2,4-D(-1-^{14}C) for 20 and 30 hours can be found in figure 29. Little radioactivity was present in apical or basal blocks after a transport period of 5 hours due to the slow rate of ^{14}C movement. A 9% and 67% reduction in ^{14}C content of apically applied blocks in experiments 1 and 2 respectively, following a 30 hour exposure to N_2, was significantly different from the air control at the 0.1% level of probability (figure 29b, treatments 1, 5).

N_2 in association with NaF in the donor and receiving blocks gave a mean reduction of 93.8% (***) after 20 hours and 77.5% (***) after 30 hours (figures 29a,b, treatments 4,8). Incorporation of the inhibitor in the donor block only produced no significant difference between the air and nitrogen treatments after 20 hours (figure 29a, treatments 3,7), but produced a 67.1% decrease in apical block content after 30 hours (figure 29b, treatments 3,7). On the other hand, application of NaF in apical receiving blocks only, yielded a 168.7% increase in receiving block content of ^{14}C after a 20 hour exposure to nitrogen (figure 29a, treatments 2,6), whilst after 30 hours no significant difference could be detected (figure 29b, treatments 2,6).

The data for 30 hour transport periods in air show that NaF in the donor and receiving blocks stimulated the level of ^{14}C in the receiving blocks (figure 29b, treatments 1,3,4), whilst its presence in receiving blocks only produced a decreased block content (figure 29b, treatments 1,2). Similarly, air/NaF in receiving + donor blocks produced a 304.2% increase (***) in receiving block content of ^{14}C after a 20 hour transport period (figure 29a, treatments 1,4). No consistent effect was detected on application of the compound under anoxic conditions (figures 29a,b, treatments 5,6,7,8).

The level of radioactivity in receiving blocks applied to the basal end of Pisum root segments was not affected by a 30 hour exposure to air or N_2 (figure 29d, treatments 1,5). Significant increases (***) in ^{14}C were detected after applying NaF in donor + receiving blocks for 30 hours in air or nitrogen (figure 29d, treatments 1,4 and 2,8). A 79.2% decrease in receiving block radioactivity was recorded after a transport period of 30 hours with the N_2/NaF in receiving block treatment (figure 29d, treatments 2,6). The remaining treatments produced no significant effect.

Extraction of radioactivity from the root segments and associated receiving blocks revealed that neither anaerobic conditions nor NaF had a significant effect on the total uptake of ^{14}C in the basipetal replicates at any time (figure 31), or in the acropetal replicates after 5 or 20 hours. After 30 hours in the acropetal replicates, however, the N_2/NaF in donor and N_2/NaF in donor + receiving block treatments produced 50% and 47%

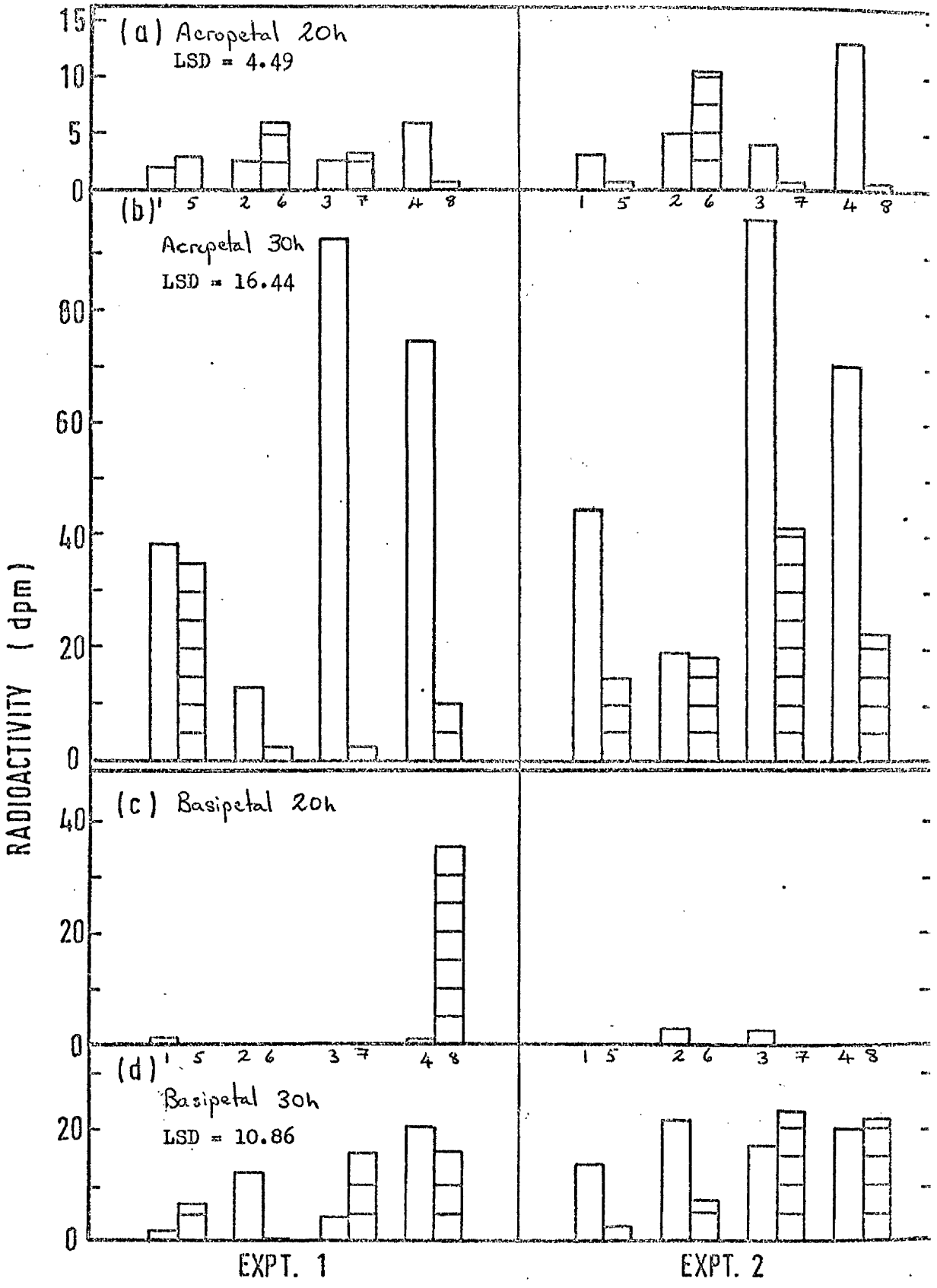
reductions respectively (figure 30c, treatments 3,7 and 4,8). These corresponded to 67% and 77% reductions in receiving block content in response to the same treatments. A 50% increase in the level of ^{14}C on application of NaF in the donor block was significant at the 0.1% level of probability (figure 30c, treatments 1,3). The remaining treatments produced no significant change in the radioactive content of the segments and receiving blocks.

These data indicate that inhibition of ^{14}C transport imposed by anoxic conditions may be antagonised by a promotory effect of NaF on the transport system.

FIGURE 29

Radioactivity in receiving blocks applied either to the apical or the basal end of Pisum root segments supplied with 1.0 µM 2,4-D(-1-^{14}C) for 20 or 30 hours at 25°C in darkness. The experiment was carried out on two different occasions using two replicate samples /treatment. Each sample consisted of four root segments supplied with communal donor and receiving blocks.

1 = air control
2 = air + NaF in receiving block
3 = air + " " donor block
4 = air + " " " + receiving block
5 = N_2 control
6 = N_2 + NaF in receiving block
7 = N_2 + " " donor block
8 = N_2 + " " " + receiving block


(a) Acropetal 20h
LSD = 4.49
(b) Acropetal 30h
LSD = 16.44
(c) Basipetal 20h
(d) Basipetal 30h
LSD = 10.86
RADIOACTIVITY (dpm)
EXPT. 1
EXPT. 2
1 5 2 6 3 7 4 8

Radioactivity in receiving blocks + root segments of acropetal replicates supplied with 1.0 μM 2,4-D(-1-^{14}C) for 5, 20 or 30 hours at 25°C in darkness. The experiment was carried out on two different days using two replicate samples/treatment on each occasion. Each sample consisted of four root segments supplied with communal donor and receiving blocks.

1 = air control
2 = air + NaF in receiving block
3 = air + " " donor block
4 = air + " " " + receiving block
5 = N_2 control
6 = N_2 + NaF in receiving block
7 = N_2 + " " donor block
8 = N_2 + " " " + receiving block

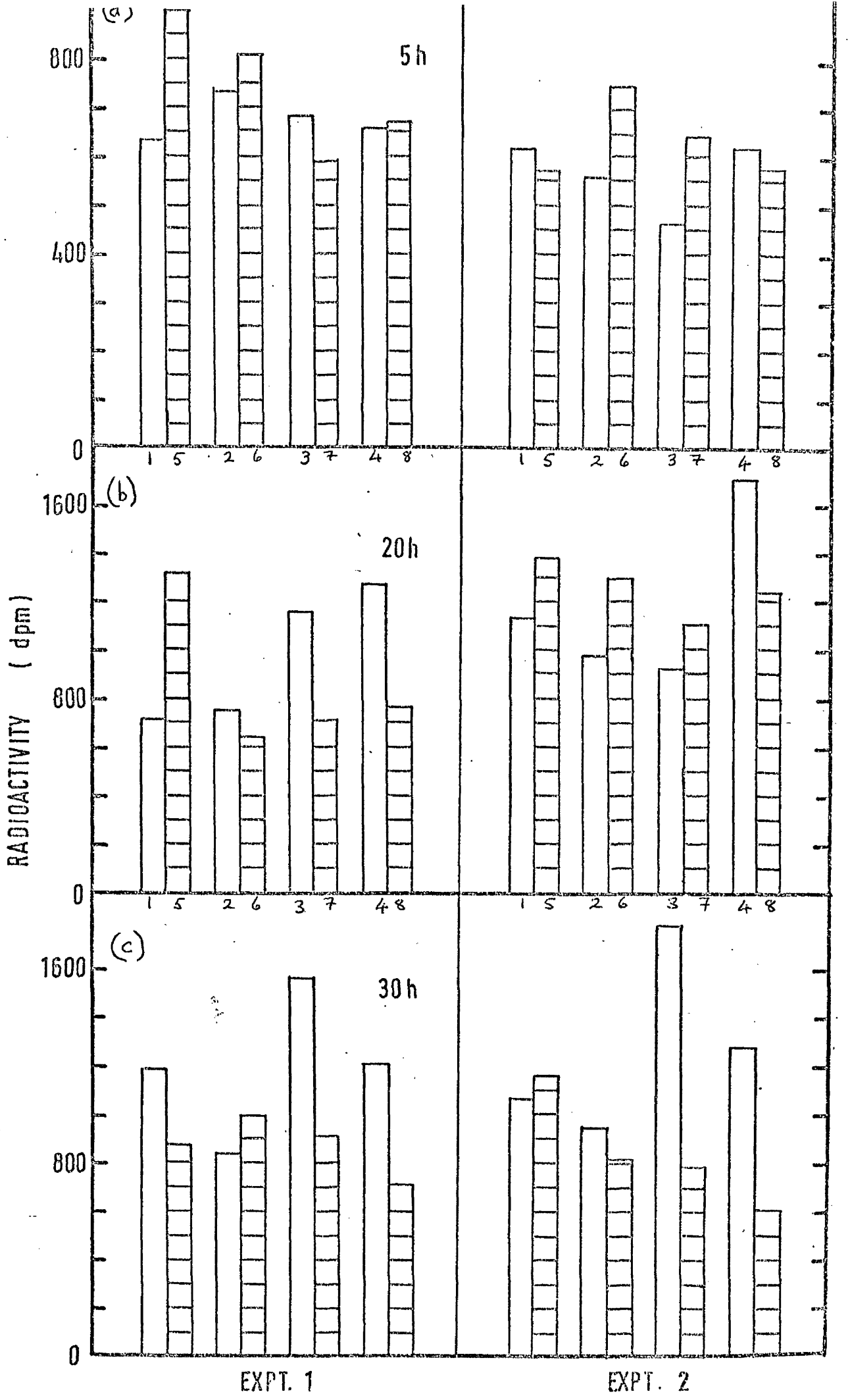

(a)
5h
800
400
0
1 5 2 6 3 7 4 8
(b)
20h
1600
800
0
(c)
30h
1600
800
0
RADIOACTIVITY (dpm)
EXPT. 1
EXPT. 2

Figure 31

Radioactivity in receiving blocks + tissue of basipetal replicates supplied with 1.0 μM 2,4-D(-1-^{14}C) for 5, 20 or 30 hours at 25°C in darkness. The experiment was carried out on two different days using two replicate samples/treatment on each occasion. Each sample consisted of four root segments supplied with communal donor and receiving blocks.

1 = air control
2 = air + NaF in receiving block
3 = air + " " donor block
4 = air + " " " + receiving block
5 = N_2 control
6 = N_2 + NaF in receiving block
7 = N_2 + " " donor block
8 = N_2 + " " " + receiving block

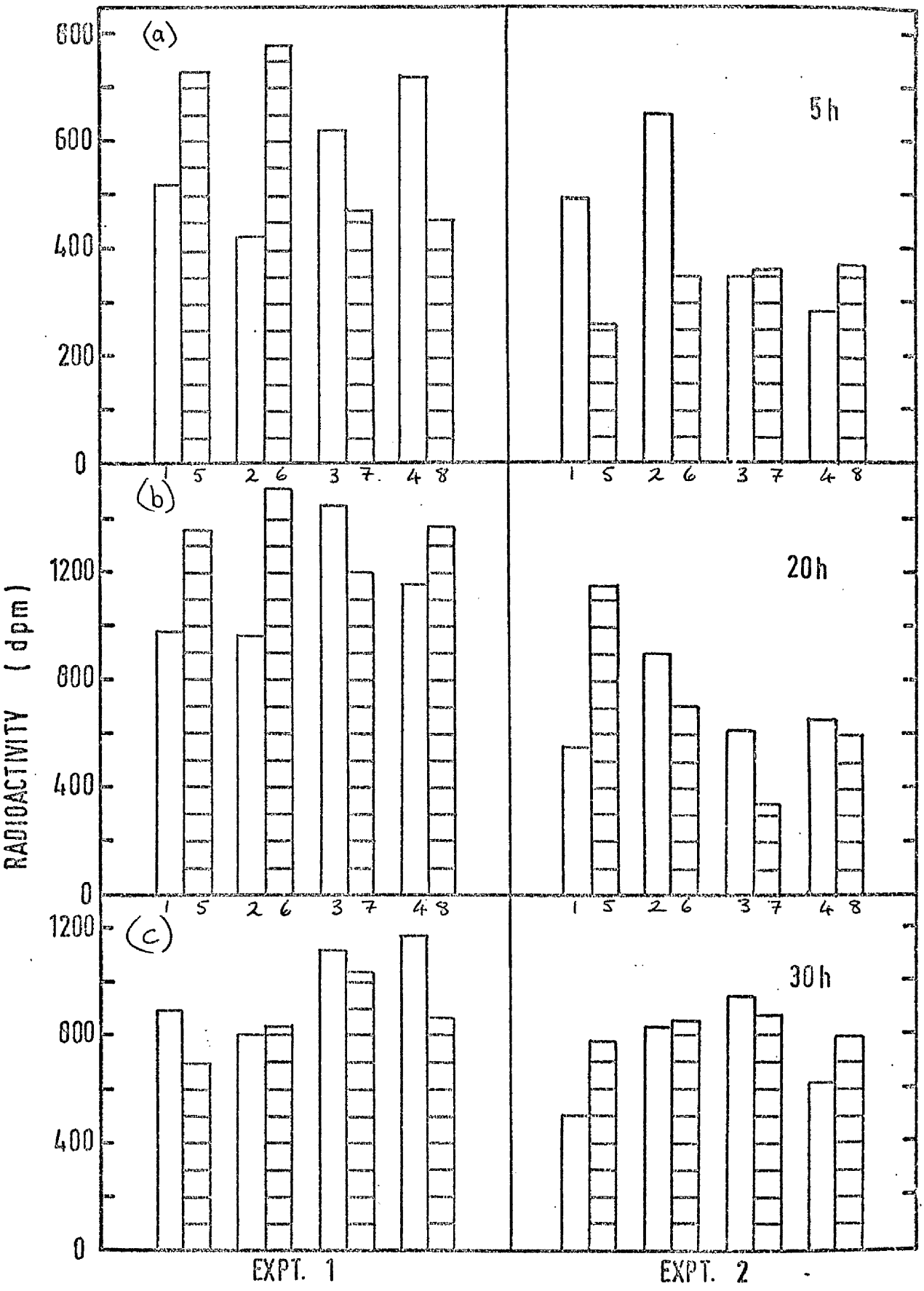

(a)
(b)
(c)
5h
20h
30h
RADIOACTIVITY (dpm)
0
200
400
600
800
1200
1 5 2 6 3 7 4 8
EXPT. 1
EXPT. 2

TABLE 18

Analysis of variance

$F_{0.001}$ = 5.23 $t_{0.001}$ on 24 DF = 3.745

(i) Acropetal-20h receiving block content

Source	DF	SS	MS	F
Between groups	7	315.0	45.00	15.90
Within groups	24	67.9	2.83	
Total	31	382.9		

SE = 1.2 LSD = 4.49

(ii) Acropetal-30h receiving block content

Source	DF	SS	MS	F
Between groups	7	27735.30	3962.18	102.68
Within groups	24	926.10	38.59	
Total	31	28661.40		

SE = 4.39 LSD = 16.44

(iii) Basipetal-30h receiving block content

Source	DF	SS	MS	F
Between groups	7	1401.70	200.20	12.1
Within groups	24	396.40	16.51	
Total	31	1798.10		

SE = 2.90 LSD = 10.86

(iv) Acropetal-5h ^{14}C content of tissue + receiving block

Source	DF	SS	MS	F
Between groups	7	151592.20	21627.46	2.98 NSD
Within groups	24	174124.10	7255.17	
Total	31	325516.30		

SE = 60.2 LSD = 225.45

(v) Acropetal-20h ^{14}C content of tissue + receiving block

Source	DF	SS	MS	F
Between groups	7	1413647.30	201949.61	4.38 NSD
Within groups	24	1106568.20	46107.01	
Total	31	2520215.50		

SE = 151.83 LSD = 568.60

(vi) Acropetal-30h ^{14}C content of tissue + receiving block

Source	DF	SS	MS	F
Between groups	7	2729072.40	389867.50	56.5
Within groups	24	165597.50	6899.90	
Total	31	2894669.90		

SE = 58.7 LSD = 219.83

(vii) Basipetal-5h ^{14}C content of tissue + receiving block

Source	DF	SS	MS	F
Between groups	7	77057.70	11008.20	0.4 NSD
Within groups	24	702196.30	29258.20	
Total	31	779254.00		

SE = 121.0 LSD = 45.31

(viii) Basipetal-20h ^{14}C content of tissue + receiving block

Source	DF	SS	MS	F
Between groups	7	736514.40	30688.10	0.1 NSD
Within groups	24	2844756.10	406393.70	
Total	31	3581270.50		

SE = 450.8 LSD = 168.82

(ix) Basipetal-30h ^{14}C content of tissue + receiving block

Source	DF	SS	MS	F
Between groups	7	367922.30	52560.30	2.50 NSD
within groups	24	500837.50	20868.20	
Total	31	868759.80		

SE = 102.1 LSD = 382.36

DF ..... degrees of freedom
SS ..... sum of squares
MS ..... mean square
F ..... variance ratio
SE ..... standard error
LSD ..... least significant difference
NSD ..... no significant difference

(iii) Effect of segment death on 2,4-D transport

A further test of whether the acropetal polarity of 2,4-D(-1-^{14}C) movement was a product of a metabolically regulated process or merely due to the physical properties of the frustum geometry was carried out using root segments killed by steam treatment.

1.0 μM labelled herbicide was supplied to the apical or basal ends of the dead segments for a period of 20 hours at 25° ± 1°C in white light. Segments were turgid at the beginning of the experiment but did not give a red colouration in a tetra-azolium chloride test, which indicated that the segments were dead. By the end of the experiment, the segments were flaccid and collapsed slightly under the weight of the receiving blocks. The data are presented in figure 32 and table 19.

Radioactivity extracted from receiving blocks applied to the apical end of the living segments of Pisum roots was 92% lower than that in blocks applied to dead segments. A value of 89% was obtained following application of the blocks to the basal end of the segments. The observation that the dead segments still maintained a highly significant acropetal polarity of ^{14}C movement was of paramount importance. In fact, the ratio of acropetal to basipetal movement in the control and dead segments was approximately 1.5 and 2.1 respectively. This indicated that the accumulation in the apical receiving blocks was more than twice that in the basal blocks.

Further examination of the data showed that the dead segments contained lower levels of radioactivity than the living segments following a donation of the carboxyl labelled compound. Segments receiving a basal application achieved only 64% of the ^{14}C content of the living segments and the value after apical donation was 70%.

It is apparent that the overall uptake of radioactivity from the donor blocks was decreased by killing the segments, whilst the flux through the tissue was increased. The important discovery was the fact that the dead segments were capable of supporting a stronger acropetal polarity of ^{14}C transport than the living segments of the control.

FIGURE 32

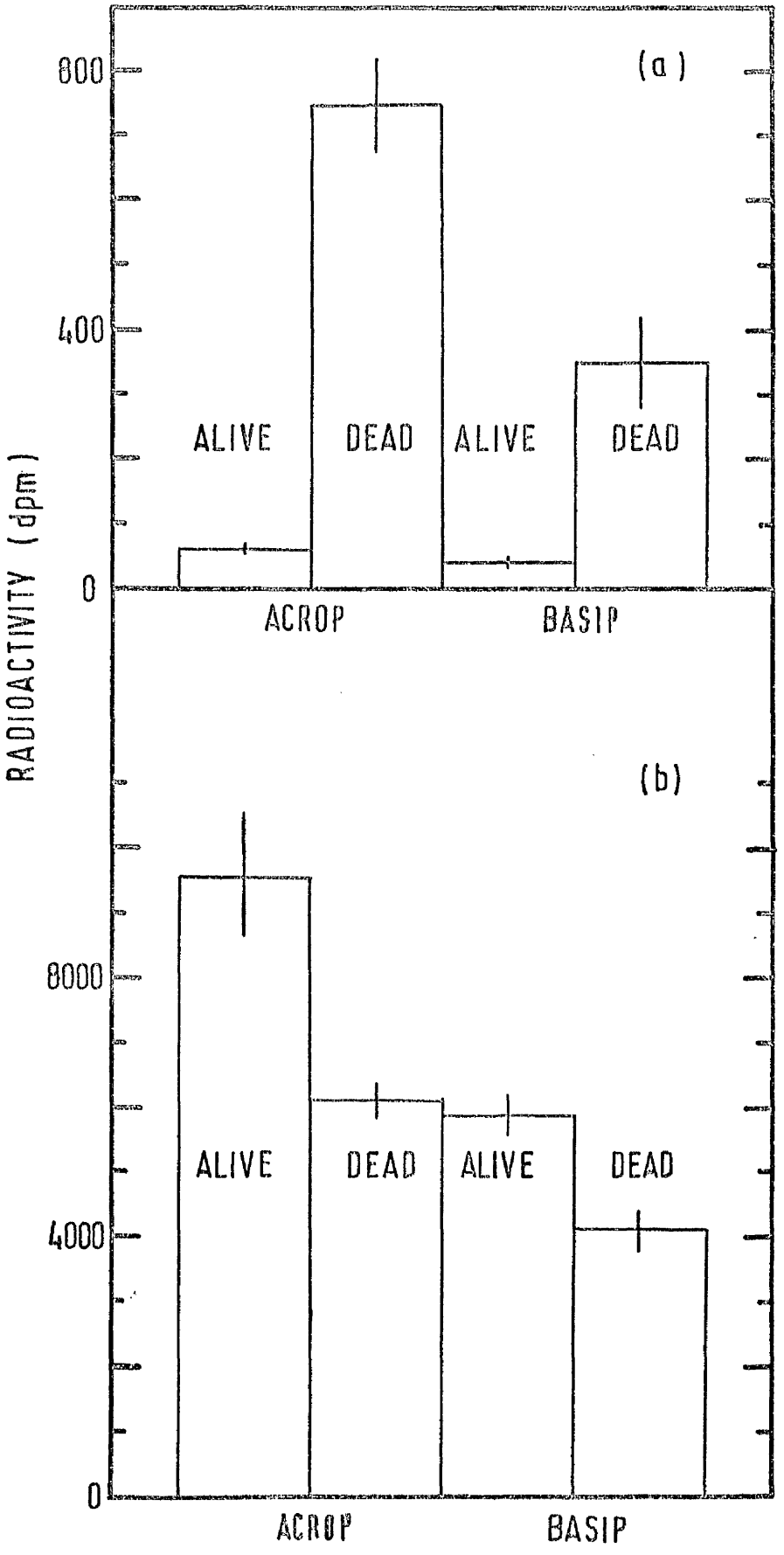
(a) Radioactivity extracted from apical and basal receiving blocks applied to living (control) or dead Pisum root segments supplied with 1.0 μM 2,4-D(-1-^{14}C) at 25°C in white light. The data are the mean of eight replicates, each consisting of four root segments.

(b) Radioactivity extracted from Pisum root segments, living or dead, supplied with radioactive 2,4-D at 25°C in white light. The data are the mean of eight independent replicates.

TABLE 19

Statistical analysis of the data presented in figure 32.
$t_{0.05}$ on seven degrees of freedom = 2.365 $t_{0.01}$ = 3.499 $t_{0.001}$ =5.405

Sample means compared	t-values	
Receiving blocks		
Acrop-control dead	***	-12.64
Basip- " "	**	-4.70
Acrop-control--basip control		2.29
Acrop dead--basip dead	**	4.57
Tissue		
Acrop-control dead	**	3.85
Basip- " "	**	4.24
Acrop control--basip control	**	4.16
Acrop dead--basip dead	**	4.48


(a)
800
400
0
ALIVE
DEAD
ALIVE
DEAD
ACROP
BASIP
RADIOACTIVITY (dpm)
(b)
8000
4000
0
ALIVE
DEAD
ALIVE
DEAD
ACROP
BASIP

(iv) Effect of an additional utilisable substrate on 2,4-D transport

Interest in the movement of the radioactive herbicide through Pisum root segments over relatively long periods of time (60hours) necessitated a further investigation into the requirement for active metabolism of the tissue during translocation. Since it could be argued that normal metabolic and translocation pathways might become less efficient due to the exhaustion of energy reserves, sucrose was added to the agar blocks as a potential source of energy.

Sucrose was incorporated into the donor and not the receiving blocks to give a final concentration of 0.76% or 1.53%, so as to exert the same osmotic pressures within the experimental system. The donor block concentration was 1.0 µM 2,4-D(-1-^{14}C) and the experiment was carried out on three different occasions on a total of eight replicate samples, each containing four root segments. As an added precaution, a further set of controls were run, in which mannitol, a non-metabolisable substrate, was incorporated into the donor blocks to provide an osmotic effect comparable to that of the sucrose. The concentrations were 0.40% and 0.80% (w/v) respectively. After experimental periods of 20 and 50 hours at 25°C the radioactivity within the root segments and the receiving blocks was determined by scintillation counting. The data are presented in figures 33,34 and table 20.

Treatment with donor blocks containing 0.76% sucrose produced a 388 dpm increase (*) inthe uptake of radioactivity through the basal end of the segments over a 20 hour period. The corresponding mannitol treatment was not significantly different from the control, indicating that the sucrose effect was most probably not caused by osmotic tension. A 411 dpm reduction (***) in uptake in response to the higher concentration of sucrose could well have been due to an osmotic effect, since the corresponding mannitol treatment was reduced similarly. These effects were reflected by the levels of radioactivity within the root segments, but no significant changes were detected in the receiving block ^{14}C content. A similar situation was found in the basipetal replicates where the low sucrose concentration produced a highly significant 42% increase in uptake, whilst the mannitol treatments showed a decrease below the level of the control (*). The higher concentration of sucrose, however, led to a 9% increase in uptake which, in common with the equivalent mannitol treatment, was not significantly different from

from the control. These trends in the pattern of uptake were reflected in the segment data, whilst no significant effect on receiving block radioactivity could be detected.

A 27% and a 10% reductionin the uptake of radioactivity from donor blocks containing 0.76% and 1.53% sucrose respectively was detected when the donation was to the basal end of the segments for 50 hours. Since the corresponding mannitol treatments gave similar significant results, the effect could have been due to the action as an osmoticum. Reductions of up to 133 dpm in the receiving block content of radioactivity in response to sucrose were not significant, whilst the 354 dpm reduction in response to mannitol was significant. In common with the 20 hour data, sucrose increased the uptake by the basipetal replicates by 57% (***), whilst the mannitol had a less marked effect. The higher concentration of mannitol did, however, give rise to the only significant decrease in receiving block radioactivity.

In conclusion, sucrose appeared to have little effect on the flux of radioactivity into the apical or basal receiving blocks of the acropetal and basipetal replicates. Increased uptake of radioactivity was detected in all the basipetal replicates, whilst an increase in the acropetal treatments occurred only at the lower concentration of sucrose following an experimental period of 20 hours.

FIGURE 33

Donor blocks of 1.0 μM 2,4-D(-1-^{14}C) containing the following compounds were applied either to the apical or the basal end of Pisum root segments at 25°C in darkness for 20 hours.

