THE DISTRIBUTION OF PHOSPHORUS, CALCIUM AND POTASSIUM IONS IN TROPICALLY STIMULATED ORGANS

by

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ABSTRACT

The distribution of ^{45}Ca, ^{42}K and ^{32}P was studied in the hypocotyls of Helianthus annus and also in the coleoptiles of Zea mays in relation to geotropic stimulation and response.

The concentration of calcium was higher in the upper side and the concentration of potassium and phosphorus was higher in the lower side of the hypocotyls placed in horizontal position. This differential distribution was correlated with the curvature.

The redistribution of ions is not connected to geotropic perception since ½ hour horizontal orientation at 25°C and subsequent exposure at 4°C did not result in any redistribution.

No differential distribution was observed in the hypocotyls placed in horizontal position when curvature was stopped by pre-treatment with NPA.

After unilateral IAA application (0.1% W/W in lanolin) the concentration of calcium was higher in the concave side whereas the concentration of potassium and phosphorus was higher in the convex side. IAA probably affects the ion pumps via differential growth which results in redistribution of ions.

In unilaterally illuminated coleoptiles illuminated (concave) side showed higher concentration of calcium, whereas shaded (convex) side showed higher concentration of potassium and phosphorus.

NPA inhibited phototropic curvature as a result no differential distribution of ion was observed.

Unilateral application of mersalyl (10^{-4}M in hydrated lanolin) resulted some movement of calcium away from the donor side and movement of potassium into the donor side. Mersalyl affects the distribution of ions by altering ion pumps and its mechanism of ion pump operation must be different from that of IAA as mersalyl does not produce a differential growth response and the IAA effect is dependent on differential growth.

Hence, the results show a correlation between differential distribution of ions and curvature. It is concluded, therefore, that differential distribution is probably caused by curvature.

CONTENTS

CHAPTER I

INTRODUCTION

Tropistic movements in plant shoots and in roots in response to unilateral light and gravity are due to differential growth on the two sides resulting from the unequal distribution of auxin. If vertically growing organs, such as shoots or roots, are placed in a horizontal position a lateral differential in auxin content occurs in the organs. In the shoots the rate of elongation is, thereby, accelerated in the lower side resulting in an upward curvature.

Many important contributions have been made by different workers in the field of auxin transport and its linkage in geotropic perception and response but very little is known about the distribution of ions in relation to tropistic responses.

Cholodny (1922) was the first to envisage the role of "microsomes" (Cholodny meant "microsomes" as particles the size of which is comparable to the size of mitochondria) as gravi-perceptors. These particles were supposed to be electrically charged and would, on sedimentation under the influence of gravity, bring about an associated migration of potassium and calcium ions. So, the differences in the ion ratio would be set up on the two sides of the cell wall. This would modify the rates of growth of these two sides. This theory has been discussed later.

Bode's (1959a) observations of the migration of potassium ions to the site of IAA application in old leaves of tomato plant are perhaps the first observations on the correlation between growth substance activation and potassium movement.

Application of IAA on the surface of old leaves of tomato plant resulted in an increase in potassium level in the leaves. This particular observation led Bode to think of an "acceptor mechanism" which proposed that at the onset of auxin action a substance is released (he called it an "acceptor") that accepts potassium at the site of auxin action. The evidence for the existence of relationship between growth substance and the distribution of potassium has been suggested by Bode (1959b) in geotropically stimulated hypocotyls of Helianthus annus. Comparison of potassium concentration on dry, fresh and ash weight basis has been made between the upper and lower halves of geotropically stimulated hypocotyls. Potassium in the lower halves was at a concentration 5% (on ash weight basis) more than that in the upper halves after 90 minutes of geotropical stimulation. Bode suggested that the increase in potassium level in the lower halves was preceded by an increased auxin activity in the lower halves of geotropically stimulated organs. A suggestion has been made by Bode that auxin probably increases permeability for potassium ions in the lower halves and the transport of potassium ions in geotropically stimulated hypocotyls is predominantly in a basal direction. However, at the end of 120 minutes of geotropical stimulation the level of potassium in the lower halves began to decrease but it always remained higher than that in the upper halves.

The other aspect of Bode's study was to compare the potassium content between apical and basal parts of geotropically stimulated hypocotyls cut transversely into equal apical and basal halves. Bode claimed that there was an increase in potassium content in the basal part as compared with the apical part in geotropically stimulated hypocotyls, the basal part

also showed a higher potassium content as compared to that of vertical control. He explained these differences in terms of a basally directed movement of potassium from cotyledons to the roots. But one can judge from the results that the increase in the basal part of geotropically stimulated hypocotyls was not equal to the decrease in the apical part Bode's idea is that the movement of potassium in the basal direction may be occurring simultaneously with the development of geotropic curvature. He doubted that there was any lateral movement of potassium during geotropical stimulation. Bode finally concluded that basally directed movement of potassium into lower half was due to increase in permeability in the cells of the lower half following auxin action. Due to greater availability of auxin in the lower half there was an increase in the capacity to accept more potassium which after geoinduction appeared to migrate in the basal direction. He studied the movement of other cations such as calcium and magnesium but the results were not clear due to anion interference in the flame photometric method. In this connection the work of Brauner and Hager (1958) may be mentioned. They suggested that "geoinduction" may be occurring at 4°C in the hypocotyls of Helianthus annus. Hypocotyls curved if they were placed in a vertical position at 25°C. They concluded that co-factor of auxin moved into lower half at 4°C. It has been suggested by these workers that this co-factor was probably a cation. But in Bode's results, it was concluded that the movement of potassium into lower half took place after auxin activity had started. Nevertheless, the results provide some evidence for a correlation of differential

distribution of potassium with geotropic response. The problem is, how this change in the permeability could bring about an increased movement of potassium? Since the movement of potassium ions is towards the electropositive side the increased movement of potassium cannot be linked with the geo-electric effect (Brauner, 1927, 1928, 1956; Woodcock and Wilkins, 1965, 1969, 1970). Hence, further investigation is needed in this field.

Following on the finding of differential distribution of potassium as outlined, Arslan-Cerim (1966) carried out some experiments on the distribution of calcium (^{45}Ca) in the geotropically stimulated hypocotyls of Helianthus annus. There has been a difference in the concentration of calcium on a dry and fresh weight basis between upper and lower halves of geotropically stimulated hypocotyls. The concentration of calcium increased with time in the upper half of a geotropically stimulated hypocotyls. The maximum increase of activity was 14% in the upper half after three hours of geotropical stimulation. She suggested that the cells of the upper half acquired the facility to accumulate more calcium probably as a result of a change in the membrane permeability. As a result of accumulation of more calcium in the upper half the plasticity decreases and the rigidity of the cell wall increases. The opposite happens in the lower half. Arslan — Cerim emphasized that the increase in the activity in the upper half was at the expense of calcium exported from roots during geotropical stimulation. The distribution of radioactivity measured as cpm per mg. dry weight between upper and lower halves in 1 and 3 hours of geotropical stimulations was found to be 7.335/6.379 and

9.025/6.801 respectively. It cannot be definitely concluded that these differences between the upper and lower halves are due to differential transport from the roots. It is not possible to say whether there has been any lateral migration of calcium from lower to upper half during geotropical stimulation.

It is worthwhile to discuss the idea put forward by Bennet — Clark (1956). It was thought that IAA could probably bring about loosening of the cell wall by removing calcium ions. This idea is no longer tenable. Cleland (1960) has shown that IAA does not remove calcium in appreciable quantity during IAA induced growth. This has been supported by Burling and Jackson (1965). Many papers are concerned with what is described as auxin-induced water uptake (Bonner et al, 1953,1956; Ordin et al, 1956). But the main conclusion from these observations would seem to be that during differential growth the total amount of osmotically active solutes increases (by greater uptake of ions) but the total molar concentration remains either constant or slightly decreases. It has also been suggested by these workers that auxin directly or indirectly stimulates salt accumulation resulting in an increase in osmotic potential of the vacuolar sap and consequently greater suction pressure results in the enlargement of the cells. These suggestions indicate probably some kind of relationship does exist between auxin activity and ion accumulation.

One may now examine the idea put forward by Cholodny (1922) which deals with the preferential distribution of ions. It was the assumption of Cholodny that "microsomes" would sediment or rise depending upon their density and that of the surrounding protoplasm. He assumed that since these particles carry

a negative charge a potential gradient would be set up across the cell which, he supposed, displaced ions such as potassium and calcium. Cholodny, therefore, believed that there would be unequal distribution of ions in the cells as a result of gravitational stimulation. Potassium ions being more mobile than calcium ions, a difference in the ion ratio would be set up on the two sides of the cell wall. This would modify the swelling properties and therefore, the permeability of the protoplasmic membranes and finally differential growth would be brought about. Various objections to this theory have been raised. Audus (1962), in a thorough analysis of the perception of gravity by plants, says that the root caps are considered as regions perceiving gravity in the root and not the elongating region proximal to the meristem (where Cholodny's changes would take place). So this theory had to fail as it supposed the idea that perception and response would take place in the same cell. Audus suggests that "microsomes" can sediment sufficiently in the known presentation time. A concentration gradient of mitochrondria could be established in the absence of protoplasmic circulation, but the establishment of equilibrium would be too slow to account for presentation times of 2 minutes or less. Furthermore, according to this theory the ratio of calcium and potassium ions would follow a similar pattern on the two sides of the cell wall in any geotropically stimulated organs such as roots and shoots, although their geotropic responses are completely opposite to one another. Hence, this theory fails to explain positive and negative responses. Electron microscopical observations of Griffiths and Audus (1964) reveal that mitochrondria do not show a significant difference in their distribution under the influence

of gravity. Hence, it is extremely unlikely that a shift in the ion ratio would be brought about on the two sides of the cell wall by the sedimentation of mitochondria in geotropically stimulated organs.

Another phenomenon, which may have some link with the ion distribution, is the geo-electric effect. When primary shoots of most plants are placed in the horizontal position, a difference in potential arises so that the lower side becomes electropositively charged with respect to the upper side. This phenomenon was first reported by Bose (1907). Bauner (1927, 1928) observed this effect in a number of plant organs and noted that it developed within a few seconds of horizontal orientation and reached a maximum of 8 - 9 mV in 10 minutes. Brauner (1942) did not consider these changes to be due to preferential sedimentation of heavier ions in relation to the lighter ions across the cell, but he concluded that these geo-electric effects could be due to some kind of modification of ion diffusion through the membranes by the action of gravity. Following on from this Grahm and Hertz (1962, 1964) have demonstrated that the geo-electric effect did not develop until 15 minutes of horizontal orientation. It has been suggested that the geo-electric effect is the result of an auxin concentration gradient between the upper and lower halves of the shoot (Grahm, 1964; Wilkins and Woodcock, 1965).

In considering the linkage of perception to auxin distribution under gravity, it was considered by different workers (Brauner, 1942; Schrank, 1945, 1947) that the increase in positive charge on the lower side could cause an electrophoretic

migration of auxin anions to the lower side and thus would result in geotropic curvature. Very recently, Woodcock and Wilkins (1969a, 1969b) have shown two different types of electric effects. The first effect develops immediately after non-decapitated organ is horizontally orientated. This process is a purely physical phenomena which arises in the static-drop electrode system. The second effect develops after an interval of 10 - 15 minutes of horizontal orientation. This process, which is called a delayed geo-electric effect, arises in the living tissue and is restricted to the non-decapitated organs (organs showing normal geotropic response by the maintenance of the supply of auxin from the tips). New developments in the study of geo-electric effects have shown that the geo-electric potential and geotropic response are due to an IAA concentration gradient resulting from the lateral transport of this substance from the upper to the lower half (Woodcock and Wilkins, 1970). This conclusion was reached from the observation which showed that unilateral application of IAA to the decapitated coleoptiles of Zea mays and Halianthus annus gave an increased electropositive potential on the side of the application. This observed potential difference was of the same order as observed in the geo-electric effects. These results led them to conclude that the geo-electric effect, at least in shoots, is not probably connected with the actual perception of gravitational stimulus but is merely a side effect arising from the lateral movement of auxin. It is very important to find out whether IAA changes the permeability with respect to particular ions or in general way.

Woodcock and Wilkins (1971) put forward a fascinating idea concerning the possible explanation for the geo-electric effect by the use of a substance called mersalyl. There are substances, commonly known as diuretics, which give rise to an increase in surface potential by virtue of their ability to inhibit or enhance ion pumps, or to bring about a change in the cell membrane to certain ions. Mersalyl, the substance used in their investigation, is the sodium salt of O - ((- 3-hydroxymercuric -2- methoxypropyl) carbonyl) phenoxy-acetic acid. The function of mersalyl in the human body has been shown to block the reabsorption of sodium from proximal kidney tubule. Probably this substance does affect a specific ion pump in the system but the effect of mersalyl on the transport of ions in plant cells is not known and requires investigation. By unilateral application of mersalyl (in aqueous lanolin emulsion pastes at a concentration of 10^{-3}M) to the decapitated coleoptile tips of Zea mays, Woodcock and Wilkins (1971) were able to show that mersalyl did result in the development of a voltage difference of about 14 mV positive on the donor after 18 minutes of application. In an experiment these workers have shown that unilateral IAA application generates electropotential differences even though the growth has been checked osmotically. This indicates that the generation of surface potential differences results probably from the modification of membrane potentials of the individual cells by IAA. How can IAA bring about such a modification? Now, it has been suggested by the above authors that IAA probably acts through the inhibition or enhancement of ion pumps, or IAA probably brings about a selective permeability of cell membrane to certain ions. This

observation led these workers to investigate whether mersalyl could possibly act in the generation of surface electropotential comparable with the potential developed during gravitational stimulation. The experiment with mersalyl led them to believe that the generation of a surface potential either by the application of IAA or mersalyl is through a common system - changes in membrane permeability or changes in an ion pump. It may be interpreted that these two substances may have the same action as far as the generation of surface potential goes. Thus, it can probably be said that the geo-electric effect arises from the influence of auxin and auxin exerts its effect through changes in membrane permeability or in an ion pump operation.

Statement of the problem and method of approach

The present study was undertaken as an extension of the work of Arslan-Cerim (1966) and of Bode (1959b). The work has been carried out by repeating the experiments of the above workers and also new experiments were conducted with the following objectives in mind.

1. Whether the differential distribution of ions has any connection with geotropic perception.
2. Whether the differential distribution of ions is correlated with the asymmetry in the growth rates of the curved organ.
3. Whether the differential distribution of ions is the result of auxin concentration gradient.

The problem has been approached by studying the distribution of calcium (^{45}Ca), potassium (ionic and ^{42}K), and phosphorus (32p) after hypocotyls had shown geotropic curvature.

From the above approach one cannot say whether there was any relationship between ion distribution and geoinduction or perception. The object was to separate geoinduction or perception from geotropic response. A stimulus was given at 25°C for ½ hour - this stimulus was enough to produce geotropic response but curvature (response) was stopped by exposure to 4°C. Any ion movement in this condition would presumably be related to geoinduction (perception), if not it would be related to geotropic response.

Another approach to the problem was the study of effect of Naphthylphthalamic acid (NPA). This substance has been used by different workers. The seeds treated with NPA show normal germination but lose their ability to direct their radicle and plumule with respect to gravity (Mentzer and Netien, 1950). McCready (1968) reported that NPA completely inhibits the basipetal transport of IAA. So NPA does inhibit the geotropic curvature through the inhibition of auxin transport, but probably does not interfere with the geotropic perception. The idea was to look into the ion distribution in the absence of curvature. The seedlings pre-treated with NPA were placed in horizontal position. Any redistribution of ion after this treatment would be related to the perception, if not, it would be related to geotropic response.

It was considered to be of much interest to study the distribution of ions after the curvature had been produced

by the unilateral application of IAA. Any differential distribution would suggest a correlation with the curvature (a result of IAA concentration gradient).

As already pointed out, recently some interest has been focussed on the possibility that the generation of surface potential differences isprobably through changes in ion pumps. The object was to study the effect of mersalyl on the ion distribution and it was thought important to find out whether these two substances (mersalyl and IAA) had the same action with regard to ion redistribution.

Thus, attempts have been made to establish any relationship between ion distribution and geotropic response.

CHAPTER II

MATERIALS AND METHODS

The aim of the work, as already discussed, was to investigate the distribution of calcium, potassium and phosphorus in seedling stems during geotropic response. The work was carried with the hypocotyls of Helianthus annus and coleoptiles of Zea mays. Seedlings were horizontally oriented after being pre-treated with radioactive solutions of calcium, potassium and phosphorus. At the conclusion of the experiment usually, the upper and lower, the left and right and the concave and convex halves were collected and assayed as necessary. Thus the distribution of ^{45}Ca, ^{42}K and ^{32}P could be ascertained. These investigations were also carried out on ionic potassium in Helianthus annus.

Other experiments included, the distribution at $4^{o}C$, the effect of NPA on the distribution, the effect of unilateral application of IAA, distribution in root-less plants during geotropic response, the effect of unilateral application of mersalyl on the distribution and the effect of unilateral illumination on the distribution of radioactivity or ions as the case may be.

1. Plant Materials

Two types of plant materials were used for the work.

(a) Helianthus annus

Seeds were soaked in running water and the temperature was maintained as near as possible at $25^{o}C$. Warm water

from a mixer tap was run into a glass jar which had a small hole at the bottom so that the water could flow out of the jar. On the following day the seeds were taken out of the glass jar and then put in the glass brick lined with a tissue paper which was soaked in water. The glass brick was covered from the top by a similar glass brick lined with a moistened tissue paper. This was left in the constant temperature room ($25^{o}C \pm 2$) under fluorescent lighting. On the following day about 80% of the soaking seeds had burst their testas so that the radicle could be clearly seen. Then seeds were selected and planted in small glass tubes (size, 3" x 1") lined with polythene bags containing vermiculite. Only one seed was planted in each tube. Seedlings were allowed to grow for 4 - 5 days in a constant temperature room. When the hypocotyls were about 4 - 5 cm tall, they were ready for the experiment.

(b) Zea mays L. Var. Gaint White Horse Tooth

Seeds of Zea mays were soaked in a beaker containing water. After soaking they were washed thoroughly in running water and were arranged in a glass brick lined with moistened tissue paper. It was covered by a similar glass brick which was also lined with moistened tissue paper. Seeds were allowed to germinate for about 24 hours. On the following day they were taken out of the dark cupboard. This stage was such that 90% of the seeds had burst their testas so that the radicles could be clearly seen. Then they were planted in glass specimen tubes as mentioned earlier. Each tube had only one seed. The tubes were placed in a dark cupboard where the seedlings were allowed to grow for about 2 days. The seedlings with their coleoptiles 3 - 4 cm long were ready for the experiment.

2. Basic Technique

The technique used throughout the investigation was basically that of Arslan-Cerim (1966) and Bode (1959b) with some modifications depending on the nature of the type of experiment.

Radioactive solution was applied to the vermiculite. Seedlings were left in a dark cupboard so as to ensure adequate uptake of radioactive substance into the seedlings. While in the dark the seedlings were placed in a horizontal position for 1, 2 and 3 hours. The orientation of the seedlings from the vertical to the horizontal position was carried out by a stand. A diagram of this stand has been shown in Fig.1.

After each horizontal exposure hypocotyls or coleoptiles were split into two halves (upper and lower) by a cutter which has been shown in the Fig.2. This cutter consists of a perspex sheet (7 mm thick) on which there were nine circular holes of varying diameter (range, 2 - 4 mm). A blade was placed on a straight line so that it passed through the diameter of the circular area of each hole. The blade was held from the sides tightly by means of two holders. Hypocotyls or coleoptiles could be inserted through the holes depending on the size and split into two longitudinal halves. Special care was taken to split the hypocotyls or coleoptiles accurately. A group of halves were placed in a planchet which was weighed and numbered before the experiment. Fresh weight was determined by weighing the planchets again. The planchets containing the material were left in an oven at 100°C for about 12 hours to determine the dry weight of the material. When the materials were completely dry they were cooled in a

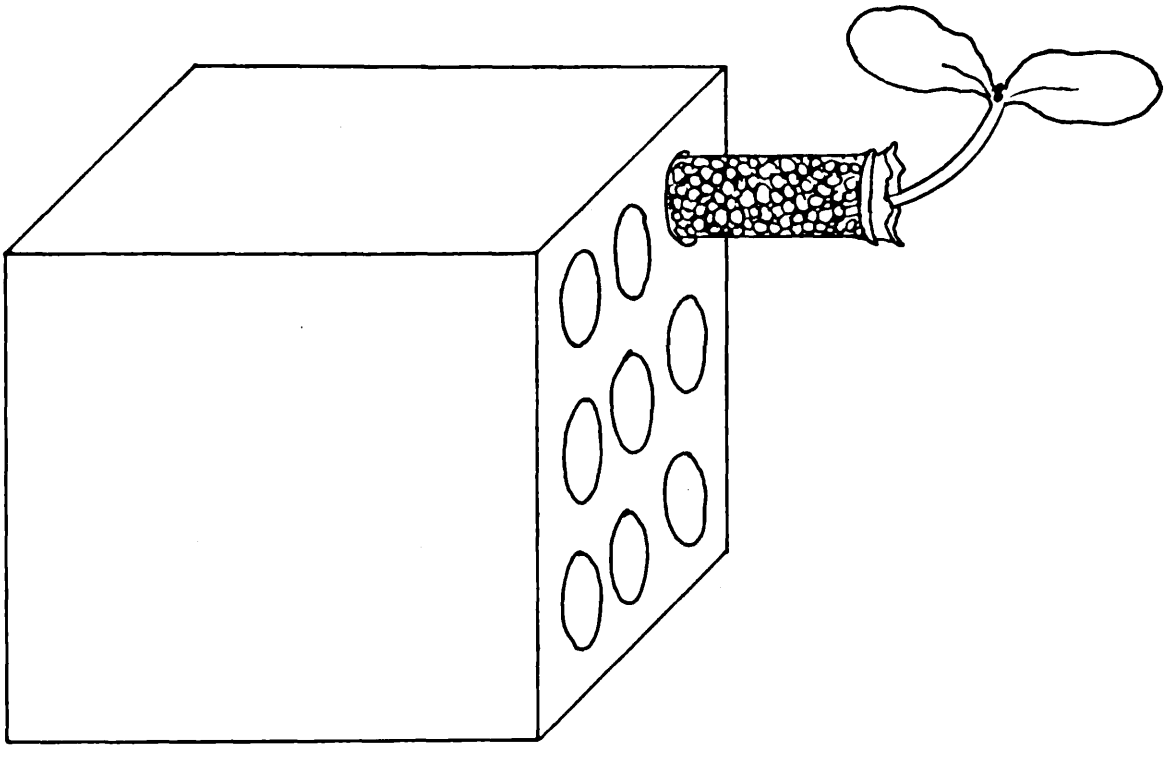


Fig. 1. A seedling of Helianthus annus in a horizontal position. Curvature is shown by the seedling.

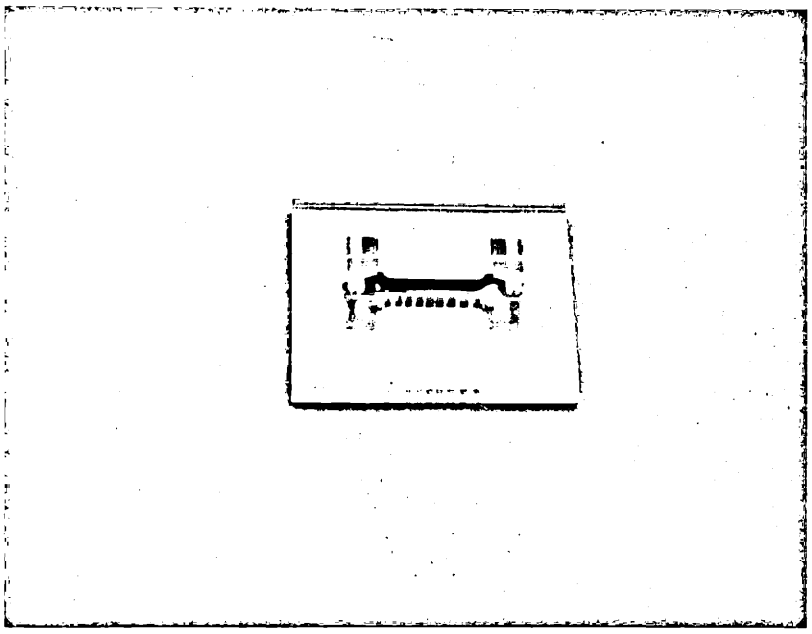


Fig. 2. A cutter

desiccator to determine the dry weight. The process of weighing and drying were repeated three times to get the constant weight. Each sample was then ground up separately if the radioactive substance was ^{45}Ca. The process of grinding was not necessary for ^{32}P and ^{42}K as these substances emit higher energy radiations and in these cases materials were directly stuck onto planchets. As ^{45}Ca emits β particles the process of grinding was carried out to apply an approximate correction factor due to tissue-thickness. Each sample was ground up separately in an agate mortar with 0.5 ml of ethanol. After grinding, the ground-up materials were carefully transferred into planchets each containing about 0.5 ml of 3% gumsolution. The samples were then dried under an infra-red lamp.. At this stage materials were placed in the counter for counting radioactivity.

3. Measurement of Curvature

After harvesting, hypocotyls or coleoptiles were left on a piece of paper and two lines were drawn, one through the top end (apical part) of the organ and the other through the main axis (basal part) of the organ. Later these two lines were extended so that they intersected each other. The angle between these was measured with a protractor and was taken as the curvature.

4. Radioactive Substances

Radioactive substances were obtained from the Radiochemical Centre, Amersham, Buckinghamshire.

$^{45}CaCl_2$

This substance was supplied in aqueous solution.

Specific activity 2 - 10 mCi/mg

^{42}KCL

This substance was supplied in sterile isotonic solution containing 11.9 mg potassium. 250 µCi in 2.5 ml.

^{32}P

This substance was supplied as orthophosphate in dilute HCl solution. pH 2 - 3, high specific activity, 1 mC in 1 ml.

5. Radioactive Assay

(a) Counting system

A fully automatic counter was used for radioactive counting. An IDL end window geiger tube (Mullard MX123) to be used in conjunction with a fully automatic sample changer (IDL Betamat type 6050). Scaler (IDL type 1700E) and progamme read-out unit (type 2007/B) were obtained. Thus 50 samples could be loaded at a time and left to count overnight.

(b) Preparation of radioactive solution

$^{45}CaCl_2$

Radioactive substance was carefully taken out by a syringe with a hypodermal needle and transferred into a polythene bottle containing Hoagland and Arnon solution. The activity was maintained in such a way so that 2.0 ml of the solution contained 1 µCi of ^{45}Ca. The pH of Hoagland and Arnon solution was previously measured and was found to be about 6.8.

^{42}KCl

The procedure for making up a radioactive solution was the same as that of ^{45}Ca. In this case the activity was maintained in such a way so that 2.0 ml of Hoagland and Arnon solution contained about 0.25 µCi of ^{42}K.

^{32}P.

This was obtained as orthophosphate. This was made up in Hoagland and Arnon solution. The pH value of the solution was maintained between 6 and 7. 2.0 ml of the solution contained about 0.5 μ Ci of ^{32}P.

(c) Preparation of radioactive samples

As already discussed in the basic technique, each sample (hypocotyl or coleoptile halves) was ground separately in an agate mortar with 0.5 ml of ethanol. This was transferred to a planchet and a very fine and uniform film of the sample was made. To this about 0.5 ml of 3% gum solution was added, and then dried under an infrared lamp. The samples could be loaded in the counter for counting. This process of grinding was necessary for the samples containing ^{45}Ca. But for substances such as ^{42}K and ^{32}P, the process of grinding was not necessary, because of hardness of the radiation.

Self absorption

^{45}Ca emits soft beta radiation which are readily absorbed by the material through which they pass. Thus a proportion of radiation will not be counted due to this self absorption. This proportion of radiation lost directly depends on the tissue thickness on the planchet. Hence, it becomes necessary to find out a correction for self absorption even when the tissue thickness varies to the slightest extent. The weight of the sample is directly proportional to the thickness of the sample. Thus, a separate correction factor had to be applied to each sample with varying weight and thickness. Self absorption also depends on the type of radiation and the nature of the material used during the

experiment. In this investigation two different types of materials used such as hypocotyls of Helianthus annus and coleoptiles of Zea mays. Hence, two separate self absorption corrections were carried out, although their correction factors varied very little against the same tissue thickness. The example which has been presented is for the hypocotyls of Helianthus annus.

Helianthus hypocotyls were cut into small segments and dried quickly in an oven at 100°C. The samples were then ground up in ethanol and ethanol was evaporated off under an infra-red lamp. Samples of this ground-up material were reweighed out in the planchet. A standard amount of stock $^{45}CaCl_2$ was added to each planchet followed by 0.5ml of 3% gum solution. The contents of each planchet were agitated to facilitate uniform spreading and the planchet was dried and reweighed. The counting rate of each sample of the same amount of stock $^{45}CaCl_2$ solution was counted alone. The correction factor for each sample was calculated as follows.

$$\text{Correction factor} = \frac{\text{Count rate of } ^{45}\text{Ca alone}}{\text{Count rate of } ^{45}\text{Ca in the material.}}$$

A graph of the count rate against thickness was then constructed which has been presented in the Fig.3. The correction for radioactive decay was not applied for ^{45}Ca as this substance has a half life for 152 days.

Corrections when assaying ^{42}K

As ^{42}K emits hard beta and gamma radiations there was no need for self absorption correction. ^{42}K was a half life for only 12 hours so a correction for radioactive decay was calculated. A correction curve has been constructed by plotting

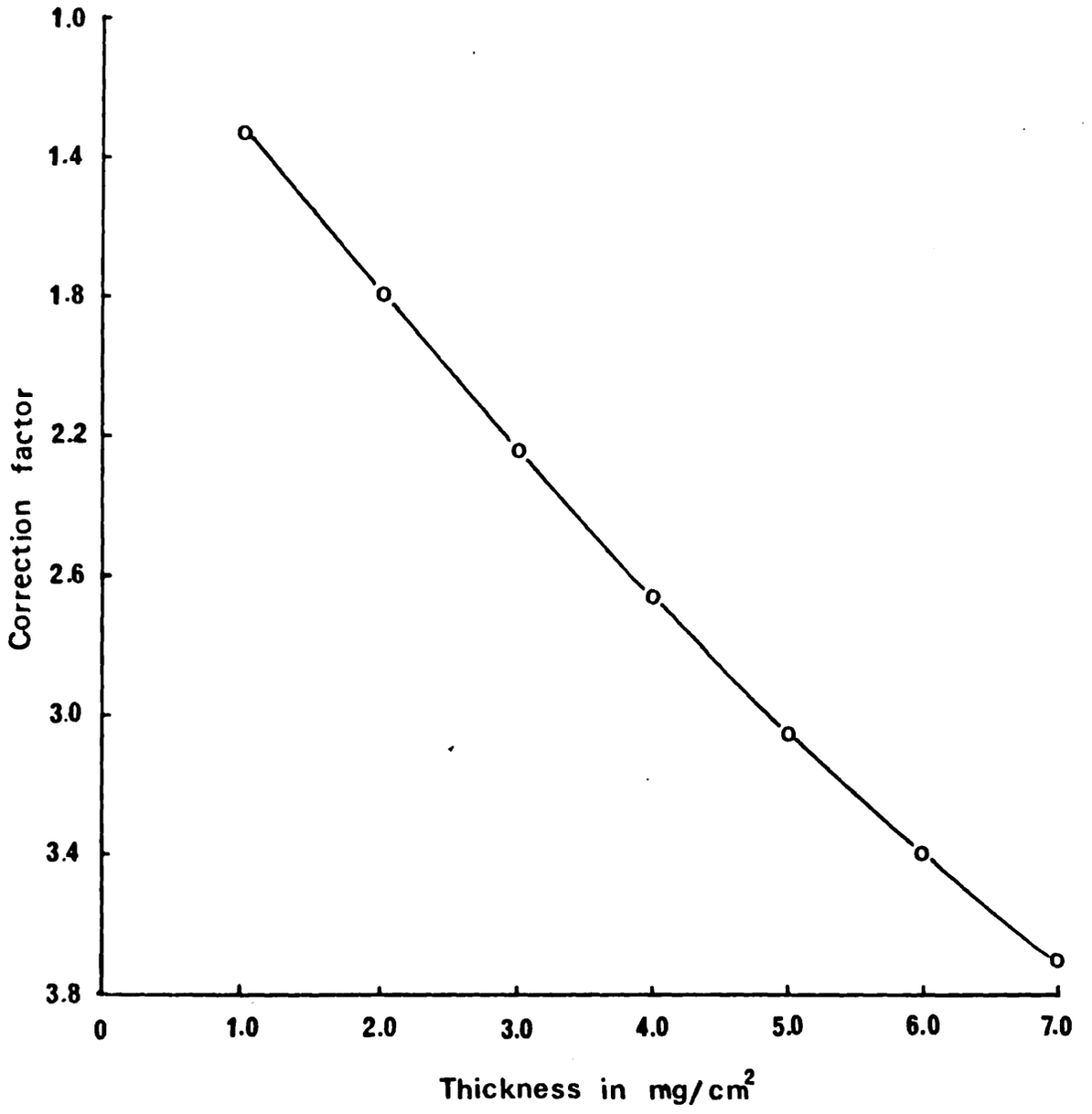


Fig.3. A self absorption curve for ^{45}Ca .

correction factor against time which has been presented in Fig.4. The values of radioactive decay in different times were obtained from the standard chart for radioactive decays. ('Isotope Tracers' by Francis, Mulligan and Wormall, Athlone Press, 1959).

Hour 0 was taken as the first hour of the experiment and all subsequent experiments were corrected out at this level. Each half containing ^{42}K was cut into pieces and the pieces were placed in an oven for drying. Thus, for example, for an experiment which was counted on hour 5 the correction factor (1.27) would be read from the figure and all the observed count rates would be multiplied by this factor.

Corrections when assaying ^{32}P.

^{32}P emits hard beta radiations like ^{42}K and so no self absorption correction was necessary. Estimates of radioactive decay after the relevant time interval were obtained from published charts ("Radiotracer methodology in biological science" by Wang and Willis, Prentice - Hall,1965). ^{32}P has a half life of 14 days thus a correction for radioactive decay was calculated which has been presented in Fig. 5. The graph was plotted in the same way as for ^{42}K. Thus for correction factor on day 6 was found to be 1.33. This correction factor was applied for the day of counting in one experiment.

6. Non-radioactive Assay

Seedlings of Helianthus annuus were grown in small glass specimen tubes (Size, 3" x 1") containing vermiculite. Seedings were grown in a constant temperature room (25°C ± 2) under fluorescent lighting. 100 seedlings were used for each

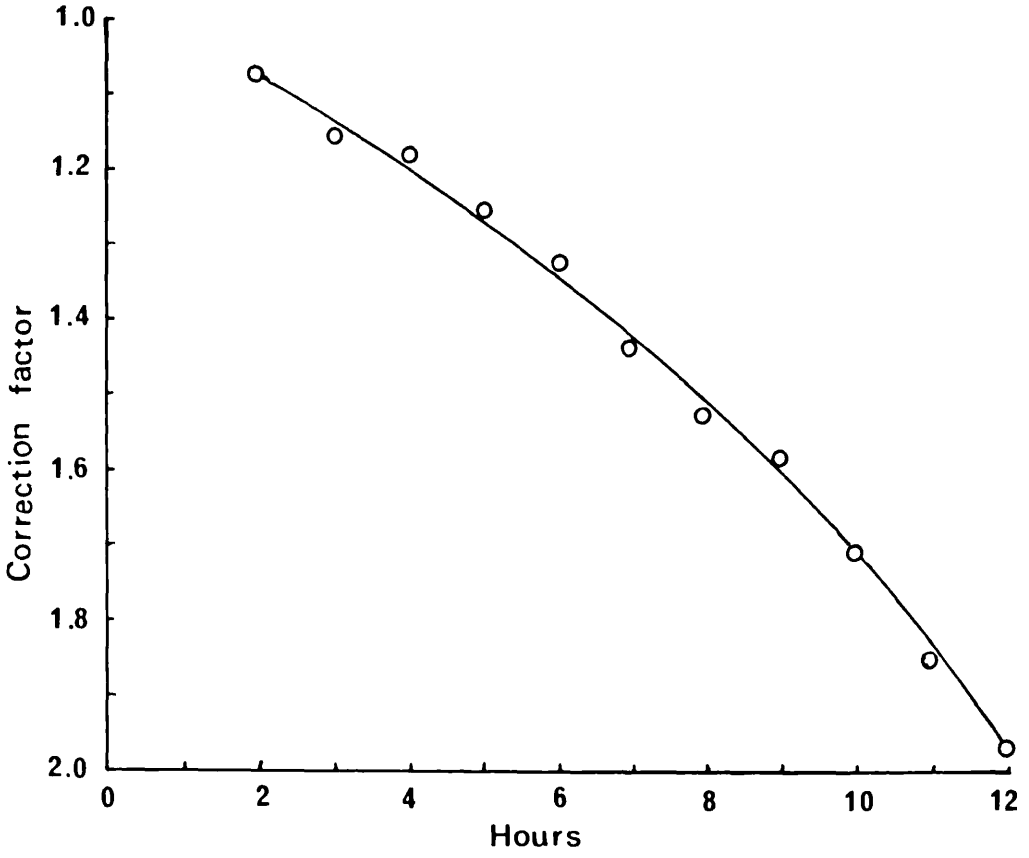


Fig.4. A decay curve for ^{42}K

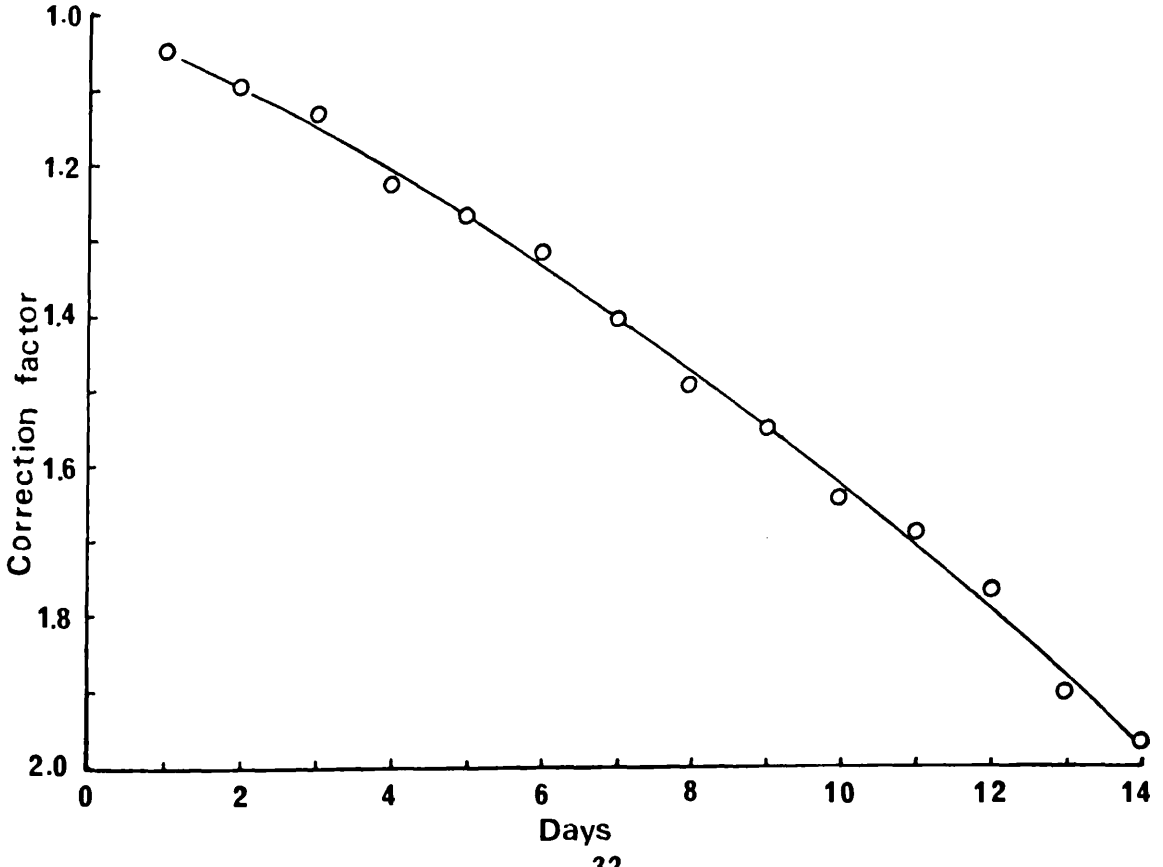


Fig.5. A decay curve for ^{32}P

horizontal exposure. So altogether 400 seedlings were required for the experiment (0 hour vertical, 1, 2 and 3 hours horizontal exposure). Seedlings were left in a dark cupboard after each was treated with 2.0 ml of Hoagland and Arnon solution. At the beginning of the experiment each hypocotyl was marked with ink on the side of the cotyledon in order to be able to distinguish between the sides. Seedlings were placed in a horizontal position for 1,2 and 3 hours in the dark cupboard. During the horizontal exposure of the seedlings the cotyledons were lying in the vertical plane. The side of the hypocotyl which was marked by ink was the upper side. Vertically growing seedlings were harvested and the hypocotyls were split centrally into left and right halves by a cutter. A group of 25 halves from each side was taken in a crucible. Hypocotyls involved in each horizontal exposure were harvested and split into upper and lower halves. Fresh weight of the halves was determined by a sensitive Torbal balance. The samples were left in an oven at 100°C for 8 hours for complete drying of the halves. When the materials were completely dry they were cooled in a desiccator. The materials inside a desiccator were dried and cooled three times until the weight of the materials was constant. After the dry weight was taken the crucibles were placed in a muffle furnace at 550 - 600°C for 12 hours. The crucibles were put back into a desiccator for complete cooling of the ash. The ash was weighed for three times to determine the actual ash weight.

Flame photometric method -

Preparation of standard solution

0.477 gms. dry "analar" quality KCl was accurately

weighed, dissolved in pure distilled water and the total volume was made up to 500 ml. To obtain 1 mg.K/100 ml the stock solution was diluted 1:50.

Calibration

The instrument was set up according to the instruction. A range of potassium chloride solution at the following concentrations were used for the calibration.

0.2; 0.4; 0.6; 0.8; 1.0 mg K/100 ml.

Potassium filter was placed in position inside the flame photometer. A standard solution of 1 mg K/100 ml. and sensitivity control was adjusted to 100 divisions. Distilled water was sprayed and the galvanometer was adjusted to get zero reading. The process of spraying standard solution and distilled water was repeated for five times. Then solutions of concentrations 0.2; 0.4; 0.6; 0.8; and 1 mg K/100 ml were sprayed and the reading was noted. A calibration curve was constructed by plotting readings against concentrations which has been presented in Fig. 6.

Determination of potassium from ash

A standard procedure was followed which was succeeded by dissolving 25 mg of ash in 100 ml. of 1.5 N.HCl. For example, if the ash weight was 12.5 mg then 50 ml. of 1.5 N HCl was needed to dissolve it. After different samples of the ash had been dissolved out separately in 1.5 N HCl, each sample was filtered so that there was no particle suspending in the solution. From each sample 5 ml was taken and transferred into separate numbered flasks. Each was diluted 20 times with demineralised water (1:20). Each sample was sprayed through the flame photometer and the amount of potassium present was calculated from the reading. Three determinations of potassium concentration were made on each sample and average

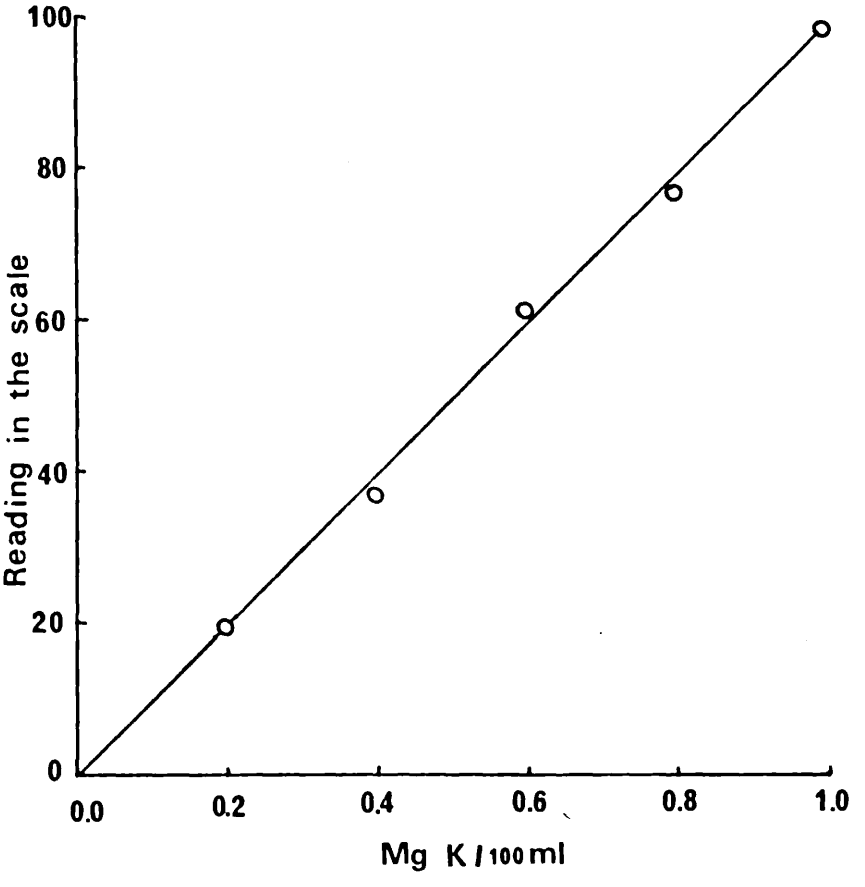


Fig.6. A calibration curve for ionic potassium

values were converted into the actual amount of potassium present in the sample.

7. Experimental Methods for Studying the Effect of Light

The effect of unilateral illumination was studied to establish whether any kind of relationship existed between ion distribution and phototropic curvature.

These investigations were carried out with the coleoptile of *Zea mays*. Coleoptiles were illuminated unilaterally for 2, 3 and 4 hours with 800 lux white light from a 150 watts incandescent bulb. The details about light measurement have been discussed later. Seedlings were treated with the radioactive substance prior to illumination. In general, at the end of each unilateral illumination, illuminated and shaded halves were collected and assayed. Thus the distribution of radioactivity in the halves was ascertained for ^{45}Ca, ^{42}K and ^{32}P.

(a) Light chamber

A light chamber - a wooden box (Size, 15" x 10" x 10") which consisted of two vertical compartments. The door of the box had two horizontal slits, each of 9 cm length and 4 mm breadth. A diagram has been drawn of this chamber (Fig.7). Seedlings were arranged in such a way so that the apical portion of the coleoptiles could be unilaterally illuminated.

(b) Light measurement

The measurement of light was carried out by means of a EEL light meter. This was conducted by holding the photocell inside the wooden box after the light had been switched on. The door of the wooden box was kept closed as much as possible during the light measurement. The entire process of light

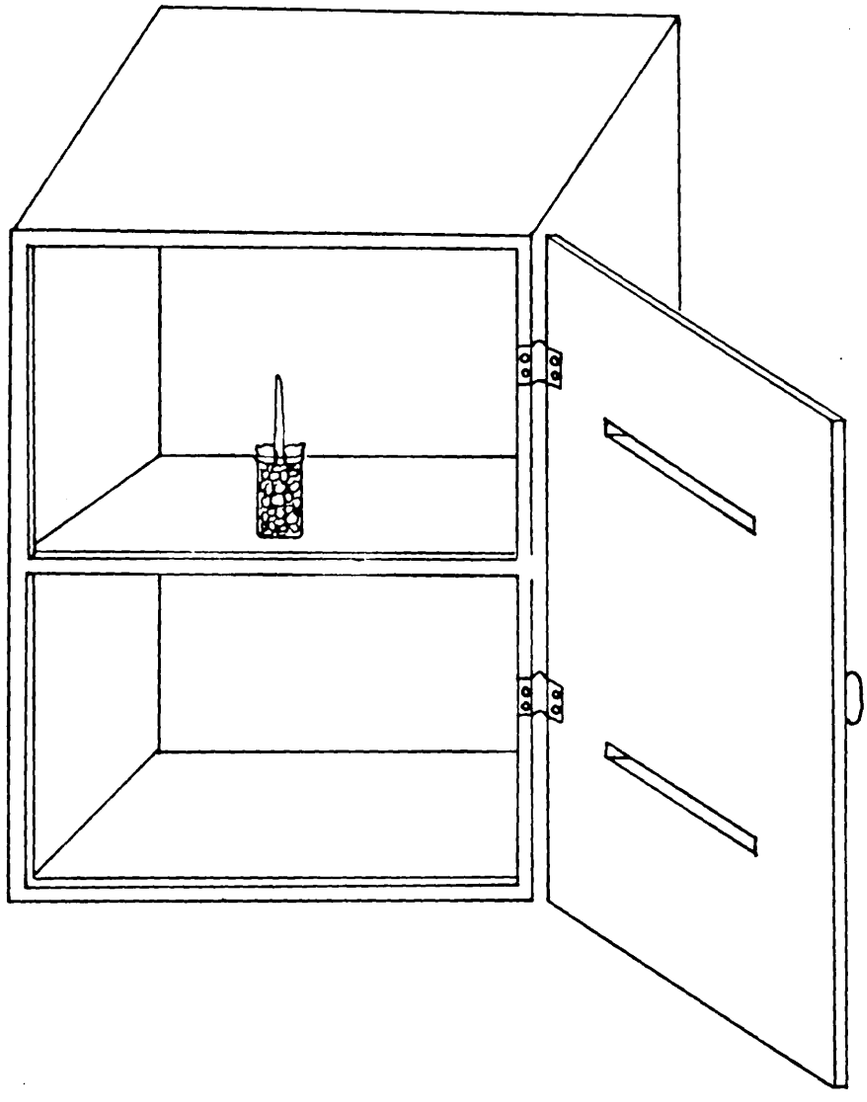


Fig.7. A light chamber

measurement was carried out inside a dark cupboard. The source of light for the phototropic exposures was a single 150 w incandescent bulb at a distance of 30 cm from the plants. The intensity of the light at this distance was about 75 lumens/sq.ft. This was converted into lux and was found to be about 800 lux.

(c) Plant material

Seedlings of *Zea mays* were used for studying the effect of unilateral illumination. The details about growing seedling have been discussed in Materials and Methods. Coleoptiles of *Zea mays* were found suitable material for the experimental purpose.

(d) Technique

Seedlings were growing in small glass tubes containing vermiculite. The seedlings were pretreated with radioactive substance when the coleoptiles were 3 to 4 cm in length. The temperature of the dark cupboard (where unilateral illumination was carried out) was maintained around $25^{o}C \pm 2^{o}C$. It was established by carrying out some experiments that with the intensity of light mentioned above the coleoptiles did produce curvature in 2 hours unilateral illumination. So curvature in degrees was measured as already discussed. Coleoptiles were unilaterally illuminated for 2, 3 and 4 hours inside the light chamber. The illuminated side was marked by ink in order to be able to distinguish from the shaded side. Coleoptiles were harvested and curvature was measured after each treatment and then they were split centrally by a cutter into two halves. Each half was put separately in numbered planchets. Determinations of fresh and dry weight were carried out as discussed

in the basic technique. Radioactive assay was followed in the same way as for the other experiments already discussed in the Basic Technique.

8. Statistical Analysis

A three factor analysis of variance was performed on the experimental results, three factors being plant (P), time (T) and side (S).

Owing to experimental design plant and time effects were partially confounded in the following way.

	S_1				S_2			
T_1	P_1	P_2	P_3	P_4	P_1	P_2	P_3	P_4
T_2	P_5	P_6	P_7	P_8	P_5	P_6	P_7	P_8
T_3	P_9	P_{10}	P_{11}	P_{12}	P_9	P_{10}	P_{11}	P_{12}
T_4	P_{13}	P_{14}	P_{15}	P_{16}	P_{13}	P_{14}	P_{15}	P_{16}

The analysis of variance combined the various factors in the way set out in the table, i.e. determined sum of squares and mean square variance for

T

S

TS

P + PT

The residual error against which the S and ST effects were tested being the combination

PST + PS

Time effects were tested against the P + PT variance.

Analysis of variance was carried out on the CDC 6600 computer by using scientific sub-routine package (Hartly, "Analysis of variance", John Wiley, New York).

The significance of the differences of individual means was tested by determining L.S.D. (least significant differences between the means) at 0·025 probability level by the following formula.

$$L.\ S.\ D. = \sqrt{\frac{msv}{n}}\ \sqrt{2}.t$$

msv = mean square variance of the residual error

n = number of replications

t = value of student's "t" at 0·025 probability level.

CHAPTER III

THE EFFECT OF HORIZONTAL ORIENTATION ON THE DISTRIBUTION OF IONS

As set out in the Introduction, the object of this study was to investigate the distribution of calcium, potassium and phosphorus in geotropically stimulated organs. The experiments have been conducted with the seedlings of Helianthus annus and Zea mays. It was hoped that this would give an indication whether the distribution of ions is connected either with the geotropic response or with the detection mechanism. In this detection mechanism all changes are occurring during the transverse action of gravity before the appearance of geotropic curvature. Growth responses do not develop immediately on the reception of the stimulus but after a lapse of period which is actually much longer than the presentation time. At the end of the presentation time of the organ a physiological polarisation of the organ develops and this leads to local differences in the rate of growth.

SECTION I DISTRIBUTION OF ^{45}Ca

Calcium is known to inhibit the growth of shoot parts by making the cell walls more rigid. According to the results presented by Arslan-Cerim (1966), there was more accumulation of radioactive calcium in the upper half of geotropically stimulated hypocotyls of Helianthus annus. There was a considerable accumulation of a radioactivity in the upper half the degree of which depended on the time of stimulation. It

seemed worthwhile, therefore, first to obtain a direct confirmation of her results by repeating the same experiment with slight modifications as a basis for an extension to other ions.

(a) Helianthus hypocotyls with roots

This experiment was carried out in the same way as was performed by Arslan-Cerim with some modification, e.g. the number of seedlings and the amount of radioactive substance used for each treatment. A group of 20 seedlings was used for each horizontal exposure and each was treated through the roots with 1 μ Ci of ^{45}Ca (made up in Hoagland and Arnon solution). This was carried out by adding 2.0 ml of radioactive solution (which contained about 1 μ Ci of ^{45}Ca) to the vermiculite. In this experiment there were three horizontal exposures (1, 2 and 3 hours). A group of 20 seedlings was used which would establish the initial distribution of radioactivity in the halves of vertically placed seedlings. All the seedlings after the treatment with ^{45}Ca were left in a dark cupboard for 6 hours to ensure adequate uptake of radioactive substance through the roots. The seedlings involved in horizontal exposure were left in a horizontal position for 1, 2 and 3 hours. This was carried out as described in the chapter Materials and Methods. After each exposure the seedlings were harvested (roots and cotyledons were removed), degrees of curvature of the hypocotyls were measured. Each hypocotyl was split centrally (by the cutter as shown in Fig.2) into the upper and lower halves. In case of vertically placed seedlings the hypocotyls were split centrally into the

left and right halves (sides). Each horizontal exposure had 4 replications, so a group of five upper and lower halves (sides) were placed separately in numbered planchets as described in the chapter Materials and Methods. The technique for the preparation of sample and radioactive assay was followed as described in the Basic Technique.

The radioactivity was recorded as cpm/mg. dry weight and cpm/10 mg. fresh weight. The degrees of curvature have been recorded in the figure. ± in the figure stands for the standard error of the mean. Analysis of variance for the results was carried out by computer programme as discussed. In Table 1 (Appendix) and all subsequent tables, the P and PT have been lumped as plant effects and provide the residual against which the time effects can be tested. The idea of the complete analysis was to eliminate the plant effects to increase the precision of the analysis. Analysis of variance shows that there has been a significant overall increase in radioactivity in the hypocotyl as a whole with time. The Table I shows that S X T effect is significant. This signifies the fact that the difference between the upper and lower halves (sides) varies significantly with time. There is a progressive difference between the side means with time which is clearly seen in Fig.8. Least significant differences (L.S.D) have been shown in the figure.

Fig.8 indicates that in 1 hour of horizontal exposure there has been a significant drop of activity in the lower side which could have been attributed to the lateral migration of activity from lower to the upper side. Graphs show that on the lower side there is first a drop and then a recovery, whereas on the upper side there is a much smaller drop (not significant) and a much greater rise. This suggests that the differences between upper and lower sides are mainly due to

Fig.8 The effect of horizontal orientation on the distribution of ^{45}Ca in the hypocotyls of *Helianthus annus*.

Time-course changes in the activity of ^{45}Ca in the sides.

Curvatures in degrees are shown at the top of the figure. $\pm$ is standard error of the mean.

A :- mean counts/min/mg. dry weight

Full circle :- upper side

Open circle :- lower side

B :- mean counts/min 10 mg. fresh weight

Full triangle :- upper side

Open triangle :- lower side

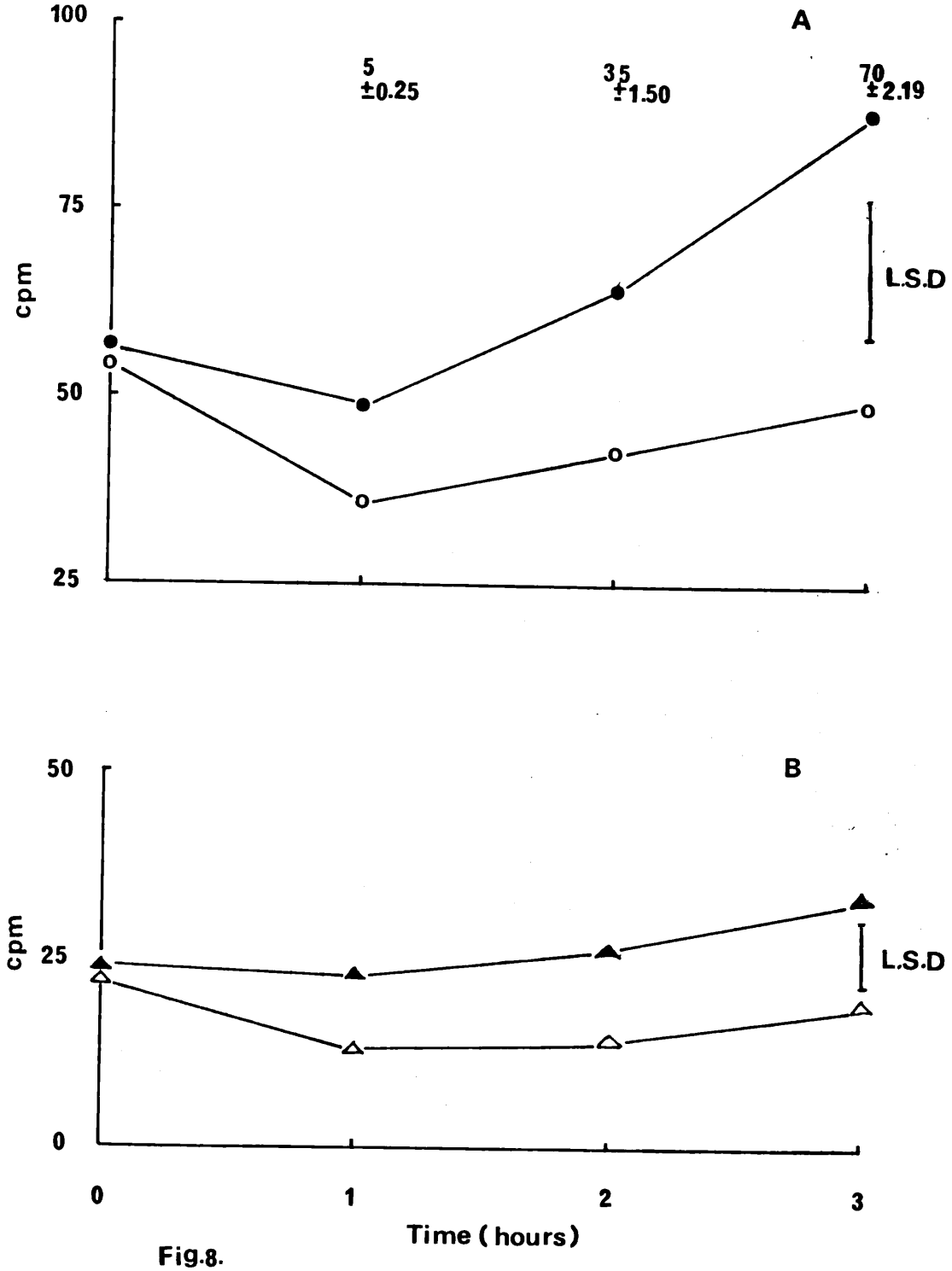

100
75
50
25
cpm
A
5
±0.25
35
±1.50
70
±2.19
L.S.D
50
25
0
cpm
B
L.S.D
0
1
2
3
Time (hours)

Fig.8.

migration from lower to upper. Subsequent movement from roots increases concentration in both sides but upper increases more than lower. Whether this is due to differential rate of movement from roots cannot be definitely concluded.

It is clear from the figure that the differences between the means of upper and lower sides are higher than L.S.D. after 2 and 3 hours of horizontal exposure.

The results strongly suggest that a concentration difference of calcium is established between upper and lower sides and is correlated with the curvature. There are three possibilities for the accumulation of more calcium in the upper side. (1) there has probably been more transport of calcium into cells of the upper side from roots during horizontal exposure. (2) there has been migration of calcium from lower to the upper side. Probably there has been combination of (1) and (2) taking place simultaneously with the beginning of horizontal orientation. It was felt necessary to clarify the situation. This was attempted by the following approach.

(b) Helianthus hypocotyls without roots

In the last experiment it was observed that there was more accumulation of radioactivity in the upper side during horizontal exposure. The object was to study the lateral migration of calcium from lower to upper side in reality.

Seedlings of Helianthus annus were treated with ^{45}Ca (1 μCi for each seedling) through the roots for 6 hours in a dark cupboard. The seedlings were harvested and roots were removed. The shoot part consisted of hypocotyl (about 3 cm long) and cotyledons. Hypocotyls (with the cotyledons) were

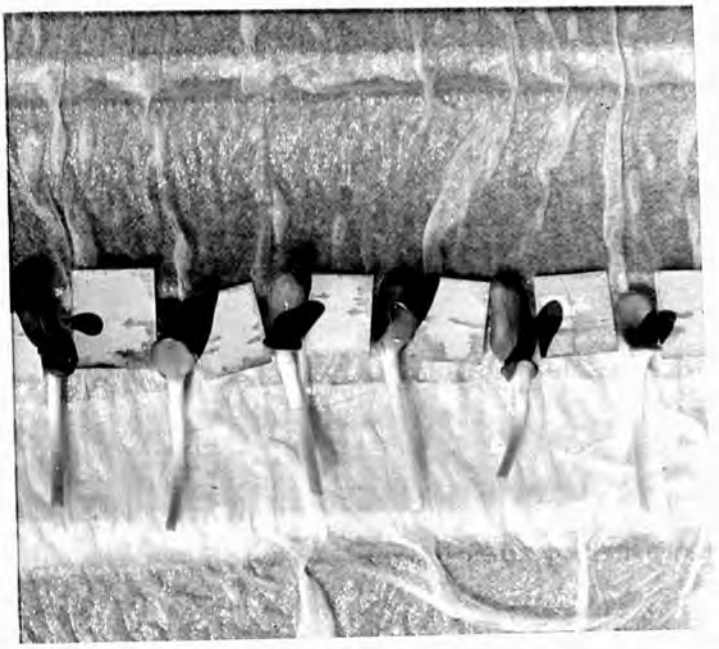


Fig. 9. Rootless seedlings of Helianthus annus in horizontal position.

Fig. 10. Rootless seedlings of Zea mays in horizontal position.

placed in a horizontal position for 1, 2 and 3 hours in a glass tray as shown in Fig.9. A special care was taken about the orientation of the hypocotyls so that they could stay in horizontal position as they curved. A glass tray had four furrows and one cotyledon of the shoot could be placed on it so that the hypocotyl was in horizontal position. On the two sides of the cotyledon (which was in the furrow), two pieces of thick paper were placed to keep the hypocotyl orientated so that it did not fall over as it curved. This glass tray was covered by a similar glass tray. Both the glass trays were lined with moistened tissue paper. A group of 20 hypocotyls (with the cotyledons) was included in each horizontal exposure. After each horizontal exposure (which was carried out inside a dark cupboard), cotyledons were removed and hypocotyls were split centrally into halves by a cutter as described. Vertical control (initial as 0 hour) would establish the initial distribution of radioactivity in the halves. Each horizontal exposure had 5 replications. Measurement of curvature, radioactive assay and preparation of sample were carried out as described in the chapter Materials and Methods.

Analysis of variance of the data was carried out which has been presented in the Table 2 (appendix). This shows that S X T effect is non-significant. But the overall S (side) effect is significant.

Graphs have been plotted both on dry and fresh weight basis. Fig.11 indicates that there has been a migration of activity from the lower to the upper side and the maximum difference was observed after 2 hours of horizontal exposure.

Fig.11 The effect of horizontal orientation on the distribution of ^{45}Ca in the hypocotyls of *Helianthus annus*.

Hypocotyls were horizontally orientated in root-less condition.

Time-course changes in the activity of ^{45}Ca in the sides.

Curvatures in degrees are shown at the top of the figure. $\pm$ is standard error of the mean.

A :- mean counts/min/mg. dry weight

Full circle :- upper side

Open circle :- lower side

B :- mean counts/min/10 mg. fresh weight

Full triangle :- upper side

Open triangle :- lower side.

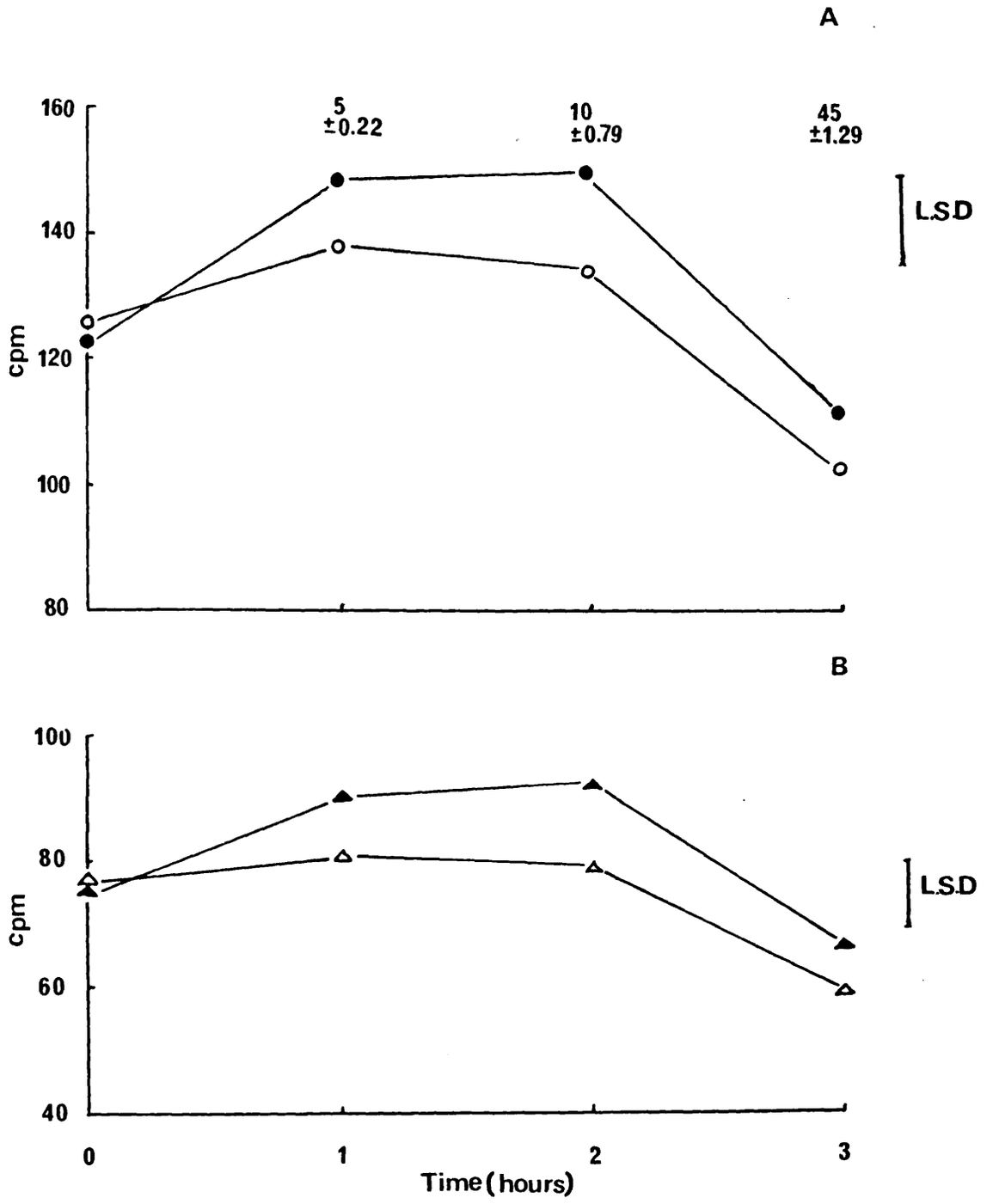

A
160
140
120
100
80
cpm
5
±0.22
10
±0.79
45
±1.29
LSD
B
100
80
60
40
cpm
LSD
0
1
2
3
Time (hours)

Fig.11.

The rises and falls on the two sides run parallel and are probably due to sampling (i.e. plant) error. That, this is so, is quite clear from the analysis of variance Table 2 (Appendix) where M.S.V. for P + P T is actually greater than M.S.V. for T. The S effect shows an overall significant increase on the upper side but no detectable change with time (i.e. as appears in the graphs all changes are established in the first hour and subsequently there is no further change). This suggests that lateral migration may take place early and accounts for much of the differences shown by rooted plants. However, it still leaves open the question whether the suggested progressive increase in the differences between upper and lower sides in rooted plants is due to a difference in transport from the roots or to a continuing migration from bottom to top.

(c) Zea coleoptiles with roots

It was thought necessary to carry out similar experiments in order to be able to bring about a direct comparison with the results of the experiment in case of Helianthus hypocotyls (with roots).

Each seedling of Zea mays was treated with 1 μCi for 6 hoursin a dark cupboard. A group of 20 seedlings was used for each horizontal exposure. Initial as 0 hour would establish the initial distribution of radioactivity in the coleoptile halves. Each exposure consisted of 5 replications. Horizontal exposures were carried out inside a dark cupboard. At the end of each exposure, seedlings were harvested (roots were removed), coleoptiles were split centrally into upper and lower halves. Inner leaves were removed from the coleoptile halves. Technique for the preparation of sample and

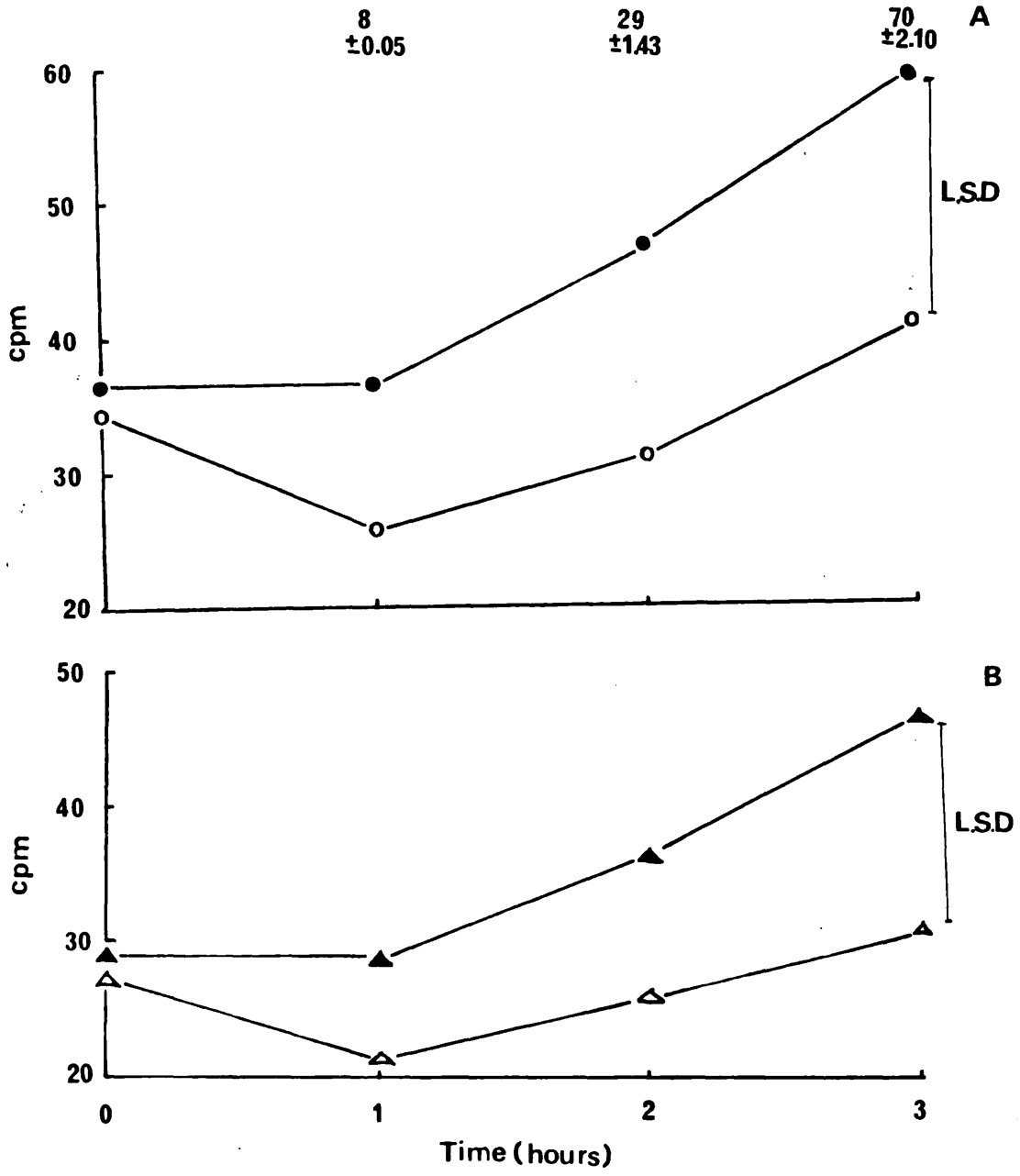


Fig.12. The effect of horizontal orientation on the distribution of ^{45}Ca in the coleoptiles of Zea mays.
Time-course changes in the activity of ^{45}Ca in the sides. Curvatures in degrees are shown at the top. ± is standard error of the mean.