1 = 2,4-D(-1-^{14}C) only
2 = " + 0.76% sucrose
3 = " + 1.53% "
4 = " + 0.40% mannitol
5 = " + 0.80% "
6 = " only
7 = " + 0.76% sucrose
8 = " + 1.53% "
9 = " + 0.40% mannitol
10 = " + 0.80% "

1-5 = acropetal
6-10 = basipetal

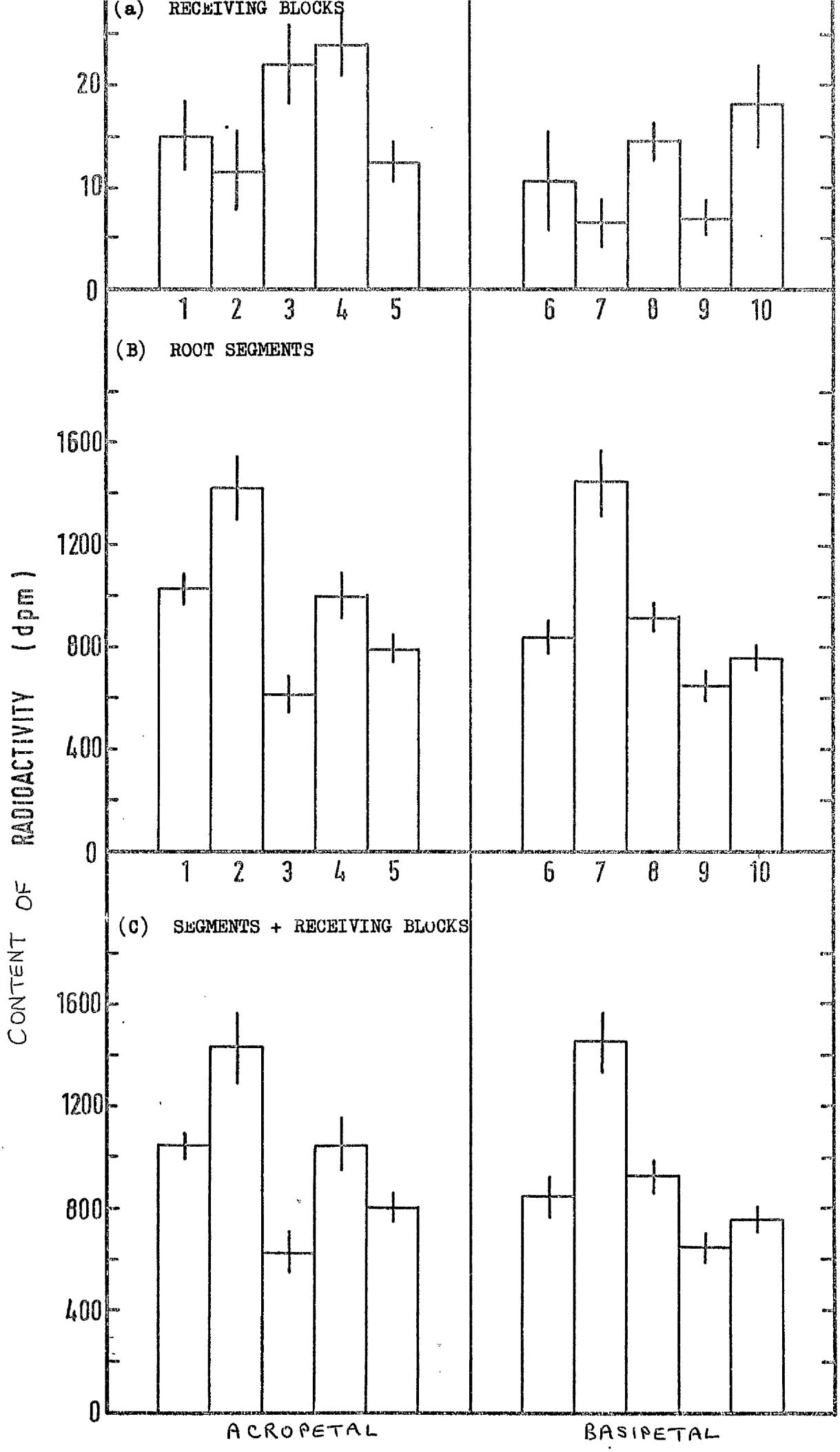

(a) RECEIVING BLOCKS
20
10
0
1 2 3 4 5
6 7 8 9 10
(B) ROOT SEGMENTS
1600
1200
800
400
0
1 2 3 4 5
6 7 8 9 10
(C) SEGMENTS + RECEIVING BLOCKS
1600
1200
800
400
0
ACROPETAL
BASIPETAL
CONTENT OF RADIOACTIVITY (dpm)

FIGURE 34

Donor blocks containing the following compounds, in addition to 1.0 µM 2,4-D(-1-^{14}C), were applied either to the apical or the basal end of Pisum root segments at 25°C in darkness for 50 hours:

1 = 2,4-D(-1-^{14}C) only
2 = " + 0.76% sucrose
3 = " + 1.53% "
4 = " + 0.40% mannitol
5 = " + 0.80% "
6 = " only
7 = " + 0.76% sucrose
8 = " + 1.53% "
9 = " + 0.40% mannitol
10 = " + 0.80% "

1-5 = acropetal
6-10 = basipetal

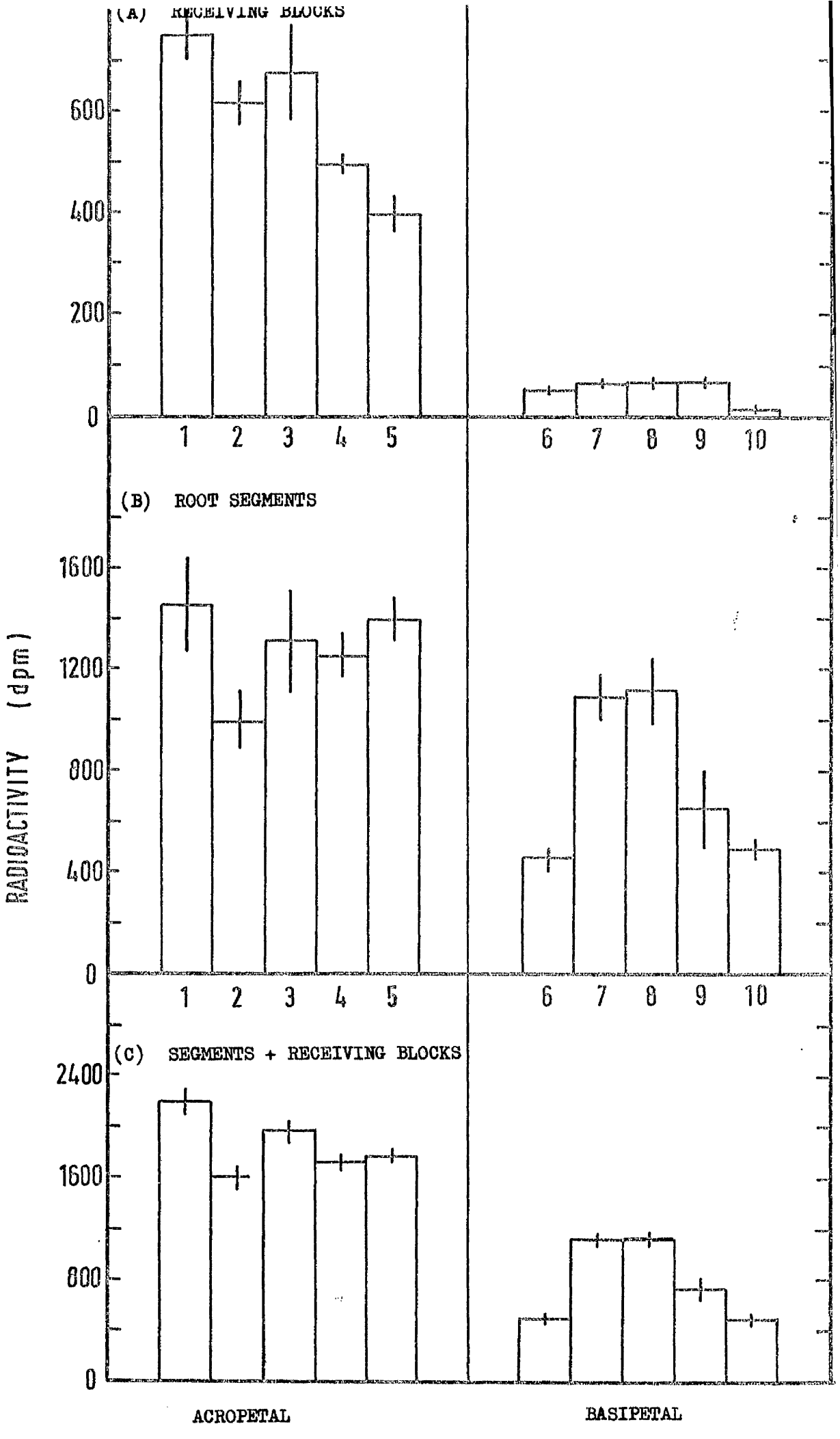

(A) RECEIVING BLOCKS
600
400
200
0
1 2 3 4 5
6 7 8 9 10
(B) ROOT SEGMENTS
1600
1200
800
400
0
1 2 3 4 5
6 7 8 9 10
RADIOACTIVITY (dpm)
(C) SEGMENTS + RECEIVING BLOCKS
2400
1600
800
0
ACROPETAL
BASIPETAL

TABLE 20

Paired t-tests of data presented in figures 33 and 34.

$t_{0.05} = 2.365$ $t_{0.01} = 3.499$ $t_{0.001} = 5.405$ (7 DF).

Time (h)	Samples compared (means)	t-values	
		Acrop	Basip
20	Receiving blocks		
	control - 0.76% sucrose	0.68	0.75
	" - 1.53% "	-1.36	-0.72
	" - 0.40% mannitol	-1.98	0.70
	" - 0.80% "	0.65	-1.21
	Tissue		
20	control - 0.76% sucrose	-2.92*	-4.69**
	" - 1.53% "	4.98**	-0.86
	" - 0.40% mannitol	0.28	2.17
	" - 0.80% "	3.12*	0.93
20	Total uptake		
	control - 0.76% sucrose	-2.82*	-4.61**
	" - 1.53% "	4.78**	-0.91
	" - 0.40% mannitol	-0.04	2.20
	" - 0.80% "	3.13*	0.83
50	Receiving blocks		
	control - 0.76% sucrose	1.98	-1.49
	" - 1.53% "	0.70	-1.42
	" - 0.40% mannitol	4.82**	-2.08
	" - 0.80% "	5.68***	5.72***
	Tissue		
50	control - 0.76% sucrose	4.31**	-12.39***
	" - 1.53% "	1.04	-10.05***
	" - 0.40% mannitol	2.01	-2.40*
	" - 0.80% "	0.57	-0.96
50	Total uptake		
	control - 0.76% sucrose	4.43**	-12.75***
	" - 1.53% "	1.51	-10.11***
	" - 0.40% mannitol	4.14**	-2.80*
	" - 0.80% "	3.65**	0.28

EFFECT OF HERBICIDE CONCENTRATION ON THE TRANSPORT OF 2,4-D

In view of the well documented herbicidal properties of 2,4-D it was decided to examine the movement of the -COOH labelled compound in Pisum root segments. Concentrations of 10.0, 1.0 and 0.1 μM herbicide were employed in experiments carried out at 25°C in darkness. The ^{14}C in each component of the system was determined at 5-hourly intervals over a period of 60 hours and the data are the mean of three experiments carried out on different occasions.

An initial period of ^{14}C uptake, lasting 15-20 hours, was recorded in both the acropetal and the basipetal replicates at each concentration tested. Thereafter, the radioactivity in the basipetal replicates decreased steadily over the subsequent 40 hours when 10.0 and 1.0 μM 2,4-D(-1-^{14}C) were used. A second phase of uptake commenced after 30 hours when 0.1 μM herbicide was used and resulted in a maximum of 230 dpm after 40 hours (figure 35). Maximal ^{14}C content of the acropetal replicates occurred after 20 and 45 hours with 10.0 μM, after 20 and 55 hours with 1.0 μM and after 15 and 45 hours with 0.1 μM 2,4-D(-1-^{14}C). It is apparent from figure 35 that acropetal and basipetal uptake was virtually identical over the initial 25 hours of the experiment at the lower herbicide concentrations. At a concentration of 10.0 μM, however, the acropetal uptake was consistently greater than the basipetal and was already 2.5 times greater after the first 25 hours of the experiment. After 60 hours, the basal uptake exceeded the apical uptake by 2.4, 3.2 and 4.6 times at concentrations of 0.1, 1.0 and 10.0 μM respectively. Table 22 revealed the only significant (*) difference at 0.1 μM, but this was probably due to the small extent of the sampling.

The radioactivity remaining in the donor blocks after each successive 5-hour period is shown in table 23. Losses of 209.0, 2726.5 and 25000.3 dpm were recorded from 0.1, 1.0 and 10.0 μM donor blocks applied to the basal end of the segments for 60 hours. In comparison, only 60.2, 968.4 and 3766.8 dpm were lost from similar blocks applied to the basipetal replicates. This indicated that a ten-fold increase in concentration led to a significant proportional increase (table 22) in the loss of radioactivity from basally applied donor blocks. When the blocks were applied to the apical end of the segments, however, an increase from 0.1-1.0 μM gave a 16 fold increase in donor-loss, whilst an increase from 1.0-10.0 μM produced only a 4-fold increase.

Figure 36 presents the ^{14}C content of plain agar receiving blocks applied to the apical or basal ends of Pisum root segments supplied with 0.1, 1.0 or 10.0 µM 2,4-D(-1-^{14}C) over a period of 60 hours at 25°C in darkness. Each point on the graph is the mean of experiments carried out on three different occasions with one sample at each time interval. A sample consisted of four root segments supplied with communal donor and receiving blocks. The data are, therefore, the total ^{14}C delivered into the receiving block from a set of four segments. 60 dpm were detected in the apically applied receiving blocks whilst no detectable activity was found in the basally applied blocks. Nevertheless, a concentration of 1.0 µM led to an accumulation of 25 dpm in the blocks applied to the basipetal replicates for 60 hours, whilst the blocks of the acropetal replicates contained approximately 26 times more ^{14}C. When the concentration was raised to 10.0 µM, however, the accumulation in the apical blocks exceeded that in the basal blocks by only 8 times. A ten-fold increase in concentration from 0.1-1.0 µM resulted in a ten-fold increase in the export of radioactivity into the apical receiving blocks by the end of the experiment. An increase from 1.0-10.0 µM 2,4-D(-1-^{14}C) gave only a three-fold increase in export in the acropetal replicates, whereas it produced a ten-fold increase in the basipetal replicates.

Examination of the polarity ratios, that is the accumulation of radioactivity in the apically applied receiving blocks compared with that in the basally applied blocks, showed that the degree of polarity was greatest with the 1.0 µM herbicide. A distinct reduction in polarity was recorded after 40 hours with 1.0 and 10.0 µM 2,4-D(-1-^{14}C) (table 24).

Ten-fold or one hundred-fold increases in herbicide concentration had little effect on the overall pattern of ^{14}C uptake into the tissue or export into the receiving blocks. An increase in concentration did, however, not automatically lead to proportional increases in uptake or export of radioactivity. In view of this, an alteration of the concentration of the herbicide led to changes in the degree of polarity of ^{14}C movement in the segment system (table 24).

FIGURE 35

Time-course for the ^{14}C-content of Pisum root segments + associated receiving blocks (ie. total uptake) supplied with donor blocks of 0.1, 1.0 or 10.0 μM 2,4-D(-1-^{14}C) at 25^{o}C in darkness. Data are the mean of experiments carried out on three different occasions using one sample/treatment. Each sample consisted of four root segments supplied with communal donor and receiving blocks.

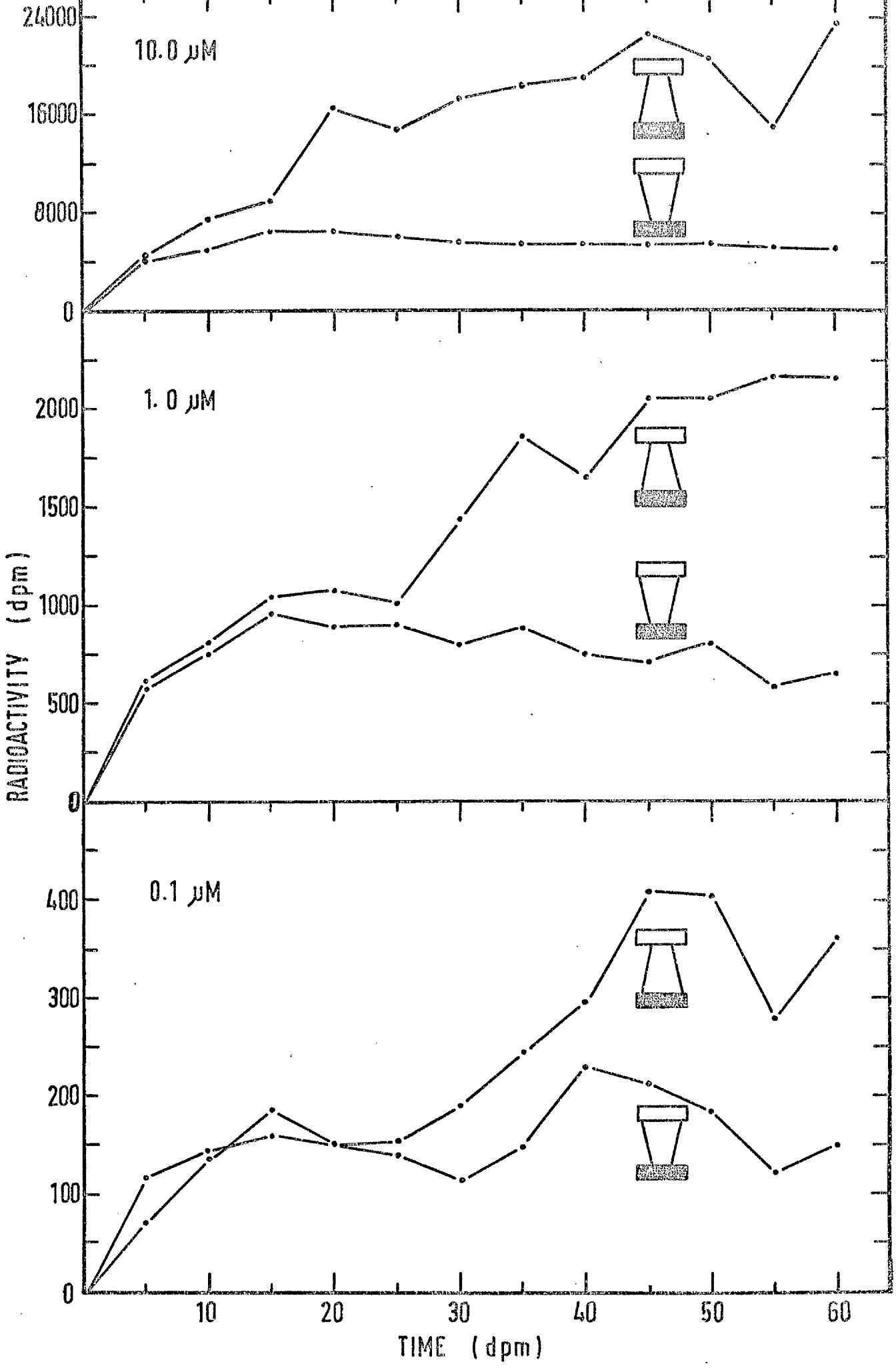

10.0 μM
1.0 μM
0.1 μM
RADIOACTIVITY (dpm)
TIME (dpm)
24000
16000
8000
0
2000
1500
1000
500
0
400
300
200
100
0
10
20
30
40
50
60

FIGURE 36

Time-course for ^{14}C content of receiving blocks applied to *Pisum* root segments supplied with 0.1, 1.0 or 10.0 µM 2,4-D(-1-^{14}C) at 25°C in darkness. Data are the mean of experiments carried out ON three occasions using one sample/treatment. Each sample consisted of four root segments supplied with communal donor and receiving blocks.

A = acropetal - 10.0 µM 2,4-D(-1-^{14}C)
B = acropetal - 1.0 µM "
C = basipetal -10.0 µM "
D = acropetal - 0.1 µM "
E = basipetal - 1.0 µM "

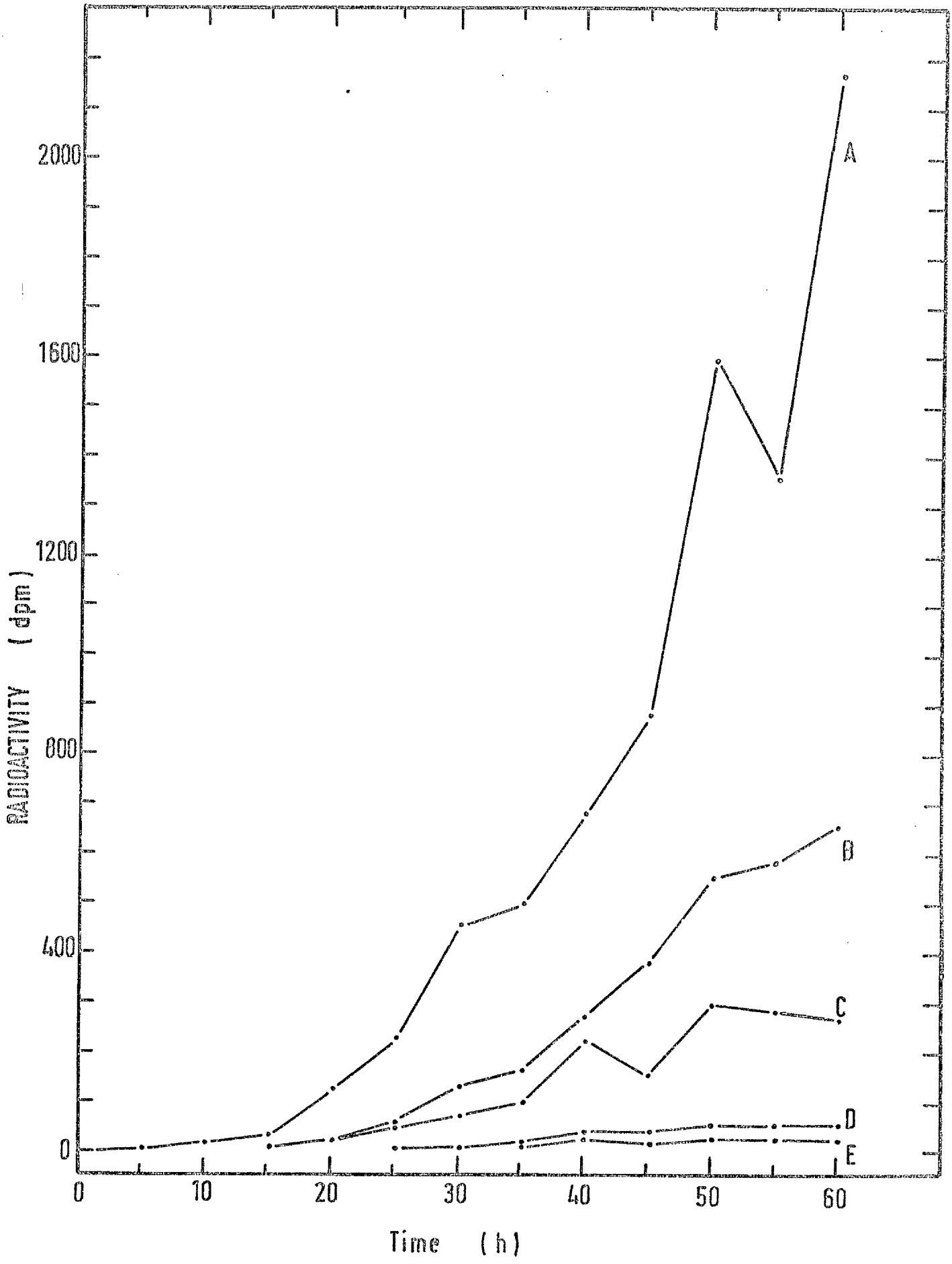

2000
1600
1200
800
400
0
RADIOACTIVITY (dpm)
A
B
C
D
E
0
10
20
30
40
50
60
Time (h)

TABLE 22

t-test for comparison of two sets of means to show statistical significance of ^{14}C-loss from donor blocks and ^{14}C-content of Pisum root segments after a transport period of 60 hours.

n = 3

Sample means compared	t - value		
	ACROP	BASIP	OTHER
(1) Donor block loss			
0.1 - 1.0 µM	-14.95***	-3.95	-
1.0 - 10.0µM	-5.57*	-17.65***	-
0.1 - 10.0µM	-12.95***	-14.32***	-
0.1 µM acrop-basip			2.47
1.0 µM " - "			2.90
10.0 µM " - "			5.09*
(2) Content of segments			
0.1 - 1.0 µM	-2.44	-15.40***	
1.0 - 10.0µM	-4.58*	-4.64*	
0.1 - 10.0µM	-4.37*	-5.21*	
0.1 µM acrop-basip			4.43*
1.0 µM " - "			2.19
10.0 µM " - "			3.76

$t_{0.001}$ = 12.941 (2DF) ***

$t_{0.01}$ = 9.925 **

$t_{0.05}$ = 4.303 *

TABLE 23

Radioactivity in donor blocks after supplying 0.1, 1.0 or 10.0 μM 2,4-D(-1-^{14}C) to Pisum root segments at 25°C in darkness. The data are the mean of three replicates, each of which consisted of four root segments.

Time (h)	BASAL DONOR BLOCKS (dpm)			APICAL DONOR BLOCKS (dpm)		
	0.1 μM	1.0 μM	10.0 μM	0.1 μM	1.0 μM	10.0 μM
0	553.2	5661.1	57062.4	553.2	5661.1	57062.4
5	509.3	5441.6	57902.6	475.6	5435.9	57076.1
10	402.0	5039.8	52546.2	396.0	4546.3	51821.7
15	464.0	4820.4	43942.0	424.9	5144.1	47427.2
20	401.3	4530.3	36214.0	410.2	4736.5	46180.8
25	445.0	4560.0	43836.9	412.8	5191.7	56466.6
30	424.3	4327.7	40968.1	438.4	5181.3	50486.2
35	432.7	4029.0	37768.0	429.5	4426.2	51415.9
40	410.1	3896.4	37723.5	463.5	4810.9	51184.7
45	423.5	3123.1	36411.2	481.4	4634.1	53753.0
50	348.5	3477.7	37448.2	450.3	4701.1	48319.1
55	393.7	3452.6	41861.0	461.1	4480.9	53669.2
60	344.9	2934.6	32062.1	493.0	4692.7	53295.6

TABLE 24

Polarity ratios were calculated as follows:

$$\text{Ratio} = \frac{\text{radioactivity in acropetal replicates}}{\text{radioactivity in basipetal replicates}}$$

Time (h)	ACROPETAL / BASIPETAL RATIO					
	0.1 µM		1.0 µM		10.0 µM	
	Uptake	Receiver	Uptake	Receiver	Uptake	Receiver
5	0.6	-	1.1	-	1.1	-
10	0.9	-	1.0	-	1.6	-
15	1.2	-	1.1	2.1	1.4	5.0
20	1.0	-	1.2	12.7	2.6	6.3
25	1.1	-	1.1	13.0	2.4	4.4
30	1.7	-	1.8	12.2	3.0	6.4
35	1.7	-	2.1	12.5	3.3	5.1
40	1.3	-	2.2	9.5	3.3	3.0
45	1.9	-	2.9	21.6	4.3	5.6
50	2.2	-	2.5	20.1	3.7	5.4
55	2.3	-	3.7	21.9	2.9	4.8
60	2.4	-	3.2	26.7	4.6	8.1

EFFECT OF WHITE LIGHT ON THE TRANSPORT OF 2,4-D

The positive geotropic behaviour of roots normally prevents them from exposure to daylight. In order to complete the characterisation of the response of the system to environmental conditions (except humidity) the effect of white light on the movement of 1.0 μM 2,4-D(-1-^{14}C) at 25°C was investigated.

The root segments were placed either in diffuse white light (radiant flux density 10^4 ergs $cm^{-2} sec^{-1}$) or in a light-proof box before being placed in a light- room at 25°±1°C. This ensured that both treatments would be subjected to the same ambient temperature and to any possible temperature fluctuations. Four replicates of each treatment were set up on two different occasions, which gave a total of 32 root segments per treatment. The experimental results are presented in figures 37,38 and table 25.

White light treatment for 20 hours resulted in a 10% enhancement of the radioactive content of the segments to which the herbicide had been supplied to the basal end. The same treatment for 50 hours gave a 17% increase, but neither of these increases proved to be statistically significant. Nevertheless, the trend towards light enhancement was apparent in the basipetal treatments. After 20 hours, for example, the light produced a significant 20% increase in ^{14}C content of the segments, but little effect was noticeable after 50 hours. Similar trends were detectable in the uptake data.

Radioactivity in receiving blocks applied to the apical ends of the segments was reduced by 53% after light treatment for 20 hours. This was significant at the 1.0% level of probability, but no significance could be attached to the 13% reduction after exposure to light for 50 hours. Conversely, the ^{14}C in basally applied blocks was increased by 11% after 20 hours and by a highly significant 48% after 50 hours treatment.

It can be concluded that exposure of Pisum root segments to white light had no consistent effect on the movement of radioactivity supplied as 2,4-D(-1-^{14}C).

FIGURE 37

The level of radioactivity in Pisum root segments and root segment + receiving blocks (ie. total uptake) after supplying 1.0 μM 2,4-D(-1-^{14}C) to the apical or basal end of the tissue in white light (L) or darkness (D) at 25°C. The data are the mean of eight replicates each consisting of fou segments supplied with communal donor and receiving blocks.