A :- mean counts/min/mg. dry weight
Full circle:- Upper side
Open circle:- Lower side

B:- mean counts/min/10 mg. fresh weight
Full triangle:-Upper side
Open triangle:-Lower side

radioactive assay was followed as described in the chapter Materials and Methods.

Analysis of variance of the data was carried out and the results are shown in Table 3 (Appendix). The analysis shows that S (side) effect is significant. This suggests an overall increase in the upper side. The analysis does not indicate any significant change with time.

Graphs for the mean counts on dry and fresh weight basis are shown in Fig.12. The differences between the sides increase slightly with time and this is correlated with the curvature. Graphs show that on the lower side there is first a drop and a recovery, whereas on the upper side there is no change and then a much greater rise. This suggests that the differences between upper and lower are mainly due to migration from lower to upper. There are indications of a subsequent increase in both sides by uptake from roots although this does not reach significant levels. Fig. 12 shows that the differences between the means of upper and lower sides are higher than L.S.D. after 3 hours horizontal exposure. These results are similar to the results obtained in case of Helianthus hypocotyls (with roots). The results discussed above imply a lateral migration from lower to upper side. The following approach was attempted to check the lateral migration.

(d) Zea coleoptiles without roots

In the last experiment it was shown that there was more accumulation of radioactivity in the upper side during horizontal exposure. The object was to get an exact idea of lateral migration of calcium from lower to the upper side.

Seedlings of Zea mays were treated with ^{45}Ca (1 μCi for each seedling) through the roots for 6 hours in a dark cupboard. The seedlings were harvested and roots were removed. Coleoptiles were placed in a horizontal position for 2 and 3 hours. Coleoptiles were horizontally orientated in a glass tray (lined with moistened tissue paper) as shown in Fig.10. Coleoptiles (about 3 cm long) were placed horizontal in the furrows. The object was to keep coleoptiles orientated so that they did not fall over as they curved. This glass tray was covered by a similar glass tray which was also lined with moistened tissue paper. A group of 20 coleoptiles were included for each exposure. Vertical control (initial as 0 hour) consisted of 20 coleoptiles. This would estabish the initial distribution of radioactivity in the halves. At the end of each exposure coleoptiles were split by a cutter into halves (upper and lower). Radioactive assay and preparation of sample were carried out as described in the chapter Materials and Methods.

Analysis of variance of the data was carried out and the results are shown in Table 4 (Appendix). The analysis shows that the main side effect (S) is significant.

Graphs have been plotted on dry and fresh weight basis shown in Fig.13. The figure shows that there is a drop on the lower side and a rise on the upper side. The differences between the sides increase with time, though this is not significant. Any increase in the upper side must be due to lateral migration from the lower side.

A comparison made with rooted plants (Fig.12) suggests that in rooted plants lateral migration takes place early and there has been progressive differences between upper and lower sides. But it cannot be concluded that in rooted plants

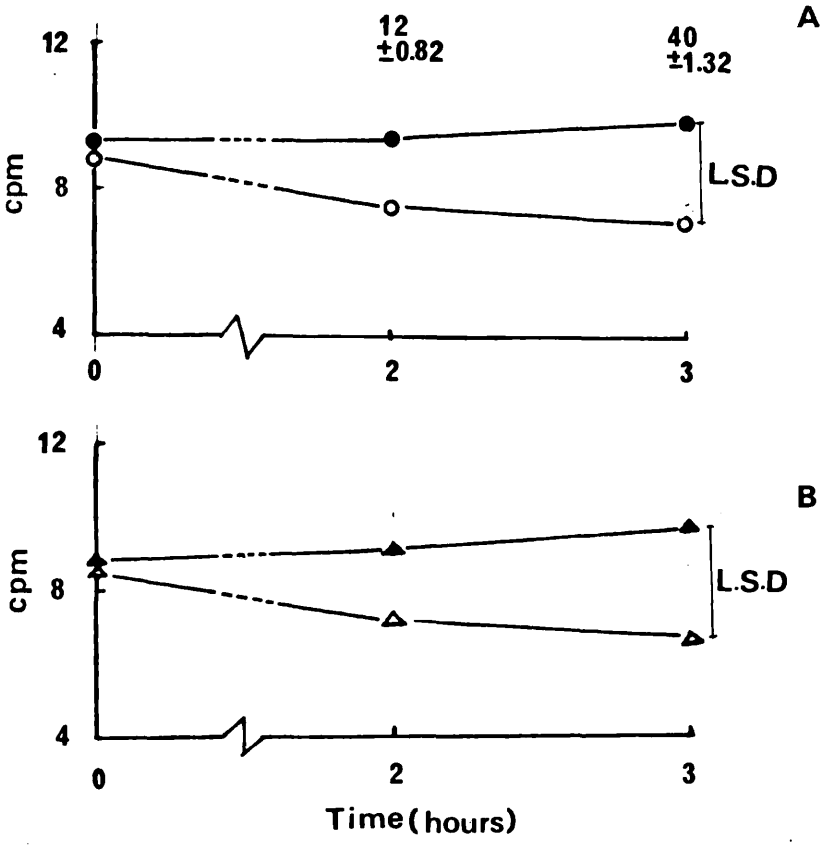


Fig.13. The effect horizontal orientation on the distribution of ^{45}Ca in the coleoptiles (without roots) of Zea mays. Curvature in degrees shown at the top. ± is standard error of the mean.

A :- mean counts/min/mg. dry weight

Full circle:- Upper side

Open circle:- Lower side

B :- mean counts/min/10mg. fresh weight

Full triangle:- Upper side

Open triangle:- Lower side

the progressive differences are due to differential transport from roots.

SECTION 2 DISTRIBUTION OF POTASSIUM

It has already been seen in the Introduction that Bode (1959b) reported an increased concentration of potassium in the lower half of geotropically stimulated hypocotyls of Helianthus annus. He suggested that the increase of potassium in the lower half is preceded by the movement of auxin and indicated that auxin probably brings about some physiological changes in the lower half which favour the movement of potassium in that direction. The idea of establishment of concentration gradient from apical to the basal part of the hypocotyl has also been suggested. So the following experiment was carried out to bring about a direct comparison with his results of distribution of Potassium in the upper and lower half of geotropically stimulated seedlings of Helianthus annus.

(a) Experiment with ionic potassium in Helianthus hypocotyls (with roots).

Seedlings of Helianthus annus were grown in glass specimen tubes containing vermiculite soaked in Hoagland and Arnon solution. The seedlings with their hypocotyls 3 cm long were placed in a stand which could be rotated from vertical to horizontal position. A group of 100 seedlings was placed in vertical position to establish the initial distribution of potassium in the halves. Horizontal exposures were carried out as described in the chapter Materials and Methods. At the

end of each horizontal exposure seedlings were harvested and roots and cotyledons were removed. Hypocotyls were split centrally into upper and lower halves by a cutter as described. Each exposure consisted of 4 replications, so each replication had 25 hypocotyls. Technique involved in this experiment has been discussed in the chapter Materials and Methods under Non-radioactive assay.

Analysis of variance of the data was carried out and the results are shown in Table 5 (Appendix). The analysis shows that S X T effect is significant. This shows that there has been progressive increase in the differences between the side means with time. Time effect is also significant (when M.S.V. for T is compared with M.S.V. for P + PT).

Graphs have been plotted based on the mean potassium values on a total ash, fresh and dry weight basis. Fig.14 clearly indicates that the increase in the amount of potassium in the lower side is directly related to the time of horizontal exposure. The figure indicates that there has been a steady increase of potassium in the lower side but the amount of potassium in the upper side remains more or less constant on ash weight basis. But when the results are considered on a fresh and dry weight basis, there is a drop in the potassium level in the upper side after 1 hour horizontal exposure. This drop in potassium level in the upper half indicates that probably there has been some lateral migration of potassium from upper to lower side during 1 hour horizontal exposure. In 2 and 3 hours there is recovery in the upper side, whereas the lower side shows a much greater rise. This clearly suggests that the differences between upper and lower are mainly due to migration from upper to lower. There is subsequent movement from roots. This increases the concentration in both but the

Fig.14 The effect of horizontal orientation on the distribution of potassium ions in the hypocotyls of <u>Helianthus annus</u>.

Time-course changes in the amount of K_2O in the sides. Curvatures in degrees are shown at the top of the figure. $\pm$ is standard error of the mean.

A :- mg. K_2O/100 mg. ash weight

Full square :- upper side

Open square :- lower side

B :- mg. K_2O/5 gm. fresh weight

Full triangle :- upper side

Open triangle :- lower side

C :- mg. K_2O/1 gm. dry weight

Full circle :- upper side

Open circle :- lower side

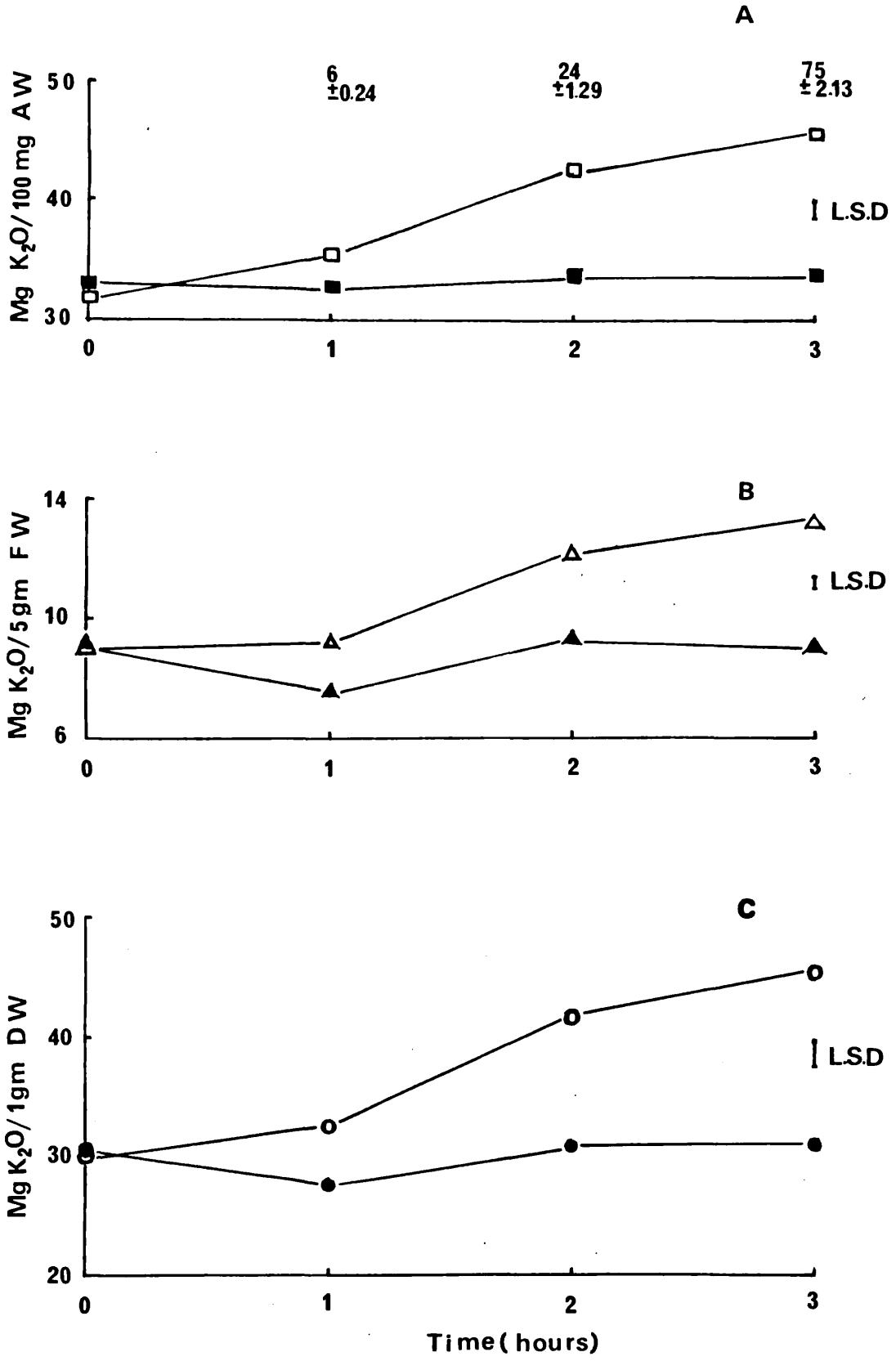

A
Mg K_2O/100 mg A W
50
40
30
6
±0.24
24
±1.29
75
± 2.13
L.S.D
0
1
2
3
B
Mg K_2O/5 gm F W
14
10
6
L.S.D
0
1
2
3
C
Mg K_2O/1 gm D W
50
40
30
20
L.S.D
0
1
2
3
Time (hours)

Fig.14.

lower increases more than the upper. It cannot be decided from the results ... whether these differences are due to differential rate of movement from roots. L.S.D. of the means have been shown in the Fig.14. It clearly signifies the fact that the increase in the lower side is significant after 2 and 3 hours horizontal exposure if mean values in the lower side are compared with L.S.D.

From the results it can be definitely concluded that a concentration difference of potassium is established between the upper and lower sides which is correlated with the curvature. The maximum K_2O values were found in the lower side after 3 hours of horizontal exposure when the curvature was found to be about 75 degrees $\pm$ 2.13. In Bode's results the maximum increase of potassium was found after 90 minutes of geotropical stimulation and this value of potassium decreased after 120 minutes. In my experiments the level of potassium increases with time and also with the curvature.

(b) Experiment with ^{42}K in the Helianthus hypocotyls (with roots)

In the last experiment it was observed that horizontal orientation did result in differential distribution of potassium ions in the hypocotyls of Helianthus annuus. The results were conclusive because the increase in the lower side was found on a total ash, fresh and dry weight basis. It was thought necessary to carry out similar experiments with ^{42}K to compare the findings of the last experiment.

Seedlings were grown in glass specimen tubes (size 3" x 1") as discussed in the chapter Materials and Methods. Seedlings were treated with ^{42}K (0.25 μCi for each seedling) through the roots for 2 hours in a dark cupboard. A group of 10 seedlings

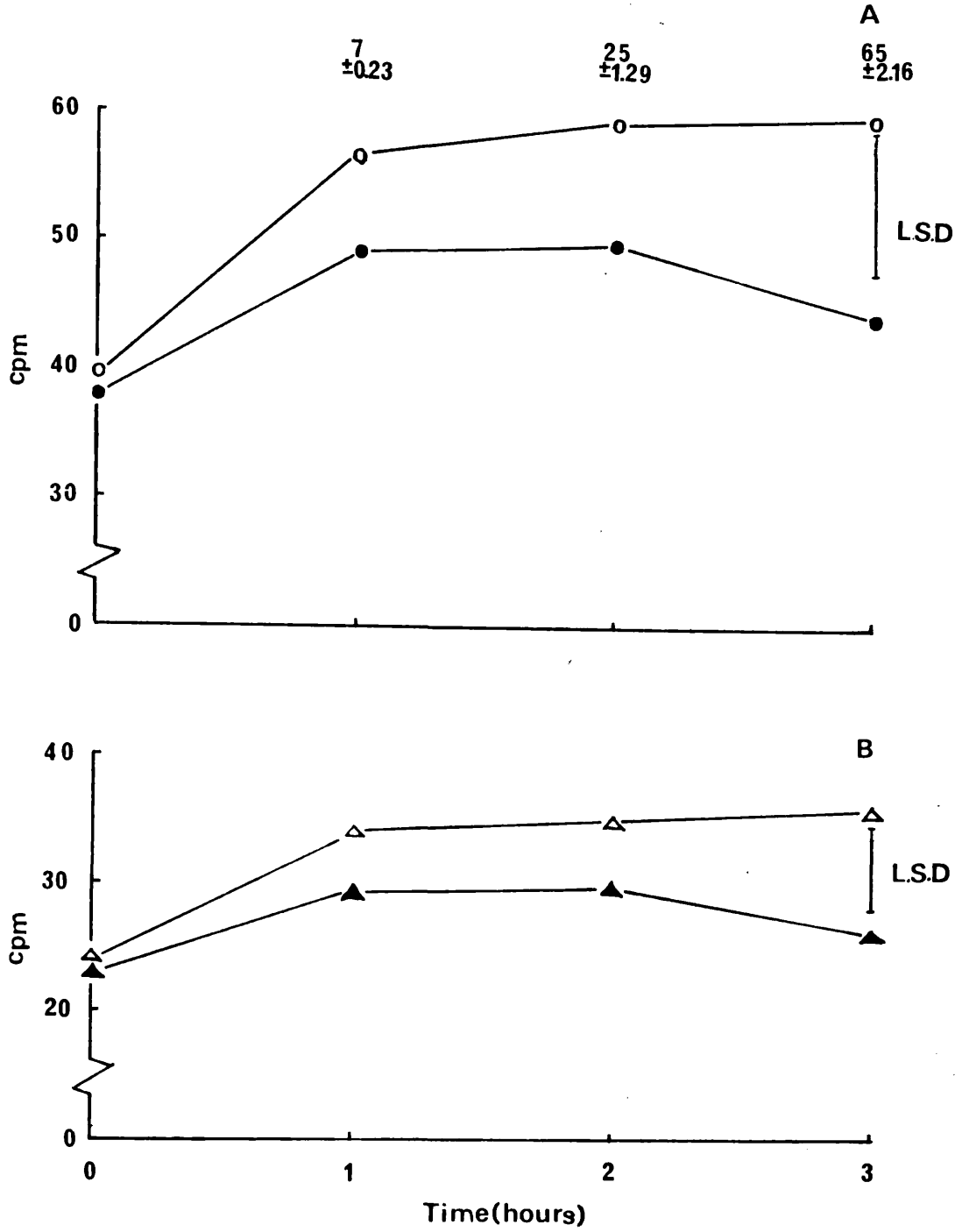


Fig. 15. The effect of horizontal orientation on the distribution of ^{42}K in the hypocotyls of Helianthus annus.

Time-course changes in the activity of ^{42}K in the sides. Curvature in degrees shown at the top. ± is standard error of the mean.

A :- mean counts/min/mg. dry weight

Full circle:- Upper side

Open circle:- Lower side

B:- mean counts/min/10mg. fresh weight

Full triangle:- Upper side

Open triangle:- Lower side

was used as a vertical (initial as 0 hour) to ascertain the initial distribution of activity in the halves. At the end of each horizontal exposure the seedlings were harvested (roots and cotyledons were removed), hypocotyls were split by a cutter. Each exposure had 5 replications, so two hypocotyls were included in each replication. Technique for the preparation of sample and radioactive assay was followed as described in the chapter Materials and Methods.

Analysis of variance of the data was carried out and the results are shown in Table 6 (Appendix). The analysis shows that side (S) effect is significant. This signifies the fact that there has been overall increase in the lower side.

Graphs have been plotted showing mean counts on dry and fresh weight basis. Fig.15 indicates an increase (but M.S.V. for P + P T is of same order as M.S.V. for T) in both sides in 1 hour horizontal exposure, but lower side shows a greater rise than the upper. The upper side shows a drop (not significant) and lower side shows a rise (not significant) after three hours horizontal exposure. This difference in activity between upper & lower sides could be due to migration from upper to lower. L.S.D. of the means have been shown in the graphs.

The results strongly suggest that a concentration difference is established between upper and lower sides which is correlated with the curvature.

(c) Experiment with ionic potassium in Helianthus hypocotyls (without roots)

This experimentwas carried out to establish the reality of lateral movement and to indicate whether the differences in rooted plants could, at least in part, be due to lateral movement.

Seedlings of Helianthus annus were harvested and roots were removed. Shoot part consisted of hypocotyl and two cotyledons. Orientation of the hypocotyls (with the cotyledons) was carried out as described in case of the experiment with ^{45}Ca in Helianthus hypocotyls (without roots), Section I (b). This has been shown in Fig.9. Number of hypocotyls, replications was same as for the experiment with ionic potassium. There were three horizontal exposures (1, 2 and 3 hours) and one vertical control. Technique for this experiment was followed as described in the Chapter Materials and Methods under Non-radioactive assay.

Analysis of variance of the data was carried out and the results are shown in Table 7 (Appendix). The analysis shows S X T effect is significant. This shows that there have been progressively increasing differences between the side means with time. The T effect is non-significant (M.S.V. for T is lower than M.S.V. for P + PT).

Graphs have been plotted showing mean potassium values on a total ash, fresh and dry weight basis.

Fig. 16 clearly indicates that there has been a lateral migration of potassium from upper to lower side during horizontal exposure. The level of potassium increases with time and is correlated with the curvature. L.S.D. of the means show that the differences between the side means are much higher than L.S.D.

By comparing the results of Fig.14 and Fig.16 it can be concluded that the differences between the side means are much higher in rooted plants than that in rootless plants. But one cannot be absolutely certain that the greater rise in the lower side is the result of differential transport as it is difficult to estimate the degree of lateral transport in rooted plants.

Fig.16 The effect of horizontal orientation on the distribution of potassium ions in the hypocotyls of Helianthus annus.

Hypocotyls were horizontally orientated in root-less condition.

Time-course changes in the amount of K_2O in the sides. Curvatures in degrees are shown at the top of the figure. $\pm$ is standard error of the mean.

A :- mg.K_2O/100 mg. ash weight

Full square :- upper side

Open square :- lower side

B :- mg. K_2O/5 gm. fresh weight

Full triangle :- upper side

Open triangle :- lower side

C :- mg.K_2O/1 gm. dry weight

Full circle :- upper side

Open circle :- lower side

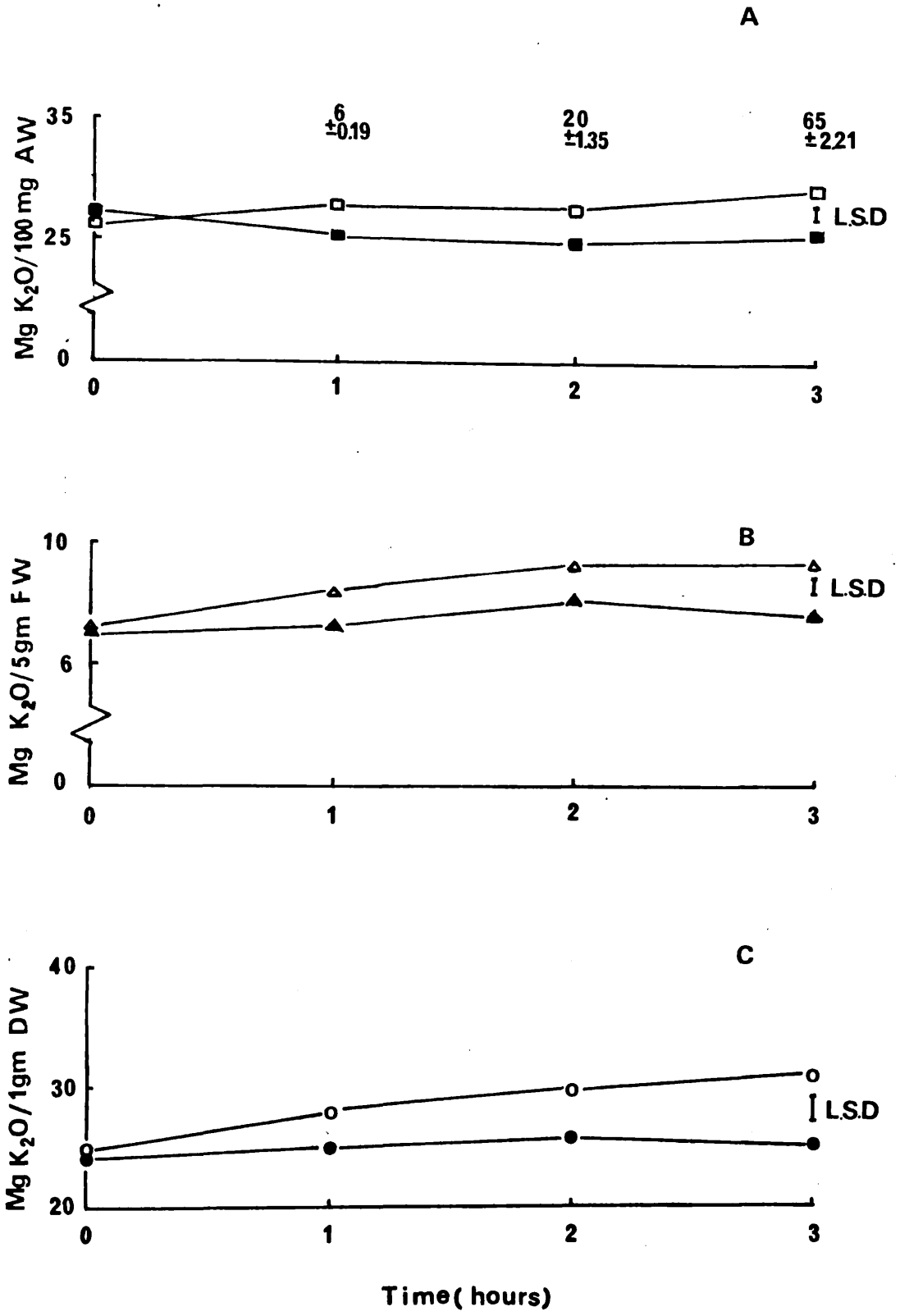

A
Mg K_2O/100 mg AW
35
25
0
6
±0.19
20
±1.35
65
±2.21
L.S.D
0
1
2
3
B
Mg K_2O/5gm FW
10
6
0
L.S.D
0
1
2
3
C
Mg K_2O/1gm DW
40
30
20
L.S.D
0
1
2
3
Time(hours)

Fig. 16.

SECTION 3 DISTRIBUTION OF ^{32}P

As already discussed, nothing is known about the connection of Phosphorus with geotropic response. But some connection between auxin and protein synthesis has been established. Nooden (1968) suggested that auxin resulted into increase in ^{32}P orthophosphate uptake which was thought to be linked with RNA synthesis during auxin-induced cell elongation.

In experiments with calcium and potassium it was observed that a correlation exists between the distribution of above ions and the curvature. It was thought necessary to look into the distribution of ^{32}P during horizontal exposure and to find out whether any correlation exists between the distribution and the curvature.

(a) *Helianthus* hypocotyls with roots

Seedlings of *Helianthus annus* were used to study the effect of horizontal orientation on the distribution of ^{32}P. A group of 20 seedlings was used for each horizontal exposure. There were three horizontal exposures - 1, 2 and 3 hours. A group of 20 seedlings was included which would establish the initial distribution of radioactivity in the hypocotyl halves of vertically (initial as 0 hour) placed seedlings. Each seedling was fed through the roots with 0.5 μCi of ^{32}P made up in Hoagland and Arnon solution. Seedlings were left in a dark cupboard for 4 hours. These seedlings were placed in horizontal position as described in the chapter Materials and Methods. There were 5 replications for each exposure. At the end each exposure

seedlings were harvested, roots and cotyledons were removed. Hypocotyls were split centrally by a cutter into upper and lower halves. Technique for radioactive assay and preparation of sample was applied as described in the chapter Materials and Methods.

Analysis of variance of the result has been presented in the Table 8 (Appendix). The analysis shows that S X T is significant. This indicates that therehave been progressively increasing differences established between the side mean with time. Time effect is also significant (as M.S.V. for T is much greater than M.S.V. for P + PT) which suggests that there has been increase in activity in the hypocotyl as a whole during horizontal exposure.

Graphs have been plotted showing mean count on dry and fresh weight basis. Fig.17 shows that on the upper side there is no significant change in 2 hours and then a rise, whereas on the lower side there is a small rise (not significant) and then a greater rise. This suggests that the differences between upper and lower may be due to migration from upper to lower which is established in 2 hours and subsequent movement from roots increases concentration in both but the upper increases more than the lower. Hence, the results suggest that the differences between the side means may be mainly due to migration from upper to lower. L.S.D. of the means are shown in the graphs which clearly indicates that the differences between the side means are significant after 3 hours horizontal exposure.

the results show that the increased accumulation of ^{32}P in the lower side is correlated with the curvature.

Fig.17 The effect of horizontal orientation on the distribution of ^{32}P in the hypocotyls of <u>Helianthus annus.</u>

Time-course changes in the activity of ^{32}P in the sides.

Curvatures in degrees are shown at the top of the figure. $\pm$ is standard error of the mean.

A :- mean counts/min/mg. dry weight

Full circle :- upper side

Open circle :- lower side

B :- mean counts/min/10 mg. fresh weight

Full triangle :- upper side

Open triangle :- lower side

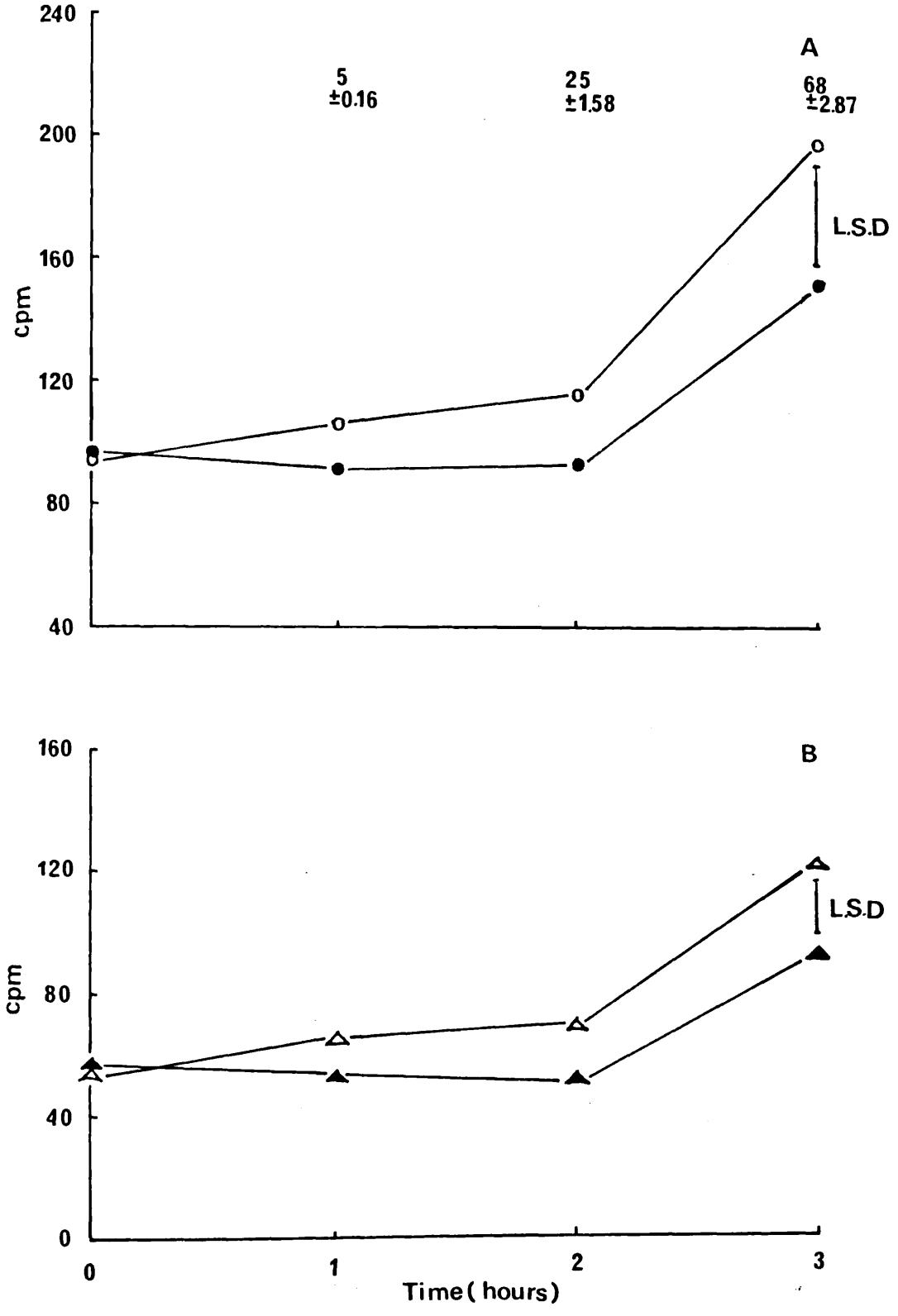

240
200
160
120
80
40
cpm
A
5
±0.16
25
±1.58
68
±2.87
L.S.D
160
120
80
40
0
cpm
B
L.S.D
0
1
2
3
Time (hours)

Fig .17.

(b) Helianthus hypocotyls without roots

In the last experiment it was observed that there was increased activity in the lower side of hypocotyls specially after 3 hours. An experiment was carried out to look into the distribution of ^{32}P in the hypocotyl halves of root-less plants. This would check whether any lateral migration of ^{32}P takes place from upper to lower side during horizontal exposure.

A group of 10 seedlings of Helianthus annus was used for each horizontal exposure. There were three horizontal exposures (1, 2 and 3 hours). A group of 10 seedlings was used as a vertical control (initial as 0 hours). This would establish the initial distribution of radioactivity in the halves. Seedlings were treated through the roots with ^{32}P (0.5μCi for each seedling) for 4 hours in a dark cupboard. Seedlings were harvested and roots were removed. Shoot part consisted of hypocotyl and cotyledons. Hypocotyls (with the cotyledons) were placed in a horizontal position as shown in Fig.9. Orientation of the hypocotyls has been discussed in detail in Section I (b). After each horizontal exposure cotyledons were removed and then hypocotyls were split into halves by a cutter. Technique for radioactive assay and preparation of sample was applied as described in the chapter Materials and Methods.

Analysis of variance of the data was carried out and the results are shown in Table 9 (Appendix). The analysis shows that side (S) effect is significant. This suggests that there is an overall increase in the lower side.

Graphs have been plotted showing means on dry and fresh weight basis. Fig.18 shows a continuing rise on

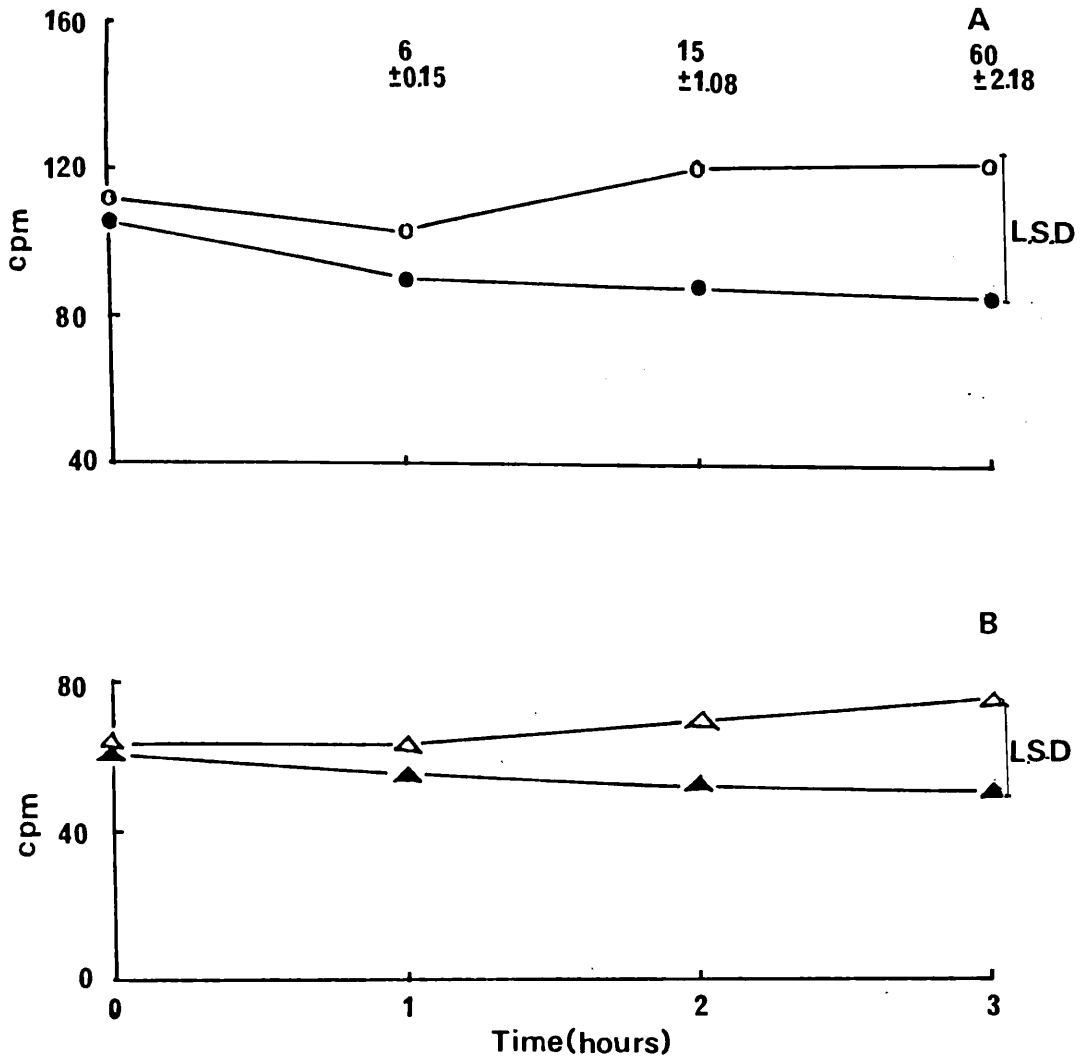


Fig. 18. The effect of horizontal orientation on the distribution of ^{32}P in the hypocotyls(without roots) of Helianthus annus.

Curvatures in degrees are shown at the top. ± is standard error of the mean.

A :- mean counts/min/mg. dry weight

Full circle:- Upper side

Open circle:- Lower side

B:- mean counts/min/10 mg. fresh weight

Full triangle:- Upper side

Open triangle:- Lower side

the lower side with continuing drop on the upper side (but both not significant). Increase in activity in the lower side is probably at the expense of migration of activity from the upper side.

By comparing the results of Fig.17 and Fig.18 it seems that since the order of difference for the two experiments is the same this would indicate that the differential distribution in the rooted plants could be explained entirely in terms of a lateral migration of phosphorus.

CHAPTER IV

THE EFFECT OF $4^{o}C$ ON THE DISTRIBUTION OF IONS IN HORIZONTAL POSITION.

In all previous experiments it was observed that horizontally orientated organs developed differential distributions of the three elements (Ca, K and P) which was correlated with the curvature. To check whether this correlation is causal would involve the study of the distribution of ions when curvature is prevented even though the seedlings receive a full geotropic stimulus. Seedlings are placed in a horizontal position for $\frac{1}{2}$ hour at $25^{o}C$. This stimulus is enough to show a normal geotropic response but curvature is stopped by subsequently putting the seedlings at $4^{o}C$. If any differential distribution is observed it would suggest the possibility of a connection between ion distribution and geotropic perception or geoinduction. This was attempted by the following approach.

SECTION I DISTRIBUTION OF ^{45}Ca.

(a) Experiment with the seedlings of *Helianthus annus.*

The experiment relating to the study of effect of $4^{o}C$ on the distribution of ^{45}Ca was divided into two groups.

Group I

(1) Seedlings were in vertical position (initial as 0 hour).

(2) Seedlings were placed in horizontal position for 1 hour at $25^{o}C$.

(3) Seedlings were placed in horizontal position for 1 hour at 25°C and subsequently placed in horizontal position for 1 hour at 4°C.

A group of 20 seedlings was included for each exposure and each exposure consisted of 4 replications.

Group II

(1) Seedlings were in vertical position (initial as 0 hour)

(2) Seedlings were placed in horizontal position for ½ hour at 25°C.

(3) Seedlings were placed in horizontal position for ½ hour at 25°C and subsequently placed for 2½ hours in horizontal position at 4°C.

A group of 20 seedlings was included for each exposure and each exposure consisted of 5 replications.

Horizontal exposures were carried out after treatment with ^{45}Ca for 6 hours in a dark cupboard. Horizontal exposure at 4°C was carried out inside a refrigerator. The details about the experimental technique, preparation of sample, and radioactive assay havebeen discussed in the chapter Materials and Methods.

Analysis of variance of the data (experiment included in Group I) was carried out and the results are shown in Table 10 (Appendix). The analysis shows that the side (S) effect is non-significant.

The results of the experiment (Group I) are shown as mean counts on a dry and fresh weight basis in Fig.19. The seedlings after 1 hour horizontal exposure at 25°C show some increase (not significant) in the upper side of the hypocotyl

Fig.19 The effect of $4^{o}C$ on the distribution of ^{45}Ca in the hypocotyls of horizontally placed seedlings of <u>Helianthus annus</u>.

Curvatures in degrees are shown at the top of the figure. $\pm$ is standard error of the mean.

A :- mean counts/min/mg. dry weight

B :- mean counts/ min/10 mg. fresh weight

0 =- Initial (vertical)

1 = 1 hour in horizontal position at $25^{o}C$

1 + 1 = 1 hour in horizontal position at $25^{o}C$

+ 1 hour in horizontal position at $4^{o}C$.

Lt :- left side

Rt :- right side

U :- upper side

L :- lower side

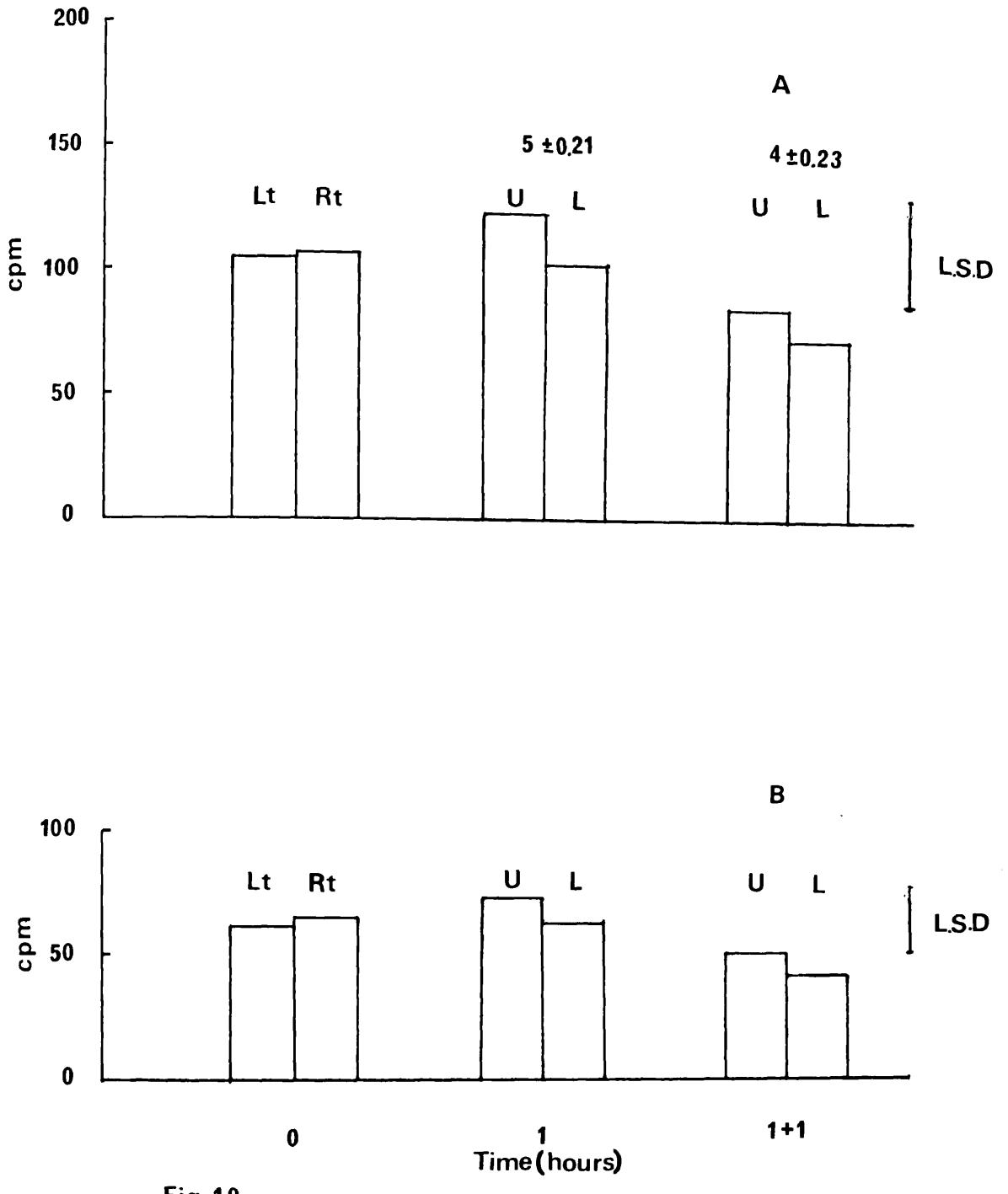

200
150
100
50
0
cpm
A
5 ±0.21
4 ±0.23
Lt
Rt
U
L
U
L
L.S.D
B
100
50
0
cpm
Lt
Rt
U
L
U
L
L.S.D
0
1
1+1
Time (hours)

Fig. 19.

Fig.20 The effect of $4^{o}C$ on the distribution of ^{45}Ca in the hypocotyls of horizontally placed seedlings of <u>Helianthus annus</u>.

Seedlings did not produce any curvature and hence curvature is shown as 0 at the top of the figure.

A :- mean counts/min/mg. dry weight

B :- mean counts/min/10 mg. fresh weight

0 = Initial (vertical)

$\frac{1}{2}$ = $\frac{1}{2}$ hour in horizontal position at $25^{o}C$

$\frac{1}{2}$ +2$\frac{1}{2}$ = $\frac{1}{2}$ in horizontal position at $25^{o}C$ +2$\frac{1}{2}$ hours in horizontal position at $4^{o}C$

Lt :- left side

Rt :- right side

U :- upper side

L :- lower side

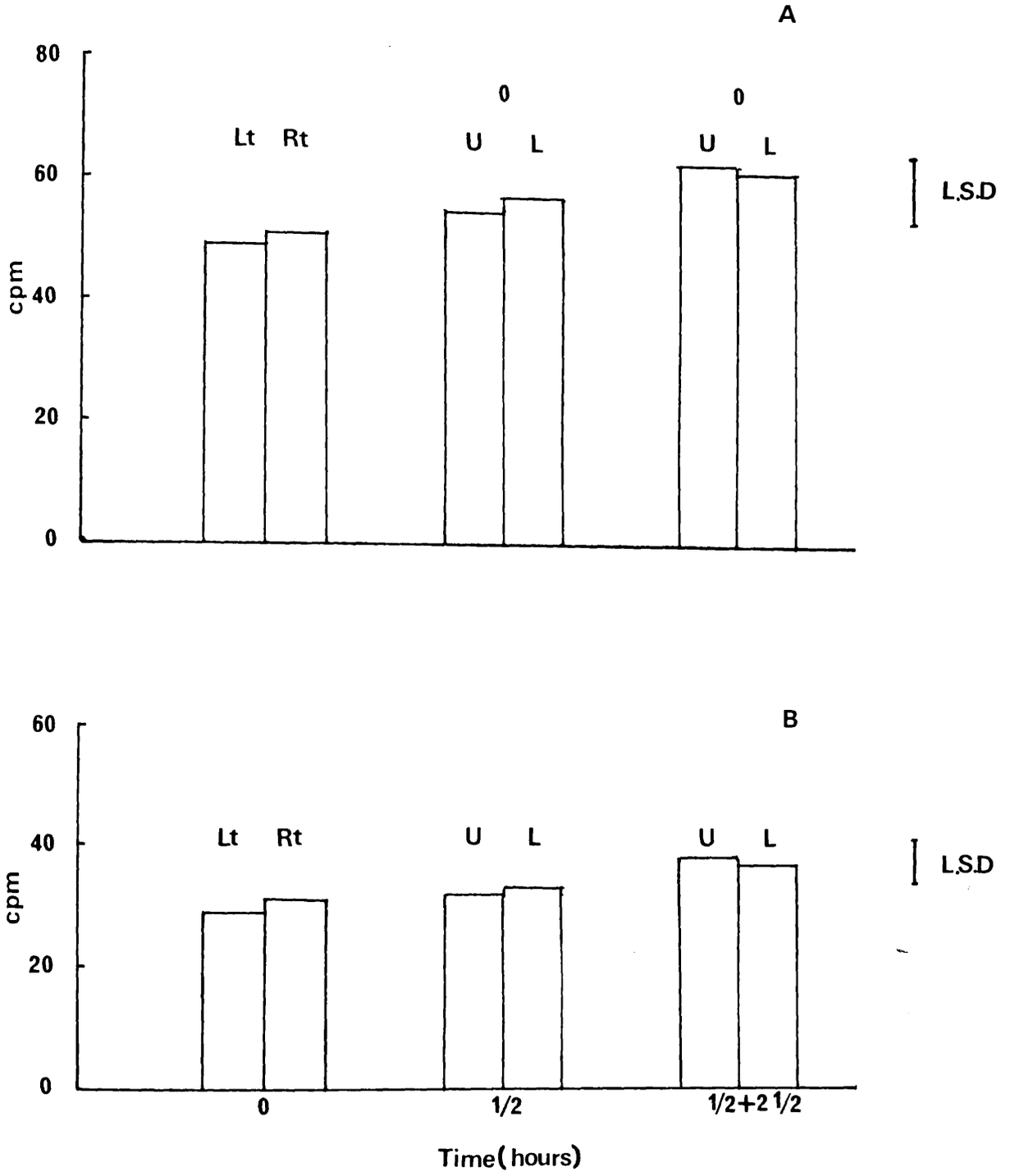

A
80
60
40
20
0
cpm
Lt
Rt
0
U
L
0
U
L
L.S.D
B
60
40
20
0
cpm
Lt
Rt
U
L
U
L
L.S.D
0
1/2
1/2+2 1/2
Time(hours)

Fig. 20.

and curvature was found to be 5 degrees ± 0.21. The exposure of 1 hour at 25°C and subsequent exposure of 1 hour at 4°C show some increase (not significant) in the upper side. This increase is slightly less as compared to the increase in 1 hour horizontal exposure at 25°C. This suggests that increase in the upper side may occur at 25°C and any further increase is stopped as soon as curvature is prevented by placing the plants at 4°C.

Analysis of variance of results (experiment included in Group II) is shown in Table 11 (Appendix). The analysis shows that there is no differential distribution in the hypocotyl.

The results of the experiment included in Group II are shown in Fig.20. Horizontal exposure of ½ hour at 25°C does not cause any increase of radioactivity in the upper side and curvature does not appear in the seedlings. In ½ hour at 25°C and 2½ hours at 4°C. the upper side does not show any increase.

(b) Experiment with the seedlings of <u>Zea mays</u>.

It was clearly observed in the last experiment that although the seedlings received an above threshold stimulus at 25°C (½ an hour), a significant increase of radioactivity was not observed in the upper side when curvature was prevented at 4°C. Increase (although non-significant) in the upper side was observed only when curvature has appeared. Furthermore, there seems to be no movement of ^{45}Ca at 4°C in the absence of curvature. To draw some kind of comparison with the previous finding an experiment

was carried with the seedlings of Zea mays.

This experiment comprises two groups.

Group I

(1) Seedlings were in vertical position (initial as 0 hour).

(2) Seedlings were placed in horizontal position for $\frac{1}{2}$ hour at 25°C and subsequently placed horizontal at 4°C for $\frac{1}{2}$ hour.

(3) Seedlings were placed in horizontal position for $\frac{1}{2}$ hour at 25°C and subsequently placed horizontal at 4°C for $2\frac{1}{2}$ hours.

Group II

(1) Seedlings were in vertical position (initial as 0 hour).

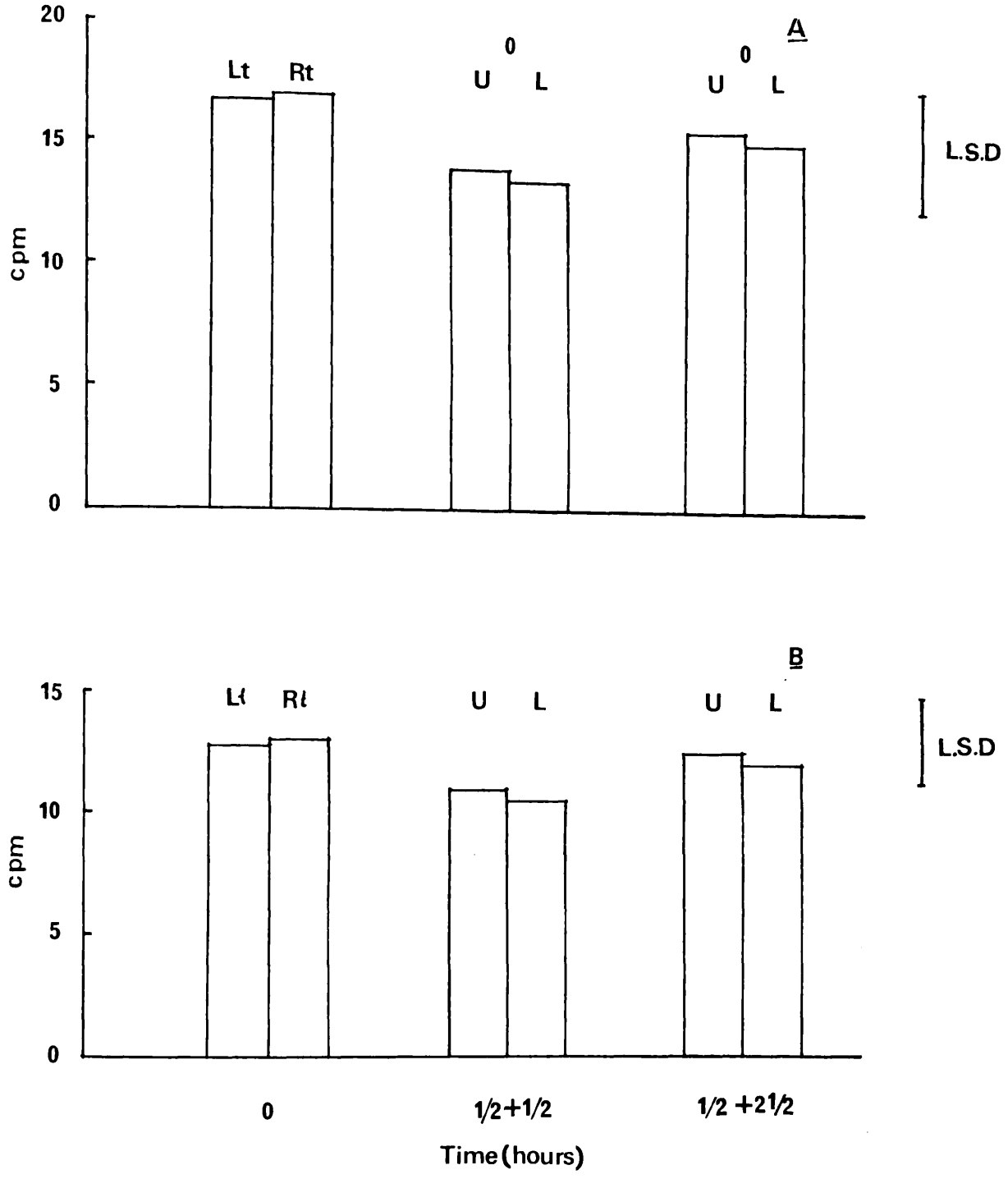
(2) Seedlings were placed in horizontal position at 25°C for 1 hour and subsequently placed horizontal at 4°C for 1 hour.

A group of 20 seedlings was included for each exposure. Each exposure consisted of 5 replications.

Horizontal exposures were carried out after treatment with ^{45}Ca for 6 hours in a dark cupboard. Technique for radioactive assay and preparation of sample was carried out as described in the chapter Materials and Methods.

Analysis of variance of the data (experiment included in Group 1) was carried out and results are shown in Table 12 (Appendix). The analysis shows that side (S) effect is non-significant.

The results of the experiment are shown in Fig. 21. It clearly indicates that there is no significant differential distribution of radioactivity in case of seedlings placed horizontal for $\frac{1}{2}$ hour at 25°C. Curvature did not appear during this exposure.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20
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10
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0
cpm
Lt
Rt
U
0
L
U
0
L
L.S.D
B
15
10
5
0
cpm
Lt
Rt
U
L
U
L
L.S.D
0
1/2+1/2
1/2 +2 1/2
Time (hours)

Fig. 21.

Fig.22 The effect of $4^{o}C$ on the distribution of ^{45}Ca in the coleoptiles of horizontally placed seedlings of *Zea mays*.

Curvatures in degrees are shown at the top of the figure. $\pm$ is standard error of the mean.

A :- mean counts/min/mg. dry weight

B :- mean counts/min/10 mg. fresh weight

0 = Initial (vertical)

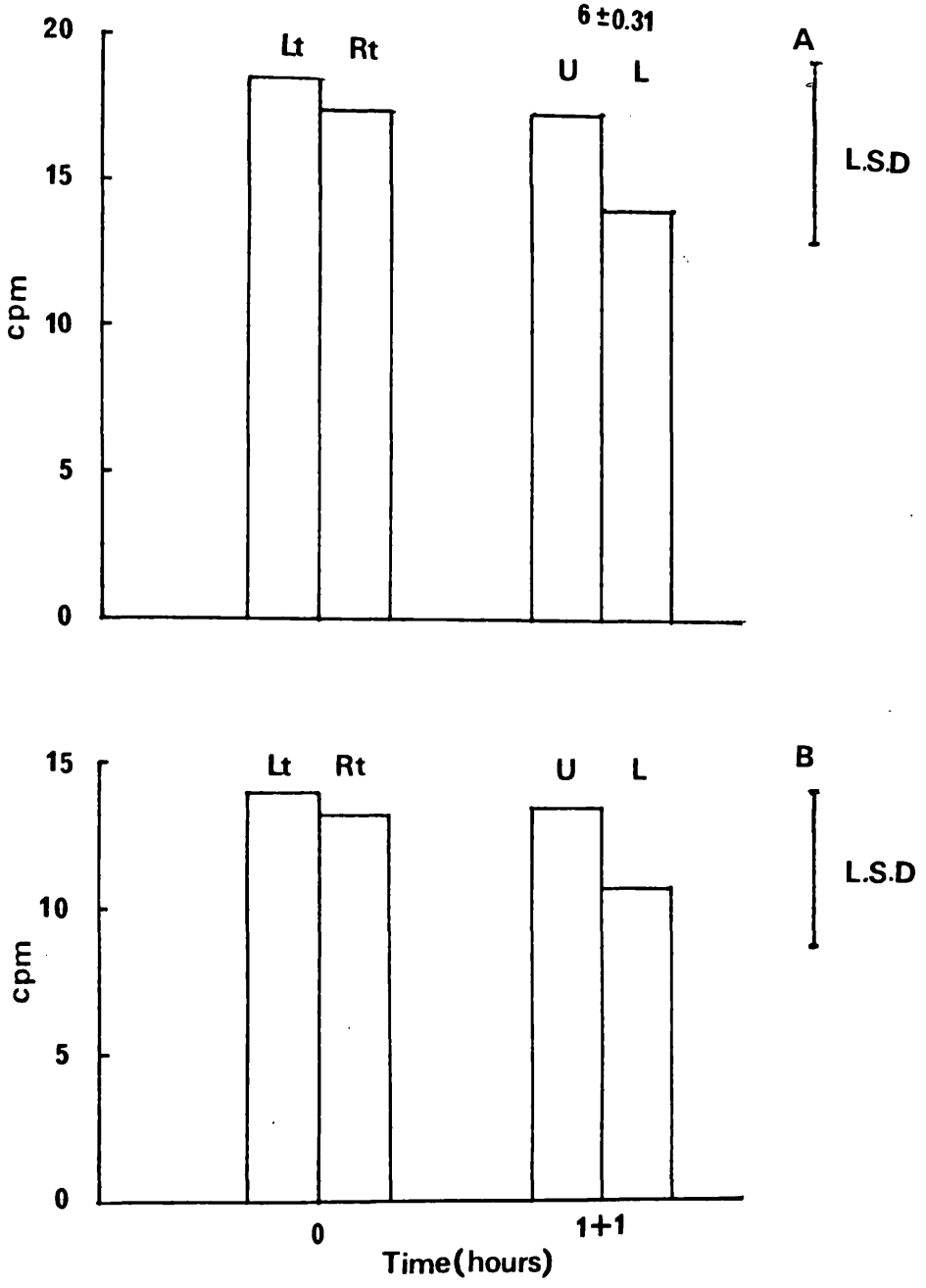
1 + 1 = 1 hour in horizontal position at $25^{o}C$ + 1 hour in horizontal position at $4^{o}C$.

Lt :- left side

Rt :- right side

U :- upper side

L :- lower side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5
0
cpm
Lt
Rt
6 ±0.31
U
L
A
L.S.D
15
10
5
0
cpm
Lt
Rt
U
L
B
L.S.D
0
1+1
Time(hours)

Fig. 22

Analysis of variance of the results (experiment included in Group II) is shown in Table 13 (Appendix). The analysis shows that the side (S) effect is non-significant.

Fig. 22 shows that there is some increase (non-significant) in the upper side after horizontal exposure of 1 hour at 25°C and 1 hour at 4°C. This increase in the upper side is correlated with the small curvature (6 degrees ± 0.31).

So there are indications that the increase in the upper side of horizontally orientated organs takes place only after the appearance of curvature.

SECTION 2 DISTRIBUTION OF ^{42}K

Experiment with the seedlings of Helianthus annus

An experiment was carried out with the object of studying the distribution of ^{42}K in the hypocotyl halves after curvature was prevented at 4°C. This 4°C exposure was preceded by ½ hour horizontal exposure at 25°C. Experiment consisted of two groups.

Group I

(1) Seedlings were in vertical position (initial as 0 hour).

(2) Seedlings were placed in horizontal position for ½ hour at 25°C.

(3) Seedlings were placed in horizontal position for ½ hour at 25°C and subsequently placed for 2½ hours at 4°C.

Group II

(1) Seedlings were in vertical position at 25°C.

(2) Seedlings were placed in horizontal position for 1 hour at 25°C and subsequently placed for 1 hour at 4°C.

A group of 10 seedlings was used for each horizontal

exposure. Each exposure consisted of 5 replications. Seedlings were treated with ^{42}K for 2 hours in a dark cupboard. Horizontal exposures were carried out as mentioned above. Technique for radioactive assay at preparation of sample was followed as described in the chapter Materials and Methods.

Analysis of various of the results (experiment of Group I) is shown in Table 14 (Appendix). The analysis shows that the side (S) effect is non-significant.

Fig. 23 shows mean counts on dry and fresh weight basis in the halves. It is clear from the figure that differential distribution does not take place during $\frac{1}{2}$ hour horizontal treatment at 25°C and hypocotyls do not produce any curvature.

Analysis of variance of the results (experiment of Group II) is shown in Table 15 (Appendix). The side (S) effect is non-significant.

Fig. 24 shows that there is some increase (non-significant) in the lower side and this is correlated with the curvature.

To Summarise: Differential distribution of ^{42}K is probably unconnected to geotropic perception since $\frac{1}{2}$ hour horizontal exposure at 25°C did not result in any subsequent differential distribution at 4°C although it would have produced a significant curvature at 25°C. Horizontal exposure of 1 hour at 25°C resulted in an increase in activity in the lower side which again is correlated with the curvature.

Fig. 23 The effect of $4^{o}C$ on the distribution of ^{42}K in the hypocotyls of horizontally placed seedlings of Helianthus annuus.

Seedlings did not produce any curvature and hence curvature is shown as 0 at the top of the figure.

A :- mean counts/min/mg. dry weight

B :- mean counts/min/10 mg. fresh weight

0 = Initial (vertical)

$\frac{1}{2}$ = $\frac{1}{2}$ hour in horizontal position at $25^{o}C$

$\frac{1}{2}$ + $2\frac{1}{2}$ = $\frac{1}{2}$ hour in horizontal position at $25^{o}C$ + $2\frac{1}{2}$ hours in horizontal position at $4^{o}C$.

Lt :- left side

Rt :- right side

U :- upper side

L :- lower side

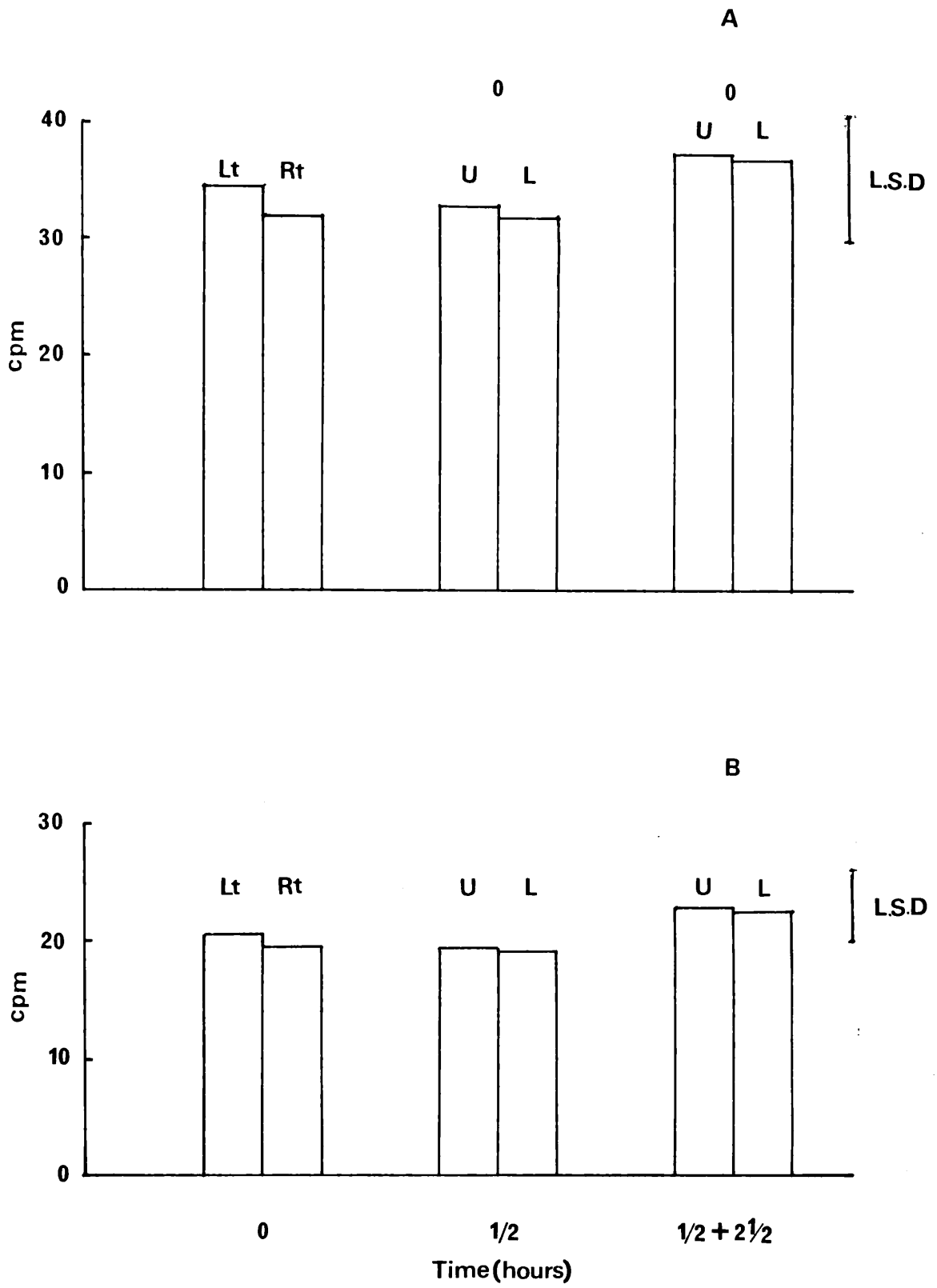

A
0
0
40
30
20
10
0
cpm
Lt
Rt
U
L
U
L
L.S.D
B
30
20
10
0
cpm
Lt
Rt
U
L
U
L
L.S.D
0
1/2
1/2 + 2½
Time (hours)

Fig.23.

Fig.24 The effect of $4^{o}C$ on the distribution of ^{42}K in the hypocotyls of horizontally placed seedlings of Helianthus annus.

Curvatures in degrees are shown at the top of the figure. $\pm$ is standard error of the mean.

A :- mean counts/min/mg. dry weight

B :- mean counts/min/10 mg. fresh weight

0 = initial (vertical)

1 + 1 = 1 hour in horizontal position at $25^{o}C$

+ 1 hour in horizontal position at $4^{o}C$.

Lt :- left side

Rt :- right side

U :- upper side

L :- lower side

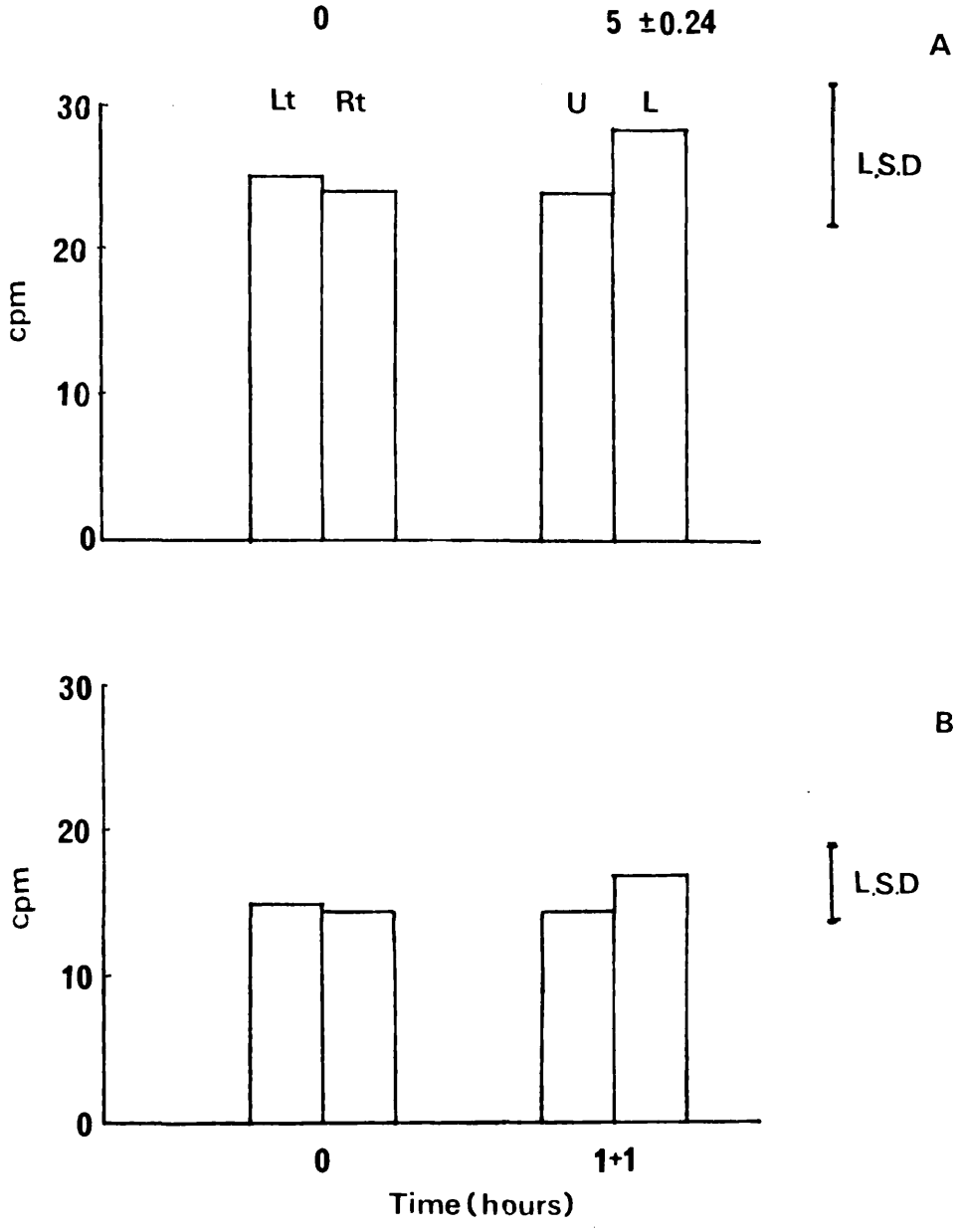

0
5 ±0.24
A
Lt
Rt
U
L
30
20
10
0
cpm
L.S.D
B
30
20
10
0
cpm
L.S.D
0
1+1
Time (hours)

Fig. 24.

SECTION 3 DISTRIBUTION OF ^{32}P

Experiment with the seedlings of Helianthus annus.

Experiment concerning the study of effect of $4^{o}C$ consisted of two groups.

Group I

(1) Seedlings were in vertical position at $25^{o}C$ (initial as 0 hour).

(2) Seedlings were placed in horizontal position for 1 hour at $25^{o}C$.

(3) Seedlings were placed in horizontal position for 1 hour at $25^{o}C$ and subsequently placed horizontal for 1 hour at $4^{o}C$.

Group II

(1) Seedlings were in vertical position at $25^{o}C$ (initial as 0 hour).

(2) Seedlings were placed in horizontal position for $\frac{1}{2}$ hour at $25^{o}C$.