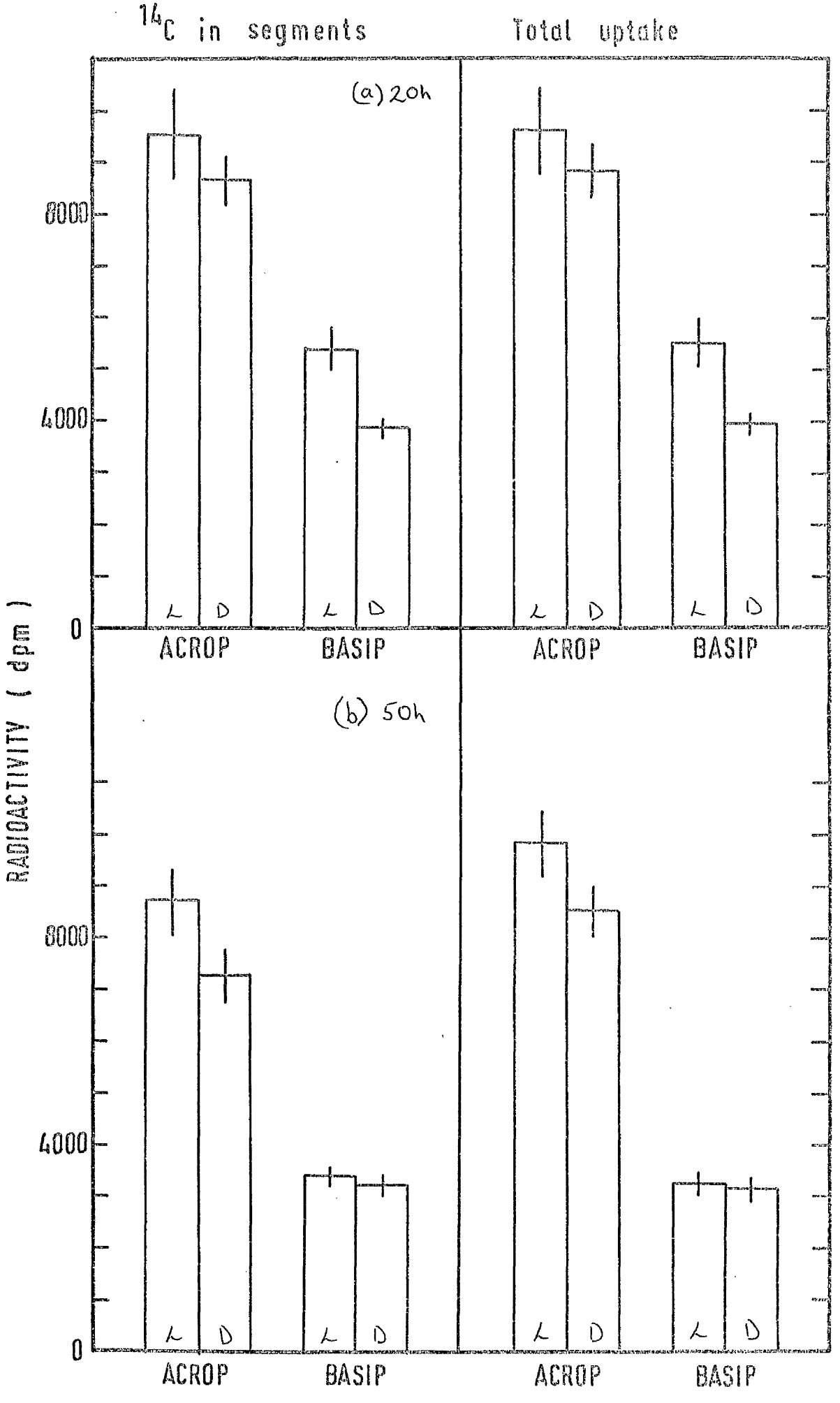

^{14}C in segments
Total uptake
(a) 20h
(b) 50h
RADIOACTIVITY (dpm)
8000
4000
0
L
D
ACROP
BASIP

FIGURE 38

Radioactivity in receiving blocks applied to the apical or basal end of Pisum root segments supplied with 1.0 μM 2,4-D(-1-^{14}C) for 20 or 50 hours in white light at 25°C. The data are the mean of eight replicates each consisting of four root segments supplied with communal donor and receiving blocks.

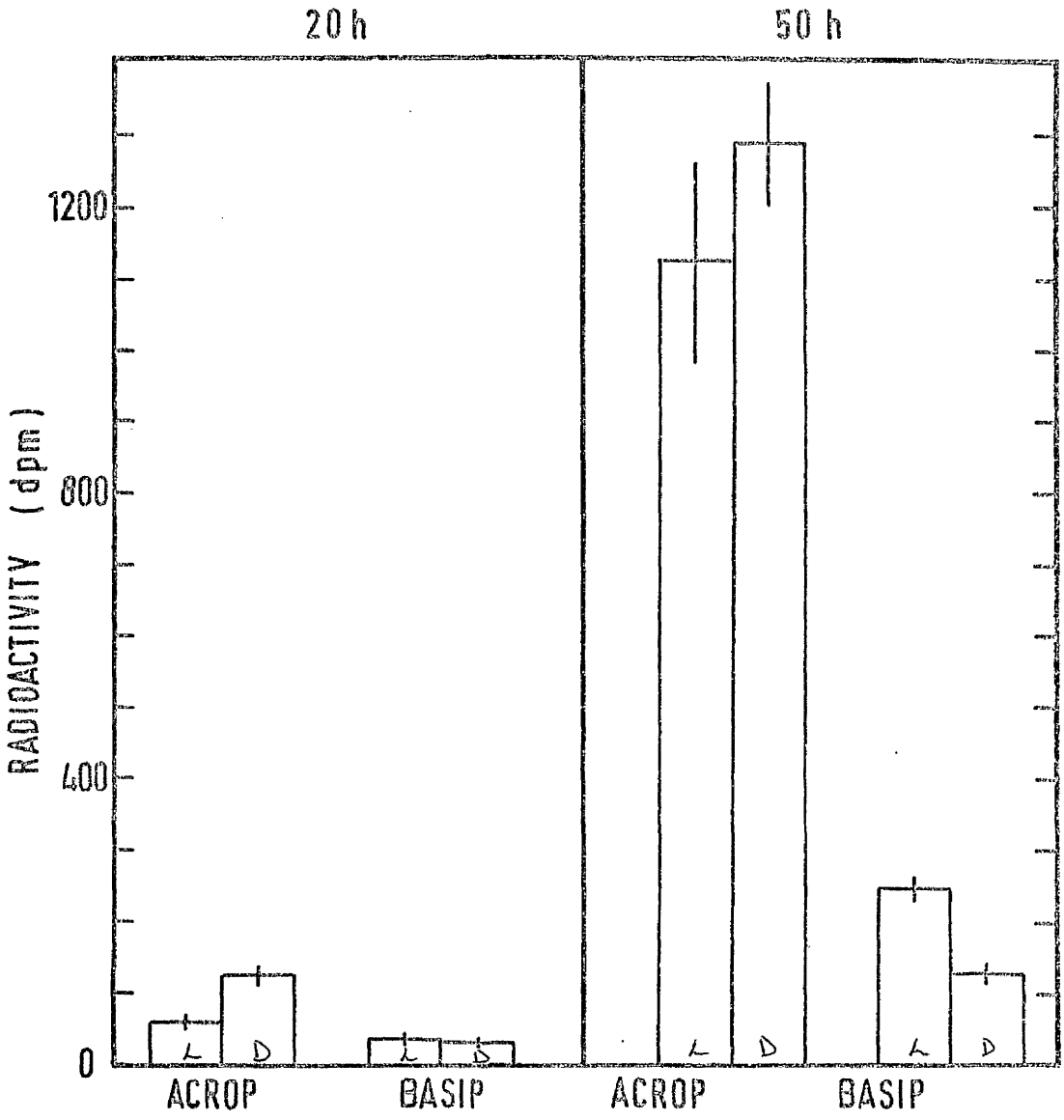

20 h
50 h
RADIOACTIVITY (dpm)
1200
800
400
0
L
D
ACROP
BASIP
ACROP
BASIP

TABLE 25

Statistical analysis of data presented in figures 37 and 38.

Time (h)	Samples compared	t - values acrop	basip
20	receiver light-dark	-5.28**	0.57
	tissue " - "	0.89	3.02*
	uptake " - "	0.80	3.03*
50	receiver light-dark	0.99	-6.44***
	tissue " - "	-1.89	-0.17
	uptake " - "	-1.57	-0.54

$t_{0.05} = 2.365$ (7 DF) *

$t_{0.01} = 3.499$ **

$t_{0.001} = 5.405$ ***

Sources of error in the isolated-segment technique

Throughout the progress of the experimental work reported in this thesis, it becme increasingly clear that several of the experimental manipulations were potential sources of artefacts. In addition, certain precautions were necessary before the data could be interpreted in a meaningful fashion.

(i) Effect of segment shape on 2,4-D translocation

There can be little doubt that the radioactivity from an application of ^{14}C-labelled 2,4-D to Pisum root segments moves preferentially towards the root apex. But could this be an artefact produced exclusively by the experimental system ? It has been suggested by Wilkins and Scott (1968b) that the frustum geometry of sub-apical root segments ie. a truncated cone, could lead to the manifestation of a polarity of growth regulator movement.

The influence of the area of cut surface of the segment in contact with the donor block, on the uptake and movement of 2,4-D was checked by the excision of segments 1-11, 12-22, 23-33 and 34-44mm behind the apex of pea roots. Table 26 presents the mean of the data from 41 independent measurements and shows that in sub-apical segments, the area of the basal end was more than three times greater than that of the apical end. However, the areas of the two ends became virtually identical as the segments were excised at increasing distance from the root tip. It was not surprising, therefore, that only the sub-apical segment proved to be a true frustum, whilst the others could be defined adequately as cylinders.

Segments from the same four regions of the root were employed in standard 24 hour experiments, in which the donor concentration was 1.0 μM 2,4-D(-1-^{14}C) and the temperature 25^{o}C. Each point on the graph in figure 39 is the mean of eight replicates, each containing four root segments. The data were collected on three different occasions. In the first three successive 1.0mm segments it was clear that the accumulation of ^{14}C in the apical receiving blocks increased significantly as the segments were excised at increasing distance from the root tip. Indeed, the accumulation in the 23-33mm segment blocks was more than double that in the 1-11mm segment

receiving blocks and this was highly significant (table 27). Little significance could be attached to the alterations in basal receiving block content of radioactivity.

The ratio of the accumulation of radioactivity in the receiving blocks appied to the acropetal and the basipetal replicates emphasised that the basal regions of the intact root possessed a more absolute polarity of ^{14}C movement than did the apical regions(table 27). For example, acropetal transport in the sub-apical segments was two times greater than the basipetal, whilst it was six times greater than the basipetal transport in the 34-44mm segments. It was clear from the 23-33mm segments that the degree of acropetal polarity did not increase linearly from the tip to the base of the intact root.

On termination of the experiment, the level of radioactivity in the segments was determined and the relevant data are plotted in figure 39 . No significant difference could be detected in the ^{14}C levels in the first three successive 1.0mm segments following either apical or basal donation. A basal donation to the 34-44mm segments led to a ^{14}C content which was significantly greater than that in any of the other segments (table 28).

In general, increasing distance from the root tip resulted in:
(a) a decrease in the base/apex surface area ratio.
(b) an increase in the degree of acropetal polarity of ^{14}C movement.
(c) little effect on the segment-content of radioactivity, except in the most basal regions of the root.

It would appear unlikely that the ratio of the cut surface areas at either end of the segments had a direct effect upon the degree of polarity. Nevertheless, the increase in area of the apical and basal ends of segments taken progressively from the root tip did increase the total uptake of ^{14}C.

FIGURE 39

The radioactive content of receiving blocks and Pisum root segments supplied with 1.0 μM 2,4-D(-1-^{14}C) to the apical or basal ends at 25°C in darkness. Segments were excised at increasing distances from the apex of the intact root. The data are the mean of eight replicates carried out on two different occasions.

Fig.39a = receiving blocks
Fig.39b = root segments

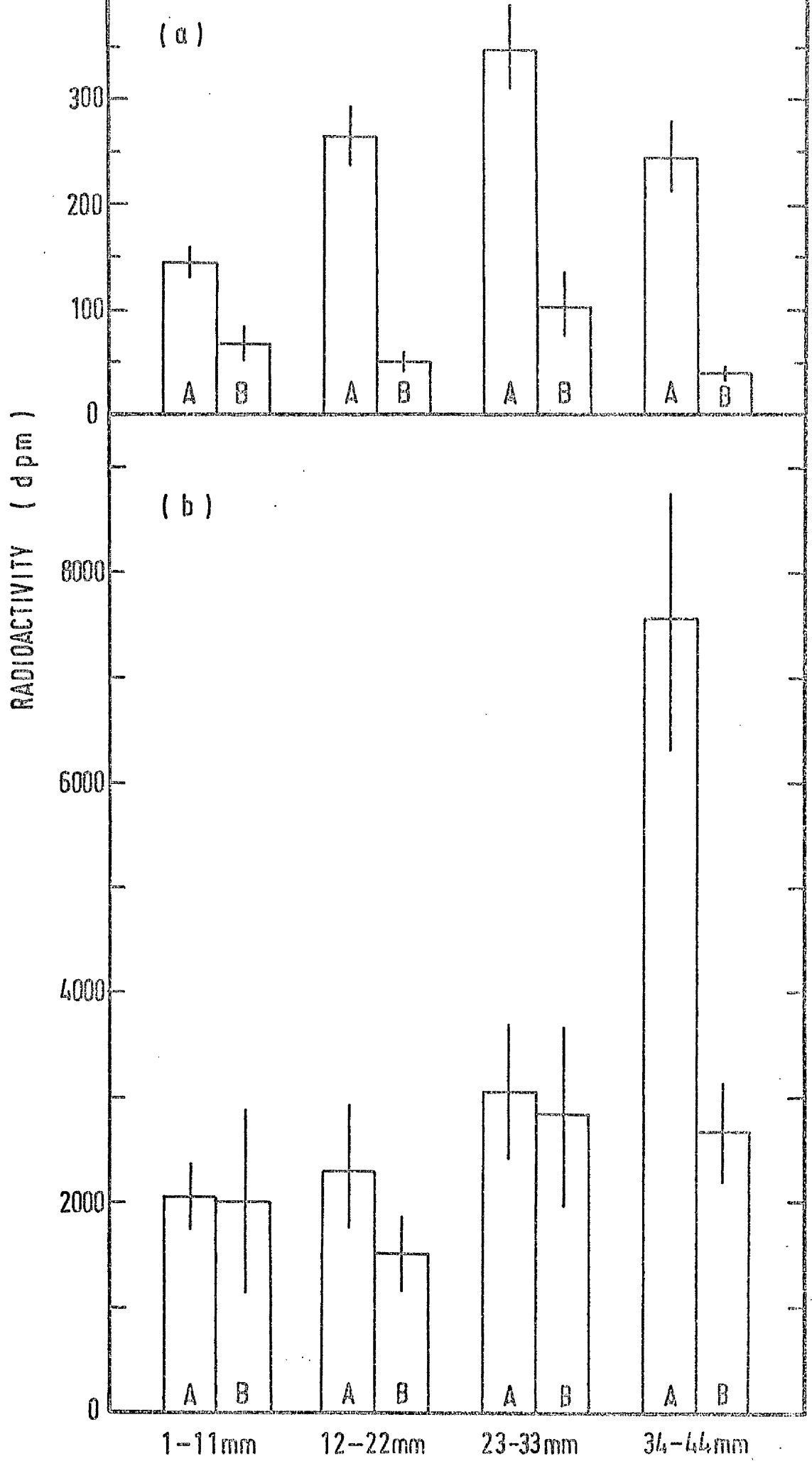

(a)
300
200
100
0
A
B
(b)
8000
6000
4000
2000
0
RADIOACTIVITY (dpm)
1-11mm
12-22mm
23-33mm
34-44mm

TABLE 26

The diameter and surface area of the apical and basal excised ends of Pisum root segments taken progressively from the apex of intact roots. Seedlings were grown in covered boxes at 25°C in darkness. The data are the mean of 41 independent measurements.

Region of root(mm)	BASAL CUT SURFACE Diameter(mm)	Area(mm^2)	APICAL CUT SURFACE Diameter(mm)	Area(mm^2)	RATIO B/A
1-11	1.04 ± 0.01	0.85	0.6 ± 0.01	0.28	3.03
12-22	1.18 ± 0.02	1.09	1.08 ± 0.02	0.92	1.18
23-33	1.54 ± 0.04	3.73	1.28 ± 0.02	3.55	1.05
34-44	1.68 ± 0.04	3.84	1.28 ± 0.02	3.55	1.08

TABLE 27

Paired t-test of the data presented in figure 39. t-values for the comparison of the means of the radioactive accumulation in apical and basal receiving blocks in the different regions of the intact root are tabulated. Acropetal/basipetal ratios are also presented.

(a) Acropetal

Region of root (mm)	t - values			
	1 - 11	12 - 22	23 - 33	34-44
12-22	-3.61**			
23-33	-4.87**	-1.76		
34-44	-2.68*	0.46	2.06	
Acrop/basip ratios	2.13	5.25	3.35	6.10

(b) Basipetal

Region of root (mm)	1 - 11	12 - 22	23 - 33	34 - 44
12-22	0.90			
23-33	-1.02	-1.69		
34-44	1.49	0.89	2.05	
Acrop/basip ratios	2.13	5.25	3.35	6.10

$t_{0.05} = 2.365$ $t_{0.01} = 3.499$ $t_{0.001} = 5.405$ (7 DF)

TABLE 28

Paired t-tests of the data presented in figure 39. The means of the radioactive content of Pisum root segments excised from different regions of the intact root have been compared. Acropetal/basipetal ratios are also presented.

(a) Acropetal

Region of root (mm)	t - values			
	1 - 11	12 - 22	23 - 33	34 - 44
12-22	-0.37			
23-33	-1.43	-0.92		
34-44	-4.46**	-4.00**	-3.33*	
Acrop/basip ratios	1.02	1.51	1.07	2.82

(b) Basipetal

Region of root (mm)	t - values			
	1 - 11	12 - 22	23 - 33	34 - 44
12-22	0.59			
23-33	-0.75	-1.46		
34-44	-0.77	-1.98	0.17	
Acrop/basip ratios	1.02	1.51	1.07	2.82

$t_{0.05} = 2.365$ $t_{0.01} = 3.499$ $t_{0.001} = 5.405$ (7 DF)

(ii) Effect of detipped segments on 2,4-D movement

The detipping of segments prior to the application of agar blocks might have a considerable effect on the characteristics of the movement of the herbicide through the segments of Pisum root tissue. It was necessary, therefore, to determine whether the root tip would act as a barrier to the uptake or export of radioactivity by the segments.

1.0 µM 2,4-D(-1-^{14}C) was supplied to intact and detipped root segments in acropetal and basipetal replicates. A total of eight replicates of each treatment, each containing four root segments, were set up on two seperate occasions, in a darkroom at 25°C. Figure 40 presents the profiles of radioactivity along the segments and table 29 the statistical analysis of the data.

The 20 hour data for the acropetal replicates showed that the presence of the root tip resulted in a 12% reduction in total uptake of radioactivity, even though the uptake was through the cut basal end of the segments, in both treatments. Slightly less ^{14}C was found in each 2mm portion of the segments with intact tips and in the associated receiving blocks, but this was not significant. A similar state of affairs was detected in the acropetal replicates after 30 hours. However, this was not the case for the basipetal replicates, where the presence of the root tip, far from being a barrier, actually increased the total uptake of ^{14}C by 30% after 30 hours. This was significant at the 0.1% level of probability, in common with the 43% increase in receiving block ^{14}C. A highly significant increase in the content of the first three successive 2mm pieces of the root tip. Similar trends detectable after only 20 hours proved to be insignificant in a t-test analysis.

There was no foundation for the proposal that the tip of Pisum root segments might act as a barrier during the uptake, or export of radioactivity derived from labelled-2,4-D. In fact, the tip was capable of enhancing the level of uptake.

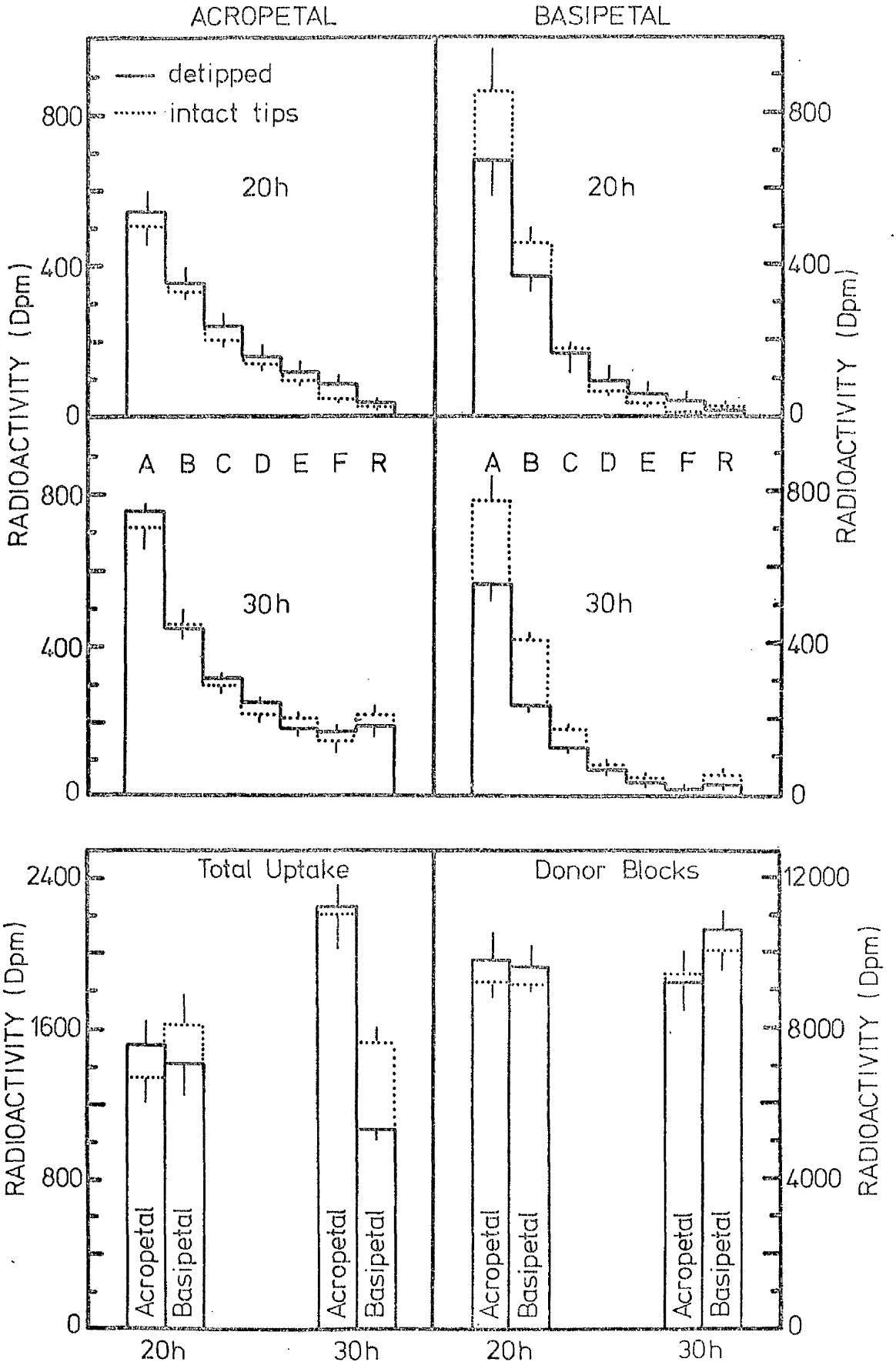
FIGURE 40

The effect of detipped segments on the uptake, distribution and accumulation of radioactivity in the tissue and receiving blocks of a 2,4-D/*Pisum* root segment system. The donor concentration was 1.0 uM 2,4-D(-1-^{14}C) and the experiment was carried out at 25^{o}C in darkness. Data are the mean of a total of eight repicates, each consisting of four root segments resting on communal donor or receiving blocks.

TABLE 29

Time (h)	Samples compared	t - values acrop	t - values basip
20	Excised tip - section A	1.51	-1.47
	- intact tip " B	0.53	-1.70
	" C	0.58	-0.26
	" D	1.25	0.80
	" E	0.61	0.88
	" F	0.71	1.30
	receiver	0.72	-0.89
	uptake	1.00	-0.85
30	Excised tip - section A	0.58	-2.83 *
	- intact tip " B	-0.83	-6.48***
	" C	1.19	-3.03*
	" D	0.65	-0.89
	" E	-0.29	-0.35
	" F	0.65	0.30
	receiver	-0.69	-4.45**
	tissue	0.14	-4.90**

$t_{0.05} = 2.365$ $t_{0.01} = 3.499$ $t_{0.001} = 5.405$ (7 DF)


ACROPETAL
BASIPETAL
detipped
intact tips
20h
30h
A B C D E F R
RADIOACTIVITY (Dpm)
0
400
800
Total Uptake
Donor Blocks
1600
2400
4000
8000
12000
Acropetal
Basipetal
20h
30h

(iii) Effect of segment inversion on 2,4-D movement

The segment systems described previously, were set up either with apical or basal ends of the root segments in contact with a lower donor block, to minimise leakage of radioactivity in response to gravity. In so doing, the acropetal treatments were arranged with root tips vertically upwards, whilst the basipetal treatments were in a more physiologically-normal orientation.

The effect of segment orientation on the movement of the herbicide was investigated by supplying 1.0 µM 2,4-D(-1-^{14}C) to four acropetal and four basipetal replicates, in which the root apices were upwards, and a second set in which the apices were downwards ie. inverted through 180°. The experiment was carried out on two occasions and the data presented in figure 41,42 and table 30 are the mean of eight replicates, each of which consisted of four root segments.

Donation of the radioactive herbicide to the basal end of the "tip-upward" segments resulted in a 27% decrease of ^{14}C in receiving blocks after 20 hours and an 18% decrease after 50 hours. Similarly, donation to the apical end of the "tip-upward" segments gave a 41% decrease after 20 hours and a 7% decrease after 50 hours. The data were not significantly different from the "tip-downward" controls.

Radioactivity within the root segments was decreased significantly when the root tip was directed downwards. After 20 hours, for example, the "tip-downwards" segments of the acropetal replicates contained 449dpm less than the corresponding "tip-upwards" segments. The only exception was the 20 hour basipetal treatments, in which the "tip-downwards" segments contained, on average, 419dpm less than the corresponding treatments.

In the light of these data, it would be reasonable to propose that in all probability, inversion of the segments produced no alteration in their ability to move the herbicide (or metabolic products formed from the herbicide). The ^{14}C content of the segments did depend upon the orientation of the segments.

FIGURE 41

The radioactive content of tissue and receiving blocks following the application of 1.0 µM 2,4-D(-1-^{14}C) to the apical or basal ends of Pisum root segments, at 25°C in darkness for 20 hours. The effect of inversion of the segments through 180° was studied. Data are the mean of eight replicates carried out on two different occasions.

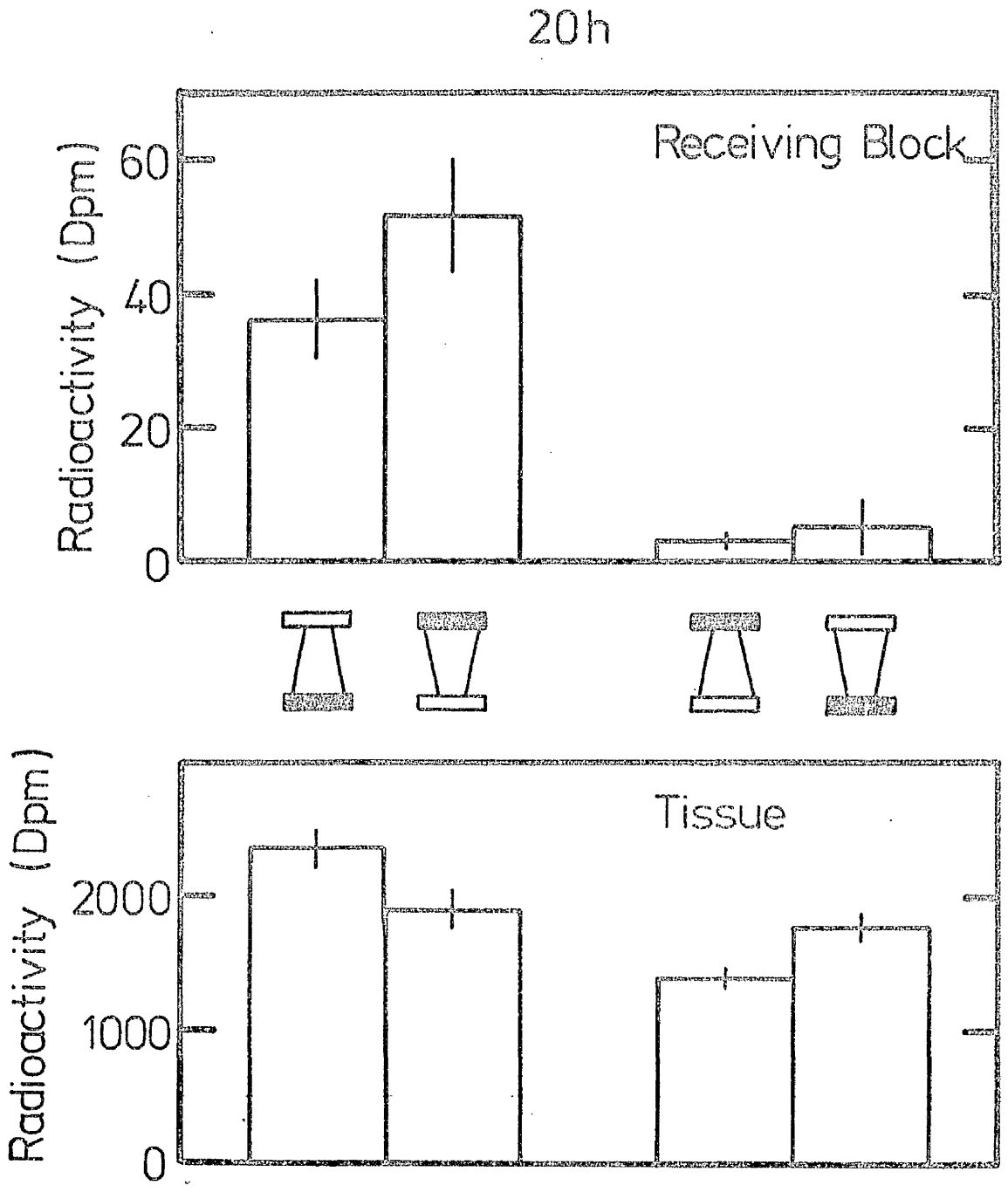

20h
Receiving Block
Radioactivity (Dpm)
60
40
20
0
Tissue
Radioactivity (Dpm)
2000
1000
0

FIGURE 42

Radioactivity in tissue and receiving blocks following the application of 1.0 μM 2,4-D(-1-^{14}C) to the apical or basal ends of Pisum root segments, at 25°C in darkness for 50 hours. The effect of segment inversion was studied and the data are the mean of eight replicates carried out on two different occasions.