(3) Seedlings were placed in horizontal position for $\frac{1}{2}$ hour at $25^{o}C$ and subsequently placed horizontal for $\frac{1}{2}$ hour at $4^{o}C$.

(4) Seedlings were placed in horizontal position for $\frac{1}{2}$ hour at $25^{o}C$ and subsequently placed horizontal for $1\frac{1}{2}$ hours at $4^{o}C$.

A group of 20 seedlings was used for each horizontal exposure. Horizontal exposures were carried out after seedlings were treated with ^{32}P for 4 hours. Each exposure

had 5 replications. Radioactive assay and preparation of sample was carried out as described in the chapter Materials and Methods.

Analysis of various of the results (experiment included in Group I) is shown in Table 16 (Appendix). The analysis shows that the side (S) effect is significant. This suggests an overall increase on the lower side.

Fig.25 shows an increase in the lower side after 1 hour horizontal exposure at 25°C. This is correlated with the curvature. In horizontal exposure of 1 hour at 25°C and 1 hour at 4°C, activity in the lower side shows an increase (not significant) and is correlated with the curvature of 4 degrees ± 0.42. There is a drop in activity in both halves in this exposure as compared to 1 hour horizontal exposure at 25°C. This drop is probably due to sampling (i.e.plant) error. This is quite clear from analysis of variance Table 15 (Appendix) where M.S.V. for P + PT is actually greater than M.S.V. for T.

Analysis of variance of the results (experiment included in Group II) is shown in Table 17 (Appendix). The analysis shows that the side (S) effect is non-significant.

Fig.26 shows the distribution of activity in the sides. This indicates that there is no significant change in the sides after ½ horizontal exposure at 25°C. The situation remains unchanged at 4°C.

Hence it can be seen that when there was no curvature after ½ hour horizontal exposure a differential distribution of ^{32}P was not observed.

Fig.25 The effect $4^{o}C$ on the distribution of ^{32}P in the hypocotyls of horizontally placed seedlings of <u>Helianthus annus</u>.

Curvatures in degrees are shown at the top of the figure. $\pm$ is standard error of the mean.

A :- mean counts/min/mg. dry weight

B :- mean counts/min/10 mg. fresh weight

0 = Initial (vertical)

1 = 1 hour in horizontal position at $25^{o}C$

1 + 1 = 1 hour in horizontal position at $25^{o}C$ + 1 hour in horizontal position at $4^{o}C$

Lt : - left side

Rt :- right side

U = upper side

L = lower side

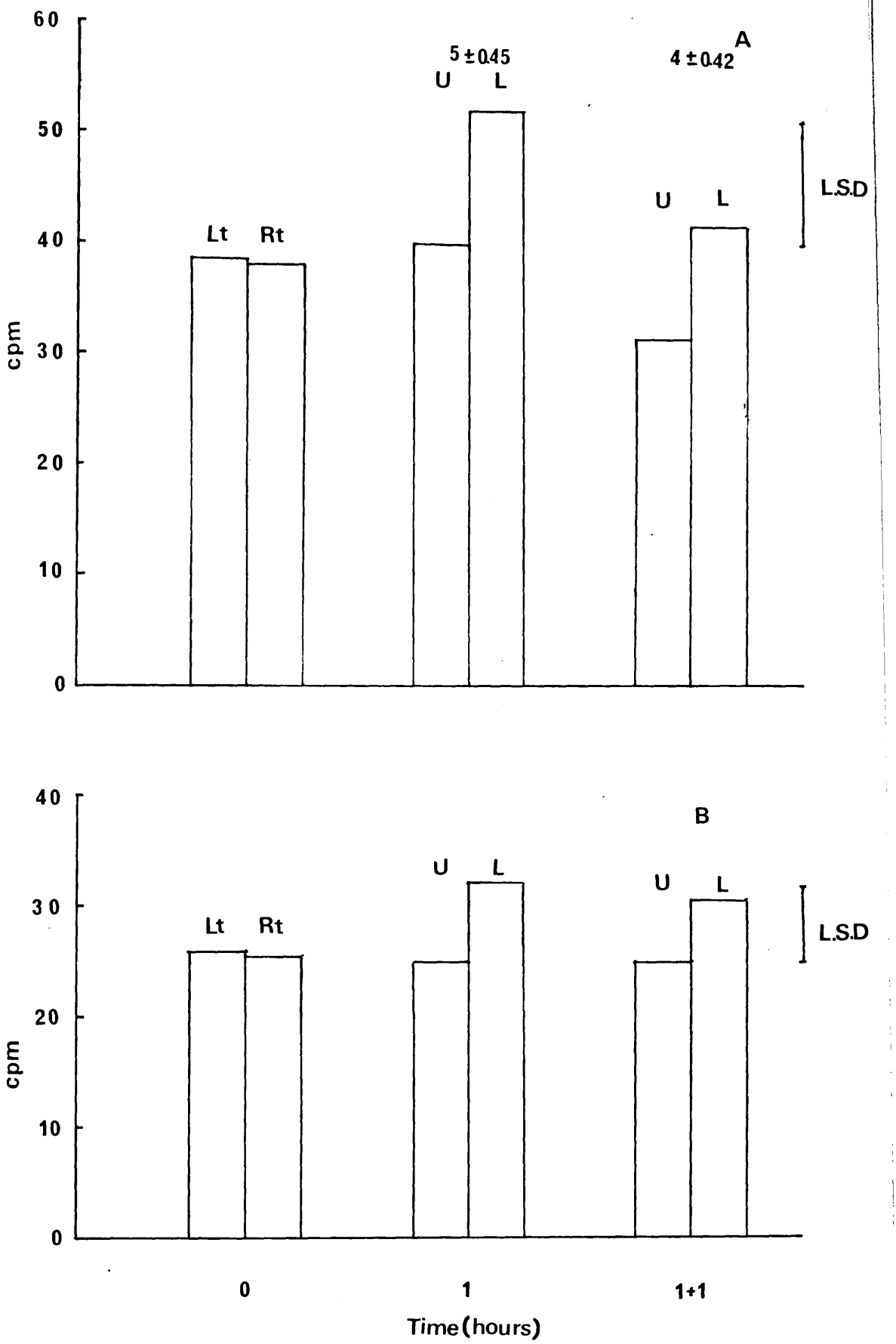

60
50
40
30
20
10
0
cpm
5 ± 0.45
U
L
4 ± 0.42
A
Lt
Rt
U
L
L.S.D
40
30
20
10
0
cpm
B
U
L
U
L
Lt
Rt
L.S.D
0
1
1+1
Time (hours)

Fig.25.

Fig.26 The effect of $4^{o}C$ on the distribution of ^{32}P in the hypocotyls of horizontally placed seedlings of <u>Helianthus annus</u>.

Seedlings did not produce any curvature and hence curvature is shown as 0 at the top of the figure.

A :- mean counts/min/mg. dry weight

B :- mean counts/min/10 mg. fresh weight

0 = Initial (vertical)

$\frac{1}{2}$ = $\frac{1}{2}$ hour in horizontal position at $25^{o}C$

$\frac{1}{2}$ + $\frac{1}{2}$ = $\frac{1}{2}$ hour in horizontal position at $25^{o}C$ + $\frac{1}{2}$ hour in horizontal position at $4^{o}C$

$\frac{1}{2}$ + $1\frac{1}{2}$ = $\frac{1}{2}$ hour in horizontal position at $25^{o}C$ + $1\frac{1}{2}$ hours in horizontal position at $4^{o}C$

Lt : - left side

Rt :- right side

U :- upper side

L :- lower side

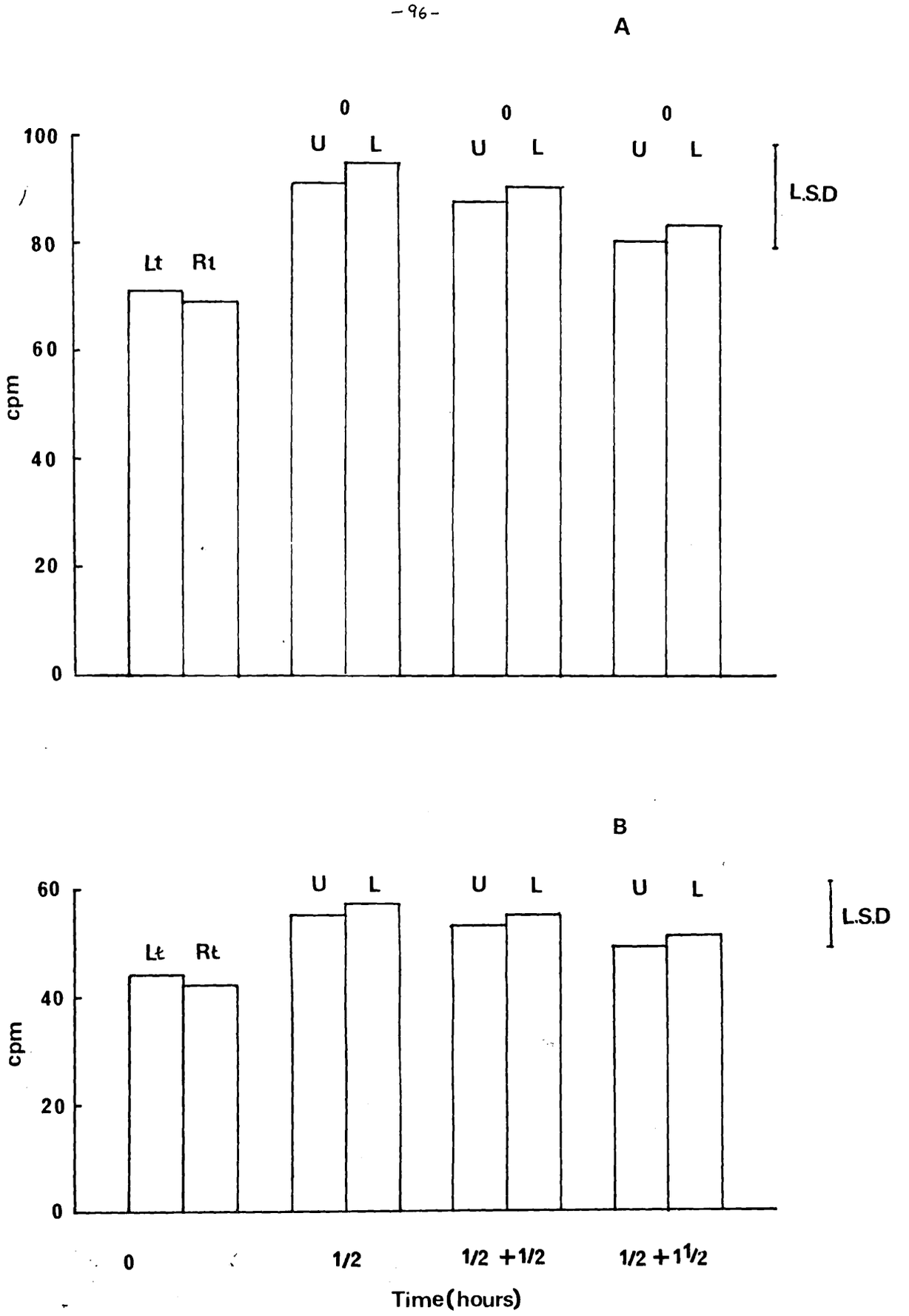

A
100
80
60
40
20
0
cpm
Lt
Rt
0
U
L
0
U
L
0
U
L
L.S.D
B
60
40
20
0
cpm
Lt
Rt
U
L
U
L
U
L
L.S.D
0
1/2
1/2 +1/2
1/2 +1 1/2
Time(hours)

Fig.26.

CHAPTER V

THE EFFECT OF NPA ON THE DISTRIBUTION OF IONS IN HORIZONTAL POSITION

In previous experiments it was clearly observed that the differential distribution of ions was correlated with the curvature but not with geoinduction. It was clearly shown in Chapter IV that $\frac{1}{2}$ hour horizontal orientation at 25°C did not result in differential distribution of the three elements (Ca, K and P) when the curvature was prevented by subsequent exposing the plants to 4°C. Horizontal orientation of 1 hour at 25°C did result into differential distribution and any further movement was stopped at 4°C. This differential distribution which occurred at 25°C was correlated with the curvature. As a further check curvature was prevented by the use of a chemical inhibitor of curvature. n-1 Naphthylphthalamic acid was first discovered as an inhibitor of geotropic response and later as an inhibitor of auxin transport. It has already been indicated in the Introduction that NPA does inhibit the polar transport of auxin at a high concentration (McCready, 1968). So NPA does inhibit the geotropic response by inhibiting the auxin transport. The object was to look into the distribution of ions in horizontally placed organs after pre-treatment with NPA. This would give an indication whether the distribution of ions is connected with geotropic response.

SECTION 2 DISTRIBUTION OF ^{45}Ca

In experiments with ^{45}Ca differential distribution of

calcium was correlated with the curvature. In the following experiments curvature was stopped by the pre-treatment with NPA and then distribution of ^{45}Ca was studied in the upper and lower halves.

(a) Experiment with the seedlings of Helianthus annus.

In this experiment NPA was used at a concentration of $10^{-4}M$ made up in Hoagland andArnon solution. Each seedling of Helianthus annus was treated with 2.0 ml of NPA containing 1 μCi of ^{45}Ca for 6 hours in a dark cupboard. A group of 20 seedlings was used for each horizontal exposure. There were horizontal exposures for 1, 2 and 3 hours. Each exposure had 5 replications. A group of 20 seedlings was also included which would establish the initial distribution (initial as 0 hour) of ^{45}Ca in the halves. Horizontal exposures were carried out as described. Technique for preparation of sample and radioactive assay was followed as described in the chapter Materials and Methods.

Analysis of variance of the results is shown in Table 18 (Appendix). The analysis shows that the side (S) effect is not significant.

Fig.27 shows mean counts on dry and fresh weight basis. It clearly shows that there has not been any differential distribution of ^{45}Ca in the hypocotyls of horizontally placed seedlings. There was no curvature after horizontal exposure.

Another experiment (without NPA) was carried out which was taken as a control for the experiment with NPA. The experimental technique for this experiment was same as for the experiment with NPA.

Fig.27 The effect of NPA on the distribution of ^{45}Ca in the hypocotyls of horizontally placed seedlings of Helianthus annus.

Time-course changes in the activity of ^{45}Ca in the sides. Seedlings did not produce any curvature and hence it is shown as 0 at the top of the figure.

Full circle:- mean counts/min/mg. dry weight in the upper side.

Open circle:- mean counts/min/mg. dry weight in the lower side.

Full triangle:- mean counts/min/10 mg. fresh weight in the upper side

Open triangle:- mean counts/min/10 mg. fresh weight in the lower side.

Fig.28 The effect of horizontal orientation on the distribution of ^{45}Ca in the hypocotyls of Helianthus annus.

This is a control experiment for the experiment with NPA.

Curvatures in degrees are shown at the top of the figure. $\pm$ is standard error of mean.

Full circle:- mean counts/min/mg. dry weight in the upper side

Open circle:- mean counts/min/mg dry weight in the lower side

Full triangle:- mean counts/min/10 mg. fresh weight in the upper side

Open triangle:- mean counts/min/10 mg. fredh weight in the lower side.

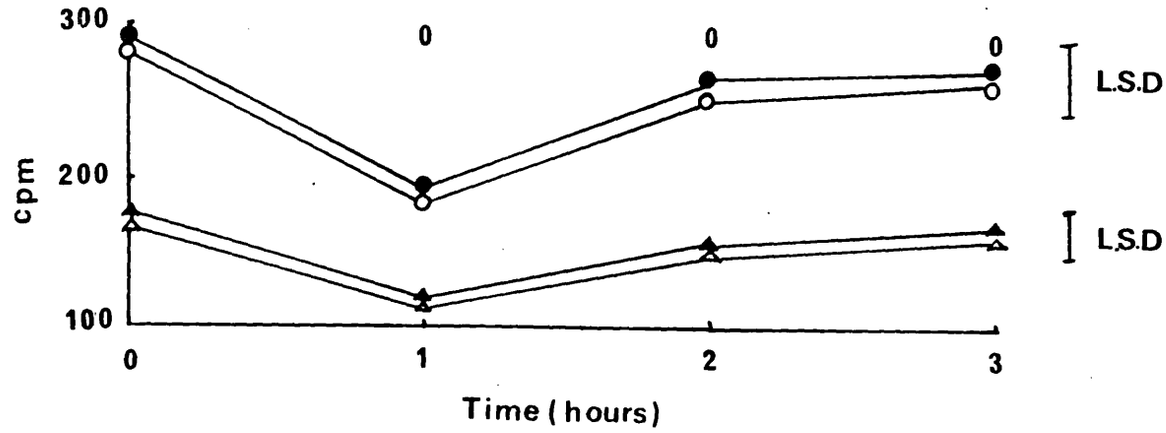

300
200
100
cpm
0
0
0
L.S.D
L.S.D
0
1
2
3
Time (hours)

Fig.27.

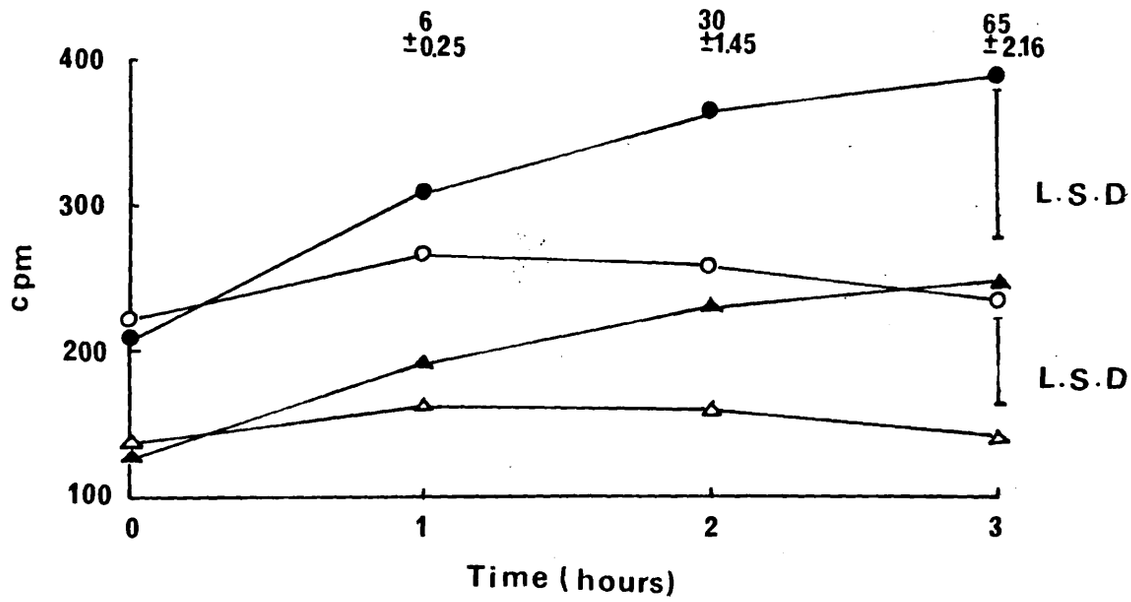

6
±0.25
30
±1.45
65
±2.16
400
300
200
100
cpm
L.S.D
L.S.D
0
1
2
3
Time (hours)

Fig.28.

Analysis of variance of the results is shown in Table 19 (Appendix). The analysis shows that S X T effect is significant. There has been progressive differences between the side means with time.

Fig. 28 shows mean counts on dry and fresh weight basis. Graphs show that in the upper side there is a steady increase with time, whereas in the lower side there is first an increase and then a decrease (non significant). This drop on the lower side may be due to migration of ^{45}Ca from lower to upper side The differences between the sides increase with time and curvature.

Hence, the results suggest that when curvature is stopped by pre-treatment with NPA, differential distribution of ^{45}Ca does not take place during horizontal exposure. It was clearly seen in the experiment without NPA that a significant increase of ^{45}Ca takes place in the upper side after the appearance of curvature. This indicates a correlation with the geotropic response.

(b) Experiment with the seedlings of Zea mays.

It was thought necessary to bring about a direct comparison with the results of the last experiment. So the following experiment was carried out with the seedlings of Zea mays.

Seedlings of Zea mays were treated with NPA (10^{-4}M) and ^{45}Ca for 6 hours in a dark cupboard. A group of 20 seedlings was included for each horizontal exposure. There were three horizontal exposures (1, 2 and 3 hours). Each exposure had 5 replications. A groupof 20 seedlings was included to establish the initial distribution of radioactivity in the

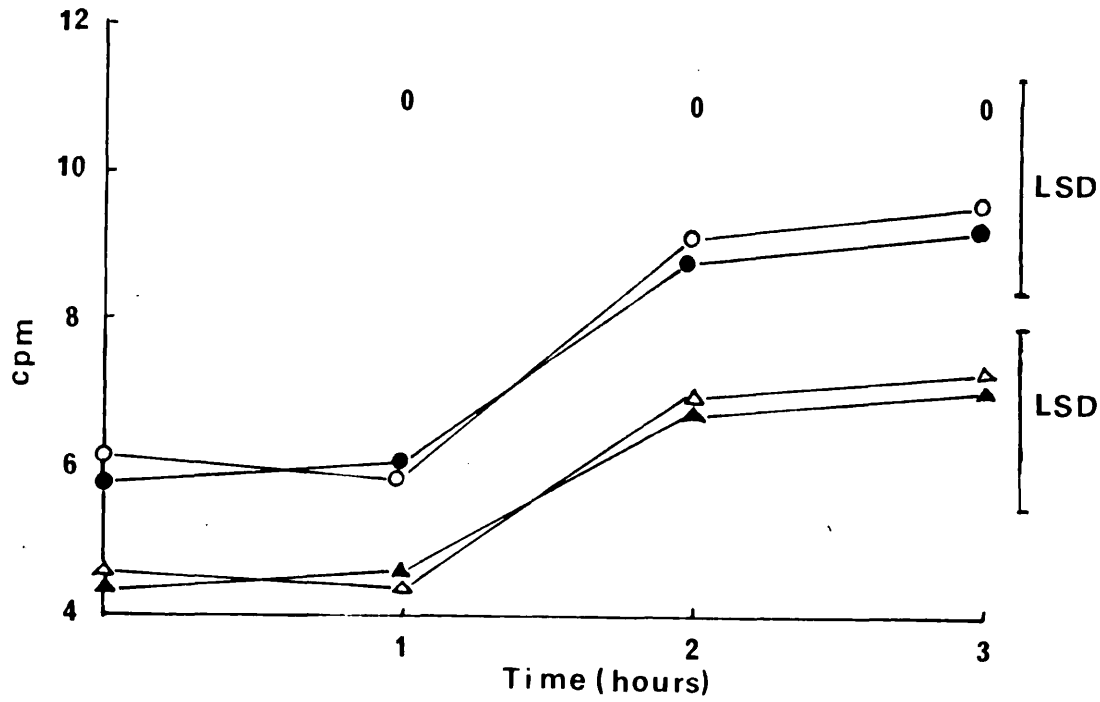


Fig. 29. The effect of NPA on the distribution of ^{45}Ca in the coleoptiles of horizontally placed seedlings of Zea mays.

Seedlings did not produce any curvature, and hence curvature is shown as 0 at the top.

Full circle:- mean cpm/mg. dry wt. in the upper side

Open circle:- mean cpm/mg. dry wt. in the lower side

Full triangle:- mean cpm/10mg. fresh wt. in the upper side

Open triangle:- mean cpm/10mg. fresh wt. in the lower side

hypocotyl halves of the seedlings in a vertical position.

Analysis of variance of the data is shown in Table 20 (Appendix). The analysis shows that the side (S) effect is not significant.

Distribution of radioactivity is shown in Fig.29. This does not indicate any significant increase of activity in any particular side. But the figure indicates a continuing transport of activity into the coleoptiles during horizontal exposure. Analysis of variance, however, shows that M.S.V. for T is of the same order as the M.S.V. for P + PT. It is, therefore, not statistically significant. L.S.D. of the means are shown in the figure. The differences between the means are lower than L.S.D.

The results of this experiment are in agreement with the results of the last experiment. This again shows that there is no differential distribution when there is no curvature.

SECTION 2 DISTRIBUTION OF POTASSIUM

In previous experiments with potassium it was observed that differential distribution was correlated with the curvature. The object was to stop the curvature by the treatment with NPA and to study the distribution of potassium in the hypocotyls of horizontally placed seedlings.

(a) Experiment with ionic potassium in the seedlings of *Helianthus annus*.

Seedlings of *Helianthus annus* were treated with NPA

Fig. 30 The effect of NPA on the distribution of potassium ion the hypcotyls of horizontally placed seedlings of Helianthus annus. Seedlings did not produce any curvature and hence curvature is shown as 0 at the top of the figure.

A :- mg K_2O/100 mg. ash weight

Full square:- upper side

Open square:- lower side.

B :- mg. K_2O/5 gm fresh weight

Full triangle:- upper side

Open triangle:- lower side

C :- mg. K_2O/1 gm dry weight

Full circle :- upper side

Open circle :- lower side

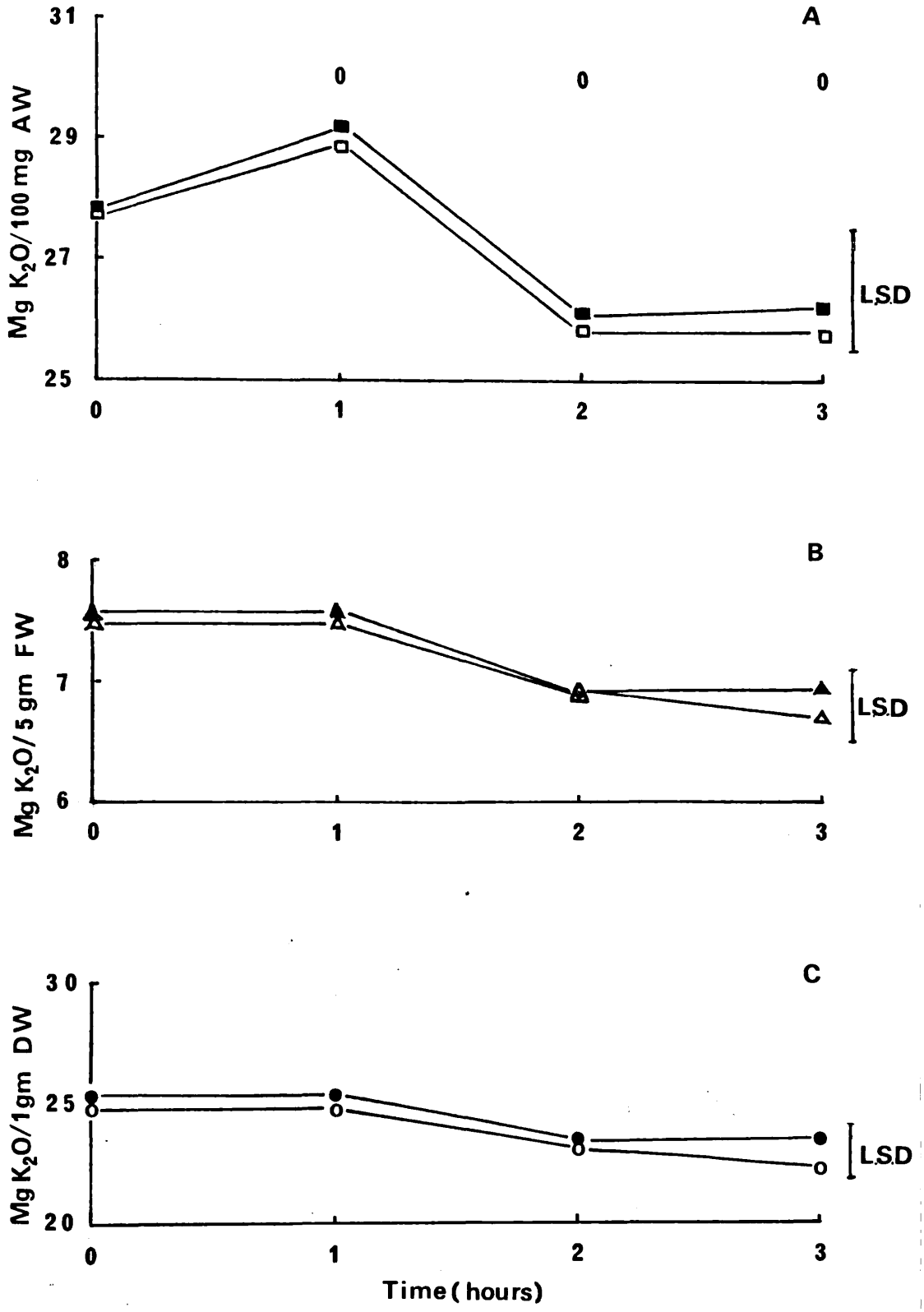

A
31
29
27
25
Mg K_2O/100 mg AW
0
0
0
LSD
0
1
2
3
B
8
7
6
Mg K_2O/5 gm FW
LSD
0
1
2
3
C
30
25
20
Mg K_2O/1gm DW
LSD
0
1
2
3
Time (hours)

Fig.30.

(conc.10^{-4}M made up in Hoagland and Arnon solution) for 6 hours in a dark cupboard. The number of seedlings, replications were the same as for experiment with ionic potassium (Chapter III, Section 2). Seedlings were placed in horizontal position for 1, 2 and 3 hours. Technique for the estimation of K_2O was followed as described in non-radioactive assay.

Analysis of variance of the results is shown in Table 21. The analysis shows that there is no effect of horizontal orientation on the distribution of potassium when curvature is stopped by NPA.

Mean K_2O values on a total ash, fresh and dry weight basis are shown in Fig.30. The figure does not indicate any significant difference in the distribution of potassium between the sides of the hypocotyls placed in horizontal position. Hypocotyls did not produce any curvature after horizontal exposures.

(b) Experiment with ^{42}K in the seedlings of Helianthus annus.

In the last experiment it was seen that differential distribution of potassium was completely inhibited when the curvature was stopped by the treatment with NPA.

An experiment was conducted to study the effect of NPA on the distribution of ^{42}K in the hypocotyls of horizontally placed seedlings of Helianthus annus. Seedlings of Helianthus annus were fed with ^{42}K and NPA through the roots. A group of 10 seedlings was used for each horizontal exposure. Seedlings were placed in horizontal position for 1, 2 and 3 hours. Each exposure had 5 replications. Technique for the preparation of sample and radioactive assay

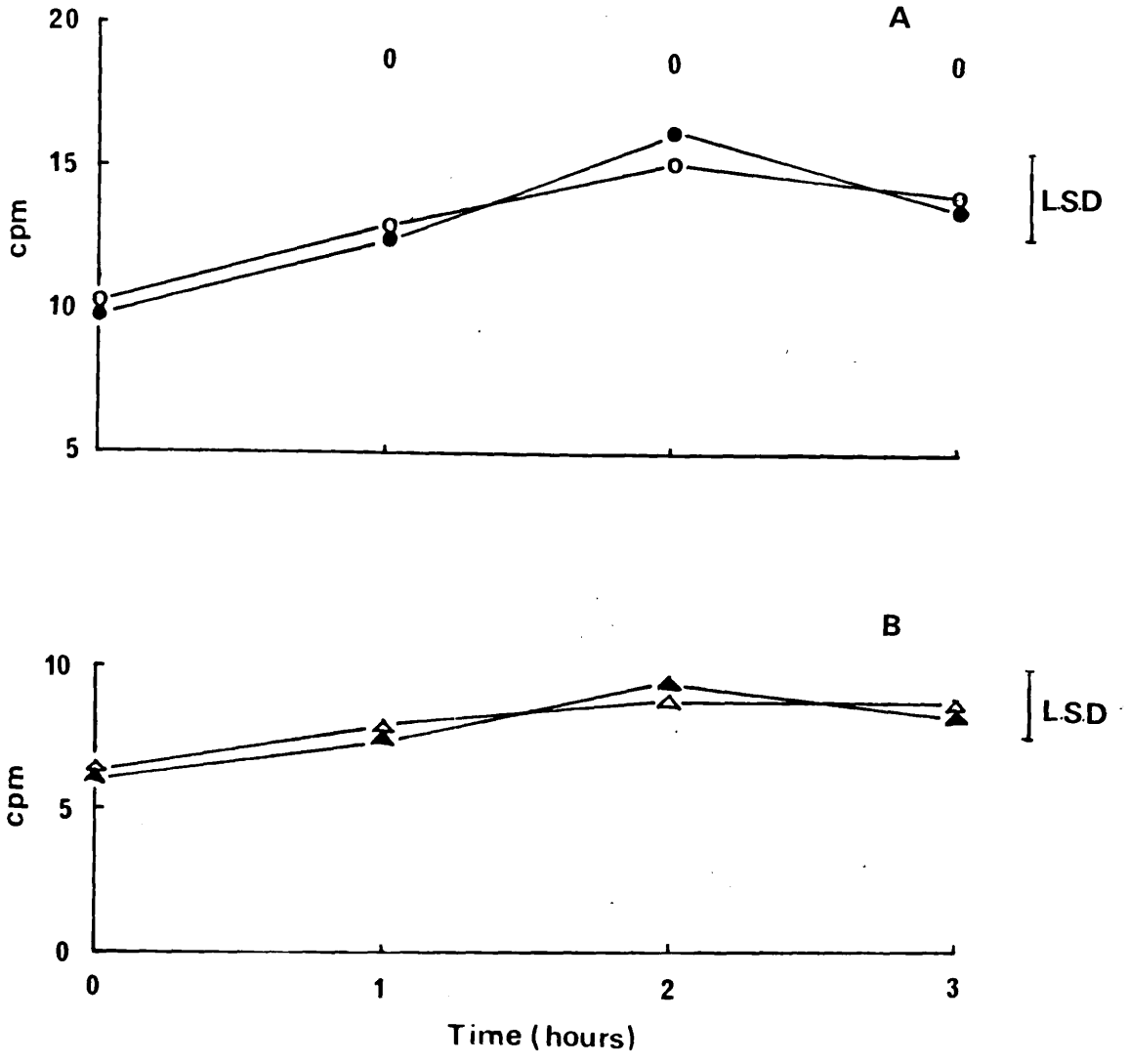


Fig. 31. The effect of NPA on the distribution of ^{42}K in the hypocotyls of horizontally placed seedlings of Helianthus annus.

Seedlings did not produce any curvature, hence it has been shown as 0.

A:- mean counts/min/mg. dry weight

Full circle:- Upper side

Open circle:- Lower side

B:- mean counts/min/10mg. fresh weight

Full triangle:- Upper side

Open triangle:- Lower side

was followed as described in the chapter Materials and Methods.

Analysis of variance of the results is shown in Table 22 (Appendix). The analysis shows that the side (S) effect is not significant.

Graphs have been plotted showing mean counts on dry and fresh weight basis (Fig.31). The figure does not indicate any significant increase of ^{42}K in any particular side. The figure suggests a continuing transport of ^{42}K into hypocotyl but this is not significant (as M.S.V. for T is of the same order as M.S.V. for P + PT).

The results are in agreement with the findings of the last experiment. It can be definitely concluded from the results that differential distribution of potassium does not take place in the absence of curvature. It does demonstrate a correlation between curvature and differential distribution. So this differential distribution is probably caused by curvature.

SECTION 3 DISTRIBUTION OF ^{32}P

In previous experiments with ^{32}P it was clearly observed that the increased accumulation of ^{32}P in the lower side was always correlated with the curvature. Hence, it was thought necessary to study the distribution of ^{32}P in the hypocotyls after curvature was stopped by the treatment with NPA.

(a) Experiment with Helianthus seedlings (with roots).

Seedlings of Helianthus annus were treated with NPA (conc.10^{-4}M) and ^{32}P for 6 hours in a dark cupboard. A group of 10 seedlings was used for each horizontal exposure. There were three horizontal exposures. A group of 10 seedlings

was used to establish the initial distribution of ^{32}P in hypocotyl halves of vertical seedlings. Seedlings were horizontally orientated for 1, 2 and 3 hours. Each exposure had 5 replications. Radioactive assay and preparation of sample were carried out as described in the chapter Materials and Methods.

Analysis of variance of the results is shown in Table 23 (Appendix). The analysis shows that the side (S) effect is not significant.

Graphs have been plotted showing mean counts on dry and fresh weight basis (Fig.32). The figure strongly indicates that there is not any significant increase in any particular side. Hypocotyls did not produce any curvature. The figure indicates a continuing transport of ^{32}P into hypocotyls during horizontal exposure. This is clearly significant since M.S.V. for T is much greater than M.S.V. for P + PT (Table 23, Appendix).

Hence the results suggest that differential distribution does not occur in the absence of curvature.

(b) Experiment with Helianthus hypocotyls (without roots)

In the last experiment it was clearly shown that differential distribution did not take place in absence of curvature. It can be interpreted that differential distribution of ^{32}P is correlated with the curvature. The continued transport of ^{32}P occurs during horizontal exposure, but does not cause an increased accumulation on any particular side. Hence, an experiment was carried out with root-less plants (pre-treated with NPA) in the absence of continuing transport from the roots.

Fig.32 The effect of NPA on the distribution of ^{32}P in the hypocotyls of horizontally placed seedlings of Helianthus annus.

Seedlings did not produce any curvature and hence curvature is shown as 0 at the top of the figure.