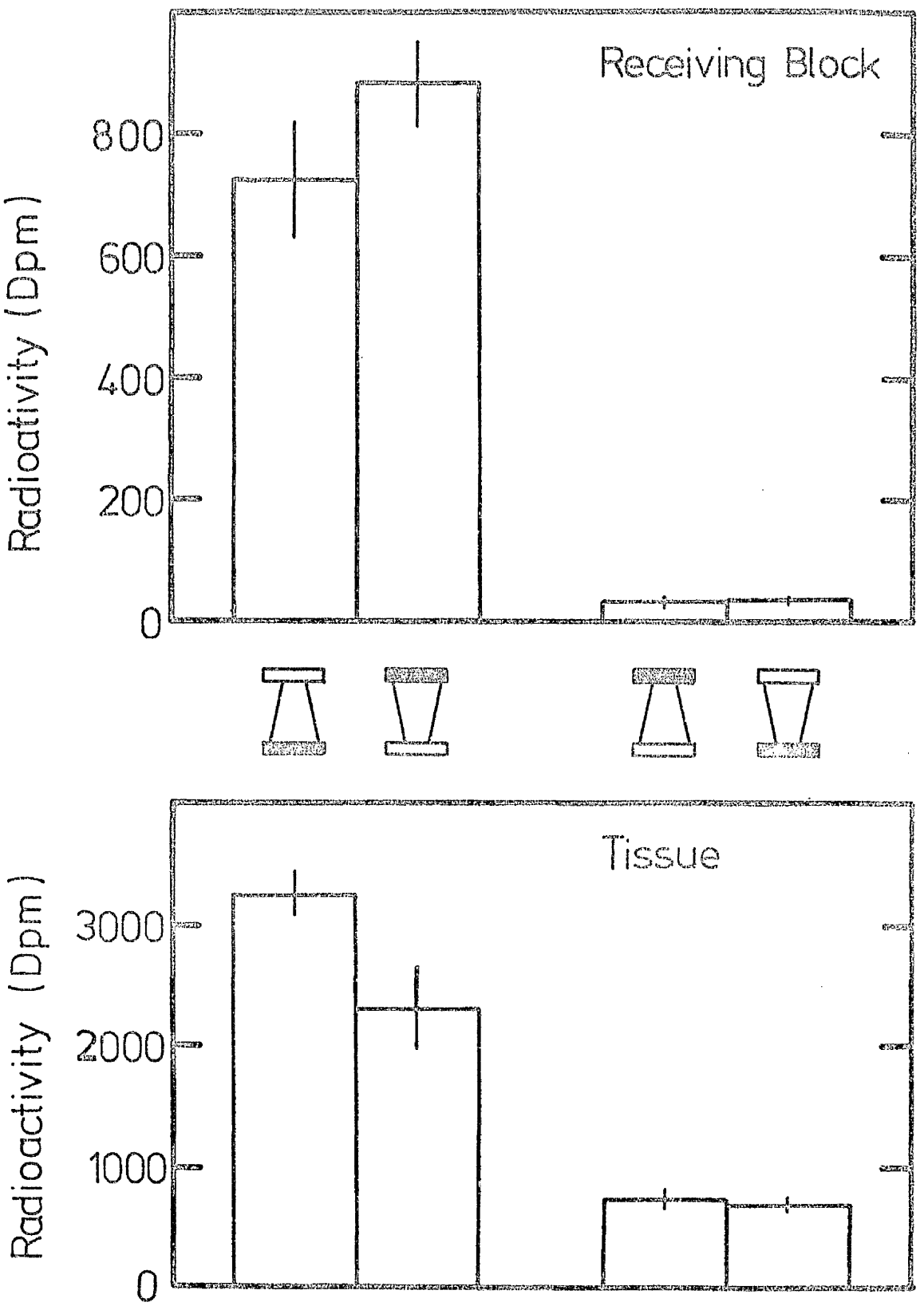

50h
Receiving Block
Radioatitivity (Dpm)
800
600
400
200
0
Tissue
Radioactivity (Dpm)
3000
2000
1000
0

TABLE 30

Paired t-tests of the data presented in figures 41 and 42.

$t_{0.05} = 2.365$ $t_{0.01} = 3.499$ $t_{0.001} = 5.405$ (7 DF)

Time (h)	Samples compared	t-values Acrop	Basip
20	Receiver tip up - tip down	-1.58	0.52
	Tissue " - "	2.37*	3.14*
50	Receiver tip up - tip down	-1.39	0.32
	Tissue " - "	2.45*	-0.62

(iv) Correction of data to give radioactivity / g dry weight of tissue

The possibility that the apparent polarity of herbicide movement might be modified on expression of the data in terms of radioactivity /g dry weight was checked by weighing the 2mm portions of the segments at the end of a 60h experiment. Appropriate correction factors by which the results of the 60h determinations of radioactive content must be multiplied are given in the following table:

Sample	ACROPETAL		BASIPETAL	
	Wt of 4 2mm pieces of tissue	Correction factor for dpm /g	Wt of 4 2mm pieces of tissue	Correction for dpm/g
A	0.0100	100.3	0.0048	208.8
B	0.0101	98.7	0.0067	149.7
C	0.0087	115.2	0.0068	146.0
D	0.0064	156.5	0.0076	130.9
E	0.0068	147.9	0.0089	112.7
F	0.0048	208.3	0.0081	122.8

It is clear that these correction factors would tend to accentuate the apparent polarity of ^{14}C distribution within the tissue, rather than mask it.

(v) Molecular degradation

Degradation of the acetic acid side-chain of the 2,4-D molecule was investigated by monitoring the evolution of radioactive gas from the experimental system.

Eighty root segments were placed with apical or basal cut surfaces in contact with donor blocks of 1.0 μM 2,4-D(-1-^{14}C) or 1.0 μM 2,4-D(-2-^{14}C), having specific activities of 34.0 and 29.0 mCi/m mole respectively. The experiments were carried out in dim green light within humidified vacuum desiccators through which air was drawn, before passing through two micro-bubblers (figure 4). Radioactivity was trapped in ethanolamine : ethylene glycol mono-methyl ether : toluene (1:8:10 v/v) and the contents of each bubbler was changed at 2-hourly intervals throughout the experiment. The data, presented in figure 43, are the total radioactivity collected in each pair of bubblers, to give total evolution of ^{14}C from eighty root segments.

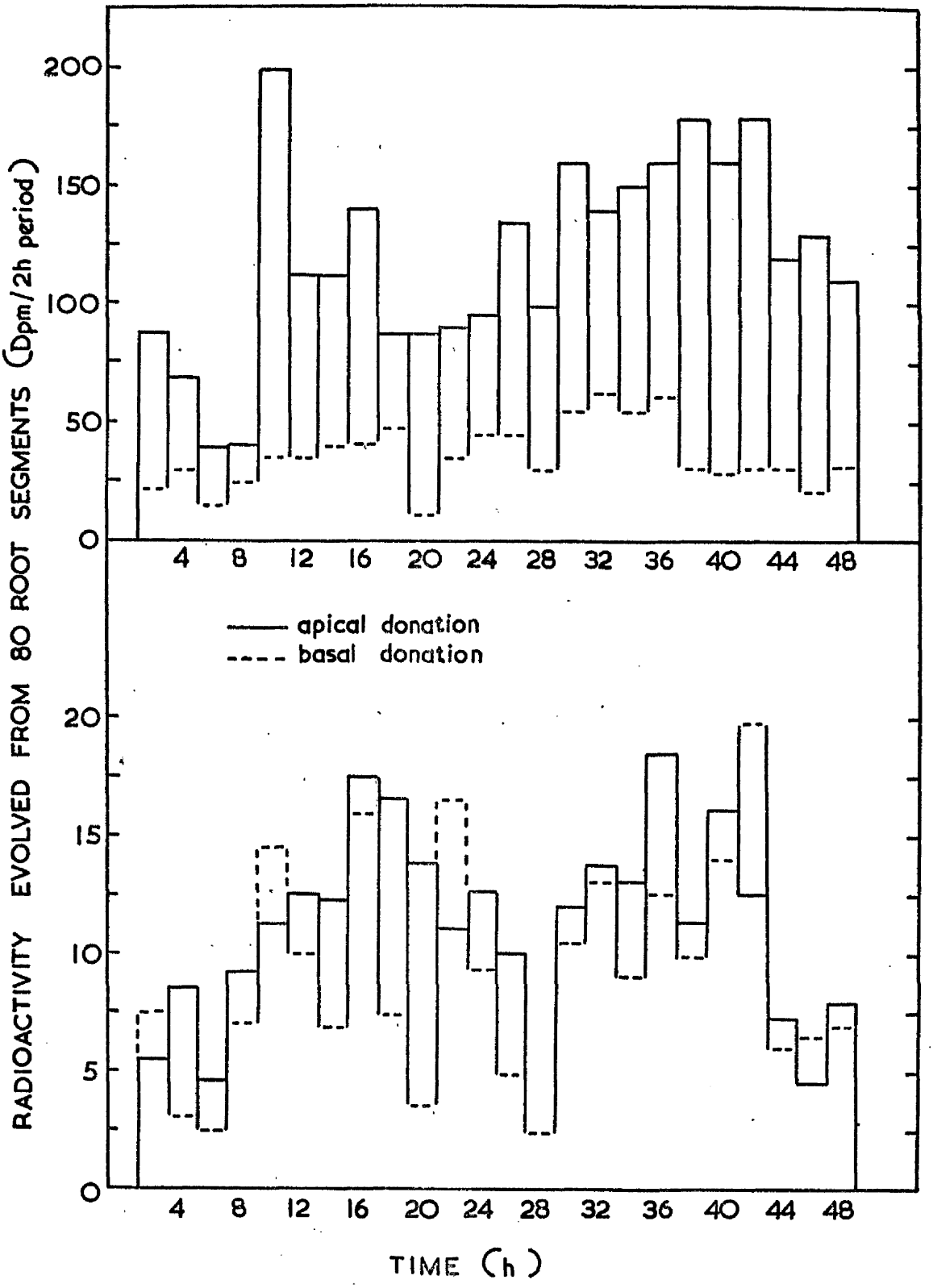
The radioactivity in the ethanolamine/toluene mixture, after supplying the -COOH labelled compound is presented in figure 43. Evolution of radioactive gas from the basipetal replicates was up to five times higher than that from the acropetal replicates. Nevertheless, the maximum output in any two-hour period was only 200 dpm/80 segments ie. less than 3 dpm from each root segment, which would amount to less than 0.1% of the initial donor supply of ^{14}C. When the -CH$_2$ labelled compound was used (figure 43) the evolution of radioactive gas was reduced ten-fold and the loss from the basipetal replicates was of the same order as that from the acropetal replicates.

It would appear, therefore, that although degradation of the acetic acid side-chain followed by the release of gaseous products was minimal, a mechanism for the preferential release of ^{14}C from the -COOH rather than the -CH$_2$ group existed.

FIGURE 43

(a) The evolution of radioactivity from 80 *Pisum* root segments supplied with 1.0 µM 2,4-D(-1-^{14}C) to the apical or basal cut surfaces. Data are presented as radioactivity evolved / 80 root segments / 2-hour period.

(b) The evolution of radioactivity from 80 *Pisum* root segments supplied with 1.0 µM 2,4-D(-3-^{14}C) to the apical or basal cut surfaces. Data are expressed as radioactivity evolved from 80 root segments / 2-hour period.


RADIOACTIVITY EVOLVED FROM 80 ROOT SEGMENTS (Dpm/2h period)
200
150
100
50
0
4
8
12
16
20
24
28
32
36
40
44
48
apical donation
basal donation
20
15
10
5
0
4
8
12
16
20
24
28
32
36
40
44
48
TIME (h)

(b) **Identification of metabolic products**

The possibility of herbicide degradation within the root segments prevented the detection of ^{14}C being used as a reliable indicator of the presence of 2,4-D. Hence, the identification of the radioactive compounds present in the segment system, employed in the previous experiments, was of paramount importance.

Experiments were run for 24 hours at 25°C in white light or darkness, with the 2,4-D molecule labelled with ^{14}C either in the -COOH or the $-CH_2$ group of the acetic acid side chain. Ethanol extracts from each component of the system were analysed on descending paper chromatograms, developed in isopropanol:ammonia:water (10:1:1), and n-butanol : propionic acid : water (21:10:14). The radioactivity on the chromatograms was determined quantitatively, by scintillation counting, and qualitatively, by autoradiography. Data obtained from scintillation counting are presented in tables 31 and 32. Stock solutions of 2,4-D(-1-^{14}C) and 2,4-D(-2-^{14}C) ran to the same Rf, namely Rf 0.7-0.8 in isopropanol : ammonia : water and Rf 0.9-1.0 in butanol : propionic acid : water. The radioactive compounds in each extract ran to the same Rf zones as the stock solutions, except in a few cases when no ^{14}C was detected due to the low level of ^{14}C movement in the basipetal replicates. As the data are so consistent, it must be concluded that there was no evidence of degradation of the radioactive compound in any of the treatments, when these extraction proceedures were employed.

Autoradiographs of duplicate chromatograms (table 33), in common with the scintillation counting data, revealed one radioactive spot running to Rf 0.88-0.95 in butanol : propionic acid : water. In isopropanol : ammonia : water, however, the major spot ran to Rf 0.75-0.83, which was identical to the pure stock solution, whilst a second compound ran to approximately Rf 0.9 in segment extracts. The appearance of this breakdown product did not depend upon the position of the radioactive label or the action of light. In addition, a third compound which was barely detectable was found at Rf 0.64-0.66. This compound occurred mainly in the donor blocks and segments of the light treatments supplied with 2,4-D(-1-^{14}C) and in two of the dark treatments after supplying 2,4-D(-1-^{14}C). It was interesting that the radioactive compounds detected

in the light and dark treatments of the acropetal and basipetal replicates were virtually identical. Furthermore, molecular degradation occurred on such a limited scale that very sensitive methods of detection were necessary.

A further series of experiments was carried out in which molecular degradation was monitored at 5-hourly intervals for 60 hours in acropetal replicates supplied with 1.0 μM 2,4-D(-1-^{14}C). The temperature was 25°C and the experiments were run in total darkness. Methanol extracts from eight replicates, each containing four root segments, were run on paper chromatograms in (a) isopropanol : ammonia : water (8:1:1), (b) n-butanol: acetic acid : water (5:1:2.2) and (c) n-butanol : acetone : water (5:2:3). The chromatograms were cut into 20 equal pieces and distribution of ^{14}C was determined by liquid scintillation counting. examination of the data in table 34 reveals that the radioactivity in the aqueous stock solution of 2,4-D(-1-^{14}C) and in the unused donor blocks ran to Rf zones 0.70-0.85, 0.85-1.00 and 0.20-0.50 in solvent systems (a), (b) and (c). The aqueous stock solution deposited a trail of ^{14}C along the chromatogram. Nevertheless, proof that 2,4-D was being supplied to the root segments came when it was shown that pure unlabelled 2,4-D, the stock solution and the unused donor block extract ran to the same Rf zone.

After a period of 18 hours, the donor blocks contained 2,4-D only, as did the half-segments in contact with the receiving blocks. Similarly, 2,4-D alone was present in the receiving block extracts when the chromatograms were run in solvent system (b), but in systems (a) and (c) an additional compound was found at Rf 0.40- 0.50 and Rf 0.60-0.80 respectively. Degradation was not detectable after 36 hours, but after48 hours the receiving block extracts left a trail of radioactivity on the chromatograms. By the end of the experiment, the donor blocks still contained pure 2,4-D(-1-^{14}C), but there was evidence of slight degradation of the translocated radioactivity. This resulted in ^{14}C running to Rf zones 0.50-0.55, 0-0.10, 0.70-0.75 and 0.90-1.00 in solvent systems (a), (b) and (c).

In general, these data provide little evidence for the breakdown of 2,4-D(-1-^{14}C) during transit through the root segments and confirm that even after 60 hours, or in the presence of light, the ^{14}C was confined mainly to the 2,4-D molecule.

Extract No.	Position of ^{14}C label	Treatment	Sample
1	-COOH	Acropetal-dark	Donor
2	"	"	Segments
3	"	"	Receiver
4	"	Basipetal-dark	Donor
5	"	"	Segments
6	"	"	Receiver
7	$-CH_2$	Acropetal-dark	Donor
8	"	"	Segments
9	"	"	Receiver
10	"	Basipetal-dark	Donor
11	"	"	Segments
12	"	"	Receiver
13	-COOH	Acropetal-light	Donor
14	"	"	Segments
15	"	"	Receiver
16	"	Basipetal-light	Donor
17	"	"	Segments
18	"	"	Receiver
19	$-CH_2$	Acropetal-light	Donor
20	"	"	Segments
21	"	"	Receiver
22	"	Basipetal-light	Donor
23	"	"	Segments
24	"	"	Receiver
25	-COOH	-	Stock soln.
26	$-CH_2$	-	Stock soln.

TABLE 31

The distribution of radioactivity from extracts applied to paper chromatograms, developed in isopropanol : ammonia : water (10:1:1). The radioactivity was estimated by liquid scintillation counting and the data are expressed as the % of the applied ^{14}C which was detected in each Rf zone.

Extract No.	% of applied radioactivity in each Rf zone									
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
1	-	-	-	-	-	-	36.1	63.9	-	-
2	-	-	-	-	-	-	15.7	83.4	0.7	-
3	-	-	-	-	-	-	-	95.6	4.4	-
4	-	-	-	-	-	0.6	61.0	38.3	-	-
5	-	-	-	-	-	0.4	53.1	44.9	1.5	-
6	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	21.4	78.4	-	-
8	-	-	-	-	-	-	3.0	96.0	1.0	-
9	-	-	-	-	-	-	-	100.0	-	-
10	-	-	-	-	-	0.2	23.2	76.6	-	-
11	-	-	-	-	-	-	14.8	84.1	1.0	-
12	-	-	-	-	-	-	-	100.0	-	-
13	-	-	-	-	-	-	2.4	94.7	2.9	-
14	-	-	-	-	-	-	17.1	80.9	2.0	-
15	-	-	-	-	-	-	-	100.0	-	-
16	-	-	-	-	-	-	2.4	96.4	1.2	-
17	-	-	-	-	-	-	3.1	92.7	2.2	-
18	-	-	-	-	-	-	-	100.0	-	-
19	-	-	-	-	-	-	2.2	95.2	2.5	-
20	-	-	-	-	-	-	12.1	85.7	2.1	-
21	-	-	-	-	-	-	-	100.0	-	-
22	-	-	-	-	-	0.5	39.9	60.0	-	-
23	-	-	-	-	-	0.5	66.7	29.2	2.9	-
24	-	-	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	8.0	92.0	-	-
26	-	-	-	-	-	-	12.5	87.5	-	-

Extract No.	Position of ^{14}C label	Treatment	Sample
1	-COOH	Acropetal-dark	Donor
2	"	"	Segments
3	"	"	Receiver
4	"	Basipetal-dark	Donor
5	"	"	Segments
6	"	"	Receiver
7	$-CH_2$	Acropetal-dark	Donor
8	"	"	Segments
9	"	"	Receiver
10	"	Basipetal-dark	Donor
11	"	"	Segments
12	"	"	Receiver
13	-COOH	Acropetal-light	Donor
14	"	"	Segments
15	"	"	Receiver
16	"	Basipetal-light	Donor
17	"	"	Segments
18	"	"	Receiver
19	$-CH_2$	Acropetal-light	Donor
20	"	"	Segments
21	"	"	Receiver
22	"	Basipetal-light	Donor
23	"	"	Segments
24	"	"	Receiver
25	-COOH	-	Stock soln.
26	$-CH_2$	-	Stock soln.

TABLE 32

The distribution of radioactivity from extracts applied to paper chromatograms, developed in butanol : propionic acid : water (21 : 10 : 14). The radioactivity was estimated by liquid scintillation counting and the data are expressed as the % of the applied radioactivity which was detected in each Rf zone.

Extract No.	% of applied radioactivity in each Rf zone									
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
1	0.6	-	-	-	3.5	-	-	-	-	95.9
2	-	-	-	-	-	-	-	-	46.1	53.9
3	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	20.3	79.7
5	-	-	-	-	-	-	-	-	25.5	74.5
6	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	77.5	22.5
8	-	-	-	-	-	-	-	-	10.4	89.6
9	-	-	-	-	-	-	-	-	52.9	47.1
10	-	-	-	-	-	-	-	-	59.0	41.0
11	-	-	-	-	-	-	-	-	18.4	81.6
12	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	73.1	26.9
14	-	-	-	-	-	-	-	-	62.3	37.7
15	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	5.3	94.7
17	-	-	-	-	-	-	-	0.5	9.3	90.2
18	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	11.4	88.6
20	-	-	-	-	-	-	-	0.6	63.2	36.2
21	-	-	-	-	-	-	-	-	-	100.0
22	-	-	-	-	-	-	-	-	28.0	72.0
23	-	-	-	-	-	-	0.5	0.7	32.6	64.0
24	-	-	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-	64.0	36.0
26	-	-	-	-	-	-	-	-	52.0	48.0

Extract No.	Position of ^{14}C label	Treatment	Sample
1	-COOH	Acropetal-dark	Donor
2	"	"	Segments
3	"	"	Receiver
4	"	Basipetal-dark	Donor
5	"	"	Segments
6	"	"	Receiver
7	$-CH_2$	Acropetal-dark	Donor
8	"	"	Segments
9	"	"	Receiver
10	"	Basipetal-dark	Donor
11	"	"	Segments
12	"	"	Receiver
13	-COOH	Acropetal-light	Donor
14	"	"	Segments
15	"	"	Receiver
16	"	Basipetal-light	Donor
17	"	"	Segments
18	"	"	Receiver
19	$-CH_2$	Acropetal-light	Donor
20	"	"	Segments
21	"	"	Receiver
22	"	Basipetal-light	Donor
23	"	"	Segments
24	"	"	Receiver
25	-COOH	-	Stock soln.
26	$-CH_2$	-	Stock soln.

TABLE 33

The distribution of radioactivity from extracts applied to paper chromatograms, developed in isopropanol : ammonia : water (10 : 1 : 1) and n-butanol : propionic acid:water (21:10:14). The radioactivity was detected by autoradioagraphy and the data are expressed as the % of the applied radioactivity which was detected in each Rf zone.

Extract No.	isopropanol:ammonia:water			butanol:propionic acid:water		
	Rf1	Rf2	Rf3	Rf1	Rf2	Rf3
1	-	0.81	-	0.89	-	-
2	-	0.81	0.98	0.90	-	-
3	-	0.81	-	0.92	-	-
4	-	0.82	-	0.92	-	-
5	-	0.83	-	0.91	-	-
6	-	-	-	-	-	-
7	-	0.77	-	0.91	-	-
8	0.64	0.76	0.91	0.88	-	-
9	-	0.76	-	0.89	-	-
10	-	0.75	-	0.89	-	-
11	0.64	0.75	0.90	0.90	-	-
12	-	-	-	-	-	-
13	0.66	0.77	-	0.90	-	-
14	0.66	0.77	0.91	0.88	-	-
15	-	0.77	-	-	-	-
16	0.66	0.77	-	0.94	-	-
17	0.66	0.76	0.91	0.94	-	-
18	-	-	-	-	-	-
19	-	0.76	-	0.95	-	-
20	-	0.76	0.89	0.95	-	-
21	-	0.76	-	0.94	-	-
22	-	0.76	-	0.91	-	-
23	-	0.77	0.89	0.91	-	-
24	-	-	-	-	-	-
25	-	0.77	-	0.90	-	-
26	-	0.77	-	0.91	-	-

The Rf value of successive spots on the autoradiographs are presented.

TABLE 34 (a)

The distribution of radioactivity from extracts applied to paper chromatograms, developed in isopropanol : ammonia : water (8:1:1). The radioactivity was estimated by liquid scintillation counting and the data are expressed as % of applied radioactivity detected in each half-Rf zone.

Time (h)	Sample	% of applied radioactivity in 0.5Rf zones							
		0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40
18	Donor	-	-	-	-	-	-	-	-
	Receiver	-	-	-	-	-	2.0	-	-
	Segment-don. end	-	-	-	-	-	-	-	-
	Segment-rec. end	-	-	-	-	-	-	-	-
36	Donor	-	-	-	-	-	-	-	-
	Receiver	-	-	-	-	-	-	-	-
	Segment-don. end	-	-	-	-	-	-	-	-
	Segment-rec. end	0.7	-	-	-	-	-	2.0	-
48	Donor	-	-	-	-	-	-	-	-
	Receiver	6.3	2.4	3.1	4.8	1.4	2.6	3.7	-
	Segment-don. end	-	-	-	-	-	-	-	-
	Segment-rec. end	-	-	-	-	-	-	1.0	-
60	Donor	-	-	-	-	-	-	-	-
	Receiver	-	-	13.7	2.9	-	-	-	-
0	Unused donors	2.0	2.6	1.2	0.9	0.3	0.7	0.6	0.1
	Stock 2,4-D(^{14}C)	1.5	-	-	0.1	-	0.9	0.7	2.3

% of applied radioactivity in each half-Rf region											
0.45	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95	1.00
-	-	-	-	-	-	15.3	69.6	14.2	0.9	-	-
23.0	34.1	-	-	-	-	10.6	30.1	-	-	-	-
-	-	-	-	-	0.6	36.1	60.8	2.3	0.3	-	-
-	-	-	-	-	-	36.1	63.8	-	-	-	-
-	-	-	-	-	1.3	33.7	60.0	4.7	0.2	-	-
-	0.6	-	-	-	4.9	21.7	70.4	2.1	-	-	-
-	-	-	-	-	-	9.9	74.7	15.3	-	-	-
2.3	-	-	1.3	-	-	4.0	48.8	40.8	-	-	-
-	-	-	-	-	0.64	22.3	68.9	7.8	0.3	-	-
5.1	-	-	3.4	2.8	0.6	8.6	42.2	8.0	2.3	-	2.5
-	-	-	-	-	-	10.5	76.8	12.7	-	-	-
-	-	-	-	-	-	-	31.3	66.1	1.5	-	-
-	0.1	-	-	-	0.8	11.0	67.4	20.3	0.4	-	-
-	-	11.7	-	-	-	-	42.3	28.9	-	-	0.4
0.4	0.7	1.6	0.5	0.6	0.9	21.5	50.5	7.5	3.4	2.0	2.0
1.7	4.5	6.0	2.1	0.7	0.5	2.0	27.4	44.3	3.4	0.6	0.9

TABLE 31 (b)

The distribution of radioactivity from extracts applied to paper chromatograms developed in n-butanol : acetic acid : water (5:1:2.2). The radioactivity was estimated by liquid scintillation counting and the data are expressed as % of applied radioactivity detected in each half-Rf zone.

Time (h)	Sample	% of applied radioactivity in each half-Rf zone							
		0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40
18	Donor	-	-	-	-	-	-	-	-
	Receiver	-	-	-	-	-	-	-	-
	Segment-don.end	-	-	-	-	-	-	-	-
	Segment-rec.end	-	-	-	-	-	-	-	-
36	Donor	-	-	-	-	-	-	-	-
	Receiver	-	-	-	-	-	-	-	-
	Segment-don.end	0.8	-	-	-	-	-	-	0.6
	Segment-rec.end	-	-	0.7	-	2.7	-	-	-
48	Donor	-	-	-	-	-	-	-	-
	Receiver	6.0	2.8	5.4	3.0	2.9	6.0	-	-
	Segment-don.end	-	-	-	-	-	-	-	-
	Segment-rec.end	-	-	-	-	-	-	-	-
60	Donor	-	-	-	-	-	-	-	-
	Receiver	29.7	2.6	-	-	-	-	-	-
0	Unused donors	-	-	0.1	0.1	0.1	0.4	0.8	0.2
	Stock soln.	0.7	1.8	0.7	1.0	3.8	3.5	2.1	1.0

	% of applied radioactivity in each half-Rf zone											
	0.45	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95	1.00
	-	-	-	-	-	-	-	0.1	2.1	43.5	53.4	-
	-	-	-	-	-	-	-	-	-	53.9	46.1	-
	-	0.3	0.2	-	-	-	-	-	10.3	77.9	11.2	-
	-	-	-	-	-	-	-	-	-	35.8	64.2	-
	-	-	-	-	-	-	-	-	0.7	15.5	83.7	-
	-	-	-	-	-	-	-	-	-	20.9	76.7	2.3
	-	-	-	-	-	-	-	-	2.8	29.1	64.7	1.9
	-	-	-	-	-	-	-	-	-	27.3	69.2	-
	-	-	-	-	-	-	-	-	2.1	39.3	57.1	1.1
	-	5.0	-	-	-	-	-	-	-	15.2	48.2	2.3
	-	-	-	0.2	-	-	-	-	2.2	29.6	67.9	-
	-	-	-	-	-	-	-	-	-	5.8	77.8	16.3
	-	0.4	-	-	-	-	-	-	0.1	4.8	60.0	34.7
	-	-	-	-	-	-	-	-	-	4.4	60.3	2.9
	0.5	1.0	2.5	1.4	1.3	0.1	0.1	0.1	0.7	9.8	70.9	9.8
	2.3	2.4	12.1	5.5	2.2	2.5	3.5	1.8	0.7	5.5	36.8	9.9

TABLE 34 (c)

The distribution of radioactivity from extracts applied to paper chromatograms developed in n-butanol : acetone : water (5:2:3). The radioactivity was estimated by liquid scintillation counting and the data are expressed as % of applied radioactivity in each half-Rf zone.

Time (h)	Sample	% of applied radioactivity in each half-Rf zone							
		0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40
18	Donor	-	-	0.2	-	-	0.9	4.5	16.7
	Receiver	-	-	-	-	-	1.9	-	-
	Segment-don.end	0.1	-	-	-	1.3	8.6	24.4	34.9
	Segment-rec.end	-	-	3.2	-	-	10.2	37.6	30.5
36	Donor	-	-	-	0.3	1.7	7.6	19.4	31.5
	Receiver	-	-	-	-	-	10.7	35.7	34.0
	Segment-don.end	3.1	1.1	-	-	0.4	6.1	20.5	33.9
	Segment-rec.end	-	-	-	-	7.8	11.4	38.2	34.4
48	Donor	-	-	-	-	1.3	8.5	27.1	36.3
	Receiver	-	-	-	-	-	-	7.7	27.5
	Segment-don.end	6.2	1.4	-	-	-	-	4.2	20.3
	Segment-rec.end	0.6	-	-	-	-	-	2.2	18.8
60	Donor	-	-	-	-	0.9	9.2	22.9	29.0
	Receiver	0.3	-	-	-	-	4.1	7.6	20.1
0	Unused donors	0.2	-	-	0.9	4.8	15.9	24.7	26.5
	Stock soln.	1.7	2.5	1.3	1.5	2.2	7.8	10.6	14.1

	% of applied radioactivity in each half-Rf zone											
	0.45	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95	1.00
	29.5	34.0	10.9	1.7	0.8	0.2	0.1	0.1	0.3	-	-	-
	7.7	31.0	16.3	-	33.2	2.5	10.5	4.5	-	-	-	-
	12.7	14.5	2.9	-	0.5	-	-	-	-	-	-	-
	16.1	2.3	-	-	-	-	-	-	-	-	-	-
	27.5	9.8	1.0	0.3	0.1	0.3	0.4	0.1	-	-	-	-
	16.5	3.0	-	-	-	-	-	-	-	-	-	-
	25.7	9.0	-	-	-	-	-	-	0.1	-	-	-
	1.1	-	-	-	-	6.9	-	-	-	-	-	-
	18.8	3.6	0.7	0.3	1.3	0.6	0.3	0.5	0.6	-	-	-
	23.2	8.3	8.9	6.0	0.2	1.6	4.7	-	-	5.6	4.4	1.9
	37.4	26.4	3.6	-	-	-	0.3	-	-	-	-	-
	35.9	31.9	10.6	-	-	-	-	-	-	-	-	-
	25.0	9.8	2.1	0.1	0.3	0.4	0.1	0.1	0.1	0.1	-	-
	17.3	28.7	3.0	0.7	-	-	10.8	-	1.2	-	3.6	2.4
	14.8	8.9	1.0	0.6	1.6	0.5	0.3	-	-	-	-	0.1
	9.4	6.6	5.0	1.4	1.8	4.6	5.0	9.2	4.3	3.8	4.7	2.2

Pulse-labelling

The experiments in the previous section demonstrated that radioactivity could be drained out of the root segments in the donor block was replaced by a non-radioactive agar block after 25 hours. Successful pulse-labelling in this system might be expected, therefore, since the movement of a well defined pulse of ^{14}C also depends on a mechanism of tissue drainage after the radioactive front passes.

A one-hour pulse-label of 1.0 µM 2,4-D(-1-^{14}C) was applied to the apical or the basal end of the segments which pretreated for 25 hours with 1.0 µM blocks of unlabelled 2,4-D for 25 hours. The pre-treatment was given since exploratory experiments showed that a pulse of radioactivity did not move in freshly excised Pisum segments. Following the one-hour pulse, a fresh set of unlabelled 2,4-D blocks replaced the radioactive donor block for 2, 5, 7 or 9 hours, after which the segments were cut into 2mm pieces and processed for liquid scintillation counting. The data are the mean of eight replicates from experiments set up on two different occasions.

Figure 44 demonstrates that the applied radioactivity did not move along the segments as a distinct pulse. Instead, the initial high ^{14}C content in the 2mm of segment in contact with the donor block spread slowly along the segments, leaving a definite proportion of radioactivity in each region. After basal donation, for example, the 1st two 2mm pieces contained 10-15% of the total ^{14}C content whilst the succeeding pieces contained approximately 9-10, 2-5, 1-5 and 0-2% (table 35). There was less evidence of ^{14}C retention near to the site of donation following apical donation and by the end of the experiment the distribution was 24, 16, 12, 5 and 2% of the total ^{14}C. The radioactivity detected in the receiving blocks applied to the basipetal replicates, in the 8 and 10 hour experiments, exceeded that in the blocks applied to the acropetal replicates. The t-test values for the comparison of the means of the acropetal and basipetal replicates at the two time intervals were -1.98 and -2.04 respectively. Since the lowest value for significance was 2.365, little importance could be attached to the results. A greater proportion of the radioactivity passed back into the replacement blocks of unlabelled 2,4-D applied to the basal end than into those applied to the apical end of the segments

(table 35). This might be expected in view of the differences in surface area at either end of the segment.

During the one-hour pulse label of the radioactive herbicide uptakes of 379 and 356 dpm were recorded in the acropetal and basipetal replicates respectively. By the end of the experiment 28.8 and 26.1% (respectively) of the initial uptake could not be accounted for by the extractable radioactivity in the tissue (table 36). The loss from the acropetal and basipetal replicates did not take place at the same rate, since after 2 hours 23.9% had been lost from the basipetal replicates whilst only 14.3% had been lost from the acropetal treatments.

It is clear from these data that under the conditions provided 2,4-D was not able to pass along the segments as a distinct pulse.

FIGURE 44

The movement of radioactivity from 1.0 μM 2,4-D(-1-^{14}C) applied as a one-hour pulse-label to the apical or basal end of Pisum root segments at 25°C in darkness. The root segments were pretreated with blocks of 1.0 μM unlabelled 2,4-D for 25 hours and the pulse label was chased by further application of unlabelled 2,4-D blocks for 0, 2, 5, 7 and 9hours. Data are the mean of eight replicates carried out on two occasions.

1 = 1 hour pulse-label + 0 hours unlabelled 2,4-D
2 = " " " + 2 " " "
3 = " " " + 5 " " "
4 = " " " + 7 " " "
5 = " " " + 9 " " "

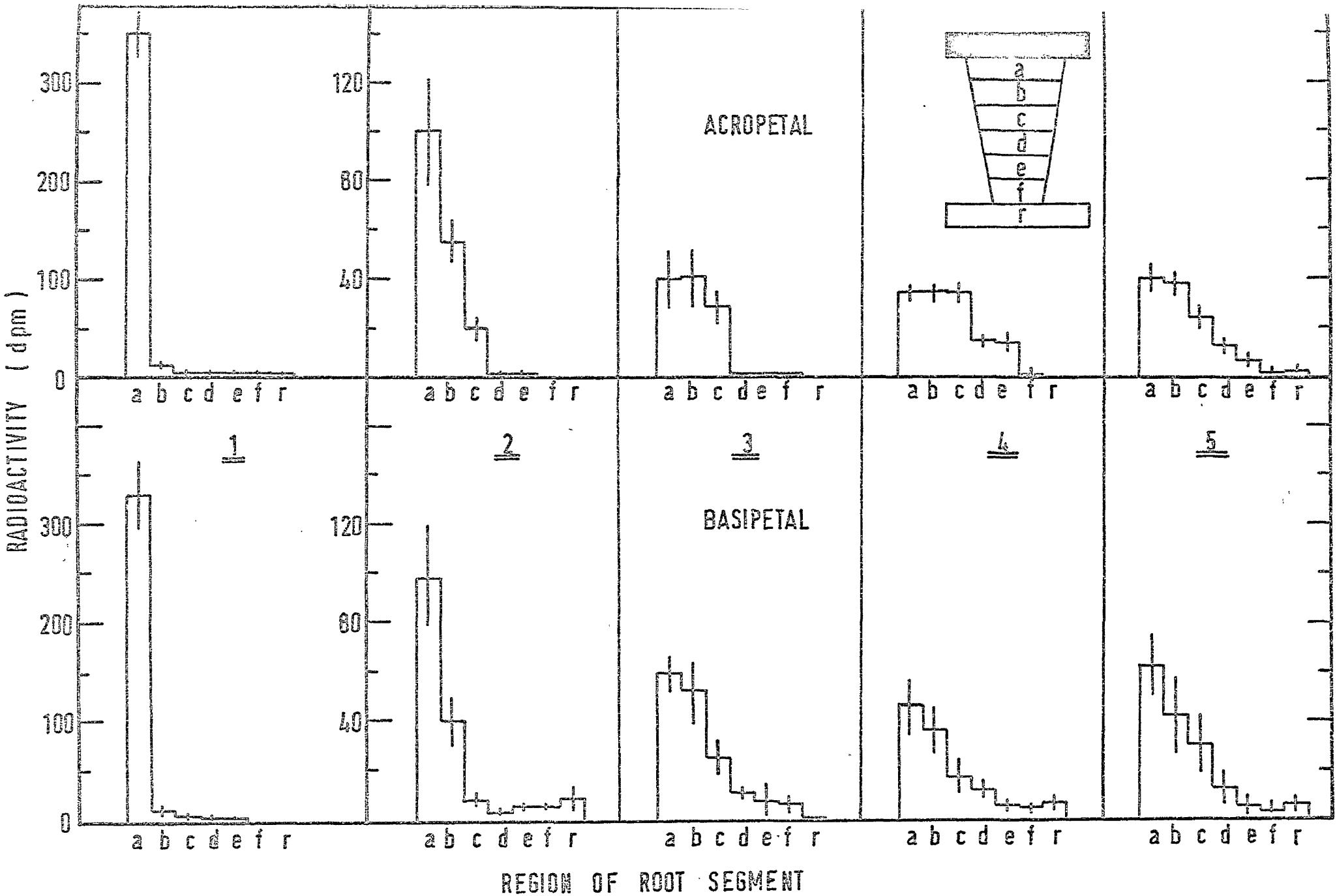

ACROPETAL
BASIPETAL
RADIOACTIVITY (dpm)
REGION OF ROOT SEGMENT
1
2
3
4
5
a b c d e f r
300
200
100
0
120
80
40
a
b
c
d
e
f
r

TABLE 35

The distribution of radioactivity along Pisum root segments supplied with 1.0 µM 2,4-D(-1-^{14}C) to the apical or the basal cut surface at 25°C in darkness. The results are the mean of eight replicates and are expressed as a percentage of the total uptake of radioactivity into the segment system.

Time of 2,4-D (h)		Treat.	Radioactivity in successive 2mm pieces of root segment (% of total uptake of dpm)							
Hot	Cold		A	B	C	D	E	F	Rec.	Repl.bl
1	0	Acrop	1.4	0.5	0.6	0.5	3.5	92.4	1.3	-
		Basip	92.9	3.4	1.7	0.6	1.1	0.1	0	-
1	2	Acrop	0.1	1.1	0.5	6.1	16.9	32.4	0	42.6
		Basip	36.3	14.8	2.7	0.9	1.9	2.2	3.5	37.4
1	5	Acrop	1.7	1.4	2.3	8.7	12.6	11.9	0.5	60.8
		Basip	24.7	21.9	10.5	4.5	3.1	3.0	0.6	31.5
1	7	Acrop	1.6	4.3	4.7	10.5	10.3	10.5	0.3	57.6
		Basip	19.5	15.2	7.4	5.1	2.5	2.1	3.1	45.0
1	9	Acrop	0.7	2.7	4.7	9.1	14.1	14.4	0.8	52.6
		Basip	24.0	16.2	11.7	5.2	2.3	1.7	2.8	35.9

Hot = radioactively labelled 2,4-D

Cold = unlabelled 2,4-D

Repl.bl = replacement block of unlabelled 2,4-D

TABLE 36

The total content of radioactivity in Pisum root segments, receiving block and replacement block of unlabelled 2,4-D, in a system supplied with 1.0 µM 2,4-D(-1-^{14}C) for a period of 1-hour at 25°C in darkness. Data are the mean of eight replicates carried out on two occasions.

Time of 2,4-D treatment (h)		Total uptake of Radioactivity (dpm)			
Hot	Cold	Acropetal	% loss	Basipetal	% loss
1	0	379.3±21.5	-	356.3±37.0	-
1	2	325.2±36.9	14.3	271.2±51.7	23.9
1	5	327.6±29.8	13.6	239.8±26.5	32.7
1	7	334.0±26.1	11.9	240.4±28.2	32.5
1	9	270.1±16.1	28.8	263.4±44.5	26.1

Hot = radioactively labelled 2,4-D

Cold= unlabelled 2,4-D

PISUM SEEDLING INVESTIGATIONS

The concluding section of the investigation was designed to determine whether the acropetal polarity of 2,4-D movement detected in the Pisum root segment system existed in the intact seedling. Donation of the radioactive herbicide was either as 1.0 µM 2,4-D(-1-^{14}C) in hollow, discoid donor blocks which encircled the root (fig.1b) or as a 1.0 µM aqueous solution into which the root tip was immersed **(fig. 1c).**

(1) Agar block donation

The discoid donor blocks were supplied, for progressively longer periods of time, to root tip or to a point approximately 5mm away from the cotyledons. Experiments were performed in green light and the periods of translocation were in darkness at 25°C. The data are the mean of three experiments, each consisting of three replicates, and the entire experimental evidence is based on 108 intact seedlings.

Histograms in figure 45 depict the level of radioactivity in the roots. Region A was the third of the radicle nearest to the cotyledons, region C represented the third nearest to the tip and region B the region of the root lying between the other two. After supplying the herbicide to region A for 3 hours the site of donation retained 67% of the total ^{14}C uptake, 27% was found in the middle region and 6% had moved into the apical region of the root. After 30 hours, only 60% remained close to the donor block whilst 13% had moved to the root tip, but by the end of the experiment a further 2% could be detected in that region. In contrast, however, application of the herbicide to the root tip (C) resulted in little movement of radioactivity towards the cotyledons. After 60 hours 96% of the applied radioactivity was confined to the site of donation, 4% had reached region B but no radioactivity could be detected in region A. Detectable movement into the other parts of the seedling ie. the shoot, occurred within 10 hours following basal donation to region A, but the plumule never contained more than 11% and the cotyledons no more than 5% of the total radioactive uptake. Donation to the root tip, however, resulted in virtually no movement into these structures (table 36).

The total content of radioactivity (figure 46) revealed that entry via the apical region of the root (C) was greater than via the basal region (A). On average, apical uptake exceeded basal uptake by 3379 dpm after an experiment of 20 hours and by 2619 dpm after 60 hours. Declines in the level of radioactivity held within the seedlings were detected after 20-30 hours and 40-60 hours, whilst increases were apparent after 10-20 and 40-50 hours. This phenomenon was not related to the site of donation employed and occurred in each of the three experiments.

At the end of each experimental period the length of the root and the shoot was measured prior to the processing of the material for liquid scintillation counting. The results are illustrated in figure 47 and show that the shoot increased in length by approximately 20mm during the 60 hour experiment and the site of donation of the herbicide on the root had little effect on the growth. Growth of the root was affected by the site of donation and the roots supplied with 2,4-D at the tip were approximately 10mm shorter than those supplied at the opposite end of the root. Examination of untreated seedlings revealed that the length of the radicle of three-day-old seedlings was 31mm (from 10 seedlings taken at random) and the zone of root hairs was 13.2mm long and started 6.4mm behind the root tip. Indian ink markers placed at 3mm intervals along the root showed that the 3mm zone at the root tip increased in length by 70% within a period of 24 hours, whilst little increase could be measured in any other zone. Application of 1.0 μM unlabelled 2,4-D to the radicle at a site near to the cotyledons, however, resulted in a 77% increase in length of the tip region, whilst no growth occurred when the herbicide was supplied to the root tip. The statistical significance of these data was not ascertained since the population sampled was not large.

These data indicate that the uptake of the radioactive herbicide was greatest following an application to the root apex, but the most rapid translocation of radioactivity was detected following an application near to the cotyledons. Since the elongation of the root took place mainly at the tip region it would be unlikely that growth would enhance apparent movement of radioacivity towards the root tip. On the other hand, the characteristics of growth could well help to confine the radioactivity to the tip of the root following a donation in that area. It is clear, however, that 2,4-D applied to the roots of Pisum sativum would find only limited avenues for translocation into the aerial structures, provided that the position of ^{14}C reflected the presence of the herbicide.

FIGURE 45a

Distribution of radioactivity in intact Pisum seedlings supplied with 1.0 μM 2,4-D(-1-^{14}C) to the region of the root nearest to the cotyledons (A). The experiment was carried out on three occasions and the data are the mean of the results from each experiment.

Region A = third of root nearest to the cotyledons
" B = " " " lying between regions A and C
" C = " " " nearest to root tip

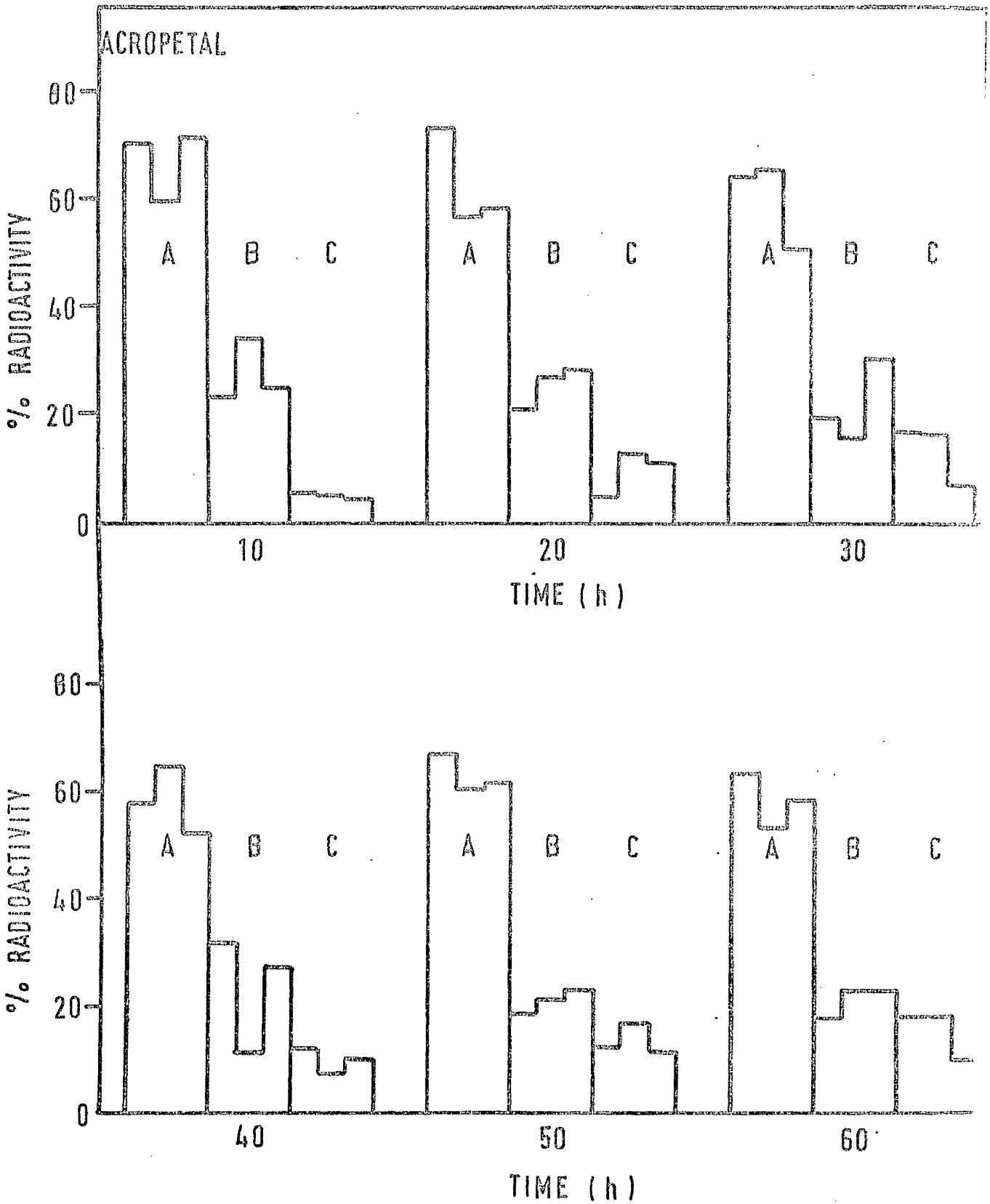
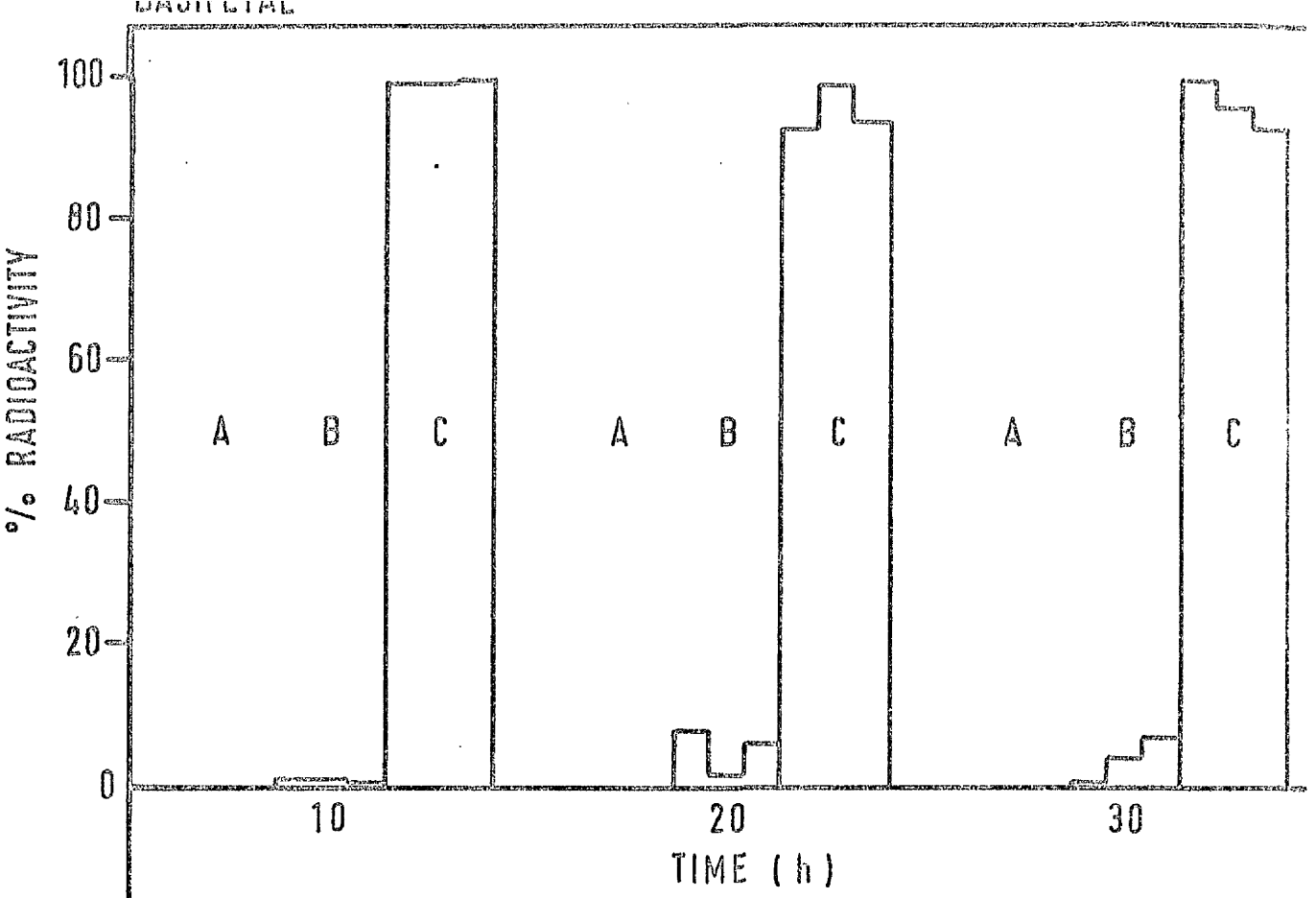

ACROPETAL
% RADIOACTIVITY
80
60
40
20
0
A
B
C
10
20
30
TIME (h)
% RADIOACTIVITY
80
60
40
20
0
A
B
C
40
50
60
TIME (h)

FIGURE 45b

Distribution of radioactivity in intact Pisum seedlings supplied with 1.0 μM 2,4-D(-1-^{14}C) to the third of the root nearest to the root tip (C). The experiment was carried out on three occasions at 25°C in darkness and the data are the mean of the results from each experiment.

Region A = third of the root nearest to the cotyledons
" B = " " " " lying between regions A and C
" C = " " " " nearest to the root tip


% RADIOACTIVITY
100
80
60
40
20
0
A
B
C
A
B
C
A
B
C
10
20
30
TIME (h)

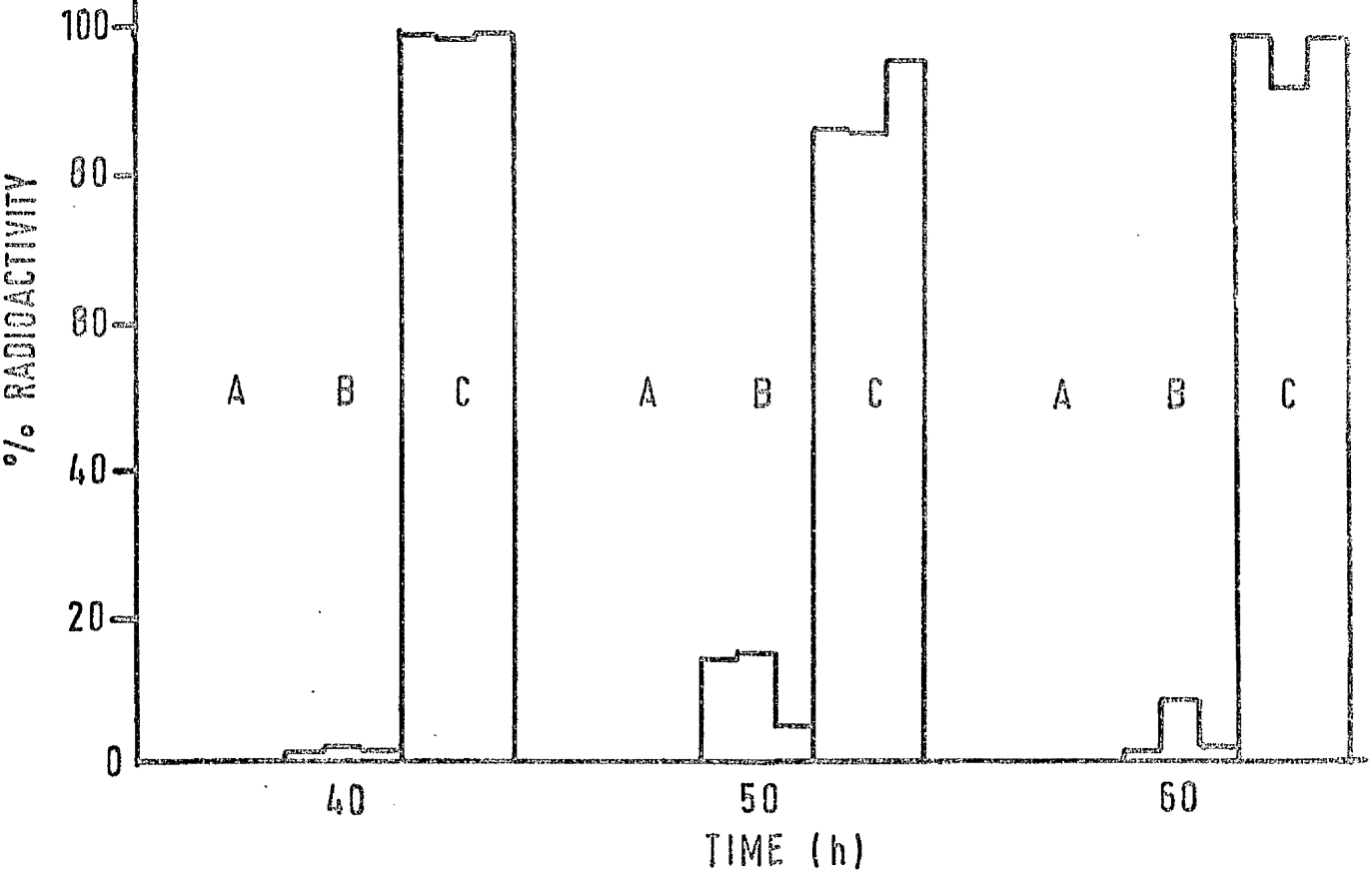

% RADIOACTIVITY
100
80
60
40
20
0
A
B
C
A
B
C
A
B
C
40
50
60
TIME (h)

TABLE 36

The level of radioactivity in the cotyledons and plumules of Pisum seedlings supplied with 1.0 μM 2,4-D(-1-^{14}C) near to the cotyledons or near to the root tip. The mean data from three independent experiments are presented separately and are expressed as % of the total uptake of radioactivity at the two sites of donation.