Full circle:- mean counts/min/mg. dry weight in the upper side.

Open circle:- mean counts/min/mg dry weight in the lower side.

Full triangle: - mean counts/min/10 mg fresh weight in the upper side

Open triangle:- mean counts/min/10 mg fresh weight in the lower side

Fig.33 The effect of NPA on the distribution ^{32}P in the horizontally placed hypocotyls of Helianthus annus.

Hypocotyls were horizontally orientated in root-less condition.

Full circle:- mean counts/min/mg. dry weight in the upper side.

Open circle:- mean counts/min/mg. dry weight in the lower side.

Full triangle:- mean counts/m n/10 mg. fresh weight in the upper side.

Open triangle:- mean counts/min/10 mg. fregh weight in the lower side.

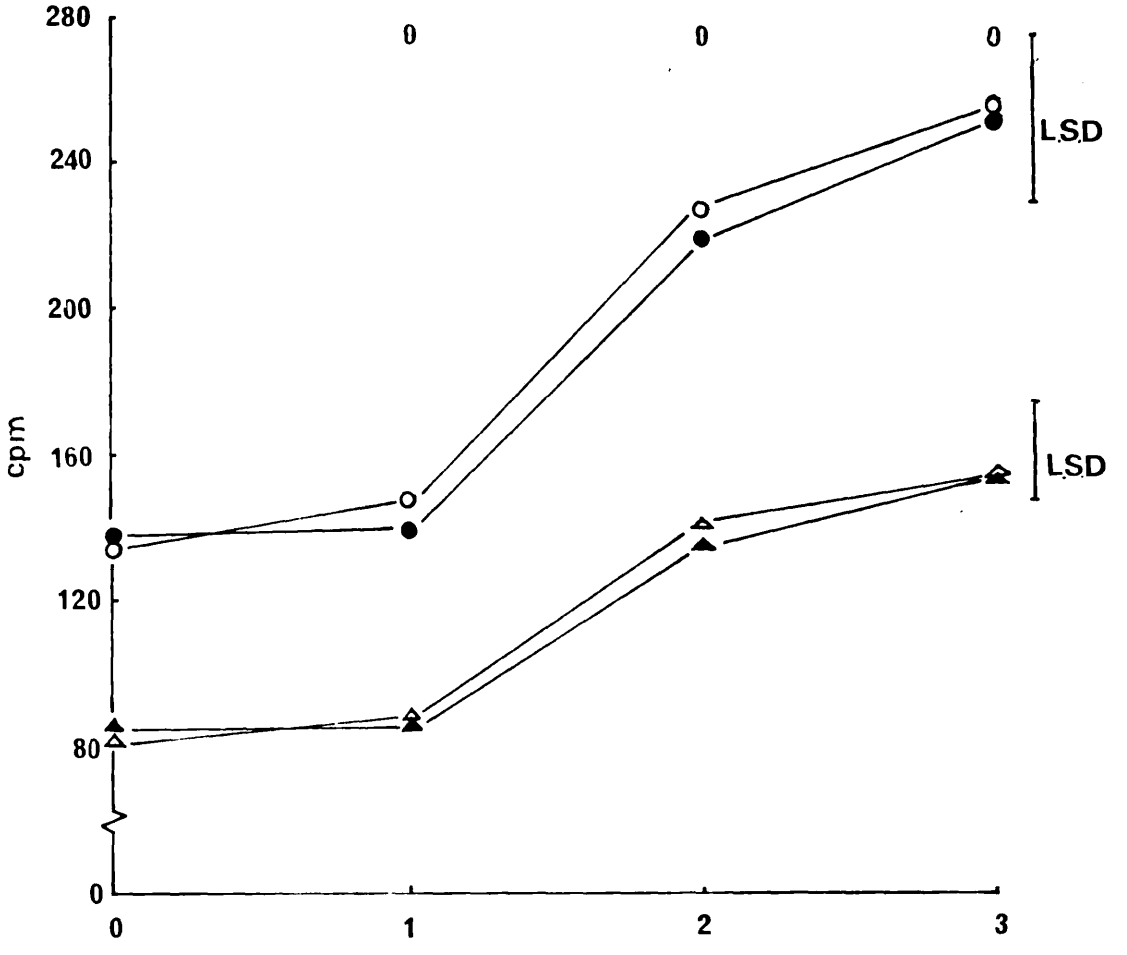

280
240
200
160
120
80
0
cpm
0
0
0
LSD
LSD
0
1
2
3

Fig. 32.

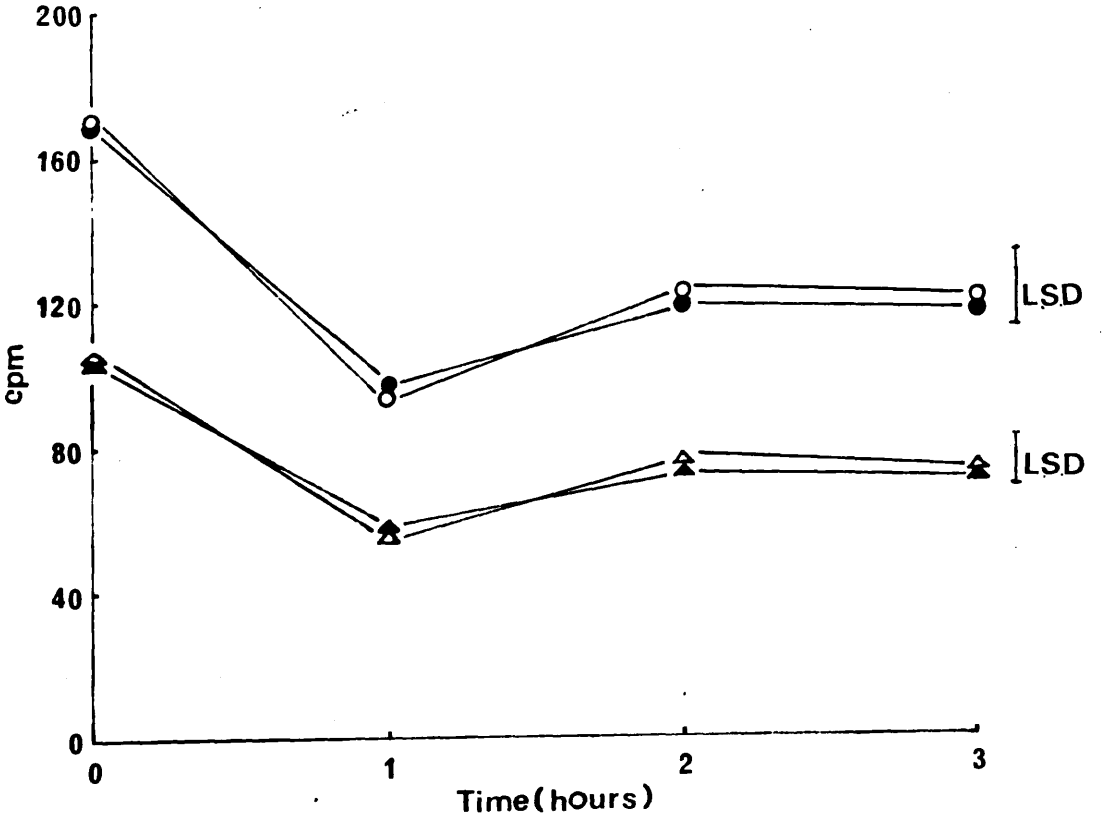

200
160
120
80
40
0
cpm
LSD
LSD
0
1
2
3
Time(hours)

Fig.33.

Seedlings of Helianthus annus were fed through the roots with ^{32}P and NPA for 6 hours in a dark cupboard. Seedlings were harvested and roots removed. Shoot part consisted of hypocotyl and two cotyledons. Shoot parts (cotyledons + hypocotyls) were placed in horizontal position in a glass tray lined with moistened tissue paper. Details about the orientation of root-less plants have been discussed in Chapter III, Section I(b). This is shown in Fig.9. There were three horizontal exposures (1, 2 and 3 hours). Each exposure had 5 replications.

Analysis of variance of the results is shown in Table 24 (Appendix). The analysis shows that the side (S) effect is not significant.

Fig.33 shows that there has not been any significant increase of activity in any particular side during horizontal exposures. The falls and rises on the two sides are probably due to sampling error. From the analysis of variance Table 24 (Appendix) where M.S.V. for T is of same order as M.S.V. for P + PT, changes with time are not significant.

Hence, the results of this experiment are in agreement with the findings of the last experiment. This demonstrates a correlation between curvature and differential distribution of ^{32}P. Redistribution is probably caused by curvature.

CHAPTER VI

THE EFFECT OF UNILATERAL APPLICATION OF IAA ON THE DISTRIBUTION OF IONS

Until now it has been observed that a correlation exists between differential distribution of ions and geotropic curvature. If redistribution is due to curvature, can it be induced in absence of a gravitational stimulus? Unilateral application of IAA would produce curvature in absence of a gravitational stimulus. Would this curvature induce redistribution of ions? It has already been discussed in the Introduction that unilateral application of IAA to the decapitated coleoptile tips results into potential differences across the coleoptile which is comparable to the geo-electric effects developed in horizontally placed organs (Woodcock and Wilkins, 1970). It has also been suggested that both geotropic response and geo-electric effects arise from auxin concentration gradient which is established between upper and lower halves of horizontally placed organs. The object was to study the distribution of ions in the organs caused to curve by the unilateral application of IAA and to see whether any correlation exists between redistribution and differential growth.

SECTION I DISTRIBUTION OF ^{45}Ca

Experiment with the seedlings of *Helianthus annuus*

In this experiment seedlings of *Helianthus annuus* were used to study the effect of unilateral application of IAA on the distribution of ^{45}Ca.

Paste of IAA (0.1% W/W in lanolin) was made up in the following way. IAA was dissolved in acetone and then molten lanolin was added to it. Acetone was evaporated off in a vacuum desiccator. When no smell of acetone was left, IAA lanolin paste was used for unilateral application.

Seedlings of Helianthus annus were treated with ^{45}Ca for 6 hours in a dark cupboard. A group of 20 seedlings was used for each unilateral IAA application. There were three unilateral applications (2, 3 and 4 hours). A group of 20 seedlings was used to determine the initial distribution (initial as 0 hour, without IAA) of ^{45}Ca. Each unilateral application had 5 replications. Seedlings pre-treated with ^{45}Ca were taken out of the dark cupboard and all cotyledons were removed except the small basal portion. This is shown in Fig.34. Cut surface was dried with a blotting paper. A small drop of IAA lanolin paste was applied to the cut basal end of one cotyledon of each seedling involved in IAA application. After IAA application seedlings were put back in the dark cupboard for 2, 3 and 4 hours. Each period of unilateral application consisted of 5 replications. At the end of each period of application seedlings were harvested and roots were removed. Small basal portion of the cotyledons was also removed. Hypocotyls were split centrally into concave and convex halves. Technique for the radioactive assay and preparation of sample was followed as described in the chapter Materials and Methods.

Analysis of variance of the results is shown in Table 25 (Appendix). The analysis shows that the side effect (S) is significant. This suggests that there has been overall increase in the concave side ("other" side). The overall increase in activity with time is significant.

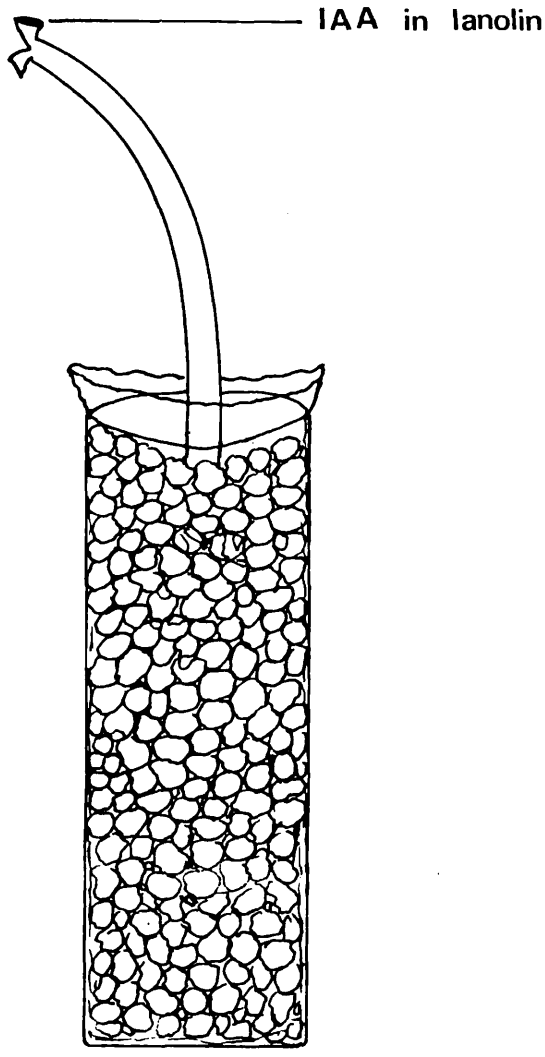


Fig.34. Unilateral IAA application to the cut basal portion of the cotyledon of Helianthus annus.
Hypocotyl has shown curvature.

Fig.35 The effect of unilateral applications of IAA (0.1% W/W in lanolin) on the distribution of ^{45}Ca in the hypocotyls of Helianthus annus.
Time-course changes in the activity of ^{45}Ca in the sides. Curvatures in degrees are shown at the top of the figure. $\pm$ is standard error of the mean.

A :- mean counts/min/mg. dry weight

Full circle:- concave (other) side

Open circle:- convex (donor) side

B :- mean counts/min/10 mg. fresh weight

Full triangle:- concave (other) side

Open triangle:- convex (donor) side

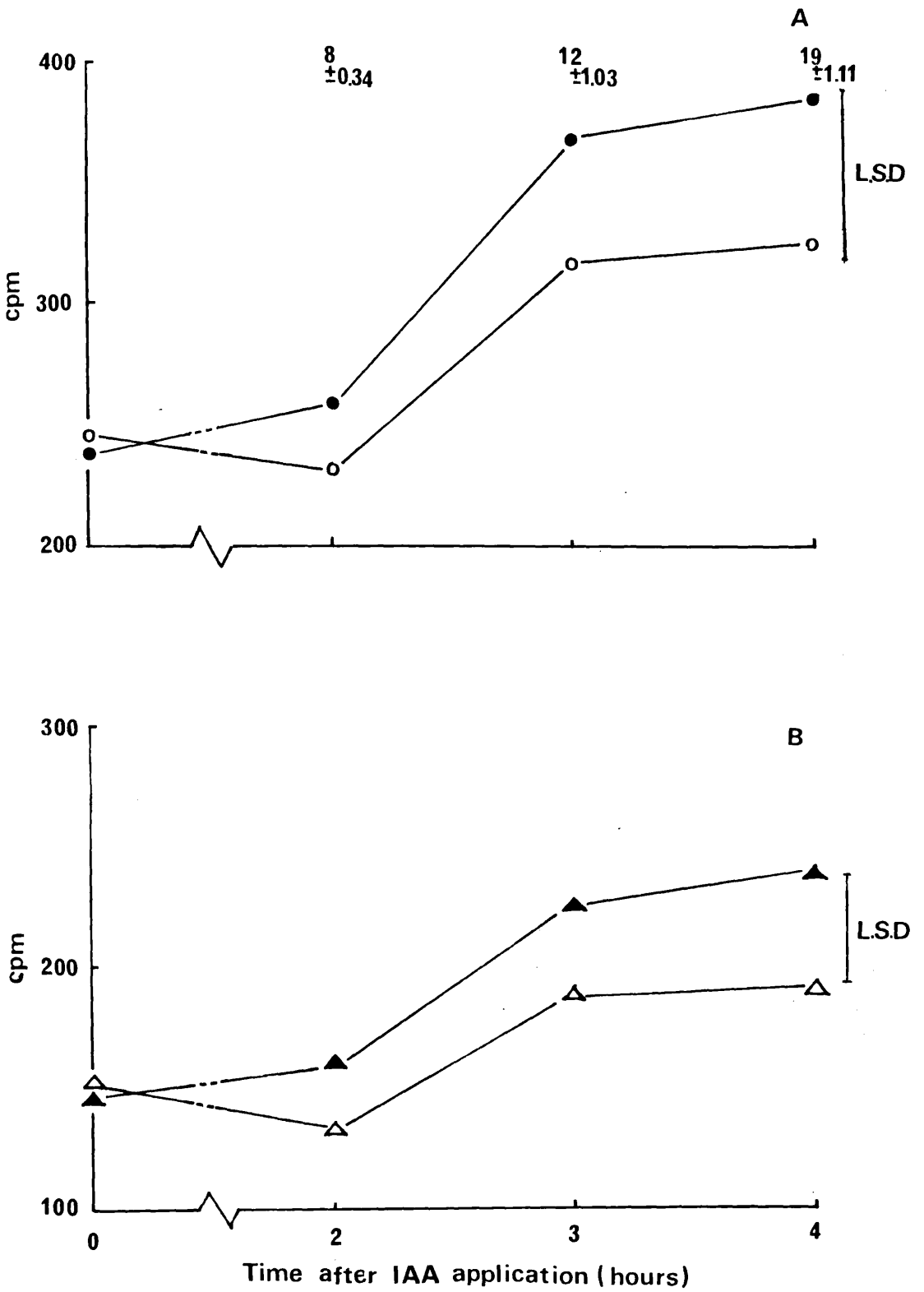

A
400
300
200
cpm
8
±0.34
12
±1.03
19
±1.11
L.S.D
B
300
200
100
cpm
L.S.D
0
2
3
4
Time after IAA application (hours)

Fig. 35.

Fig. 35 shows mean counts on dry and fresh weight basis. The figure indicates that on the convex side ("donor" side) there is first a drop (not significant) and then a recovery, whereas on the concave side there is first a rise (not significant) and then a much greater rise. This suggests that there has been migration of ^{45}Ca from convex to concave side at an early period. Continuing transport from roots increases concentration in both sides (as M.S.V. for T is much greater than M.S.V. for P + PT). Increase in the concave side is greater than that in convex side.

Maximum difference between the sides is observed after 4 hours of IAA application when curvature has been recorded as 19 degrees $\pm$ 1.11. L.S.D. of the means are shown in the graphs which clearly suggest that the differences between the means are significant after 4 hours IAA application.

It can be definitely concluded from the results that a concentration difference of ^{45}Ca is established between concave and convex sides. It has been observed in preceding experiments that the increased accumulation is correlated with the curvature and the increase is always on the concave side in geotropic response also. Hence, the results are in agreement with the previous findings.

SECTION 2 DISTRIBUTION OF POTASSIUM

The foregoing type of experiment (Page 113) was repeated for potassium.

(a) Experiment with ionic potassium in Helianthus annuus seedlings.

Seedlings of Helianthus annuus were used to study the effect of unilateral application of IAA on the distribution of potassium. There were three unilateral IAA applications (2, 3 and 4 hours). The number of seedlings and replications for each IAA application was same as in experiment with ionic potassium (Page 56). Unilateral application of IAA was carried out in the same way as described in page 114 . This is shown in Fig. 34. The technique for the estimation of potassium was carried out as described in non-radioactive assay.

Analysis of variance of the results is shown in Table 26 (Appendix). The analysis shows that S X T effect is significant. This suggests that there have been progressive differences between the side means with time. The overall increase in potassium with time is also significant on an ash weight basis.

Fig. 36 shows that on the concave side ("other" side) there is a significant drop, then a recovery and again a drop, whereas on the convex side ("donor" side) there is first a drop (not significant) and then a rise till 3 hours, but after 3 hours there is not much change. This suggests that there has been migration of potassium from concave to convex at an early period (2 hours). Increased movement from roots after 2 hours could account for the rise in both sides, but convex increases more than the concave side.

The maximum curvature was 25 degrees ± 1.38. L.S.D. of means have been shown in the figure which suggests that differences between the means are higher than L.S.D.

Fig.36. The effect of unilateral application of IAA (0.1% W/W in lanolin) on the distribution of potassium ions in the hypocotyls of *Helianthus annus*.

Time-course changes in the amount of K_2O in the sides.

Curvatures in degrees are shown at the top of the figure. $\pm$ is standard error of the mean.

A :- mg. K_2O/100 mg. ash weight

Full square:- concave (other) side

Open square:- convex (donor) side

B :- mg. K_2O/5 gm. fresh weight

Full triangle:- concave (other) side

Open triangle:- convex (donor) side

C :- mg. K_2O/1 gm dry weight

Full circle:- concave (other) side.

Open circle:- convex (donor) side

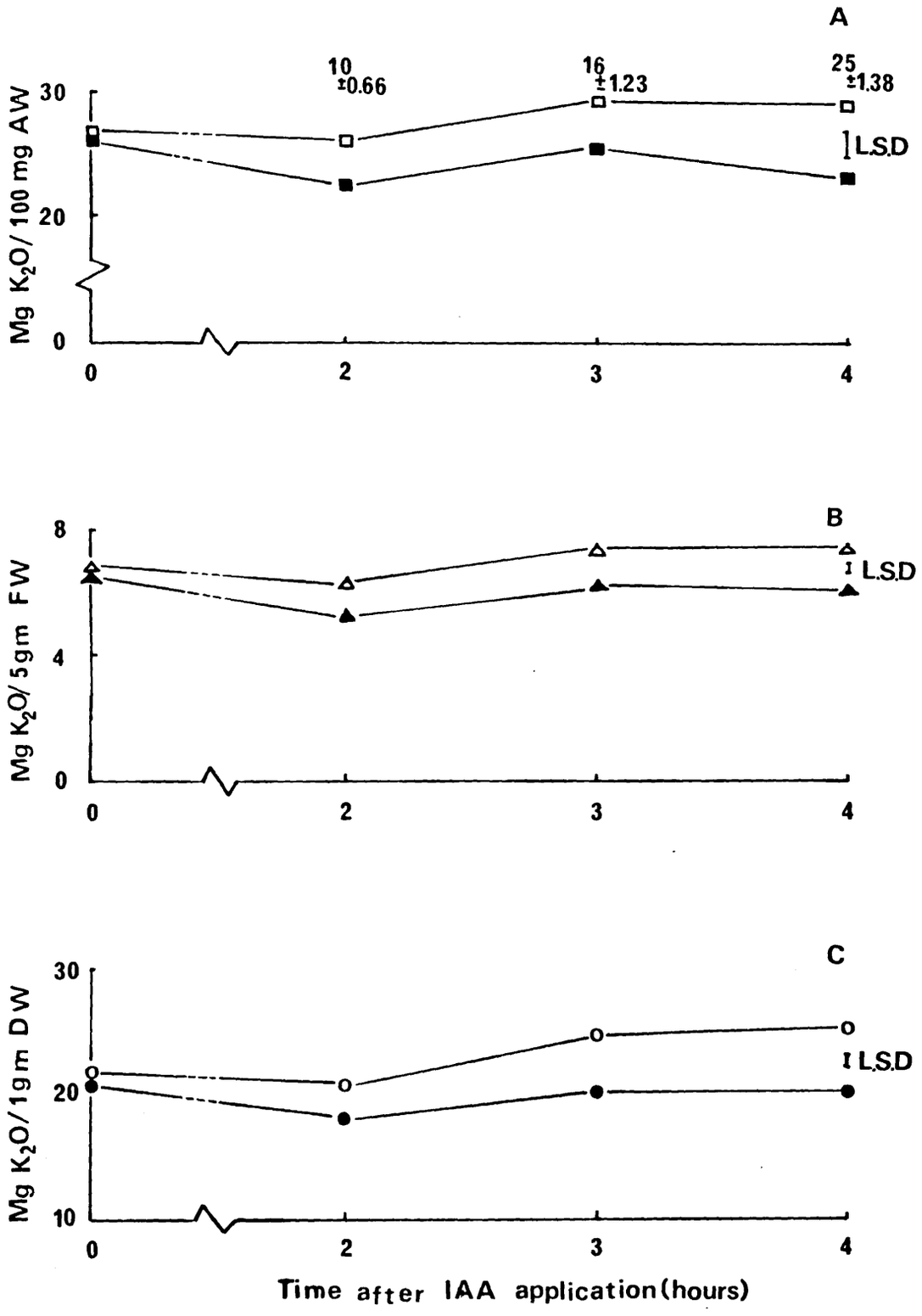

A
10
±0.66
16
±1.23
25
±1.38
L.S.D
Mg K_2O/ 100 mg AW
30
20
0
0
2
3
4
B
L.S.D
Mg K_2O/ 5gm FW
8
4
0
0
2
3
4
C
L.S.D
Mg K_2O/ 1gm DW
30
20
10
0
2
3
4
Time after IAA application (hours)

Fig.36.

The results show a correlation between differential distribution and curvature (result of IAA application) and the increase is always on the convex side in geotropic response also.

(b) Experiment with ^{42}K in Helianthus annuus seedlings.

An experiment was carried out with ^{42}K to compare the findings of the last experiment. There were two unilateral applications of IAA (2 or 3 hours). A group of 10 seedlings was included for each application. Each period of unilateral application consisted of 5 replications. Seedlings were treated with ^{42}K for 2 hours in a dark cupboard. Application of IAA (0.1% W/W in lanolin) was carried out as described in Page 114 . This is shown in Fig.34. Technique for the preparation of sample and radioactive assay was followed as described in the chapter Materials and Methods.

Analysis of variance of the results is shown in Table 27 (Appendix). The analysis shows that the side (S) effect is significant. This suggests that there has been overall increase in the convex side after IAA application.

Fig.37 shows that on the concave side there is a drop after 3 hours (not significant) whereas on the convex side there is a rise. This drop on the concave side could be explained in terms of a lateral movement. This suggests that there has been migration of ^{42}K from concave to convex side during IAA application. This increase in the convex side is correlated with the curvature. The differences between the means are higher than L.S.D. after 3 hours of IAA application. Hence, the results of this experiment are in agreement with findings of the last experiment.

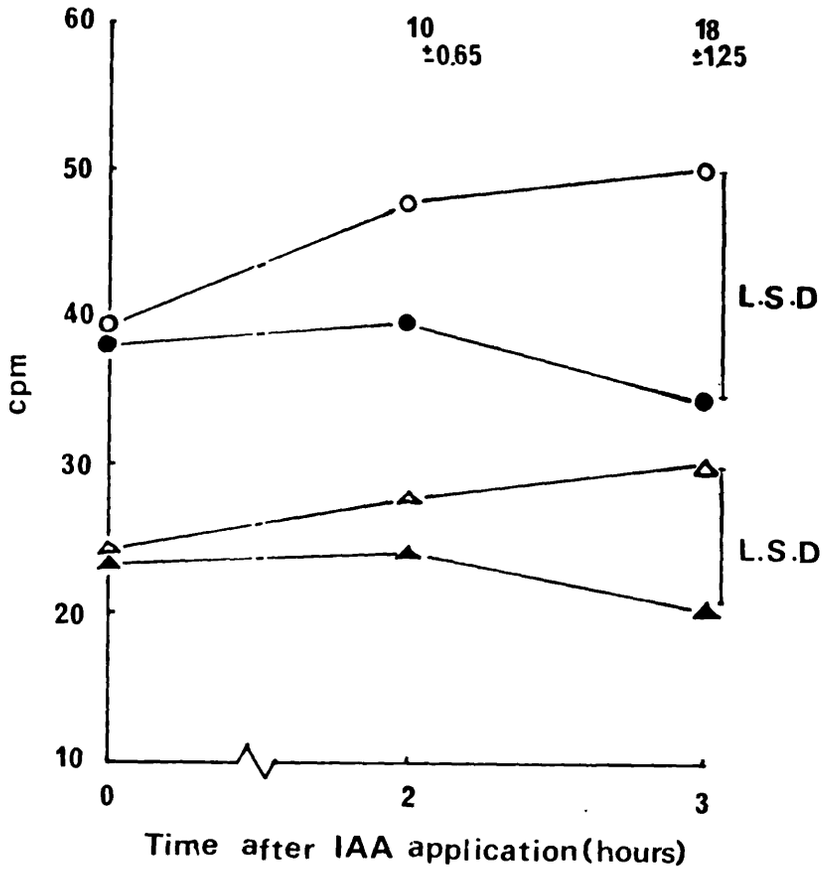


Fig. 37. The effect of unilateral application of IAA on the distribution of ^{42}K in the hypocotyls of Helianthus annus.

Curvatures in degrees are shown at the top.

± is standard error of the mean.

Full circle:- mean cpm/mg. dry wt. in the concave side

Open circle:- mean cpm/mg. dry wt. in the convex side

Full triangle:- mean cpm/10mg. fresh wt. in the concave side

Open triangle:- mean cpm/10mg. fresh wt in the convex side

SECTION 3 DISTRIBUTION OF ^{32}P

The foregoing type of experiment (Page 113) was repeated for ^{32}P.

Experiment with ^{32}P in Helianthus annus seedlings.

Seedlings of Helianthus annus were used to study the effect of unilateral applications of IAA on the distribution of ^{32}P. Seedlings were treated with ^{32}P for 4 hours in a dark cupboard. A group of 10 seedlings was used for each unilateral IAA application. There were two applications (1 and 2 hours). Each application consisted of 5 replications. Unilateral application of IAA was carried out in the same way as described in page 114 . This is shown in Fig.34. Technique for the preparation of sample and radioactive assay was followed as described in the chapter, Materials and Methods.

Analysis of variance of the results is shown in Table 28 (Appendix). The analysis shows that the side (S) effect is significant.

Fig.38 shows that in the convex side there is an increase with time, whereas in the concave side there is first an increase and then a decrease (not significant). The figure suggests that there has been continuing transport from roots, although T effect is not significant (as M.S.V. for T is of the same order as M.S.V. for P + PT). L.S.D. of the means have shown in the figure which suggests that the difference between the means is higher than L.S.D. on dry weight basis after 2 hours IAA application.

Hence, the results show that the increase in the convex side is correlated with the curvature and the increase is always in the convex side in geotropic response also.

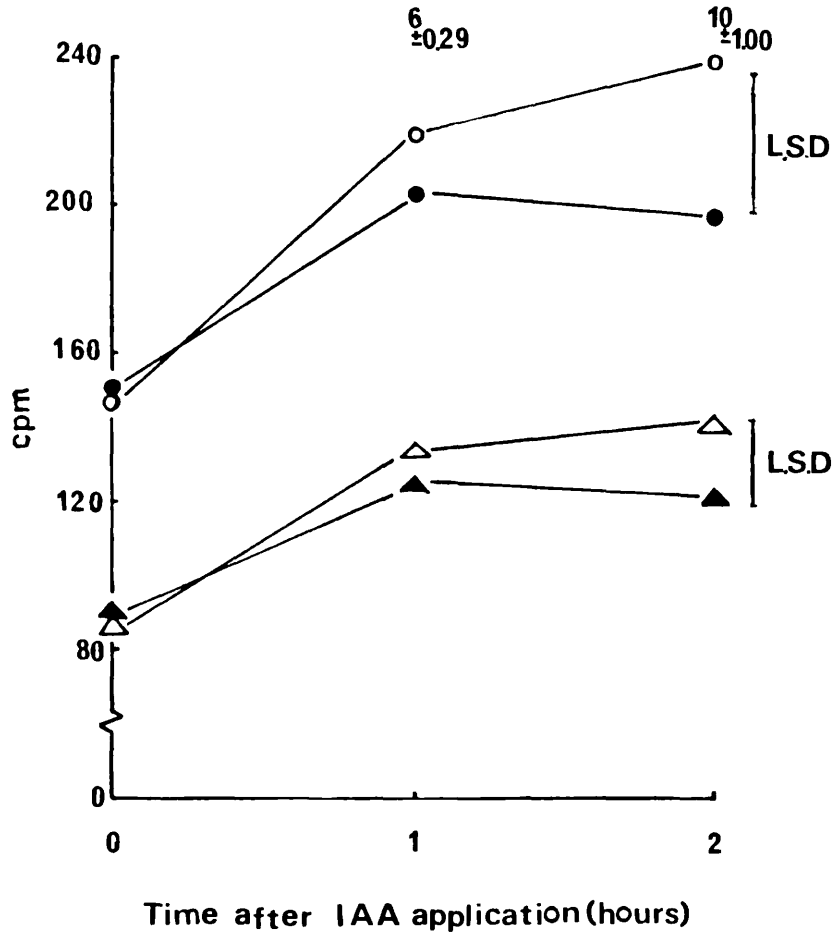


Fig. 38. The effect of unilateral application of IAA on the distribution of ^{32}P in the hypocotyls of Helianthus annuus.

Curvatures in degrees are shown at the top.

± is standard error of the mean.

Full circle:- mean cpm/mg. dry wt. in the concave side

Open circle:- mean cpm/mg. dry wt. in the convex side

Full triangle:- mean cpm/10mg. fresh wt. in the concave side

Open triangle:- mean cpm/10mg. fresh wt. in the convex side

CHAPTER VII

THE EFFECT OF UNILATERAL ILLUMINATION ON THE DISTRIBUTION OF IONS

The curvature of the shoot towards unilateral light is well known phenomenon and the growth curvature of the organs in relation to directional light stimulus is known as phototropism. In all experiments it was clearly observed that the differential distribution of ions is correlated with the curvature. The parallelism between geotropism and phototropism may be drawn from the results of Naqvi and Gordon (1966) who suggest that gravity and light probably modify the basipetal transport of auxin during growth response. The work of Pickard and Thimann (1964) has shown that there is asymmetry of auxin distribution taking place during phototropic curvature. So a close similarity exists between positively phototropic and negatively geotropic organs in the process of producing curvature. Thus, work has been carried out to see whether any kind of correlation exists between ion distribution and phototropic curvature.

SECTION I DISTRIBUTION OF ^{45}Ca

In this section experiments have been carried out with coleoptiles of *Zea mays* (with and without roots) pre-treated with ^{45}Ca. The activity has been ascertained in the illuminated and shaded halves after different periods of unilateral illumination.

(a) Experiment with the seedlings of *Zea mays* (with roots) (vascular bundles transversely orientated, i.e. one

vascular bundle was nearer the illuminated and the other was nearer the shaded side as shown in Fig.39).

Seedlings of Zea mays were grown in a dark cupboard as described in Materials and Methods. Seedlings were treated with ^{45}Ca for 6 hours in a dark cupboard. A group of 10 seedlings was used for each period of unilateral illumination. There were three different periods of illumination (2,3 and 4 hours). Each period of unilateral illumination consisted of 5 replications. A group of 10 seedlings was used to determine the initial distribution of radioactivity in the coleoptile halves. Each period of unilateral illumination had 5 replications. Seedlings after treatment with ^{45}Ca were placed inside a phototropic chamber as shown in Fig.7. Special care was taken about the arrangement of the seedlings so that one vascular bundle was nearer the illuminated and the other was nearer the shaded side, as shown in Fig.39.. Then seedlings were unilaterally illuminated for2, 3 and 4 hours. The amount of light available to the coleoptiles was about 800 lux. Details about the experimental technique have been discussed in the chapter Materials and Methods. After each period of unilateral illumination seedlings were harvested and coleoptiles were split into illuminated and shaded halves. Preparation of sample and radioactive assay were carried out as described in Materials and Methods.

Analysis of variance of the results is shown in Table 29 (Appendix). The analysis shows that the side (S) effect is significant. This suggests that there is overall increase in the illuminated (concave) side. The S X T effect is significant on a fresh weight basis. This indicates that there has been progressive differences between the means of

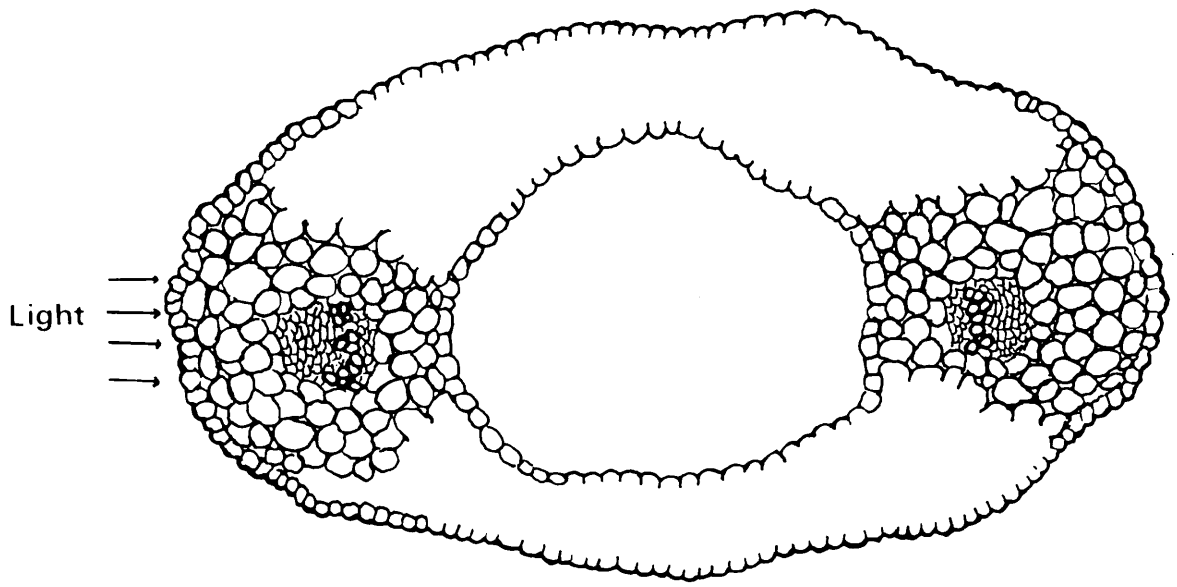


Fig.39. Vascular bundles of Zea coleoptiles are shown transverse to the direction of illumination.