Time (h)	Donation near to cotyl. % of total uptake found in cotyl.			plumules			Donation at tip of root % of total uptake found in cotyl.			plumules		
	1	2	3	1	2	3	1	2	3	1	2	3
10	1.6	2.5	0.2	0	0.1	0.1	0	0	0	0	0	0
20	1.1	4.3	0.4	0.5	0.2	1.9	0	0	0	0	0	0.1
30	0	1.7	1.8	0	0.9	10.5	0	0	0	0	0	0.1
40	0.5	0.8	0.4	0.1	2.0	1.7	0	0.1	0	0	0.1	0
50	0.8	1.3	0.1	1.8	0.7	3.3	0	0	0	0	0	0.1
60	0.3	1.0	1.3	0.4	4.7	7.7	0	0	0.1	0	0.1	0.1

FIGURE 46

Radioactivity extracted from Pisum seedlings supplied with 1.0 μM 2,4-D(-1-^{14}C) at (A) a site in the basal region of the root near to the cotyledons and (B) a site near to the root tip. The experiment was carried out on three occasions at 25°C in darkness and 1, 2 and 3 indicate the mean result from each experiment.

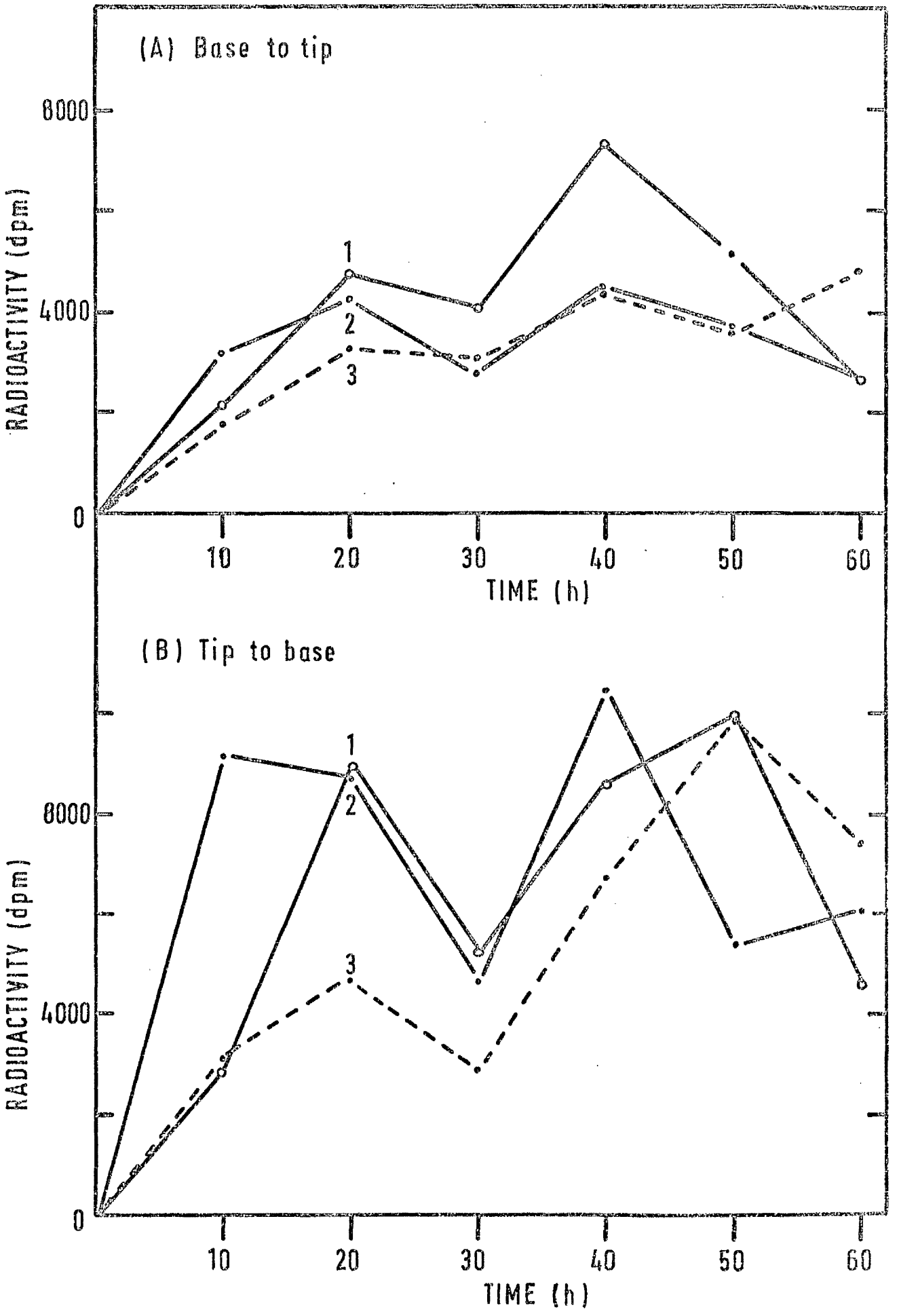
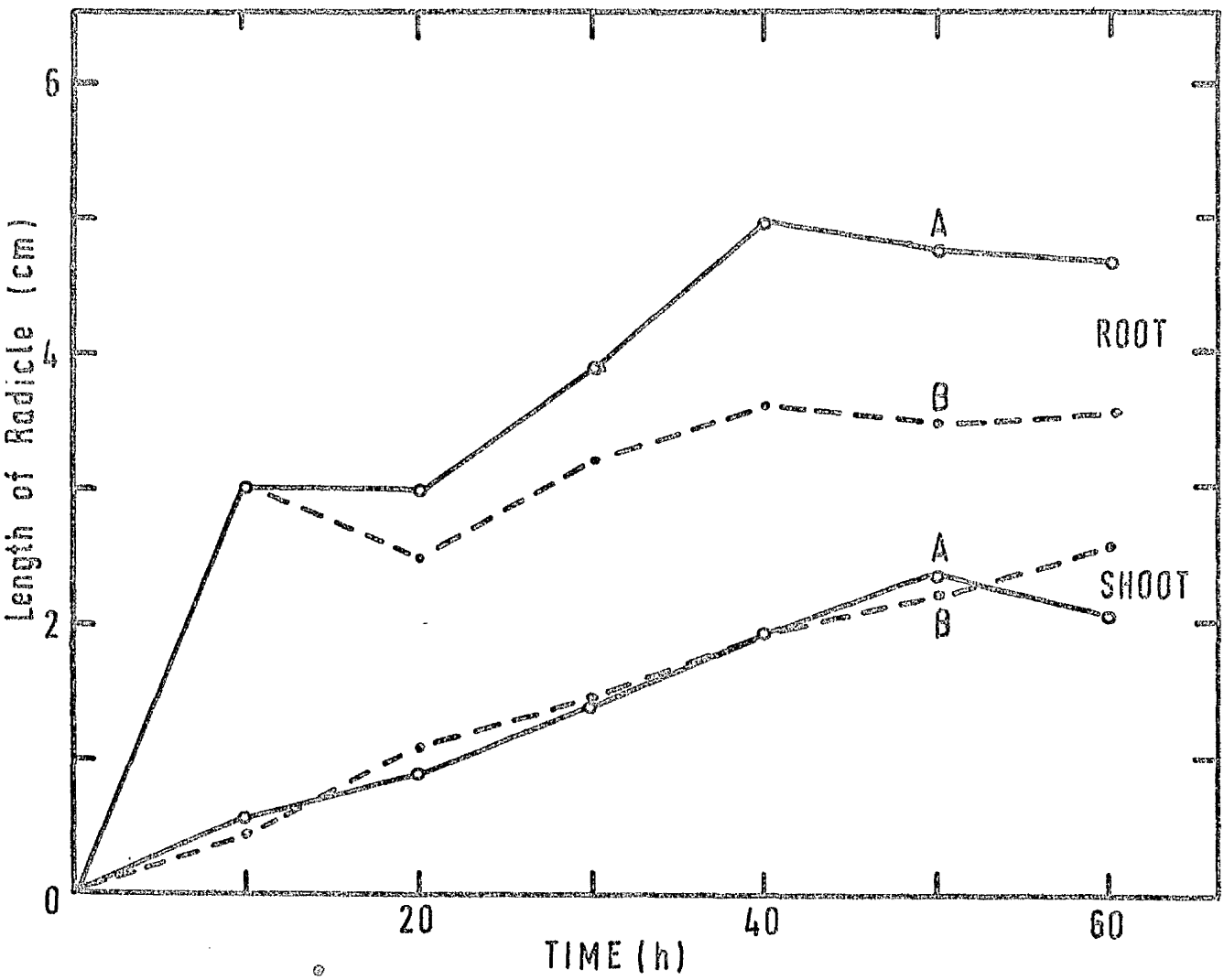

(A) Base to tip
8000
4000
0
RADIOACTIVITY (dpm)
1
2
3
10
20
30
40
50
60
TIME (h)
(B) Tip to base
8000
4000
0
RADIOACTIVITY (dpm)
1
2
3
10
20
30
40
50
60
TIME (h)

FIGURE 47

Elongation of the root and shoot of three-day-old Pisum seedlings supplied with 1.0 μM 2,4-D(-1-^{14}C) at (A) a site near to the cotyledons and (B) a site near to the root tip. The data are the mean of experiments carried out on three occasions at 25°C in darkness.


Length of Radicle (cm)
0
2
4
6
20
40
60
TIME (h)
A
B
ROOT
A
B
SHOOT

(2) Aqueous donation

Three-day-old Pisum seedlings were placed with the apical 3mm of the root tip immersed in 5ml of 1.0 μM 2,4-D(-1-^{14}C) solution for 0.5, 1.0, 3.0, 4.0 or 5.0 hours at 25°C in white light or darkness (figure 1c). Transport periods were run in humidified boxes in the white light or darkness in the same growth room and the experimental manipulations were carried out on three different occasions with three independent replicate seedlings for each time interval. Thus, each point on the graphs is the mean of nine readings and the experimental work necessitated the use of 90 intact seedlings.

A significant difference in the level of radioactivity in the light and dark treated replicates could not be detected in the first three hours of the experiment (figure 48). During the remainder of the experiment a rapid increase in the rate of uptake resulted in 8950 dpm in the dark treated seedlings and only 4048 dpm in the light. This was significant at the 1% level of probability (table 37). Reduction in uptake in response to light (*) was obvious in the three regions of the root (A,B & C), the cotyledons and the plumule after 4 or 5 hours (figure 49). Within one hour, 250-500 dpm could be extracted from the region (A) of the root nearest to the cotyledons, whilst the level in the plumule and cotyledons was 325-450 dpm and 100-200dpm respectively in light and darkness. During the initial 30 minutes of the experiment 325-375 dpm were translocated into the plumule. This indicated a velocity of at least 8 cm h^{-1}, since the plumule was approximately 4 cm away from the site of donation.

These data strongly suggest that the movement of ^{14}C from the root apex into the plumule was extremely rapid following an aqueous donation of the herbicide to the root tip. In addition, white light resulted in an overall reduction in the level of radioactivity in the seedlings. It was surpriding, however, that an application of the radioactive compound in an agar block to the root tip resulted in little detectable basipetal movement of ^{14}C. A further control experiment was performed to determine whether a water deficit within the plant might account for the ease of distribution of the aqueous solution.

A 1.0 μM solution of 2,4-D(-1-^{14}C) was supplied to the root tip of three-day-old Pisum seedlings by the method described in the previos section (figure 1c), following a 5-hour pretreatment with the root tip immersed in water. The control seedlings had the root tip immersed in the radioactive herbicide solution immediately. Table 38 presents the data from three representative seedlings and show that the water pretreatment had little effect on the subsequent uptake of the ^{14}C. It is unlikely, therefore, that the rapid uptake and distribution of the radioactivity was associated with a rapid influx of water into the root to satisfy any internal deficit.

FIGURE 48

The level of radioactivity in Pisum seedlings supplied with 1.0 μM 2,4-D(-1-^{14}C) to the root tip as an aqueous solution at 25°C in darkness or light. The results are the mean of nine independent determinations.

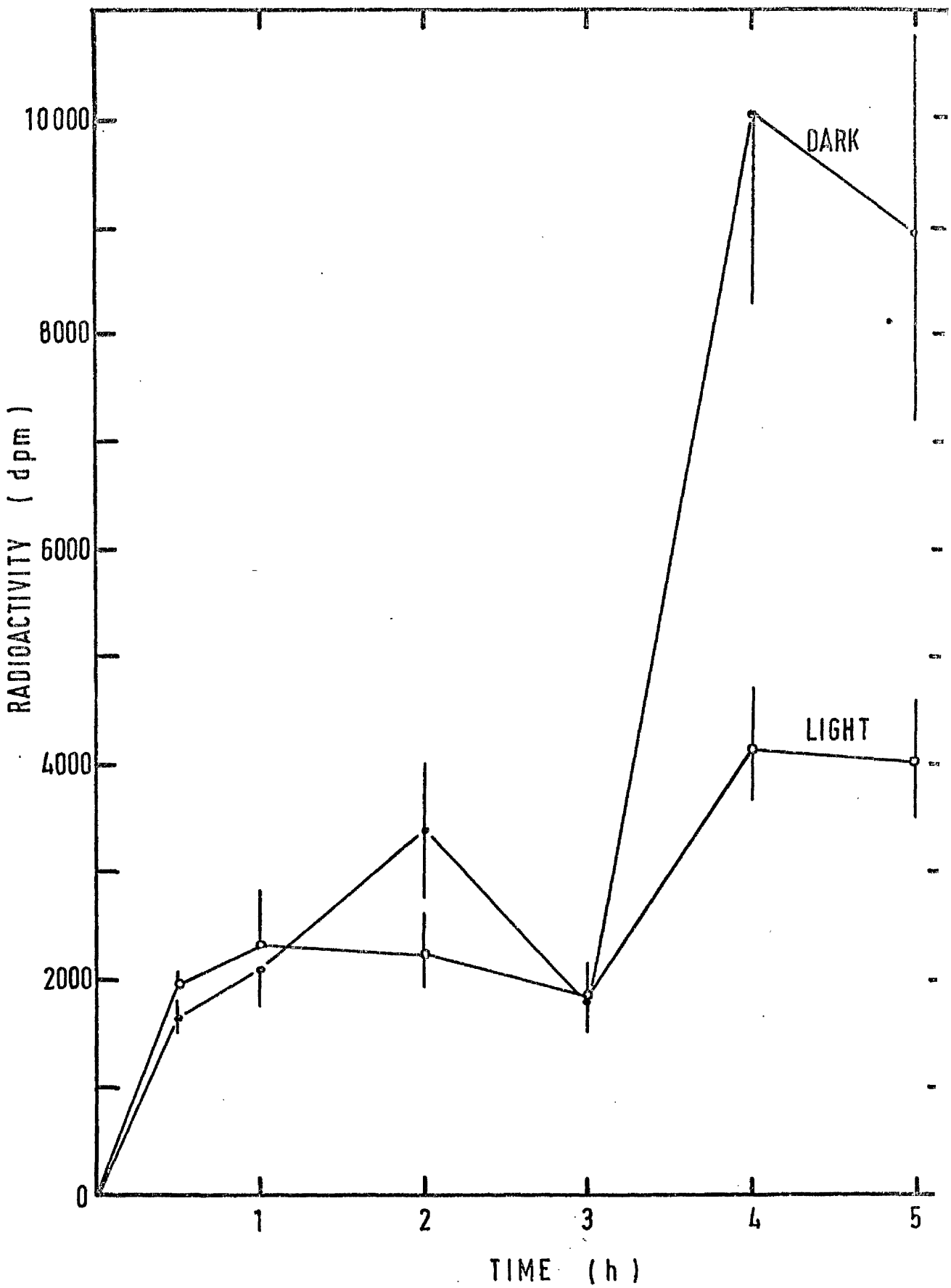

10000
8000
6000
4000
2000
0
RADIOACTIVITY (dpm)
DARK
LIGHT
1
2
3
4
5
TIME (h)

FIGURE 49

Radioactivity in (a) the three regions of the intact root, (b) the cotyledons and (c) the plumule of *Pisum* seedlings supplied with 1.0 μM 2,4-D(-1-^{14}C) at 25°C in white light or darkness. The data are the mean of nine independent determinations.

Region A = third of root nearest to the cotyledons
" B = " " " lying between regions A and C
" C = " " " nearest to the root tip

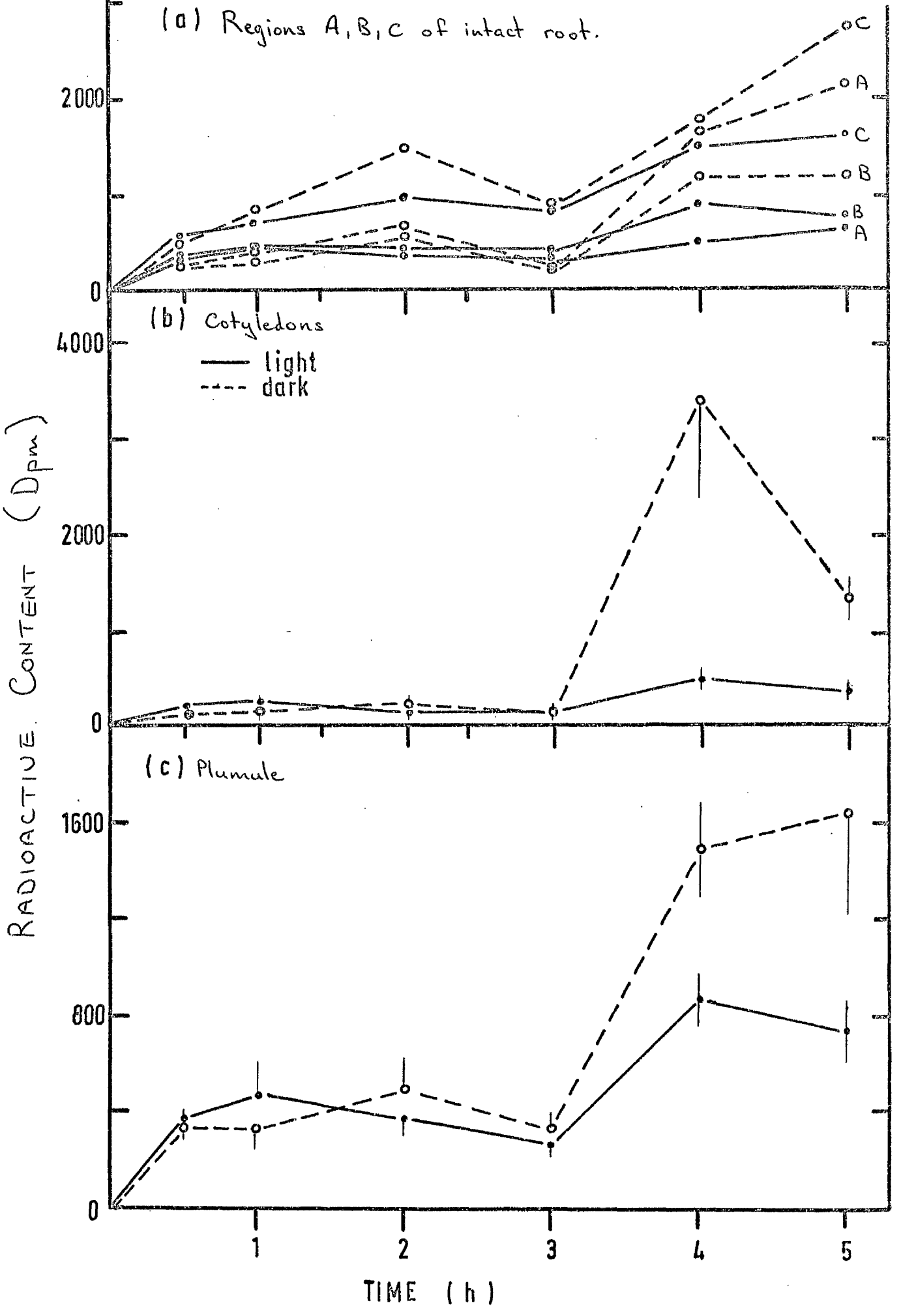

(a) Regions A, B, C of intact root.
2000
0
C
A
C
B
B
A
(b) Cotyledons
light
dark
4000
2000
0
(c) Plumule
1600
800
0
1
2
3
4
5
TIME (h)
RADIOACTIVE CONTENT (Dpm)

TABLE 37

t-tests of paired comparisons ie. light vs. dark, on data presented in figures 48 and 49.

Sample	Means compared.	S.E. of difference	t-value
Total ^{14}C content (fig. 48)	3h light-dark	1165.89	0.056
	4h " "	59221.98	-2.993 *
	5h " "	47569.66	-3.091 *
^{14}C in plumule (fig. 49)	3h " "	1716.07	-1.013
	4h " "	9906.96	-1.887
	5h " "	9937.39	-2.760 *
^{14}C in cotyledons (fig. 49)	3h " "	1250.66	-0.794
	4h " "	32657.50	-2.675 *
	5h " "	16361.46	-1.776

$t_{0.001}$ (8DF) = 5.041

$t_{0.01}$ (8DF) = 3.355

$t_{0.05}$ (8DF) = 2.306

TABLE 38

Distribution of radioactivity in Pisum seedlings supplied with 1.0 µM 2,4-D(-1-^{14}C) to the tip of roots pretreated with water for 5 hours prior to the application of the herbicide. Controls were run in which the radioactivity was supplied to the root tip immediately, without pretreatment in water. The experiments were carried out at 25°C in darkness and the results from three representative seedlings are presented.

Seedling regions (dpm)	RADIOACTIVITY (dpm) CONTROL			WATER PRETREATMENT		
	1	2	3	1	2	3
Root region C	1569.2	1572.7	1408.3	1838.4	1064.9	1333.4
" " B	434.6	362.1	212.8	631.4	358.1	247.8
" " A	372.8	217.8	127.0	77.5	152.2	27.0
Cotyledons	145.2	132.7	44.2	35.5	31.1	26.3
Plumule	224.6	200.6	106.5	141.8	74.2	134.7
Total uptake	2746.4	2485.9	1898.8	2724.6	1680.1	1669.2

(3) Tissues involved in 2,4-D translocation in Pisum seedlings

Experiments were designed to determine if any specific tissue was involved in the translocation of the herbicide in Pisum root tissue.

In the first experiments, a 5mm portion of the primary root situated 5mm below the cotyledons was either (a) steam girdled or (b) had the cortex removed. The latter process was simple and was achieved by gently bending the root until the outer tissues of the root were torn, the epidermis and cortex were then peeled away from the central vascular tissue and removed. Figure 50 presents the mean data from nine replicates, from experiments carried out on three independent occasions, at 25°C in darkness for 15 hours.

Steam girdling had no effect on the total quantity of radioactivity which was extractable from the seedlings, but ^{14}C accumulated in the area of the girdle making the level approximately twice that detected in the controls. The control plumules contained 254 dpm more than those from the treated plants but this was not great enough to be significant (table 39). Removal of the cortex from the vascular system resulted in a 22% reduction in total ^{14}C uptake which was not significant at the 5 % level of probability. A 36% reduction in radioactivity into the plumules was recorded after theremoval of the cortex.

If the steam girdling data were considered in isolation, it would appear that the living cells of the intact root played only a minor role in the translocation of radioactivity from the root tip. However, the result of the cortex removal experiments revealed that considerable movement of radioactivity might, in fact, occur via the living cells.

In the second experiment, the Pisum roots were supplied with the radioactive herbicide for a period of 24 hours, after which the steam girdling or the cortex removal operation was carried out and the root tips were replaced into distilled water. The radioactivity in region C was determined after a further period of 24 hours, in order that ^{14}C would be able to drain into the distilled water. Table 40 presents the data from six replicate seedlings treated on two different occasions and show that removal of the cortex had little effect on the ^{14}C in region C whilst the steam treatment resulted in a 6-fold decrease in content. It would appear, therefore, that the living tissues of the vascular system were necessary

for acropetal movement of radioactivity in the intact root.

In the third experiment, the radioactive herbicide was applied to region A of the root and the movement of ^{14}C past the girdled or the region lacking cortex was monitored as the radioactive content of region C, 24 hours later. The data presented in table 41 revealed that a 14-fold reduction of content resulted from the steam treatment and the content was approximately half that detected after removing the cortical tissues. These data indicate that both the cortex and the living vascular tissue were involved in the movement of radioactivity in the intact root of Pisum seedlings, which were exposed to white light.

(4) Identity of radioactive compounds in roots of intact Pisum seedlings

In order to determine whether the ^{14}C in the root system of the intact seedlings was still confined to the 2,4-D molecule methanol extracts were analysed by paper chromatography in three different solvent systems. The position of radioactivity on the chromatograms was located by the use of a Panax chromatogram scanner and the results are presented in table 42. It is clear that the radioactivity extracted from the root system reflected the position of 2,4-D, under the conditions employed. However, the slight alteration of the Rf value of the radioactive compound extracted from the roots which had been drained into unlabelled 2,4-D solution might indicate that the roots contain relatively immobile radioactive metabolites.

In summary, 2,4-D or metabolites of 2,4-D moved towards the apex of intact Pisum seedlings in a polar manner under the conditions tested using the agar block technique. This was not true for the treatments in which the herbicide was supplied to the root apex as an aqueous solution. In this case a strong basipetal movement of radioactivity could be detected. The presence of a functional vascular system was essential.

FIGURE 50

Effect of cortex removal or steam girdling region B of the root of intact Pisum seedlings supplied with an aqueous solution of 1.0 µM 2,4-D(-1-^{14}C) to the root tip. The data are the mean of nine replicates from three experiments carried out at 25°C in darkness, with a translocation period of 15 hours.

1 = Root apex (region C)
2 = Root region B
3 = Root region C
4 = Cotyledons
5 = Plumule
6 = Total uptake of radioactivity

RADIOACTIVITY (dpm)
0
1000
2000
3000
6000
9000
10000
CONTROL
CORTEX REMOVED
STEAM GIRDLED
1
2
3
4
5
6

TABLE 39

Paired t-test of the data presented in figure 50

$t_{0.05} = 2.306$ $t_{0.01} = 3.355$ $t_{0.001} = 5.041$ (8 DF)

Total uptake values compared	t-values
Control - cortex removed	2.24
" - steam girdled	-0.64
Cortex removed - steam girdled	-0.83

TABLE 40

Following a 24 hour donation of 1.0 μM 2,4-D(-1-^{14}C) to the root apex of intact Pisum seedlings, the root was either steam girdled or the cortex was removed and the root tip transferred to distilled water for a further 24 hours. The data for six representative replicates from two independent experiments are presented.

Rep. No.	RADIOACTIVITY IN REGION C OF ROOT (dpm) Control	Cortex removed	Steam girdled
1	221.0	223.1	53.7
2	363.8	323.6	60.3
3	404.5	573.2	65.2
4	460.7	227.0	63.4
5	431.1	201.6	59.7
6	310.3	337.5	56.1
Mean	365.3	314.3	59.7

TABLE 41

Radioactivity detected in region C following an agar block application of 1.0 μM 2,4-D(-1-^{14}C) to region A of the root of intact Pisum seedlings. The data for six replicates carried out at 25°C in light are presented.

Rep. No.	RADIOACTIVITY IN REGION C OF ROOT (dpm) Control	Cortex removed	Steam girdled
1	1840.8	126.2	125.7
2	1486.7	202.8	143.0
3	750.0	123.1	102.0
4	1791.2	262.9	80.7
5	1395.6	68.2	53.1
6	894.9	190.2	64.6
MEAN	1359.9	162.2	94.8

TABLE 42

Determination of the identity of the radioactive compounds extracted from the roots of intact Pisum seedlings supplied with 1.0 μM 2,4-D(-1-^{14}C) at 25°C in the light. In addition, extracts were taken from roots which had been supplied with the radioactivity for 24 hours and subsequently "drained" into unlabelled 2,4-D solution or distilled water.

Solvent system 1 = n-butanol:acetone:water (5:2:3)
" 2 = n-butanol:acetic acid:water (5:1:2.2)
" 3 = iso-propanol:ammonia:water (8:1:1)

Extract	Solvent system	Run of solvent (mm)	Run of spot (mm)	Rf
Radicle	1	165	123	0.74
	2	210	192	0.91
	3	271	227	0.84
Radicle - unlabelled 2,4-D	1	156	117	0.75
	2	191	172	0.90
	3	278	250	0.90
Radicle - distilled water	1	166	120	0.72
	2	215	188	0.87
	3	275	230	0.84
Donor solution	1	17	12	0.71
	2	20	17	0.87
	3	28	23	0.84
Stock radioactive solution	1	16	11	0.69
	2	19	17	0.88
	3	28	24	0.85

DISCUSSION

The research programme was based upon an assessment of the use of isolated root segments as an acceptable technique in the study of the movement of 2,4-dichlorophenoxyacetic acid in plant tissue. Since a cursory appraisal of the system revealed several potential artefacts each component was studied in detail in order to define the roles and the interrelationships within the complete technique. Clearly, the usefulness of the technique would be directly related to the ease and accuracy with which the data could be extrapolated to the intact plant.

Preliminary investigations were designed to determine a suitable working concentration of the herbicide for use in the Pisum root segment system. The choice depended upon the relative phytotoxicity and specific activity of the radioactive compound and a compromise between the two factors was to be necessary. Two bioassays were employed and flotation of the segments in solutions of unlabelled 2,4-D indicated that concentrations of 0.1 nM to 10.0 nM inhibited least, whilst inclusion of the herbicide in agar blocks applied to the ends of the segments revealed little change in potency over the entire range. The lack of compatibility between the two sets of data could well be related to the absorptive powers of the root, in which case flotation of the segments would, perhaps, aid penetration, uptake and accumulation of the herbicide in phytotoxic concentrations. This was supported by the fact that all concentrations of 2,4-D tested in the flotation experiments produced a reduction in elongation of the segments below the level of the control. Elongation in the agar block method, however, was greater than in the controls and was probably caused by slow penetration of the compound through the two cut surfaces of the segments. These findings would substantiate those of Audus (1948) that the initial rate of inhibition of root growth in Pisum and Lepidium was limited by the rate of entry of low concentrations of the herbicide. Promotion of segment elongation by sub-optimal concentrations within the tissue might be mediated through mechanisms similar to those operating in auxin stimulation of root growth (Thimann, 1936, 1956). In view of these results, 1.0 μM 2,4-D(-1-^{14}C) was chosen as a working concentration possessing an acceptable level of radioactivity and phytotoxicity.

An acropetal polarity in the movement of 2,4-D labelled with ^{14}C in either the -COOH or the $-CH_2$ group of the acetic acid side chain was detected after supplying the radioactive compound to either end of the sub-apical segments of Pisum roots. In other words, the applied radioactivity moved preferentially towards the root apex. After a lag-phase of 10-15 hours accumulation of a mobile radioactive component was approximately 32 times greater in agar receiving blocks applied to the apical end than in those applied to the basal end of the segments when 2,4-D(-1-^{14}C) was donated and 13 times greater when 2,4-D(-2-^{14}C) was supplied (fig 6). The polarity was not absolute and after 60 hours a maximum 50 dpm were detected in the blocks from basipetal replicates supplied with compound labelled in either position. Several workers, including Wilkins and Scott (1968) and Kirk and Jacobs (1968) have reported a similar polarity in the movement of IAA in sub-apical root segments of several monocotyledonous and dicotyledonous species. A faster velocity of IAA than of 2,4-D movement was indicated by the relatively short lag-phases whenever the indole compound was used. The acropetal/basipetal ratio of IAA transport in Zea, Avena, Triticum, Lens and Helianthus depended upon the species and ranged from 7.8 to 37 during a 6 hour experiment. Iversen and Aasheim (1970), however, claimed that accumulation of ^{14}C in receiving blocks supplied to basipetal replicates was only 25% smaller than in those supplied to acropetal replicates of Helianthus roots. No other evaluation of IAA movement revealed such a pronounced basipetal component, although Hillman and Phillips (1970) reported that the basipetal transport in Pisum root segments could amount to 50% of the acropetal movement.

A disturbing feature of the receiving block data was the minimal level of ^{14}C accumulation. Indeed, Yeomans and Audus (1964) and Bonnett and Torrey (1964) were forced to abandon the estimation of radioactivity in the blocks since the accumulation was so small. The problem was not so acute in the 2,4-D/Pisum system where 30% of the total uptake of ^{14}C could be extracted from apical blocks at the end of a 60 hour experiment, but this only accounted for 12% of the initial donor supply. Pilet (1964) found that 6.1% of the radioactivity supplied to Lens root segments was present in the blocks after only 2 hours. However, accumulation in apical blocks

applied to Lens for 6 hours; Pisum for 24 hours and Zea for 18 hours accounted for only 0.81, 3.3 and 7.0 % of the total ^{14}C uptake respectively (Kirk and Jacobs, 1968; Hillman and Phillips, 1970; Wilkins et al, 1972). The values would be very much smaller if expressed as a proportion of the initial donor content. These data indicate clearly that (a) the root segments had a great capacity for the retention of radioactivity, (b) the radioactive molecules were subjected to a massive degradation with a subsequent loss of ^{14}C from the system or (c) ^{14}C transport was minimal.

The distribution of radioactivity along the Pisum root segments revealed, without any doubt, the polar behaviour in the movement of 2,4-D(-1-^{14}C) (figure 10). By the end of the experiment, 4-6% of the applied radioactivity had arrived in the 2mm of the segment adjacent to the receiving blocks of the acropetal replicates, whilst only 1-2% was present in the basipetal replicates (figures 10 and 11). This closely paralleled the findings when radioactive IAA was supplied to roots of Brassica, Convolvulus, Vicia, Pisum, Lens and Zea. Accumulation in the apical end of these segments was up to 7 times greater than that at the opposite end, but was dependent upon the region of the root from which the segment was excised, the length of the segment and the concentration of the auxin supplied (Iversen and Aasheim, 1970; Bonnett and Torrey, 1965; Yeomans and Audus, 1964; Kirk and Jacobs, 1968; Wilkins etal, 1972). Iversen and Aasheim (1970) must be criticised at this point for not providing evidence on which they based a statement that the polarity was detectable in Helianthus tissue. Wilkins et al (1972) claimed that expression of the Zea data as proportions of the total uptake of radioactivity tended to obscure the polarity effect, but this was not substantiated by the results for 2,4-D presented in figure 11 of this thesis. It should be pointed out, however, that although chemically related compounds might be transported by the same system, it would be by no means obligatory. Furthermore, since the figures comprise both the immobile and mobile components of the radioactivity extractable from the tissue they cannot be taken as definite indicators of polarity because the capacity for immobilisation at the two ends of the segments might well be different. Nevertheless, the acropetal polarity in the movement of radioactivity previously established in the receiving block data, could be demonstrated at the receiving block end of the segments.

Preferential movement of radioactivity towards the root apex was evident also in the steady increase in the ^{14}C content of each successive 2mm piece of the segments, in the acropetal replicates, as the experiment progressed (figure 10 and 18). The basipetal replicates, however, exhibited increasing levels of ^{14}C over the first 10-15 hours before declining during the remainder of the experiment and this was reflected in the proportion of radioactivity retained in the 2mm portion of the segment adjacent to the donor blocks. In view of the differences in terminal surface area of the segments it was surprising that the level of radioactivity in the 2mm adjacent to the block was almost identical in the acropetal and basipetal replicates over the initial 15-25 hours of the experiment. By the end of a 60 hour experiement, almost 24% of the applied ^{14}C was retained in the basal region of the acropetal replicates following a donation of the -COOH labelled herbicide, whilst 47% was retained by the basipetal replicates. Although the percentage of the total radioactive uptake held in the same region of the acropetal replicates fell steadily for 40-45 hours (figure 11), the absolute level of radioactivity increased slowly over the same period (figure 10). This showed that rapid distribution of ^{14}C along the segment was accompanied by a continued uptake from the donor block. In contrast, the proportion held in the corresponding region of the basipetal replicates fell from 80% to 50% during the 60 hour experiment and was accompanied by a decrease in the absolute level of radioactivity. This could explain, to a certain extent, the preferential movement of radioactivity towards the root apex. It is likely that some basic property of the transport system was effective in confining the radioactivity to the apical region of the basipetal replicates, thus preventing rapid distribution along the segments. Several reports in the literature, dealing with the movement of IAA in root segments of Vicia, Convolvulus, Lens, Brassica and Pisum, confirmed the validity of the finding, although the precise result depended on the location of excision and the concentration of the auxin supplied (Yeomans and Audus, 1964; Bonnett and Torrey, 1965; Kirk and Jacobs, 1968; Iversen and Aasheim, 1970; Hillman and Phillips, 1970).

In common with the ^{14}C retained in the segment adjacent to the donor block, little difference in the total uptake of radioactivity could be detected for 30 hours with 2,4-D(-1-^{14}C) and 20 hours with 2,4-D(-2-^{14}C) (figure 7). This fact was not compatible with the suggestions of Yeomans and Audus (1964) and Kirk and Jacobs (1968), that the lack of homogeneity

in surface area and cellular composition at either end of the segment could lead to differences in rates of diffusion, uptake, and ultimately transport. The idea was further questioned by data showing the greatest polarity of ^{14}C movement in the 2,4-D/Pisum system was present in segments with identical terminal surface areas ie. excised from the 34-44mm zone (figure 39). The weakest polarity was exhibited by 1-11mm segments, yet the basal surface area was more than three times greater than that at the apical end. Furthermore, data on page 135 revealed that expression of the data as radioactivity/g dry weight of segment would, undoubtedly, increase the apparent polarity in the distribution of ^{14}C, but tend to reveal differences in contentof the terminal 2mm of the segments. These findings corroborated those of Wilkins and Scott (1968a) and Wilkins et al (1972), which led the authors to believe that no relationship existed between the uptake of radioactivity and the surface area of the segment exposed to the donor blocks. A simple correlation of surface area to degree of polarity was not possible because of the variation in age and differentiation of the tissue from one region to the next.

After transport periods of 30 hours the acropetal replicates contained, on average, 1.7 times more radioactivity than the basipetal replicates (see acrop/basip ratios in figure 7). By the end of the experiment the differences in ^{14}C content were 4.9 times after donation of the -COOH labelled compound and 3.5 times with the $-CH_2$ labelled compound. It is difficult to determine whether the observed polarity of ^{14}C transport was a result of differential uptake by the two ends of the segments during the later stages of the experiment, or whether the differential uptake was a result of a polar translocation mechanism which limited the rate of diffusion into the segments.

Since the uptake of radioactivity into the apical and basal ends of the Pisum root segments was in a definite polar manner after the first 30 hours donation, differential loss from the donor blocks might be expected. The data presented in table 2 and figures 8 and 9 revealed that the loss of ^{14}C from basal blocks exceeded that from apically-applied blocks after a transport period of 15 hours at 25°C in darkness. The extent of the differential loss increased as the experiments progressed. Donation of 2,4-D(-1-^{14}C) and 2,4-D(-2-^{14}C) gave closely similar acrop/basip ratios, with the exception of the 60 hour point when the ratios differed by 8%. The position of the ^{14}C labelling would

be unlikely to affect the extent of the loss to any great extent. Comparable data for the loss of IAA from donor blocks supplied to Phaseolus and Pisum were published by Kirk and Jacobs (1968) and Hillman and Phillips (1970). Similarly, the data presented by Yeomans and Audus (1964) and Kirk and Jacobs (1968) for Vicia and Lens also revealed a greater loss from the basal than from the apical blocks. The loss from the 1-5mm zone of Vicia root supplied with IAA(-1-^{14}C) was exponential, but in the 4-8 and 8-12mm zones the rate of loss mirrored the rate of uptake and neither was constant. Similarly, figure 8 illustrated that a mirror-image relationship existed between the ^{14}C lost from the donor blocks and the radioactivity extractable from the Pisum root segments + receiving blocks.

Throughout the studies into the movement of 2,4-D in Pisum root segments, fluctuations in uptake, distribution and export of ^{14}C were recorded with such regularity that they could not be dismissed immediately as artefacts of the technique. Clearly, any error in the preparation of standard donor blocks might lead to fluctuations, so the 8% error reported earlier ie. 15,432 ± 1250 dpm, could lead to spurious peaks of uptake. Furthermore, since expression of the data for the ^{14}C content of successive 2mm sections of tissue (figure 11) as a % of the total ^{14}C content tended to erase the peaks of absolute activity (figure 10), it would appear that ^{14}C content might be a definite proportion of the donor supply.

Error in donor supply appeared to be an unlikely explanation of the fluctuations on consideration of figure 7 illustrating the total radioactivity extracted from the root segments and receiving blocks following donation of 2,4-D(-1-^{14}C) or 2,4-D(-2-^{14}C). Peaks of ^{14}C content occurred in every experiment 1-8 after 10-20 and 35-40 hours and was followed by a further increase in radioactivity towards the end of the experiment. The pattern was clearly visible in the ^{14}C content of the basal 2mm of the segments in contact with the donor block (figure 13). Of greater importance, however, was the synchrony of the fluctuations in independent acropetal and basipetal replicates, which indicated that erroneously 'hot' donor blocks would be an unlikely explanation on the sixteen occasions. Further support came from evidence (figure 8) that uptake of ^{14}C was the mirror image of increases and decreases in activity of the donor supply. It might be concluded, therefore, that the level of ^{14}C in the donor blocks increased at intervals and on these occasions it was rare to find high levels of uptake.

Clearly, attempts to pin-point accurately a fluctuation by using 5-hour sampling intervals was difficult since any given peak could be displaced by up to 10 hours by a lack of precision associated with the lengthy sampling intervals. It was considered worthwhile to use 2-hour sampling intervals to assess the fluctuations more precisely and to determine whether an increase in the number of sampling points increased the number of fluctuations. Figure 14A & B revealed that a series of fluctuations in the ^{14}C content of the acropetal replicates were detectable and that several of the troughs and peaks were significantly different from the fitted quadratic curves. It is suggested that peaks 1-4 in experiments A and B possessed a visual similarity, although displaced by approximately 8 hours.

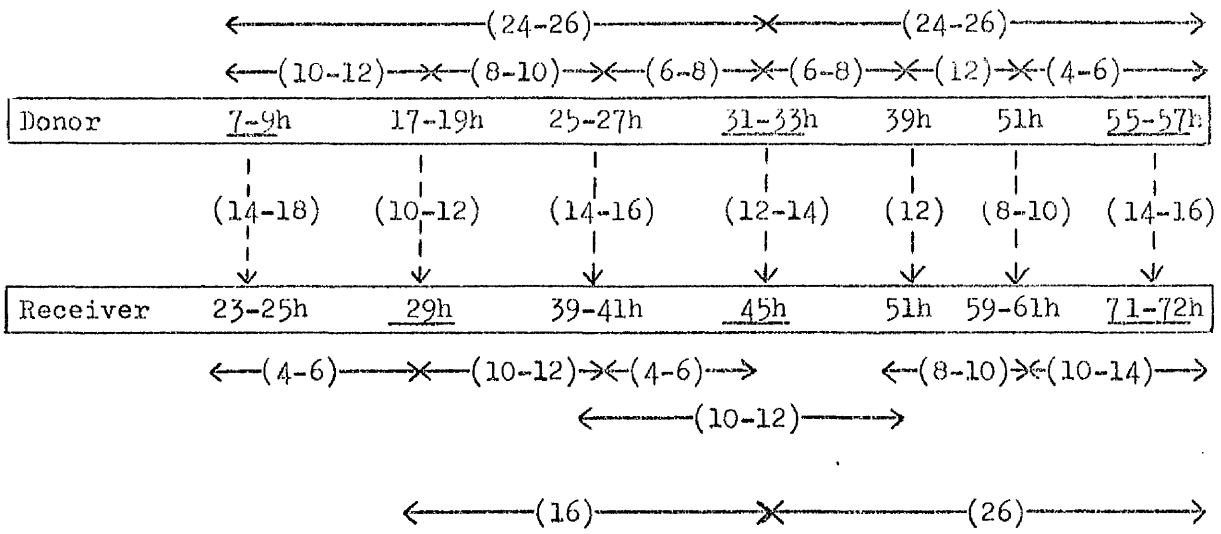
It was interesting that the first major peak of ^{14}C content of receiving blocks occurred after 30 hours in experiment A and 38 hours in experiment B (figure 15) ie. a delay of 8 hours, which corresponded to the delay recorded in the uptake data.

These data were consistent with the view that non-random fluctuations occurred and supported the conclusion presented for 2,4-D(-1-^{14}C) and 2,4-D(-2-^{14}C) (previous page) that peaks were always recorded after 10-20, 35-40 and towards the end of the experiment. A similar conclusion was possible from a visual appreciation of figure 18, but analysis revealed that the fluctuations of ^{14}C level in section A of the acropetal replicates were not significantly different from the fitted curves.

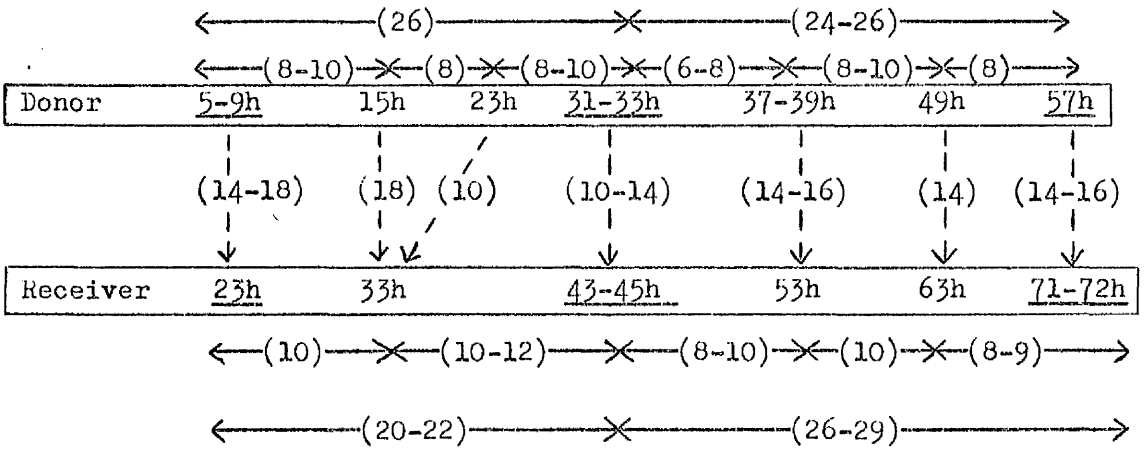
Investigations using one set of root segments throughout an experiment were carried out in order that the innate variability of the destructive sampling techniques could be reduced. The receiving blocks or the donor blocks were renewed by fresh blocks every two hours and the ^{14}C-content of the blocks assessed by liquid scintillation counting. In this way it was hoped to minimise error normally introduced by the use of a different set of roots for each sampling point. Points in which ^{14}C-loss from apical and basal donor blocks was greatest occurred after 7-9 hours and at intervals of 22-26 hours thereafter (figure 16) (table 43). Corresponding periods of ^{14}C export into the receiving blocks were detected, in general, 12-16 hours later than the peaks of donor-loss ie. the time taken for radioactivity to pass from one end of the segments to the other. The involvement of translocation and/or

TABLE 43

ACROPETAL



BASIPETAL



Figures in brackets are the number of hours by which peaks of donor loss or receiver increase in ^{14}C content were separated, or the time taken for the peak to appear at the opposite end of the segment.

export processes proceeding at rates different to that of uptake could not be ruled out.

In conclusion, therefore, the data presented in this thesis have shown that polynomial regression analysis did not support the possibility of consistently significant fluctuations in ^{14}C level with time. However, a visual appreciation of the graphs indicated that peaks of ^{14}C content in the tissue occurred after 10-20, 35-40 hours and towards the end of the experiments reported.

Explanation of the fluctuations in terms of an oscillation of some environmental factor was unlikely since:

(a) Dark room temperature was monitored by a thermograph and found to be accurate to within $\pm$ 1°C. Furthermore, any fluctuation would be buffered by the thick walls of the closed cupboard in which the humidified chambers were stored during the transport periods.

(b) Routine washing of the humidity chambers on termination of each experiment removed any possibility that particular points on the time-course were associated with any particular chamber.

(c) Experiments 1-4 were carried out at the School of Agriculture, Nottingham, whilst experiments 5-8 (in the first section of results) were carried out in the Garscube Research Laboratories of Glasgow University, using fresh supplies of equipment and experimental material.

(d) The time of setting up over a 9 hour period had little effect on the level of ^{14}C transported (table 5) and nothing was detected which could explain the magnitude of the fluctuations.

It is important, therefore, to speculate further about possible mechanisms to which the fluctuations might be linked. A periodic loss of ^{14}C from the acetic acid side chain of the radioactive molecule would explain a complete loss of radioactivity from the experimental system. Figure 43 revealed that ^{14}C evolution was greater in the basipetal than in the acropetal replicates, indicating a greater capacity for degradation by the cells in the apex of the segment. The rate of evolution did not

appear to be constant throughout the experiment and highest rates were recorded after approximately 10-18 hours and 36-42 hours. ^{14}C was lost preferentially from the -COOH than from the $-CH_2$ group of the side chain but the low level of evolution from the segments made it unlikely for degradation to be the cause of the fluctuations. It could, however, be an indicator of an underlying mechanism. The preliminary nature of these investigations precluded the formation of definite theories and further studies of gaseous evolution are required. Nevertheless, it is quite clear that the oscillations of ^{14}C levels must take place within the system and not by a loss of radioactivity from the segments. Hillman and Phillips (1970) postulated that a decrease in ^{14}C content of the receiving blocks might be related to a loss of a volatile product from the system, whilst Wilkins et al (1972b) revealed that the loss of $^{14}CO_2$ from IAA(-1-^{14}C) and IAA(-2-^{14}C) could be relatively high. However, ^{14}C loss was unlikely to account for the fluctuations observed by these workers.

Circumstantial evidence presented on page 36 indicated that the level of radioactivity within the donor block might increase at intervals during the transport period. Clearly, this could occur only if a reversal of the major acropetal flux returned a quantity of ^{14}C to the donor block. This theory was supported by data for the ^{14}C level in plain agar blocks and blocks containing unlabelled 2,4-D used to replace the normal donor blocks of 2,4-D(-1-^{14}C) after 25 hours (figures 21,22). A polynomial regression analysis (table 16) confirmed that radioactivity was exported into these blocks for 10-20 hours following the change-over. Thereafter, the level of ^{14}C declined prior to further accumulation during the final hours of the experiment. An exact correlation of the phase of this oscillation with that of the donor blocks, or the total ^{14}C content of the segments was not possible, but it is likely that the block change-over could influence processes already in progress. Since the activity in the replacement blocks never exceeded 600 dpm, the losses of 1000 dpm from the segments could not be explained entirely in terms of re-translocation into the donor blocks. Error in preparation of standard donor blocks and the possible loss of $^{14}CO_2$ could account for much of the difference. It is obvious, however, that re-translocation of ^{14}C into the donor blocks could, to a certain extent, help to explain fluctuations in the ^{14}C level of the root segments.

The greater accumulation of radioactivity in the replacement blocks supplied to the apical end of the segments (figures 21;22) emphasised the fact that part of the radioactivity entering the basipetal translocation system was diverted by the acropetal system. In this way, radioactivity would be confined to the region of the segment adjacent to the donor block. A clear acropetal polarity would result since a smaller proportion of the ^{14}C supplied to the basal end of the segments would be diverted by the weaker basipetal translocation system.

Removal of the radioactive donor supply after 25 hours resulted in the uptake of ^{14}C by the acropetal control exceeding that of the replicates supplied with unlabelled 2,4-D or plain agar blocks (figure 24). The uptake by basipetal replicates, at the same stage of the experiment, was such that the controls contained no more ^{14}C than the segments subjected to donor replacement. A reduction in the level of radioactivity held by the controls and the plain agar treatment was noted after the 25 hour point of the experiment, whilst the level in the unlabelled 2,4-D treatment remained virtually constant. This phenomenon might be attributable to an exchange of labelled for unlabelled herbicide molecules at immobilisation sites, where normal extraction was not completely effective in releasing the radioactivity. In other words, the unlabelled compound might act as a flushing agent.

Removal of the radioactive donor supply did not produce a dramatic reduction in radioactivity accumulating in apical receiving blocks, in fact, the only significant effect on the acropetal replicates was detectable in the 50-60 hour period. In view of the finding that the uptake by the acropetal replicates was severely reduced on removal of the donor supply after 25 hours, it was surprising to detect such a negligible effect on receiving block content. Replacement of an apical donor block with an unlabelled 2,4-D donor resulted in an increased receiving block content of ^{14}C, possibly caused by the proposed flushing action of the unlabelled compound. The level of activity in the control and the plain agar treatment was almost identical. It is obvious that the export of radioactivity applied to Pisum root segments was not dependent upon continued uptake from a donor block, once the 25 hour point of the experiment had passed. This indicated that only a small proportion of the ^{14}C was being moved towards the receiving block by the transport system.

Replacement of the donor block by either plain agar or unlabelled 2,4-D blocks after 25 hours resulted in a rapid drainage of radioactivity from the segments (figures 19,20). By the end of the experiment the level of ^{14}C distributed along the segments of the acropetal replicates was constant, whereas the 2mm regions of the basipetal segments retained a definite level of radioactivity. The reason for the different patterns of drainage was not apparent, but the rapidity and effectiveness of the process indicated that the segments might be capable of translocating a clear-cut pulse of 2,4-D. It was found, however, that a one-hour pulse-label of 2,4-D(-1-^{14}C) supplied to the segments, pretreated with unlabelled 2,4-D for 25 hours, merely spread along the tissue. The lack of a clearly defined movement of an IAA(-1-^{14}C) pulse through Zea root segments was reported by Wilkins et al (1972). The present finding that 2,4-D(-1-^{14}C) was distributed most rapidly along the segments of the basipetal replicates supported the findings of Kirk and Jacobs (1968) and Wilkins and Cane (1970). They proposed that a transient basipetal polarity of IAA movement could result from a small, rapidly moving flux of ^{14}C moving away from the root tip, whilst the major acropetal transport consisted of a large flux with a slower velocity. The results of the pulse investigations presented in this thesis would support this theory.

The literature contains several references to oscillations in the rates of uptake and export of IAA and 2,4-D, though the authors rarely commented on the significance of the phenomenon. Indeed, in some cases fluctuations clearly illustrated graphically were not aknwledged in the text. Perhaps this indicated a reluctance of the workers to accept the data as anything other than artefacts. The radioactive content of receiving blocks applied to Pisum root segments supplied with IAA(-2-^{14}C) decreased after an experimental period of 18 hours and Hillman and Phillips (1970) suggested that the data could be explained as a loss of radioactivity from the system. Kirk and Jacobs (1968) and Scott and Wilkins (1968) detected similar decreases in Phaseolus root segments after 8 hours and in Zea root segments after 6 or 18 hours, depending on the concentration of the auxin used. Wilkins et al (1972b) revealed that a decrease in radioactivity in receiving blocks applied to the apical end of Zea root segments supplied with IAA(-1-^{14}C) was a metabolically controlled process which commenced after transport periods of at least 8 hours at 25°C. Most of the "resorbed" ^{14}C remained in the apical 2-4mm of the segment.

Shen-Miller (1973) reported a 20 minute period for a rhythmic fluctuation in ^{14}C content of decapitated coleoptiles of Zea and Avena seedlings following a one-minute pulse-label of IAA(-1-^{14}C) or IAA(-2-^{14}C). He concluded that the data could only be explained in terms of a reversal of the acropetal flux of IAA through the tissue. This appeared to be similar to a 20-30 minute rhythm in the transport of IAA in Zea and Avena coleoptiles reported by Hertel and Floxy (1968).

Pilet (1965) claimed that a reduced content of receiving blocks applied to stem segments of Lens culinaris could be related to a reduction in the velocity of ^{14}C-loss from the donor blocks rather than a depletion of the donor block reserves of radioactivity. It is apparent from the data for 2,4-D transport in this thesis and by McCready (1963) and McCready and Jacobs (1963) that theonset of the initial phase of decline in receiving block radioactivity occurred much later than in the IAA experiments. This probably reflected the observation that IAA movement was up to 10 times faster than that of 2,4-D. The possibility exists, therefore, that further fluctuations in the IAA experiments might have been detected had the transport periods been longer. In support of this, Bottrill and Hanson (1968) found that a decrease in the rate of uptake of 2,4-D, after the initial 100 minutes of the experiment, was replaced by a second phase of rapid uptake in Zea roots. Blackman (1961) claimed that the phenomenon was confined to dicotyledonous species susceptible to the action of 2,4-D and that resistant monocotyledonous species did not exhibit the renewed phase of uptake. It is necessary to examine these data with caution, however, since the infrequency of the sampling intervals, especially in the later stages of the experiments, could well have resulted in the lack of detection of uptake. Reinhold (1954) and Johnson and Bonner (1956) suggested that this initial phase of rapid uptake could be caused by a physical adsorption process within the system. Since the subsequent phases possessed a temperature coefficient of 2.6 and were sensitive to cyanide, arsenite and diethyl-dithiocarbamate, a metabolically-controlled process was implicated.

Pittendrigh (1954) proposed an ideal code to which a fluctuation must be closely related before being classed as an endogenous rhythm.

The conditions were:

(1) The rhythm must persist in a constant environment.

(2) The rhythm should be initiated by a single stimulus.

(3) The phase of the rhythm should be able to be adjusted by suitable treatment.

(4) The phase of the rhythm should be delayed by an interruption of metabolism of the tissue.

(5) The rhythm should not be exactly 24 hours.

The 2,4-D/Pisum root segment system satisfied rule 1, since experiments were carried out at constant temperature and humidity in growth rooms. The effects of fluctuations in gravitational or cosmic stimulation were not counteracted. The nature of the experimental technique necessitated the use of an unreasonably high number of replicates if the rules were to be investigated fully. Rule 2 was not satisfied since it was difficult to determine the initiating stimulus. It was possible that the observed oscillations merely reflected an endogenous rhythm, but the initiation of a de novo rhythm could not be ruled out. The stimulus might have occurred during:

(a) Exposure of the dark-grown material to green light.

(b) Excision of the root segments.

(c) Application of the donor and/or receiving blocks.

As the three operations were separated by less than 1-2 minutes, the individual effects would be difficult to distinguish. Thus, although it would appear likely that a rhythmic phenomenon was involved or associated with the mechanisms responsible for the movement of 2,4-D in root segments the complex nature of the experimental system precluded any precise monitoring of the fluctuations.

The fluctuations reported in the 2,4-D/Pisum root segment system might well reflect the endogenous periodicity found in root pressure and exudation and in the uptake of ions (Huck et al, 1962, 1970; Hanson and Biddulph, 1953; Vaadia et al, 1960).

Progressing from the basic acceptance of an acropetal polarity in the movement of 2,4-D in isolated segments of Pisum roots, was the question of whether the movement was dependent upon a supply of metabolic energy from the segments or was merely passive. A clue came from a series of experiments in which a range of concentrations of 2,4-D was applied to apical or basal ends of the segments. The ratio of uptake of radioactivity by the acropetal and basipetal replicates (table 24) increased with time at each concentration and was due to the cessation of uptake into the apical end of the segments after 15 hours. Uptake into the basal end of the segments was continuous. An increase in donor concentration from 1.0µM to 10.0 µM 2,4-D(-1-^{14}C) resulted in a proportional increase in uptake through the basal ends. For example, after 20, 40 and 60 hours the increases were from 1100 to 16,500 dpm, 1650 to 19,000 dpm and 2150 to 23,500 dpm. The uptake at 0.1 µM was greater than would be expected on a purely proportional basis and values of 150, 300 and 370 dpm were recorded after 20, 40 and 60 hours respectively. A similar situation was found when the uptake by basipetal replicates was studied. Calculation of the polarity ratio from the receiving block data revealed that an increase in donor concentration from 1.0-10.0 µM resulted in a marked loss of polarity of. 26.7 to 8.1 after 60 hours (table 24). This was due to a proportional increase in radioactivity in the receiving blocks of the basipetal replicates in response to the ten-fold increase in donor concentration, whilst only a 3-4 fold increase in counts was detected in the acropetal replicates. McCready (1963) reported a similar disproportionate increase in the acropetal movement of 2,4-D(-1-^{14}C) through segments of Phaseolus petioles, but quite the reverse was detected in Convolvulus and Zea root segments by Bonnett and Torrey (1965) and Scott and Wilkins (1968). It is clear that a passive process such as diffusion would be greatly dependent on concentration of the growth regulator supplied to the segments. On the other hand, a process dependent on the provision of metabolic energy might be concentration dependent only over a limited range and become saturated at higher concentrations when the capacity of the system was exceeded. It is possible, therefore, that the data in table 24 provide circumstantial evidence for acropetal transport of 2,4-D in Pisum root segments being more dependent on metabolism than the basipetal movement. The fact that the export of radioactivity into the receiving blocks (figure 36), exhibited no saturation effects at the higher concentrations would not be compatible with this theory, nor would the

claim that concentrations of IAA up to 10.0 μM did not saturate a Zea root segment system (Scott and Wilkins, 1968). It is conceivable that a further increase in concentration might have produced the required result. Even more surprising was the fact that Iversen and Aasheim (1970) were unable to detect any polarity in the movement of IAA at concentrations lower than 0.1 μMolar.