Fig.40 The effect of unilateral illumination on the distribution of ^{45}Ca in the coleoptiles (vascular bundles transverse) of Zea mays.

Time-course changes in the activity of ^{45}Ca in the sides. Curvatures in degrees are shown at the top of the figure. $\pm$ is standard error of the mean.

A :- mean counts/min/mg. dry weight

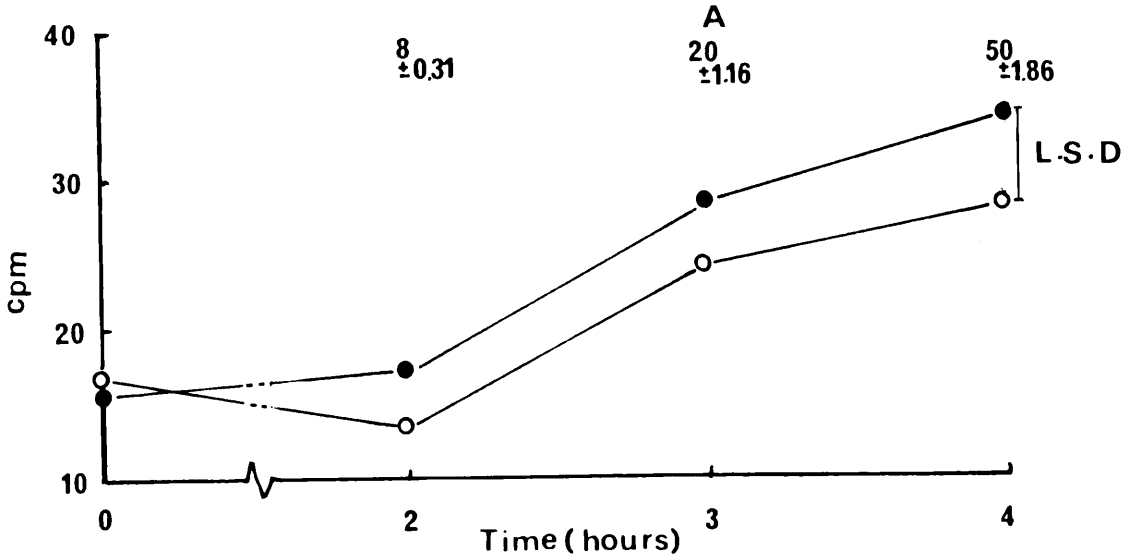
Full circle:- illuminated (concave) side

Open circle:- shaded (convex) side

B:- mean counts/min/10 mg. fresh weight

Full triangle:- illuminated (concave) side

Open triangle:- shaded (convex) side.


A
40
30
20
10
cpm
8
±0.31
20
±1.16
50
±1.86
L.S.D
0
2
3
4
Time (hours)

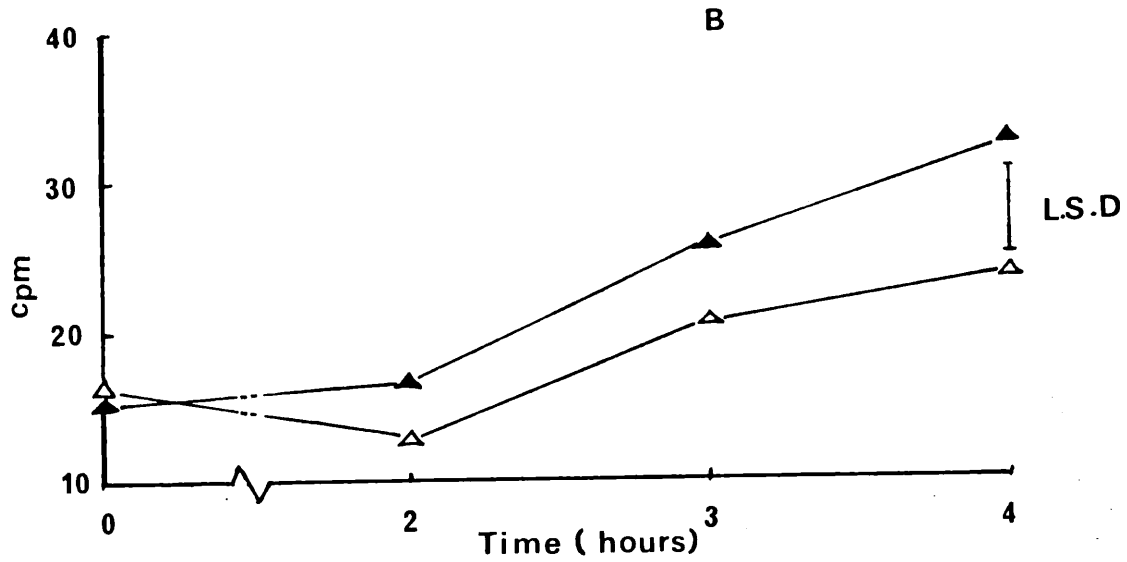

B
40
30
20
10
cpm
L.S.D
0
2
3
4
Time (hours)

Fig.40.

illuminated and shaded sides with time.

Fig. 40 shows mean counts on dry and fresh weight basis in the illuminated (concave) and shaded (convex) sides. Graphs show that in the shaded (convex) side there is first a drop (not significant) and then a steady recovery, whereas in the illuminated (concave) side there is first a small rise (not significant) and then a much greater rise. This suggests that the lateral migration of ^{45}Ca is established at an early stage (2 hours). There has been increase in transport from roots with both sides (as M.S.V. for T is much greater than M.S.V. for P + PT), but illuminated (concave) side increased more than the shaded (convex). Hence, it seems from the results that differential distribution may be due to migration from convex to concave. With the increase in the time of unilateral illumination differences between the sides increase and the maximum is found after 4 hours when curvature recorded as 50 degrees $\pm$ 1.86.

Hence, the results suggest that a concentration difference is established between illuminated (concave) and shade (convex) sides. This is correlated with the curvature.

(b) Experiment with <u>Zea</u> coleoptiles (without roots) (vascular bundles transversely orientated as shown in Fig.39).

In the last experiment it was observed that the accumulation of more radioactivity in the illuminated side was accompanied with the curvature. In this experiment roots were

Fig.41 The effect of unilateral illumination on the distribution of ^{45}Ca in the coleoptiles of (vascular bundles transverse) of Zea mays.

Coleoptiles were unilaterally illuminated in root-less condition.

Curvatures in degrees are shown at the top of the figure. $\pm$ is standard error of the mean.

A :- mean counts/min/mg. dry weight

B :- mean counts/min/10 mg. fresh weight

0 = Initial (not illuminated)

4 = 4 hours unilateral illumination

Lt:- left side

Rt:- right side

I :- illuminated (concave) side

S :- shaded (convex) side

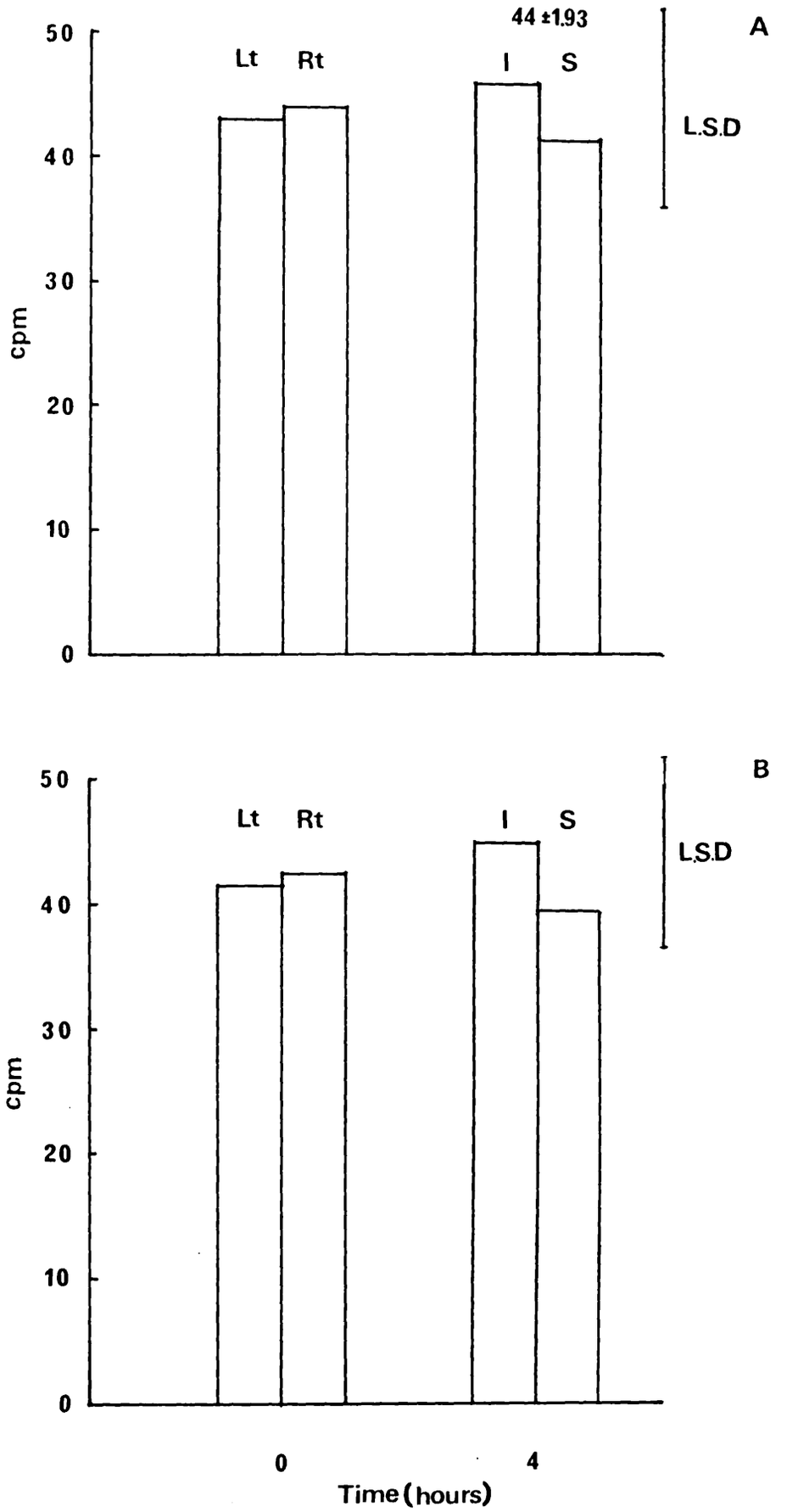

44 ±1.93
A
50
40
30
20
10
0
cpm
Lt
Rt
I
S
L.S.D
B
50
40
30
20
10
0
cpm
Lt
Rt
I
S
L.S.D
0
4
Time (hours)

Fig.41.

removed prior to illumination to eliminate any possible effect due to differential uptake for roots.

A group of 10 coleoptiles was used for the period of unilateral illumination (4 hours). Seedlings were treated with ^{45}Ca for 6 hours in a dark cupboard and then roots were removed. Position of vascular bundles was marked by ink and coleoptiles were placed carefully in small glass tubes containing some distilled water. Coleoptiles were unilaterally illuminated for 4 hours.

Analysis of variance of the results is shown in Table 30 (Appendix). The analysis shows that the side (S) effect is not significant.

Fig.41 indicates some migration of activity from the shaded (convex) to the illuminated (concave) side. The activity in the illuminated side increases with a corresponding decrease in the shaded side. But this is not significant. The results suggest that the differences are greater when roots are present than when they are not.

(c) Experiment with the seedlings of <u>Zea mays</u> (with roots) (vascular bundles were parallel as shown in Fig.42)

This experiment was carried out to investigate the effect of orientation of the vascular bundles during unilateral illumination. In the experiment of transverse orientation of vascular bundles (rooted plants) the results suggest a greater increase of ^{45}Ca in the illuminated (concave) side. There has probably been differential transport and this could be one reason for the differential distribution. In case of rooted plant (vascular bundles transverse) one vascular bundle was

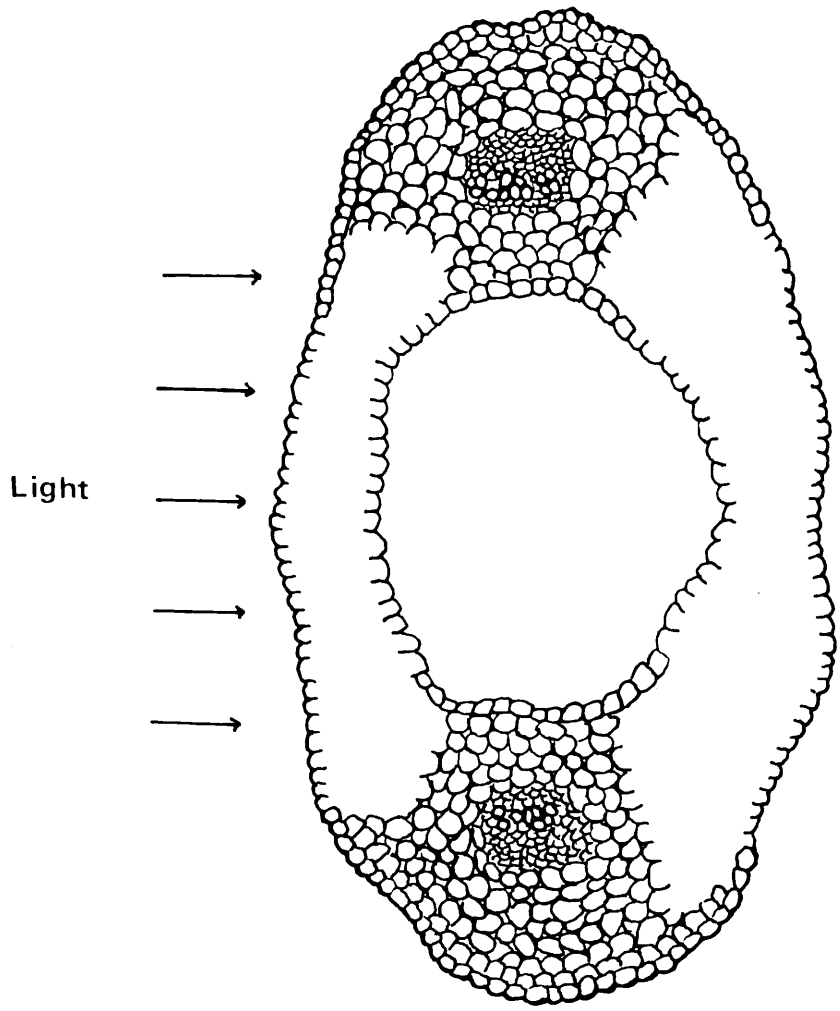


Fig.42. Vascular bundles of Zea coleoptiles are shown parallel to the direction of illumination

Fig.43 The effect of unilateral illumination on the distribution of ^{45}Ca in the coleoptiles (vascular bundles parallel) of Zea mays.

Curvature in degrees are shown at the top of the figure. $\pm$ is standard error of the mean.

A :- mean counts/min/mg. dry weight

B :- mean counts/min/10 mg. fresh weight

0 = Initial (not illuminated)

4 = 4 hours unilateral illumination

Lt :- left side

Rt :- right side

I :- illuminated (concave) side

S :- shaded (convex) side.

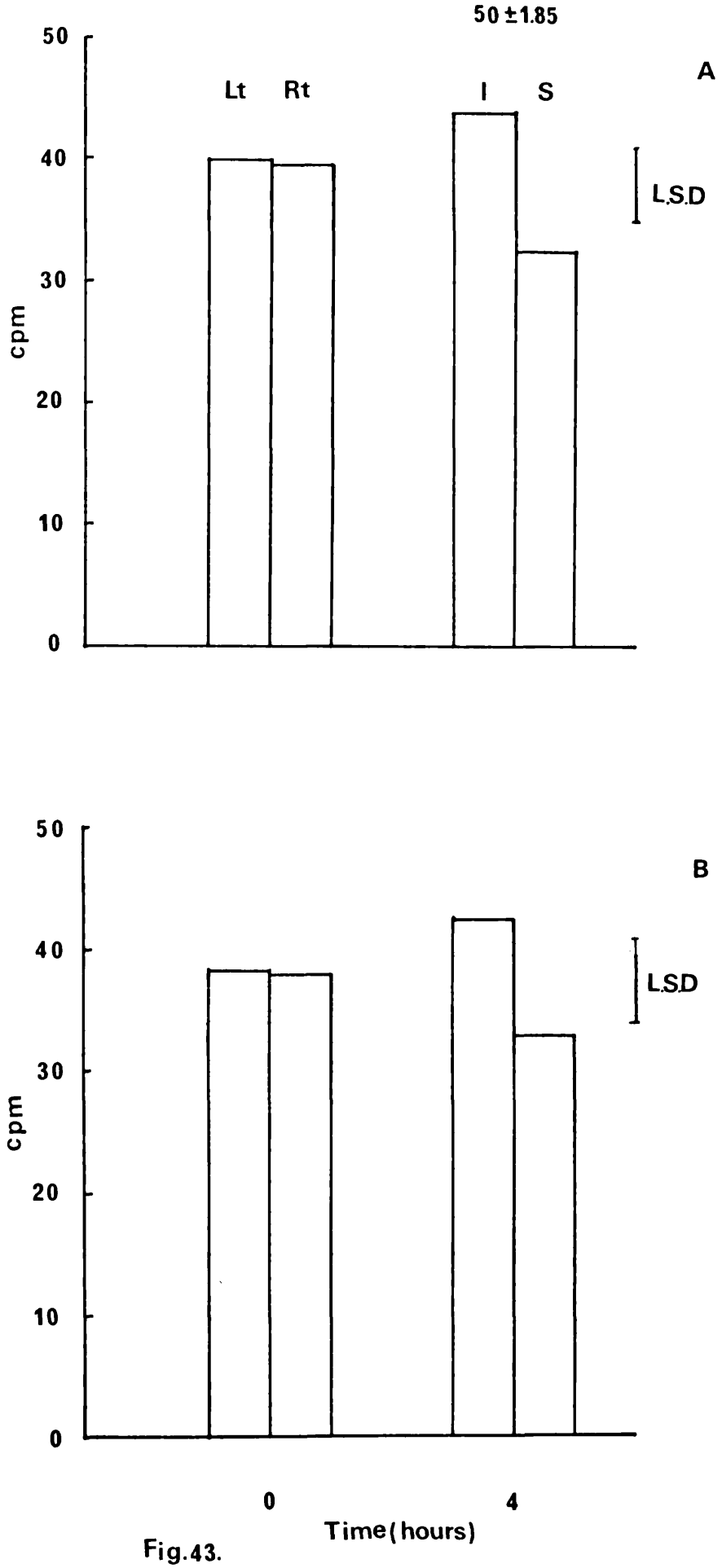

50 ±1.85
A
Lt
Rt
I
S
L.S.D
cpm
50
40
30
20
10
0
B
L.S.D
cpm
50
40
30
20
10
0
0
4
Time(hours)

Fig.43.

in the shaded side and the other in the illuminated side. One can interpret that differential transport in the vascular bundle on the illuminated and on the dark sides might be accounting partly for the differential distribution. In this experiment vascular bundles were parallel to the direction of illumination (Fig.42) so that they might be expected to be affected equally by illumination.

A group of 10 seedlings (pre-treated with ^{45}Ca) was unilaterally illuminated (vascular bundles were parallel) for 4 hours.

Analysis of variance of the results is shown in Table 31 (Appendix). The analysis shows that S X T effect is significant.

Fig. 43 indicates that there has been increase in the activity in the illuminated (concave) side. The figure shows that there has not been any transport of activity from the roots during unilateral illumination, rather it suggests that the difference in activity between illuminated and shaded sides is probably due to lateral migration alone from the shaded side. L.S.D. show that the means of the illuminated and shaded sides are significantly different from one another.

Hence, the results suggest that the differential distribution of ^{45}Ca is due entirely to lateral migration and in no way to differential longitudinal transport in the vascular bundles.

(d) Experiment with <u>Zea</u> coleoptiles (without roots) (vascular bundles were parallel as shown in Fig.42).

In this experiment roots were removed after pre-treatment with ^{45}Ca. Coleoptiles were unilaterally illuminated for 4 hours.

Fig.44 The effect of unilateral illumination on the distribution of ^{45}Ca in the coleoptiles (vascular bundles parallel) of Zea mays.

Coleoptiles were unilaterally illuminated in rootless condition.

Curvatures in degrees are shown at the top of the figure. ± is standard error of the mean.

A :- mean counts/min/mg. dry weight

B :- mean counts/min/10 mg. fresh weight

0 = Initial (not illuminated)

4 = 4 hours unilateral illumination

Lt :- left side

Rt :- right side

I :- illuminated (concave) side

S :- shaded (convex) side.

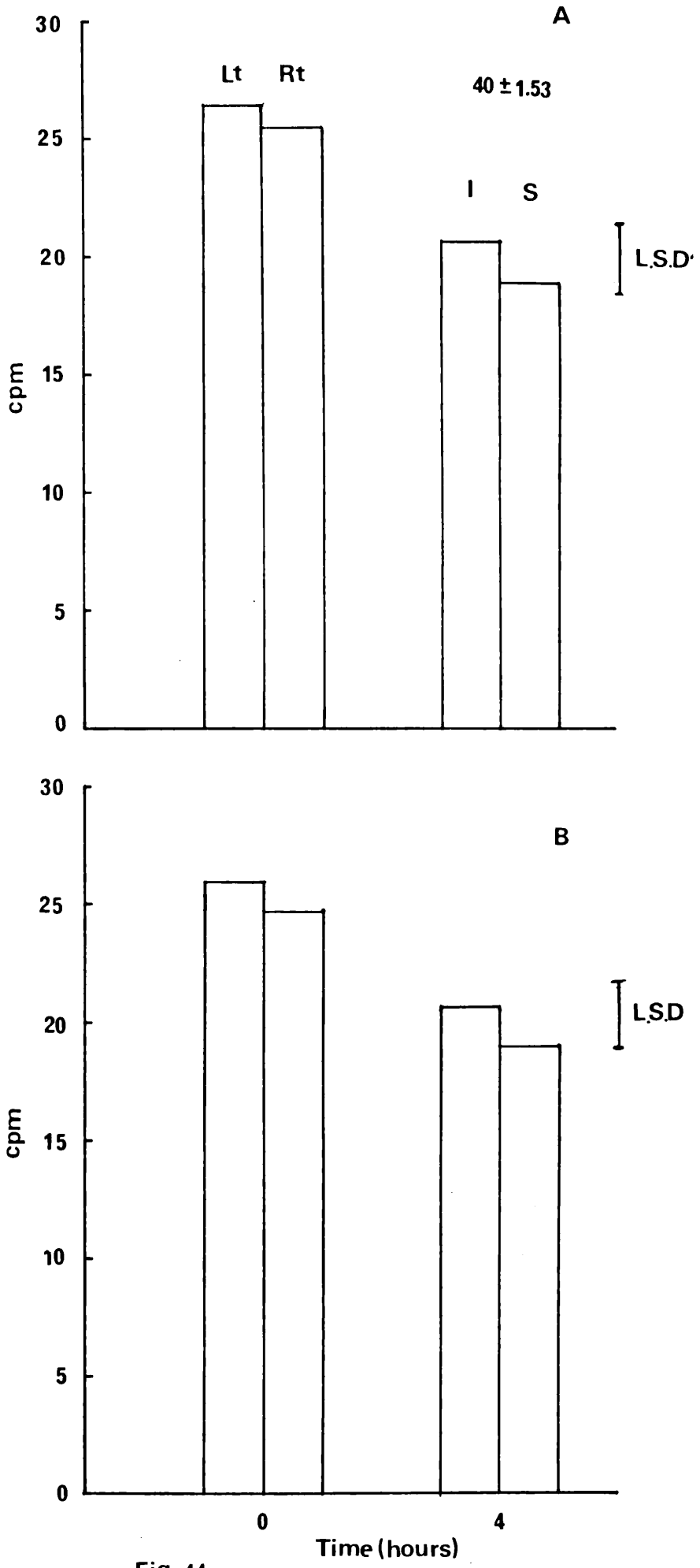

A
Lt
Rt
40 ± 1.53
I
S
L.S.D
cpm
30
25
20
15
10
5
0
B
L.S.D
cpm
30
25
20
15
10
5
0
0
4
Time (hours)

Fig. 44.

Analysis of variance of the results is shown in Table 32 (Appendix). The analysis shows that the side (S) effect is not significant.

Fig. 44 suggests that there has been some migration (not significant) of activity from the illuminated to the shaded side, but the difference in activity between the sides drops considerably as compared to the rooted plants.

If all the results in this section are considered it seems probable that the differential distribution is due to lateral migration from the shaded (convex) to the illuminated (concave) side. It was clearly shown in the experiment where the plane of vascular bundles of the coleoptiles was parallel to the direction of illumination. All the results suggest that increase in the illuminated side is correlated with the curvature.

SECTION 2 DISTRIBUTION OF ^{42}K

(a) Experiment with the seedlings of *Zea mays* (with roots)

Seedlings of *Zea mays* were treated with ^{42}K for 2 hours in a dark cupboard. There were 2 unilateral illuminations (3 and 4 hours). Each consisted of 10 seedlings. Each period of unilateral illumination had 5 replications. A group of 10 seedlings was also treated with ^{42}K to establish the initial (not illuminated) distribution of ^{42}K in the coleoptile halves. Coleoptiles were unilaterally illuminated (with vascular bundles transverse as shown in Fig.39) as described in Materials and Methods.

Fig.45 The effect of unilateral illumination on the distribution of ^{42}K in the coleoptiles of *Zea mays*. Curvatures in degrees are shown at the top of the figure. $\pm$ is standard error of the mean.

A : - mean counts/min/mg. dry weight

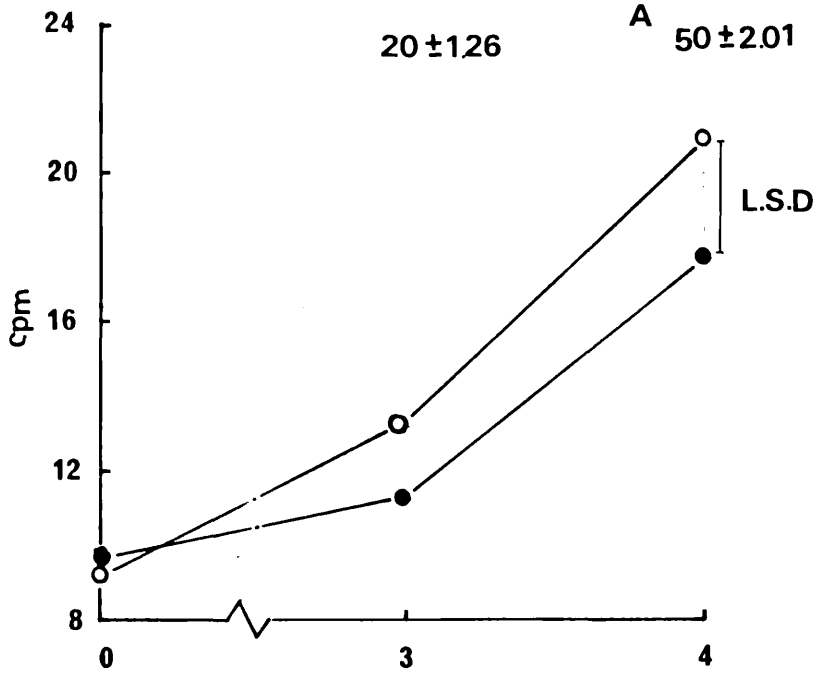
Full circle:- illuminated (concave) side

Open circle:- shaded (convex) side

B :- mean counts/min/10 mg. fresh weight

Full triangle:- illuminated (concave) side

Open triangle:- shaded (convex) side


A
20 ± 1.26
50 ± 2.01
L.S.D
cpm
24
20
16
12
8
0
3
4

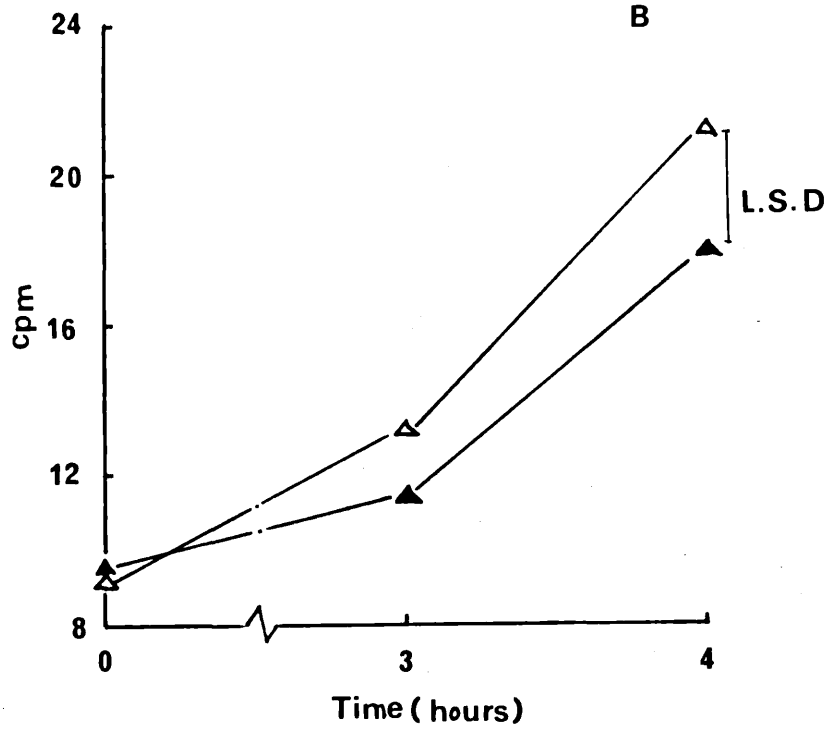

B
L.S.D
cpm
24
20
16
12
8
0
3
4
Time (hours)

Fig.45.

Analysis of variance of the results is shown in Table 33 (Appendix). The analysis shows that the side (S) effect is significant. When the results are considered on fresh weight basis S X T effect is significant. This suggests that there has been a progressive increase in the difference between the side means with time.

Fig.45 shows that there is increase in the activity in both sides but shaded (convex) increases more than the illuminated (concave) in 3 hours of unilateral illumination. In 4 hours unilateral illumination the activity in both sides increases (M.S.V. for T is greater than M.S.V. for P + PT) and the differences between the side means are higher than L.S.D.

It cannot be concluded from the results that the differences between the sides are due to differential longitudinal transport.

The results show that the differential distribution of ^{42}K is correlated with the curvature.

(b) Experiment with coleoptiles of <u>Zea mays</u> (without roots)

An experiment was carried out with the object of studying the distribution of ^{42}K in root-less coleoptiles. Any possible effect due to differential uptake from roots was eliminated.

Seedlings of <u>Zea mays</u> were treated with ^{42}K for 2 hours in a dark cupboard and then roots were removed. Coleoptiles were unilaterally illuminated (vascular bundles were transverse) for 3 and 4 hours. A group of 10 coleoptiles were used for each unilateral illumination. Technique was followed as described in <u>Materials and Methods</u>.

Fig.46 The effect of unilateral illumination on the distribution of ^{42}K in the coleoptiles of Zea mays. Coleoptiles were unilaterally illuminated in root-less condition.

Curvatures in degrees are shown at the top of the figure. $\pm$ is standard error of the mean.

A :- mean counts/min/mg. dry weight

Full circle:- illuminated (concave) side

Open circle:- shaded (convex) side

B :- mean counts/min/10 mg. fresh weight

Full triangle:- illuminated (concave) side

Open triangle:- shaded (convex) side

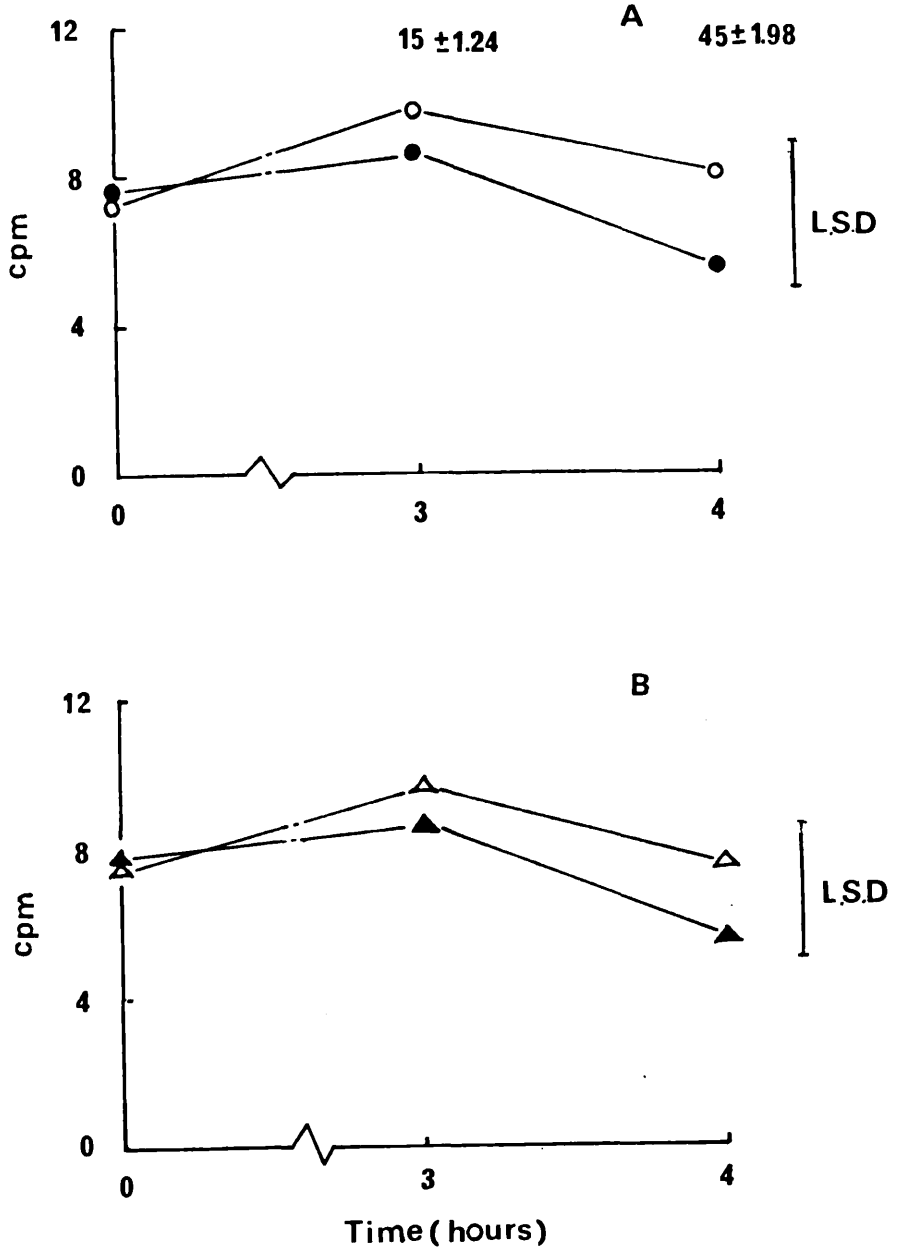


Fig. 46.

Analysis of variance of the results is shown in Table 34 (Appendix). The analysis shows the side (S) effect is not significant.

Fig.46 shows mean counts on dry and fresh weight basis. It indicates that there has been some migration (not significant) of ^{42}K from the illuminated (concave) to the shaded (convex) side. The rises and falls on the two sides are probably due to sampling (i.e. plant) variations. It is quite clear from the analysis of variance Table 34 (Appendix) where M.S.V. for P + PT is greater than M.S.V. for T.

Although the increase in the shaded (convex) side is not significant, the increase is in the shaded side in both periods of illumination and also of the same order as those in the experiments with rooted plants. Non-significance is clearly due to greater variability of material, and perhaps due to the effects of root surgery.

SECTION 3 DISTRIBUTION OF ^{32}P

Experiments similar to the preceding were also carried out for ^{32}P

(a) Experiment with the seedlings of *Zea mays* (with roots)

Seedlings of *Zea mays* were treated with ^{32}P for 6 hours in a dark cupboard. A group of 10 seedlings was used for each period of unilateral illumination. Each period of unilateral illumination consisted of 5 replications. There were three unilateral illuminations (2, 3 and 4 hours) and the orientation of the vascular bundles was transverse as shown in Fig.39. A group of 10 seedlings was treated with ^{32}P

Fig.47 The effect of unilateral illumination on the distribution of ^{32}P in the coleoptiles of Zea mays. Time-course changes in the activity of ^{32}P in the sides.

Curvatures in degrees are shown at the top of the figure. $\pm$ is standard error of the mean.

A :- mean counts/min/mg. dry weight

Full circle:- illuminated (concave) side

Open circle:- shaded (convex) side.