Using a different approach, Niedergang-Kamien and Leopold (1957), Goldsmith (1967,1968) revealed that the velocity and polarity of auxin movement in segments excised from the aerial structures of several species was dependent on aerobic respiratory processes. A related technique was employed to establish the presence or absence of a metabolic dependence in the 2,4-D/Pisum system. Segments were exposed to an anaerobic environment, to prevent aerobic metabolism, and to NaF to inhibit anaerobic metabolism by complexing with the magnesium cofactor of the enzyme 'enolase' in the glycolytic pathway. The inhibitor was supplied to the root segments at a concentration of 2 mM incorporated into donor and/or receiving blocks by the method of Wilkins andWhyte (1968). Results from two experiments revealed that the radioactivity in the apical receiving blocks was reduced by an average of 38% in N_2 alone, 67% by the N_2/NaF in donor block treatment and 77% by the N_2/NaF in donor and receiving block treatment. These reductions were computed using the data from the corresponding 30 hour controls and were all significant at the 0.1% level of probability. Incorporation of the inhibitor in the receiving blocks only gave no significant alteration of the level of radioactivity in the blocks (figure 29 and table 18). Exposure of the basipetal replicates to anoxic conditions for 30 hours gave a reduction in the N_2/NaF in receiving block treatment only. These data indicated that the transport of ^{14}C from 2,4-D was dependent on the aerobic and anaerobic metabolism of the segments and supported reports of reduced movement of IAA on exposure of corn coleoptiles and root segments to anoxic conditions (Goldsmith, 1967a,b; Wilkins and Martin, 1967; Wilkins and Scott, 1968; Wilkins and Whyte,1968). Since the N_2/NaF in donor and receiving block treatment of the Pisum acropetal replicates was more effective than the N_2/NaF in donor block treatment, it might be concluded that:

(a) The segments received an inadequate endogenous concentration of the inhibitor from the donor block-only donation, or

(b) The inhibitor effectively reduced ^{14}C movement at both the donor and the receiving block end of the segments ie. inhibited the uptake and export processes, in the presence of N_2.

The latter view was substantiated by the 30 hour basipetal N_2/NaF in receiving block treatment in which a reduction in ^{14}C content possibly indicated an inhibition of the export of radioactivity into the receiving block. This was supported by the N_2/NaF in donor and the N_2/NaF in donor and receiving block treatments which gave 50% and 47% reductions in the total ^{14}C content of the segments, whilst the receiving block contents were reduced by an additional 17% and 30% respectively. The theory was not sound in the case of the 30 hour NaF/receiving block treatment which exhibited no change in receiving block content on exposure to N_2.

Another interesting phenomenon was the massive increase in receiving block radioactivity whenever NaF was present in the donor blocks of the air-controls of the acropetal replicates but not the N_2 treatments. A similar stimulatory effect was detected in both air and N_2 treatments of the basipetal replicates when NaF was supplied in donor and receiving blocks for 30 hours. No explanation of the stimulatory effect of NaF could be offered and no reports in the literature could be found. Wilkins and Whyte (1968) concluded that the lack of effect of the inhibitor under aerobic conditions demonstrated the insignificance of glycolysis under normal environmental conditions. The inhibitor had no stimulatory effect on the uptake of radioactivity from IAA(-1-^{14}C) when supplied to Zea root segments as an aqueous pretreatment (Wilkins and Scott, 1968b). It is conceivable that the use of longer experimental pretreatment periods might have revealed a stimulatory effect in the Zea/IAA system. Other inhibitors of auxin transport eg. tri-iodobenzoic acid (TIBA), naphthylphthalamic acid, p-chloromercuri benzoate and p-dinitrophenol are known to increase the IAA content of shoot segments. The effect of TIBA was attributed to a reduction in export of radioactivity into the receiving blocks, leading to an accumulation of ^{14}C in the segments. Export into the receiving blocks was found to be more sensitive to inhibition than was the uptake of ^{14}C from the donor blocks, which would

support the theory presented earlier for the 2,4-D/Pisum root segment system (Christie and Leopold, 1965a,b; Winter, 1967). It is clear that further work would be valuable in the evaluation of the stimulatory effect of NaF.

The reasons for the unacceptable level of variability in the datafrom the 2,4-D/Pisum root segment system, under anoxic conditions (figure 30,31) were difficult to pinpoint. It was disturbing, however, that identical techniques with other plant species provided dependable data. Absence of total anaerobiosis would result in the continuance of a finite level of metabolism, but this error seemed unlikely since replicates within the same experimental chamber gave divergent results. Another potentially important factor was the effect of anaerobic conditions on the IAA-oxidase/peroxidase system of the pea roots (Janssen, 1969, 1970). If the enzyme system was active in the degradation of 2,4-D molecules under normal conditions, the imposition of an oxygen depleted atmosphere would prevent the oxidase activity and facilitate the movement of a greater flux of unaltered herbicide through the segments. A similar effect in intact Helianthus seedlings was caused by flooding of the root system, resulting in an inactivation of the oxidase system with a consequent increase in auxin level (Phillips, 1964). It is obvious that the expected inhibitory effect of N_2 and NaF would be antagonised by the oxidase effect and might account for the inconsistency of the results in the 2,4-D/Pisum root segment system. Furthermore, the stimulatory effect of NaF on the uptake and movement of ^{14}C could act in opposition to the expected inhibitory activity under anoxic conditions.

As roots normally develop in the soil the optimum temperature for metabolic and metabolically-controlled processes might be lower than the 25°C employed routinely in the previous experiments. Figure 27 A shows that the velocity at which ^{14}C from the radioactive herbicide moved from base to apex or apex to base of the segments increased steadily as the temperature was increased. The dramatic increase from 35-40°C must be regarded with suspicion since the segments became flaccid after 20-30 hours at this temperature. It is likely, therefore, that the determination was the rate of diffusion through a dead or dying segment rather than a true estimation of transport velocity. The same criticism must be levelled against a reduction in the velocity of IAA movement through Zea root segments from a maximum of 8mm h^{-1} at 31°C to 1mm h^{-1} at 50°C (Wilkins and Cane,1970). In view of this, 25°C appeared to be the optimum temperature for basipetal transport, in the 2,4-D/Pisum system, with a velocity of 1.1mm h^{-1}. A clear optimum temperature for acropetal transport was not found but the increase in velocity with increasing temperature had levelled off at the 25° and 35°C points to give a maximum of 0.8mm h^{-1}. Determinations of velocity of ^{14}C movement, such as those reported in this thesis, computed from segment length and the time taken for the first radioactive molecules to arrive in the receiving blocks may be inaccurate due to the possible hindering effects of ^{14}C immobilisation at the cut surfaces and at the cellular level. Since it is probable that the effects on acropetal and basipetal transport would be similar there can be little doubt that the velocity of basipetal ^{14}C movement in Pisum root segments exceeded that of acropetal movement.

The quantity of radioactivity moving through the Pisum segments/unit time ie. the flux, was computed from the gradient of the fitted regression lines in figure 26. 35 dpm h^{-1} moved towards the apical end of the segments at the optimum temperature of 35°C, whilst the flux in the opposite direction was only 1 dpm h^{-1}. In common with the velocity calculations, the data for 40°C must be considered with caution as should the 50°C data for IAA in Zea roots presented by Wilkins and Cane (1970). The maximal flux of IAA in the Zea system at 15°C had no counterpart in the 2,4-D/Pisum system.

It is obvious that the basipetal transport of ^{14}C from 2,4-D(-1-^{14}C) was smaller but faster than the acropetal transport. This supported the claim by Wilkins and Cane (1970) that a transient basipetal polarity of IAA movement in Zea roots at low temperature and 50°C was directly attributable to a low density-high speed ^{14}C movement.

Examination of the receiving block data revealed that little difference in block content existed after acropetal or basipetal transport at 5° or 15°C. Indeed, by the end of a 60 hour experiment the level of ^{14}C in the basal blocks at 5°, 15°, 25° or 35°C did not exceed that in the apical blocks at 5° or 15°C. It is proposed that this was indicative of the purely diffusional nature of the movement and that the increased apical block content at 25° and 35°C resulted from a stimulation of a metabolically-controlled translocation system. The data for Zea root segments under anaerobic conditions (Wilkins and Cane, 1970), however, would suggest that the acropetal, in contrast to the basipetal transport retained a metabolic component at low temperatures.

A more complex situation was detected when the total ^{14}C content of the Pisum segments and receiving blocks was considered (figure 26). After a 60 hour transport period the basipetal replicates at 1°-35°C contained virtually the same level of ^{14}C as the 1°-15°C acropetal replicates. This indicated little involvement of metabolism in the basipetal replicates since a 10°C rise in temperature is normally associated with a doubling of the rate of a metabolic process. The 15°- 25°C increase produced this relationship in the acropetal replicates. After 20 hours, the level of ^{14}C in the basipetal replicates exhibited a clear stepwise increase (approx. 1.5 times) as the temperature increased from 5°-15°, 15°-25°, and 25°-35°C indicatingthe possible involvement of metabolic processes in the initial stages of basipetal transport. The decline in ^{14}C level in the basipetal replicates after the 25-30 hour point might be related to the cessation of the metabolic component at that time. On the other hand, the continued uptake in the acropetal replicates at 25° and 35°C could well indicate the continued involvement of the metabolic component.

Figure 28 provides a further illustration of the suggested metabolic component in acropetal transport at 25° and 35°C. ^{14}C loss from donor blocks applied to the basal ends of Pisum root segments was greatest at 25° and 35°C whilst losses at 1°-15°C were at the same level as those from blocks applied apically at 1°-35°C.

Reports in the literature of temperature comparisons in transport studies have put forward varied viewpoints on the dependence of the system on a supply of energy from metabolism. Keitt and Baker (1967) investigating ^{14}C export in to receiving blocks in an IAA/Phaseolus internode segment system claimed the implication of a physical process eg. diffusion ,in the uptake and

export of radioactivity, whilst Christie and Leopold (1965) and Pilet (1968) found that acropetal and basipetal transport in shoot segments were metabolically dependent. McCready (1968a,b) disagreed and claimed that a reduction in temperature in an IAA/Phaseolus petiole segment system resulted in a decreased basipetal movement but an increased acropetal movement. Keitt and Baker (1967) expounded the same theory that a decrease in the activity of the metabolically dependent process ought to produce an increase in the physical process by a cessation of reverse transport by the active stream. A similar phenomenon could not be detected in the 2,4-D/Pisum root segment system (figure 26), which might indicate a less pronounced metabolic component. Wilkins and Cane (1970), however, found a reversal of the normal acropetal polarity of ^{14}C movement in an IAA/Zea root segment system when the temperature was less than $15^{o}C$. This basipetal polarity also became apparent at $50^{o}C$ when the segments would, presumeably, be dead and could easily be the result of a lack of active acropetal translocation. It was surprising that these workers detected such a marked metabolic dependence of acropetal ^{14}C movement at $1^{o}C$, but metabolism accentuated the basipetal polarity.

Thus, experiments with 2,4-D(-1-^{14}C) carried out over a range of concentrations and temperatures, or in an anaerobic environment with a metabolic inhibitor have demonstrated a greater metabolic dependence for acropetal than for basipetal transport.

The effect of white light on the Pisum root segment system was not clearly defined (figures 37,38 and table 25). Apical receiving block ^{14}C content was significantly reduced by exposure to white light for 20 hours, whilst a 50 hour treatment had no effect. On the other hand, a 50 hour treatment gave a significant increase in basally applied blocks whilst the 20 hour treatment had no effect. The total ^{14}C content of the segments and the receiving blocks ie. the total uptake, exhibited slight enhancement in response to light in all cases, but analysis revealed that only the 20 hour basipetal treatment had been increased significantly. These data contrast sharply with those published by Scott and Wilkins (1969), in which white, red and blue light exposure of Zea root tissue supplied with IAA(-1-^{14}C) increased the ^{14}C content of apical receiving blocks by up to 100%. Accumulation in the basal receiving blocks was not affected. A comparison of tissue data was

not possible since the relevant results were not published by Scott and Wilkins. Nevertheless, both the 2,4-D and the IAA systems were stimulated in the presence of light and the equal effectiveness of the three different wavelengths prompted the suggestion that a chlorophyll pigment could be involved in the trapping of light energy. A similar proposal came from Thimann and Waxddlew (1963) after finding a reduction in the polarity of IAA movement in Alaska pea internode segments was due to the promotion of the acropetal flux by exposure to light. Reductions in the basipetal movement in response to white/and/or blue light in the aerial structures of Avena, Zea and Helianthus were reported by Lam and Leopold (1964), Shen-Miller et al (1969), Shen-Miller and Gordon (1966) and Thornton and Thimann (1967). The level of endogenous auxin readily diffusible from Zea coleoptile tips was reduced on exposure to white light (Naqvi and Gordon, 1967). Koevenig and Jacobs (1972) claimed that planchette counters were not sensitive enough to detect increased export of ^{14}C into receiving blocks in response to blue, red and far-red irradiation of Coleus stem segments. Experience has shown the higher efficiency of scintillation counting might not be so useful since the variability in the background radiation of the vials hindered the detection of small differences in count rate.

That light was capable of stimulating the mechanisms by which growth regulators are transported through root segments whilst inhibiting the transport through shoot segments was surprising, since shoots possess a well-established energy trapping pigment system. The literature revealed, however, that chloroplasts and chlorophyll were not entirely absent from root structures. Fadeel (1962) discovered chloroplasts, containing grana of the same size as those found in leaf chloroplasts, mainly in the inner regions of the cortex of wheat, barley and flax roots. Björn (1965) demonstrated that blue light was more effective than red light in stimulating the synthesis of chlorophyll a and b in excised wheat roots, whilst a combination of the two resulted in an enhancement of synthesis. This synergism was not found in cucumber and pea roots, where

blue light was the sole stimulating agent (Bjorn and Odhelius, 1966). Clearly, the involvement of blue and red irradiation in the enhancement of chlorophyll synthesis and auxin translocation in roots indicated a close relationship between the two phenomena, probably in the reception and transfer of energy. Thimann and Wardlaw (1963) concluded that the light effect was fairly localised within the plant and was due to the local availability of energy from photosynthesis. A further possibility, ofcourse, would be the involvement of the red/far-red phytochrome system since measurable quantities of the pigment have been extracted from the roots of Alaska pea and implicated in lateral root initiation (Furuya and Torrey, 1964).

Criticisms that an exhausted energy supply in root segments subjected to 60 hour experimental periods might influence the results, prompted a series of investigations in which an exogenous supply of potential energy was provided (fig.33,34and table 21). Statistical analysis revealed that 20 or 50 hour sucrose or mannitol treatments produced no significant effect on the level of ^{14}C in apical or basal receiving blocks. The total ^{14}C content of the segments and receiving blocks of the basipetal replicates was significantly increased (***) by inclusion of 1.53% sucrose in the donor blocks. This was not attributable to an osmotic effect since the 0.8% mannitol control (non-metabolisable osmoticum) was unchanged. Alterations in the acropetal replicates in response to sucrose were accompanied by correspondingly significant changes in response to the mannitol controls. These data indicate that sucrose had little overall effect on the uptake or movement of ^{14}C following a donation of 2,4-D(-1-^{14}C) except in the 50 hour basipetal treatment. This might indicate that the reduced level of uptake detected in the basipetal replicates after the 25-30 hour point of the experiments in figure 7 and page 174 could be due to exhausted energy reserves. It is unlikely, however, that fluctuations of ^{14}C uptake into the basal ends of the segments, reported throughout this work, could be related to a periodic availability of endogenous substrates.

In order to reduce the possibility of radioactive contamination of the humidified chambers, acropetal and basipetal replicates were set-up with apical and basal ends of the Pisum segments in contact with a lower donor block. Clearly, it was essential to check whether or not this refinement of the technique influenced the nature of the results by a gravity effect. Increased levels of ^{14}C could be extracted from the "tip-upward" segments, with the exception of the 20 hour basipetal replicates in which a significantly reduced level was found (figure 41,42). The orientation of the segments had no influence on the level of radioactivity present in the receiving blocks after transport periods of 20 or 50 hours. This supported reports in the literature that gravity had little effect on transport in IAA/Lens and IAA/Zea root segment systems (Pilet, 1965). In contrast, Naqvi and Gordon (1965) and Little and Goldsmith (1967) detected an inhibition of basipetal transport of IAA whenever Zea or Avena coleoptile segments were inverted with respect to the normal orientation. It is clear, however, that whilst the orientation of Pisum segments influenced the level of ^{14}C within the tissue, there was no effect on the movement into receiving blocks.

The practice of decapitating shoot segments has been employed extensively in physiological studies to provide segments lacking an endogenous supply of auxin. Since the root tip has never been shown to be a definite site of auxin synthesis, presumeably detipping guaranteed an unimpeded entry of the applied compounds. The use of these segments (fig. 40) revealed that the root tip presented no barrier either to the uptake of ^{14}C by the basipetal replicates or the export of ^{14}C into the apically applied receiving blocks of the acropetal replicates. Scott and Wilkins (1968), however, claimed that the presence of Zea root apices prevented the export of radioactivity, from IAA(-1-^{14}C), into apical receiving blocks. A 30% enhancement of uptake was recorded in the basipetal replicates of the 2,4-D/Pisum root segment system when the tip remained intact. Most of the additional radioactivity was found in tissue nearest to the donor block. This retention was most likely related to the small flux of radioactivity moving through the basipetal treatments, which would be unable to cope with the translocation of higher levels of ^{14}C. Alternatively, immobilisation of radioactive molecules might be greater in the apical cell division zone of the root, but this proposal was not supported by the fact that acropetal movement through the same region was unimpeded. Calculation of the acropetal/basipetal ratios for the 30 hour data showed that the polarity in uptake was reduced from 6.3 to 4.2 when the tips were intact. Accumulation of ^{14}C in receiving blocks was also less polar, with the ratio changing from 2.1 to 1.4. Clearly, the effect on polarity was due directly to the stimulation of the basipetal uptake and translocatory mechanisms.

There can be little doubt that the detailed appraisal of the Pisum root segment system presented in this thesis has uncovered several factors capable of influencing the precise nature of the polarisation of herbicide movement. Experiments were carried out in order to determine whether the the movement detected in the segments adequately reflected the pattern of movement within the intact seedling

Application of radioactive donor blocks either to the root tip or to root/hypocotyl region demonstrated a clear polarity of 2,4-D movement toward the apex of the intact root (fig. 45). The herbicide arrived at the apex within ten hours' of application, indicating a minimum velocity of 3.0mm h^{-1}, and continued accumulationequalised the levels in the central and apical $\frac{1}{3}$ regions of the root. In contrast to a report by Scott and Morris (1970) who immersed pea roots in herbicide solution, a build-up of ^{14}C in the apical region of the root was not detected. Although the radioactivity supplied to the root tip was confined to the zone of donation, possibly by the elongation of the root, an appreciable movement from the root/hypocotyl region into the plumule and cotyledons was found. This accounted for up to 10.5% and 4.3% of the total uptake in the plumule and cotyledons respectively. The rapid growth of the shoot during the experiment would facilitate even passive movement of molecules into the aerial structures.

When the herbicide was supplied to the root tip of the Pisum seedlings as an aqueous solution, a rapid basipetal movement, verified by chromatography, took place in white light and darkness (figure 48). Radioactivity could be detected throughout the entire seedling within 30 minutes, at which time the combined root and shoot length in light and darkness were 35 and 38 mm respectively, indicating a velocity of at least 70mm h^{-1}. A significant difference in seedling content of ^{14}C existed at the 4 and 5 hour determinations when the dark treatment was 2.2-2.5 times greater than the light treatment. Similarly, light-dark differences in the ^{14}C content of the plumule or cotyledons was detectable after 4 or 5 hours. The reason for the reduced content following light exposure was not apparent but hinderance of the ^{14}C flow by photosynthates translocated from shoot to root was possible, although unlikely over the short time scale. The feasibility of this proposition was supported by the indirect evidence for the movement of the herbicide in both the phloem and the xylem (fig. 49, table 40,4). An interchange

of 2,4-D molecules from the xylem to the phloem, in the manner described by Bowen and Wareing (1969) for kinetin and GA in Salix viminalis, would permit interaction of movement. Kendall et al (1971) discovered that ^{14}C from labelled-IAA solution applied to the root tips of Pisum seedlings accumulated in the cotyledons which, in common with the present study, indicated a movement against a counter current of metabolites. The majority of the radioactivity was confined to the roots and the first internode, whilst Davies and Mitchell (1972) could detect no movement into the aerial structures following a donation to the root tip of Phaseolus coccineus. Donation of the radioactive compounds 20mm away from the root tip , however, resulted in basipetal movement of ^{14}C into the shoot and acropetal movement into the root, which confirmed the findings for 2,4-D. A build-up, in the root apex, of ^{14}C and ^{3}H from labelled IAA applied to Pisum and Phaseolus was not typical of the data for 2,4-D (Konings and Gayadin, 1971; Davies and Mitchell, 1972; Iversen et al, 1971). The same workers calculated a velocity of approximately 7mm h^{-1} for the movement of ^{14}C, but Davies and Mitchell (1972) claimed a reduction to 3mm h^{-1} after 6 hours, which was identical to that of 2,4-D. Konings and Gayadin (1971), however, reported that the velocity of acropetal movement of IAA in pea roots would be several cm h^{-1} but the validity of the results were questionable due to the use of red light during the experimental manipulations. On the basis of the root segment experiments the velocity of IAA movement would be expected to be greater than that of 2,4-D (McCready and Jacobs, 1963).

The rapid degradation of the IAA molecule within the tissue made the interpretation of the data and comparison with the 2,4-D data very difficult. Iversen et al (1971), for example, found that 40% of the ^{14}C present in Phaseolus roots after 1 hour was lost after 20 hours, but they neglected to report that only 0.4% of the 374,000 dpm injected into the root could be accounted for at the 1 hour point. It was possible that part of the activity could have been translocated into the plumule, which the authors did not sample, but more likely that part of the massive 10 µl injection dripped away from the root.

Most workers agree that indole acetyl aspartic acid and indole-3-aldehyde are formed and translocated readily within the plant (Morris et al, 1969; Morris, 1969; Iversen, 1971; Kendall et al, 1971). Basipetal movement of ^{14}C in Phaseolus coccineus consisted exclusively of radioactive

metabolites, whilst the acropetal movement was mainly that of the IAA donated, indicating a differential degradation in the two translocatory systems. It was obvious that a detailed chromatographic analysis would be necessary to achieve a clear understanding of IAA and IAA-metabolite movement in intact seedlings. The extreme stability of the 2,4-D molecules (table 45), however, ensured that the detection of ^{14}C in Pisum seedlings reflected the presence of the herbicide molecules and the calculation of velocities was not affected by degradation to such a great extent.

Reports in the literature show that 6-17% of the 2,4-D(-1-^{14}C) applied to Vicia roots and Phaseolus leaves reppectively was released as $^{14}CO_2$ in 3-4 days (Fang et al, 1951; Canny and Markus, 1960). A release of 22% of the original ^{14}C within 13 days was recorded in Phaseolus seedlings with 2,4-D(-1-^{14}C) but an application of 2,4-D(-2-^{14}C) resulted in a three-fold reduction in evolution (Weintraub, 1952). Application of the -CH_2 labelled compound produced a two-fold reduction of evolution in Sorghum and Gossypium (Morgan and Hall, 1963). This pointed to a sequential degradation of the acetic acid side chain of the molecule commencing at the -COOH group, in a similar manner to that reported in this thesis for the Pisum root segment system. In contrast, however, Canny and Markus (1960) found no difference in ^{14}C evolution on applying the herbicide, labelled in either position, to the roots of Vicia. The possibility that microbial action could lead to the release of CO_2 prompted Kendall et al (1971) to examine evolution from pea seedlings cultured under sterile conditions. Root tips were placed in solutions of IAA but loss of radioactivity still occurred under these controlled conditions. They detected a 36% and 17% loss of the initial donation after 2 days in white light and darkness respectively. Therefore, a release of $^{14}CO_2$ from the Pisum seedlings provided with 2,4-D(-1-^{14}C), of the same order as the examples, would give a substantial biological and experimental error.

Chromatographic analyses of methanol or ethanol extracts from root segments or intact roots of Pisum sativum supplied with 2,4-D(-1-^{14}C) showed the compound to be relatively stable within the plant system. Autoradioagraphs of paper chromatograms, run in iso-propanol:ammonia:water (10:1:1), revealed that 1-2% of the applied radioactivity appeared as radioactive compounds running immediately in front of and behind the major 2,4-D spot (table 28). These compounds were not detected by scintillation counting, but this was not surprising since low levels of radioactivity might easily be masked by the variability in background radiation of the glass vials. The presence of the compounds after using the herbicide labelled with ^{14}C in either position on the side chain would indicate that sequential degradation of the acetic acid moiety was not operative. This was not supported by the data for ^{14}C evolution from the segment system (fig.51b) which demonstrated a ten-times greater loss after using the -COOH labelled compound than after using the -CH_2 labelled compound. Analysis of extracts from further experiments carried out over periods up to 60 hours revealed no substantial evidence for metabolic degradation of 2,4-D, but occasionally the compounds from the segments and receiving blocks were not purely 2,4-D (table 29). A similar absence of degradation was found when extracts of roots of intact Pisum seedlings were prepared (table 45). However, extracts of roots supplied with the radioactive herbicide for 24 hours followed by drainage into distilled water or unlabelled-2,4-D revealed the possible presence of a metabolite. Interpretation of these results must be undertaken with caution since it is conceivable that the nature of the compounds could be altered during the extraction proceedure or during the running of the chromatograms. The latter point appeared to be likely judging from the fact that radioactivity which, in theory, had been applied to the chromatograms could not be recovered completely on termination of the experiment.

Identification of the radioactive metabolites was not attempted, but it was probable that the compounds extracted from root segments and intact roots could be conjugation products with amino acids, proteins, sugars etc. Reports of such metabolites in the literature were numerous and revealed that mild hydrolysis released the free acid from extracts of whole plants treated with the herbicide (Jaworski and Butts, 1952; Jaworski et al, 1955; Bach, 1961; Butts and Fang, 1955; Canny, 1960; Morgan, 1963; Holley, 1952; Thomas, 1964b). McCready (1963), however, found little evidence of 2,4-D

breakdown in receiving blocks applied to petiole segments of Phaseolus and only a minor metabolite in the tissue. Thus, it would appear that 2,4-D was relatively stable against the degradatory mechanisms of plant tissue segments.

It was surprising that 2,4-D underwent so little transformation in the Pisum experiments, since similar experiments with IAA, reported by several authors, revealed a great capacity for degrading exogenous compounds, in this particular tissue. Hillman and Phillips (1970), for example, had great difficulty in interpreting the results for the transport of IAA(-2-^{14}C) in Pisum root segments due to the confounding effects of spontaneous breakdown within the donor blocks in addition to enzymic degradation within the tissue. Using intact Pisum seedlings, Kendall et al (1971) concluded that light had little effect on the uptake or distribution of ^{14}C, but tended to increase the decarboxylation of IAA(-1-^{14}C) whilst a ring-labelled IAA was not decarboxylated. A similar view was asserted by Iversen and Aasheim (1970), who claimed that IAA degradation could be stimulated by non-biological factors such as phosphate or heavy metal ions or by biological enzyme systems. This paper must be criticised for the lack of data on which the conclusions were based. The products of IAA degradation in these works were similar , the most common being indole acetyl aspartate which indicated that amino acid/ growth regulator complexes were produced by the Pisum system.

There can be little doubt that adequate interpretation of the movement of ^{14}C-tracer compounds is possible only when a detailed knowledge of the carrier molecule is available. Since 2,4-D underwent little degradation within the whole plant or in root segments, the presence of ^{14}C was an accurate indication of the location of the herbicide molecules.

The present investigations have defined several aspects in which the root segment system proved to be more complex than was appreciated by many of the earlier workers studying the physiology of growth-substance transport.

Acropetal polarisation of herbicide movement evident in the root segments of Pisum had a counterpart in the roots of the intact seedlings, provided that the compound was supplied in agar blocks. An aqueous donation on the other hand, resulted in a rapid basipetal movement of 2,4-D from the intact root apex into the plumule. This emphasised the inevitable difference in transport characteristics revealed on application of the compound by different techniques. Furthermore, a difference between the intact seedling and the segment system must be expected after altering the normal properties of the phloem and xylem by excision of the segments.

Any indication of transport polarity given by the root segment system must, therefore, be interpreted with caution. Although the technique might be considered acceptable in the identification of the general pattern of ^{14}C movement, it could never be used as a quantitative extrapolation of the mechanisms present within the whole plant. This is not surprising if the system is regarded in true perspective as a damaged segment of plant tissue separated from two agar blocks by a layer of dead or injured cells.

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