B :- mean counts/min/10 mg. fresh weight

Full triangle:- illuminated (concave) side

Open triangle:- shaded (convex) side

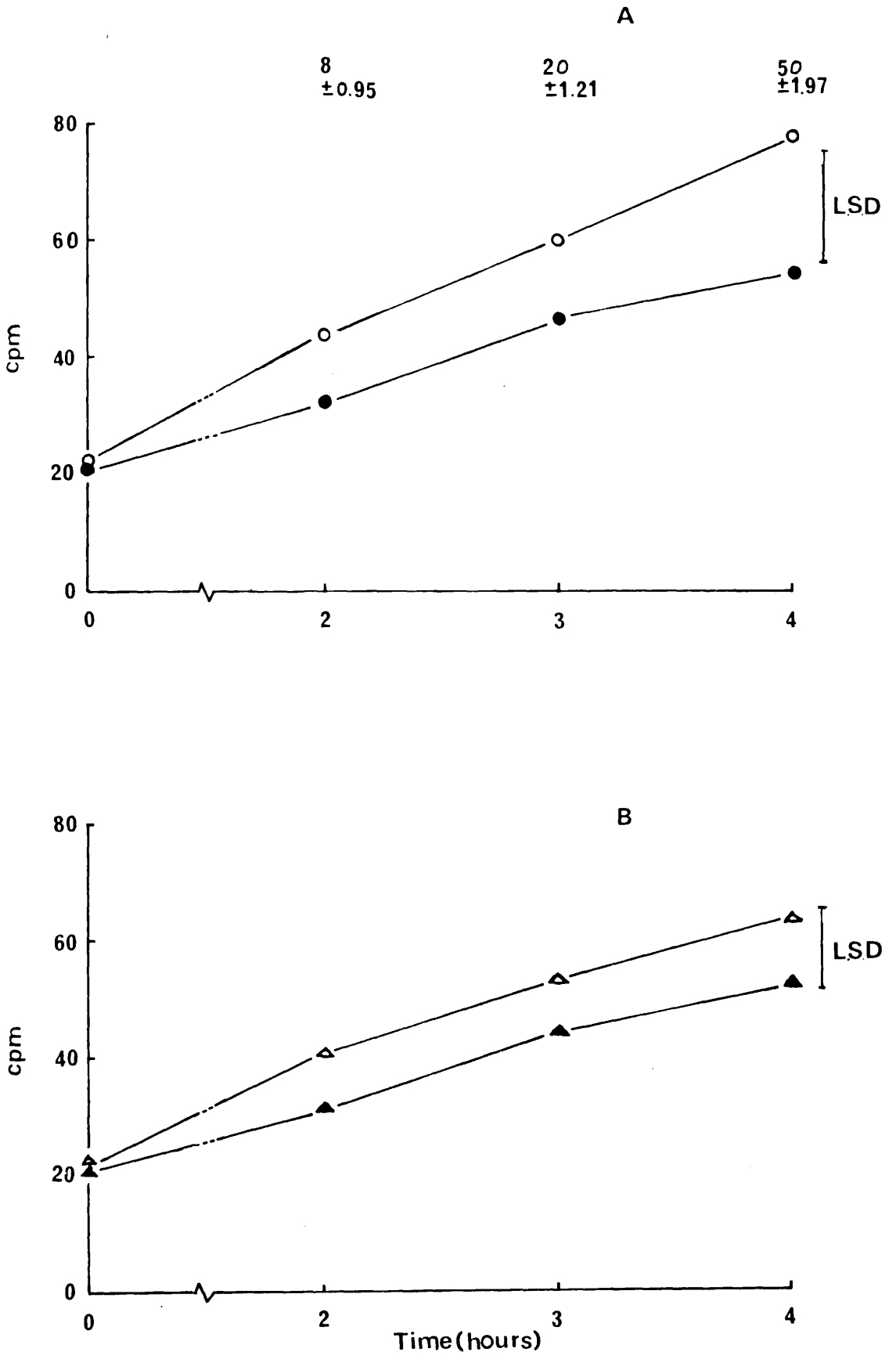

A
8
±0.95
20
±1.21
50
±1.97
80
60
40
20
0
cpm
LSD
0
2
3
4
B
80
60
40
20
0
cpm
LSD
0
2
3
4
Time (hours)

Fig.47.

to establish the initial distribution of radioactivity in the coleoptile halves. Radioactive assay and preparation of samples were carried after unilateral illumination.

Analysis of variance of the results is shown in Table 35 (Appendix). The analysis shows that the side (S) effect is significant which suggests an overall increase in the side.

Fig. 47 shows that there is increase in the shaded (convex) side which is correlated with the curvature. The maximum increase of activity in the shaded (convex) side was found after 4 hours when curvature is 50 degrees $\pm$ 1.97. The figure indicates a continuing transport (M.S.V. for T is greater than M.S.V. for P + PT) from the roots during the period of unilateral illumination.

Hence, the results show that the differential distribution of ^{32}P is correlated with the curvature.

(b) Experiment with <u>Zea</u> coleoptiles (without roots)

An experiment was carried out with root-less coleoptiles. The object was to study the distribution of ^{32}P in absence of continuing transport from roots. This would give an indication about the lateral migration of activity from the illuminated (concave) to the shaded (convex) side.

Seedlings were harvested after pre-treatment with ^{32}P for 6 hours in a dark cupboard. Roots were removed and coleoptiles were unilaterally illuminated for 2, 3 and 4 hours (vascular bunldles were transverse). A group of 10 coleoptiles was used for each period of unilateral illumination. A group of 10 coleoptiles (not illuminated) was used to establish the initial distribution of radioactivity in the halves.

Analysis of variance of the results is shown in Table 36 (Appendix). The analysis shows that the side (S) effect is

significant.

Fig. 48 shows that there has been migration of activity from the illuminated (concave) to the shaded (convex) side. Maximum difference is observed after 4 hours unilateral illumination which is accompanied with the curvature of 40 degrees $\pm$ 1.73. The rises and falls in the graphs are probably due to sampling (i.e. plant) variation. It is clear in the analysis of variance Table 36 (Appendix) where M.S.V. for T is about the same order as M.S.V. for P + PT.

By comparing the results of experiments with rooted and root-less plants it seems likely that the differential distribution is due entirely to lateral migration.

Hence, the results strongly suggest that redistribution of ^{32}P is correlated with the curvature.

Fig.48 The effect of unilateral illumination on the distribution of ^{32}P in the coleoptiles of Zea mays. Coleoptiles were unilaterally illuminated in root-less condition.

Curvatures in degrees are shown at the top of the figure. $\pm$ is standard error of the mean.

A : - mean counts/min/mg. dry weight

Full circle:- illuminated (concave) side

Open circle:- shaded (convex) side

B :- mean counts/min/10 mg. fresh weight

Full triangle:- illuminated (concave) side

Open triangle:- shaded (convex) side

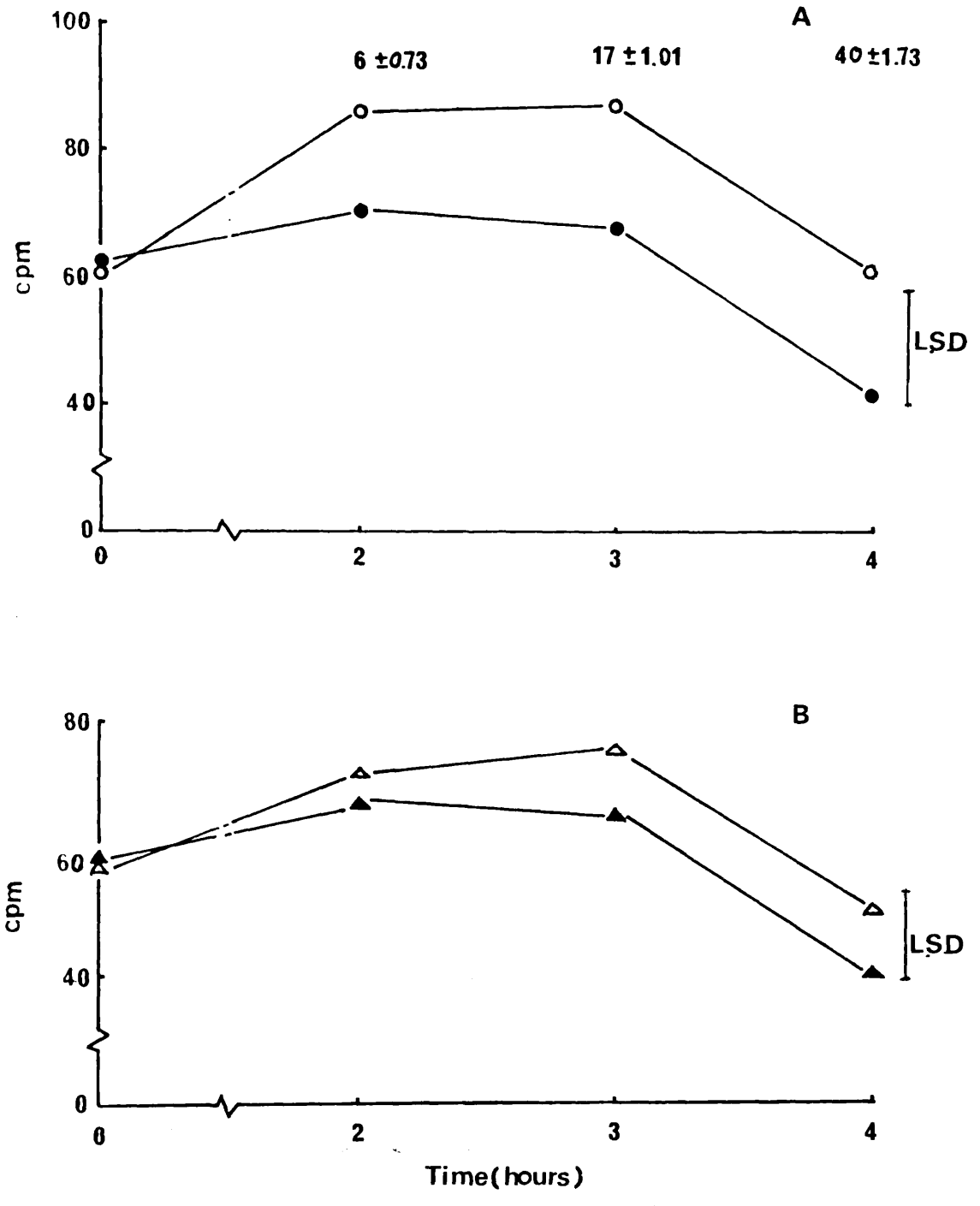

A
100
80
60
40
0
cpm
6 ±0.73
17 ±1.01
40 ±1.73
LSD
0
2
3
4
B
80
60
40
0
cpm
LSD
0
2
3
4
Time(hours)

Fig.48.

CHAPTER VIII

THE EFFECT OF NPA ON THE DISTRIBUTION OF IONS IN UNILATERALLY ILLUMINATED COLEOPTILES

In all preceding experiments of phototropism it was clearly shown that differential distribution of radioactivity (^{45}Ca, ^{42}K and ^{32}P) was correlated with the curvature. It was thought, therefore, to use NPA in the hope that it would inhibit the phototropic curvature. The object was to allow experiments to run parallel to those performed under geotropic stimulation. So the following experiments were carried out with ^{45}Ca, ^{42}K and ^{32}P to study the distribution of radioactivity in unilaterally illuminated coleoptiles when curvature was stopped by the pre-treatment with NPA.

SECTION I DISTRIBUTION OF ^{45}Ca

In this section experiments were carried out with rooted and root-less coleoptiles of *Zea mays* after pre-treatment with ^{45}Ca and NPA.

(a) Experiment with the seedlings of *Zea mays* (with roots)

In this experiment NPA was used at a concentration of 10^{-4}M made up in Hoagland and Arnon solution. Each seedling of *Zea mays* was treated with 2.0 ml of NPA (containing 1 μCi of ^{45}Ca) for 6 hours in a dark cupboard. A group of 10 seedlings was used for each period of illumination. The side of the coleoptile (which would be illuminated) was marked by ink in order to be able to distinguish from the shaded side. Coleoptiles

were unilaterally illuminated for 2 and 3 hours. In this experiment the vascular bundles were transverse to the direction of illumination as shown in Fig.39. A group of 10 seedlings was used to establish the initial distribution of radioactivity halves of coleoptiles (not illuminated). Technique for radioactive assay and preparation of sample was followed as described in the chapter Materials and Methods.

Analysis of variance of the results is shown in Table 37 (Appendix). The analysis shows that the side (S) effect is not significant.

Fig.49 shows mean counts on dry and fresh weight basis in the sides. This does not indicate any differential distribution of radioactivity during unilateral illumination. There were no curvatures in the coleoptiles. The figure indicates that there has probably been transport of ^{45}Ca from the roots during 3 hours unilateral illumination.

These results are in agreement with results of the experiment with NPA in horizontally orientated organs. Hence, no differential distribution occurs in absence of curvature.

(b) Experiment with *Zea* coleoptiles (without roots)

In this experiment roots were removed after pre-treatment with ^{45}Ca and NPA. A group of 10 coleoptiles was used for each period of unilateral illumination. There were three unilateral illuminations (2,3 and 4 hours). Each period had 5 replications. A group of 10 coleoptiles was used to establish initial distribution of radioactivity in the coleoptile halves.

Analysis of variance of the results is shown in Table 38 (Appendix). The analysis shows that the side (S) effect is not significant.

Fig.49 The effect of NPA on the distribution of ^{45}Ca in the unilaterally illuminated coleoptiles of Zea mays. Seedlings did not produce any curvature and hence curvature is shown as 0 at the top of the figure.

A :- mean counts/min/mg. dry weight

Full circle:- illuminated side

Open circle:- shaded side

B :- mean counts/min/10 mg. fresh weight

Full triangle:- illuminated side

Open triangle:- shaded side

Fig.50 The effect of NPA on the distribution of ^{45}Ca in the unilaterally illuminated coleoptiles of Zea mays. Coleoptiles were unilaterally illuminated in the root-less condition.

A :- mean counts/min/mg. dry weight

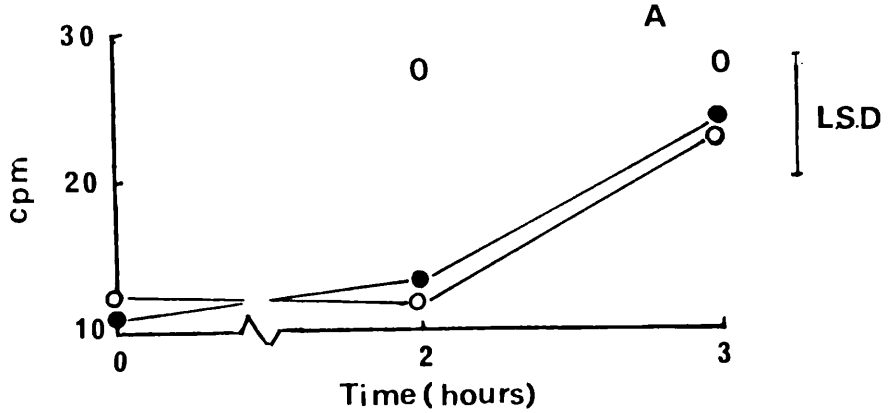
Full circle:- illuminated side

Open circle:- shaded side

B :- mean counts/min/10 mg. fresh weight

Full triangle:- illuminated side

Open triangle:- shaded side.


A
30
20
10
cpm
0
0
LSD
0
2
3
Time (hours)

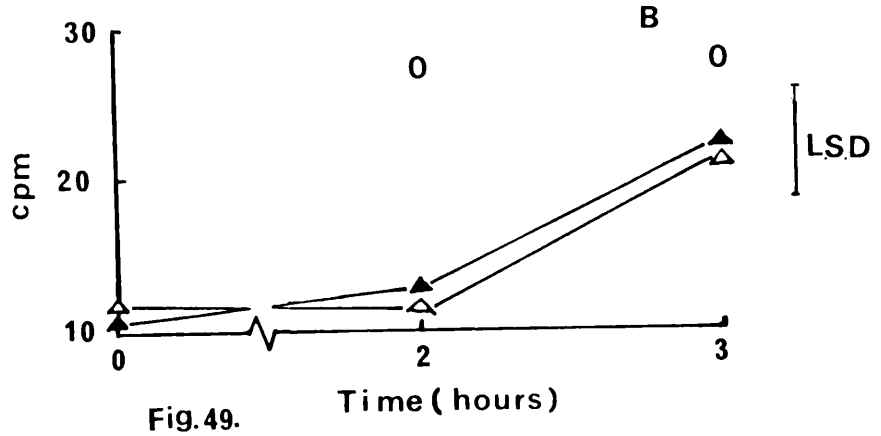
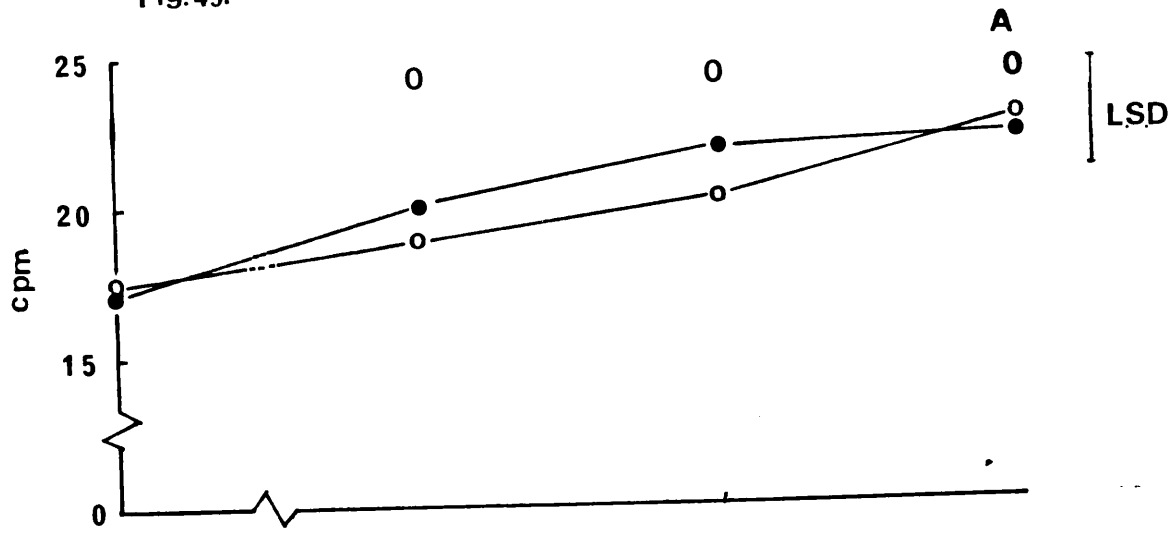

B
30
20
10
cpm
0
0
LSD
0
2
3
Time (hours)

Fig. 49.


A
25
20
15
0
cpm
0
0
0
LSD

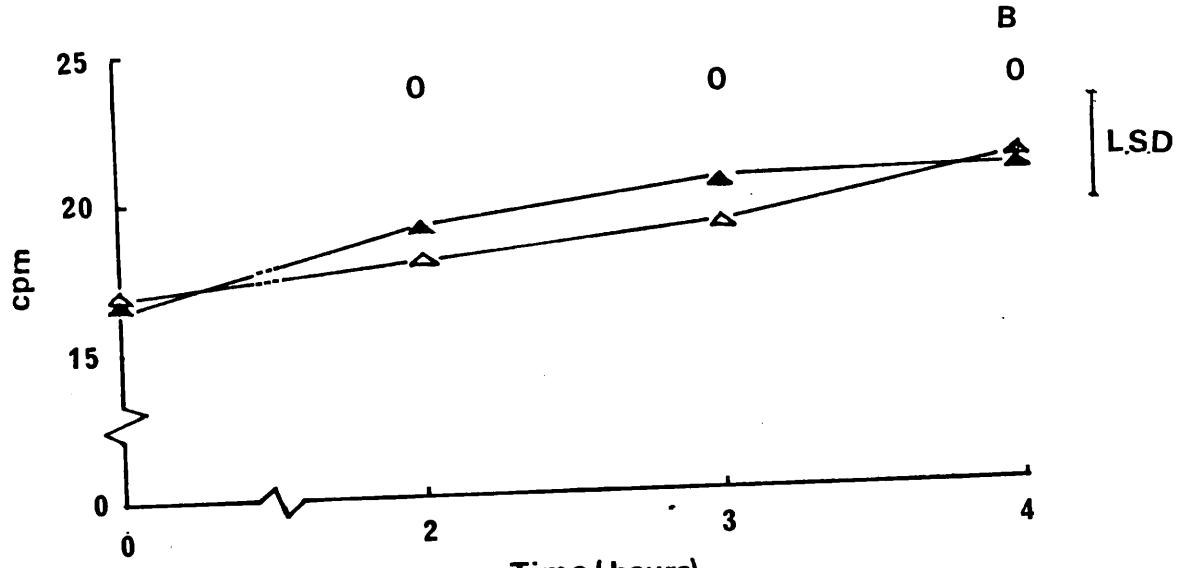

B
25
20
15
0
cpm
0
0
0
LSD
0
2
3
4
Time (hours)

Fig. 50.

Fig.50 shows mean counts on dry and fresh weight basis in the sides. It clearly indicates that there has not been any differential distribution of radioactivity in the coleoptiles. There were no curvatures in the coleoptiles. The increase in activity in both sides with time is probably due to sampling (i.e. plant) error. It is clear in analysis of variance Table 38 (Appendix) where M.S.V. for T is about the same order as M.S.V. for P + PT.

The results strongly suggest that differential distribution of ^{45}Ca does not occur in the absence of curvature which is in agreement with the last experiment. This redistribution is probably caused by curvature.

SECTION 2 DISTRIBUTION OF ^{42}K

Experiments similar to the preceding were also carried out for ^{42}K.

(a) Experiment with the seedlings of *Zea mays* (with roots)

Seedlings of *Zea mays* were treated with NPA (conc.10^{-4}M) and ^{42}K for 6 hours in a dark cupboard. A group of 10 seedlings was used for each period of unilateral illumination. There were three periods of unilateral illumination (2, 3 and 4 hours). A group of 10 seedlings was used to establish the initial distribution in the coleoptile halves. Each unilateral illumination had 5 replications. Coleoptiles were unilaterally illuminated. Radioactive assay and preparation of sample were carried out as described in Materials and Methods.

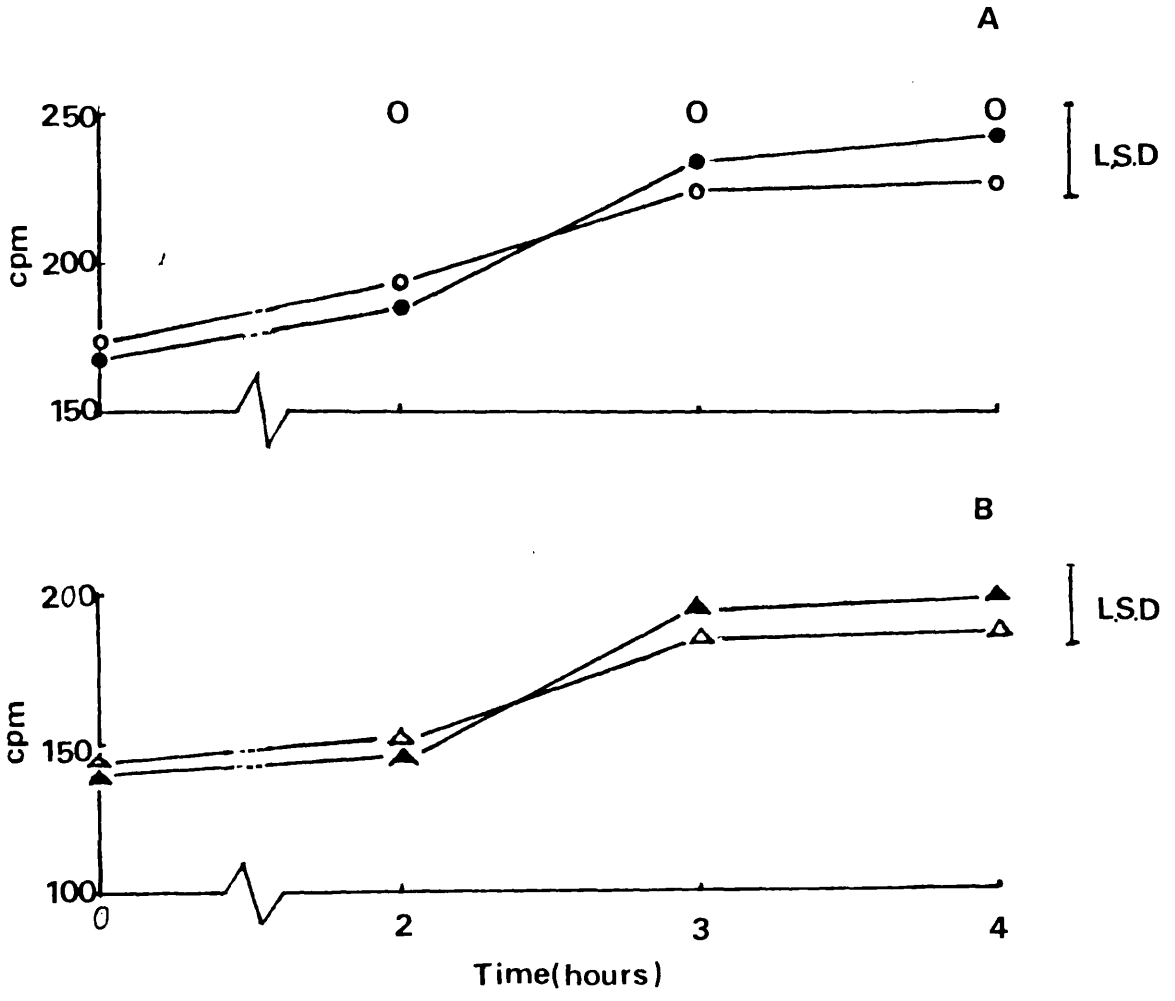


Fig. 51. The effect of NPA on the distribution of ^{42}K in the unilaterally illuminated coleoptiles (with roots) of Zea mays.

Coleoptiles did not produce any curvature and hence, it is shown as 0 at the top.

A :- mean counts/min/mg. dry weight

Full circle :- illuminated side

Open circle :- shaded side

B :- mean counts/min/10mg. fresh weight

Full triangle :- illuminated side

Open triangle :- shaded side

Analysis of variance of the results is shown in Table 39 (Appendix). The analysis shows that the side (S) effect is not significant.

Fig. 51 shows distribution of radioactivity in the sides. The figure does not indicate any significant increase in any particular side. There were no curvatures in the coleoptiles. The figure shows some increase in transport of ^{42}K with coleoptiles during unilateral illumination, but this is not significant (as M.S.V. for P + PT is actually greater than M.S.V. for T).

Hence, the results suggest that there has not been any differential distribution of ^{42}K which shows a correlation with the curvature.

(b) Experiment with Zea coleoptiles (without roots)

This experiment was carried out with a view to eliminate any transport of ^{42}K during unilateral illumination.

Seedlings of Zea mays were treated with ^{42}K and NPA for 6 hours in a dark cupboard. Roots were removed and coleoptiles were unilaterally illuminated for 2, 3 and 4 hours. A group of 10 coleoptiles was used for each period of unilateral illumination. Each period consisted of 5 replications. A group of 10 coleoptiles (not illuminated) was used to establish the initial distribution of activity in the halves.

Analysis of variance of the results is shown in Table 40 (Appendix). The analysis shows that the side (S) effect is not significant.

Fig. 52 shows mean counts on dry and fresh weight basis. It does not indicate any increase of radioactivity in any particular side. The differences between the side means are lower than L.S.D. There were no curvatures after unilateral

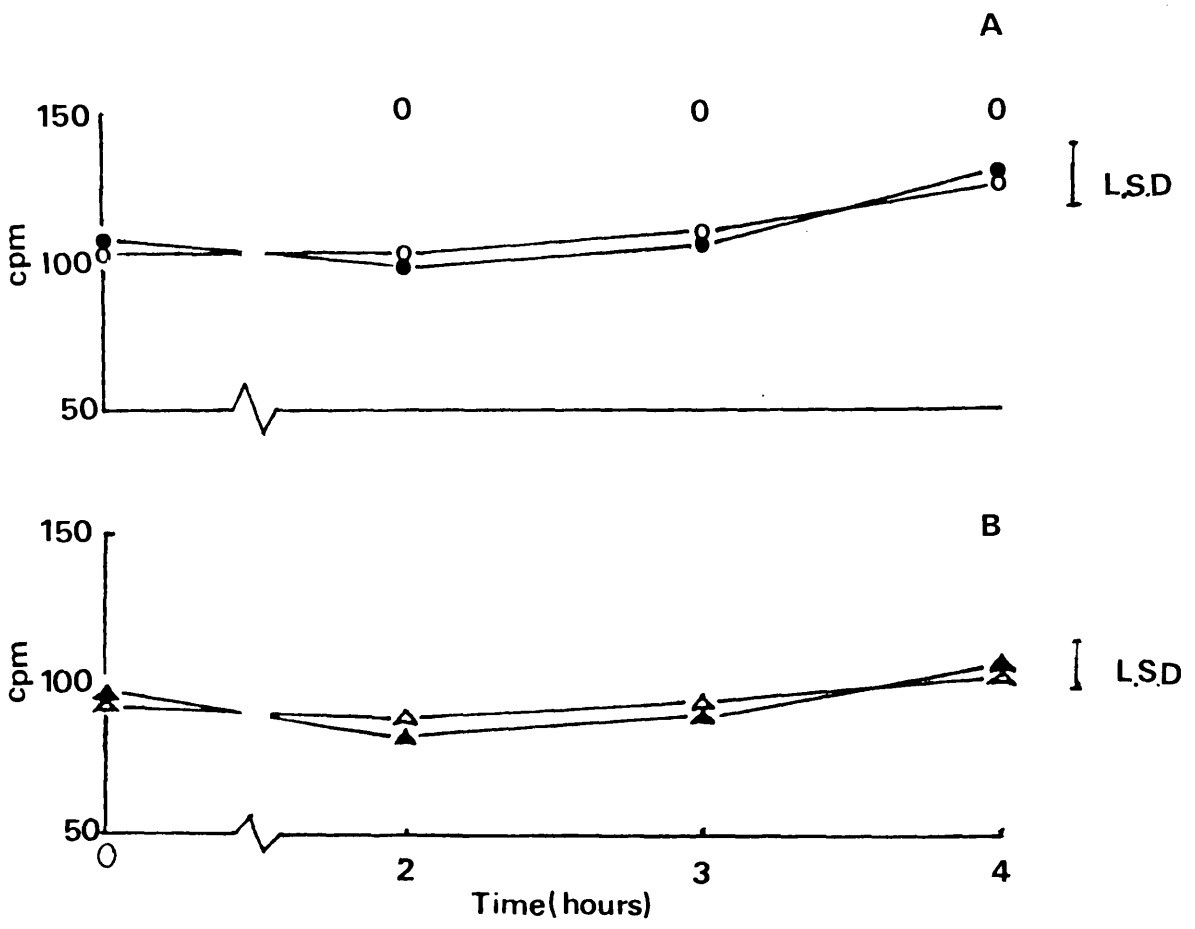


Fig. 52. The effect of NPA on the distribution of ^{42}K in the unilaterally illuminated coleoptiles (without roots) of Zea mays.

Coleoptiles did not produce any curvature and hence, it is shown as 0 at the top.

A :- mean counts/min/mg. dry weight

Full circle :- illuminated side

Open circle :- shaded side

B :- mean counts/min/10mg. fresh weight

Full triangle :- illuminated side

Open triangle :- shaded side

illumination.

Hence, the results are in agreement with the results of the last experiment which do not suggest any differential distribution of ^{42}K due to the lack of curvature. This differential distribution is the result of curvature.

SECTION 3 DISTRIBUTION OF ^{32}P

Experiments similar to the preceding were also carried out for ^{42}K.

(a) Experiment with the seedlings of Zea mays (with roots)

Seedlings of Zea mays were treated with ^{32}P and NPA (conc. $10^{-4}M$) for 6 hours in a dark cupboard. A group of 10 seedlings was used for each period of unilateral illumination. Coleoptiles were unilatterally illuminated for 2, 3 and 4 hours. Each period consisted of 5 replications. A group of 10 seedlings was also included to establish the initial distribution of radioactivity in the halves.

Analysis of variance of the results is shown in Table 41 (Appendix). The analysis of variance shows that the side (S) effect is not significant.

Fig. 53 shows mean counts on dry and fresh weight basis in the sides. It does not indicate any differential distribution of radioactivity. The figure shows some early increase in activity in the coleoptile as a whole, but this is not significant.

Hence, the results strongly suggest that differential distribution of ^{32}P does not occur in absence of curvature.

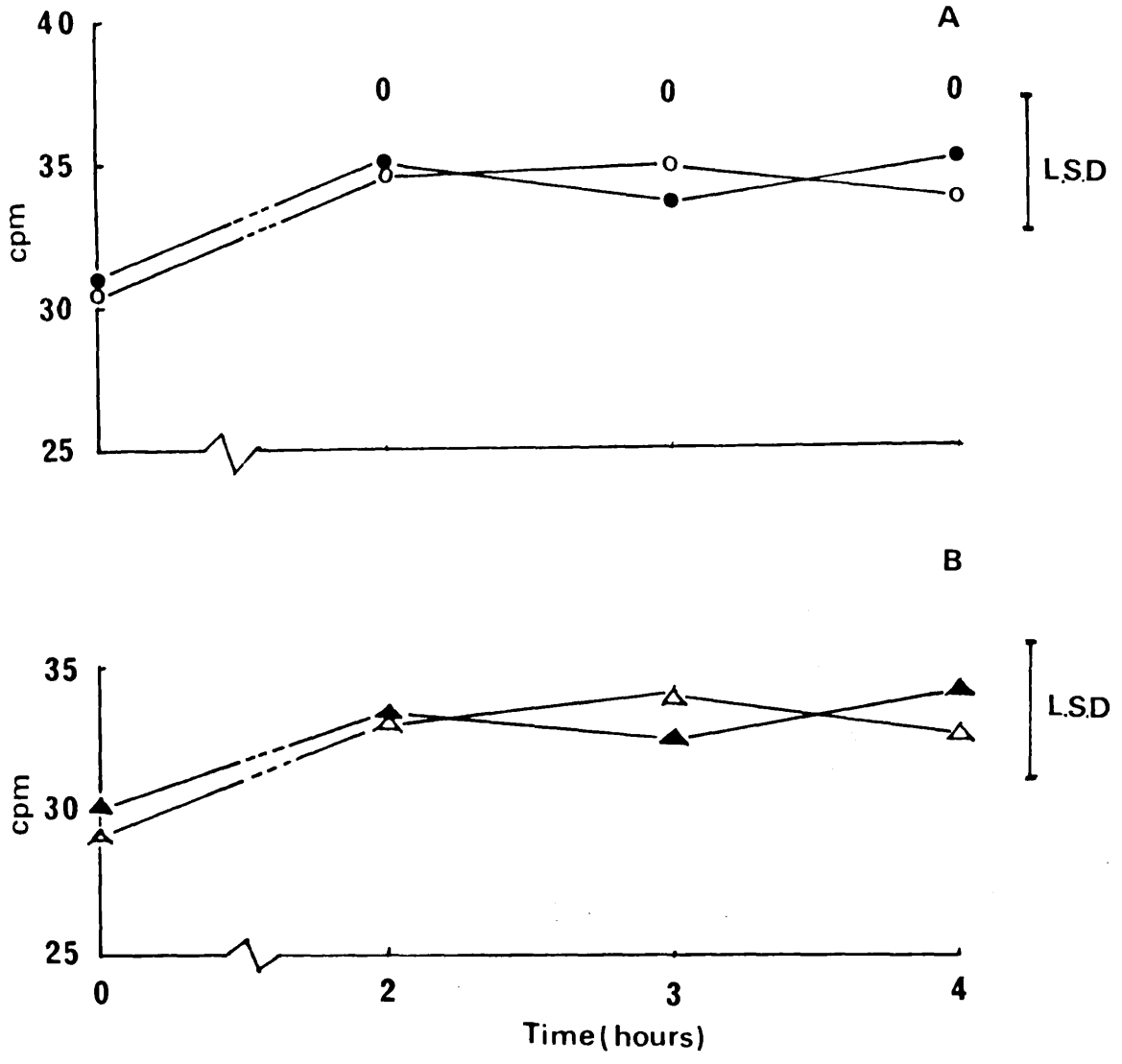


Fig. 53. The effect of NPA on the distribution of ^{32}P in the unilaterally illuminated coleoptiles (with roots) of Zea mays.

Coleoptiles did not produce any curvature and hence, it is shown as 0 at the top.

A :- mean counts/min/mg. dry weight

Full circle :- illuminated side

Open circle :- shaded side

B :- mean counts/min/10mg. fresh weight

Full triangle :- illuminated side

Open triangle :- shaded side

(b) Experiment with Zea coleoptiles (without roots)

In this experiment roots were removed prior to illumination so as to ensure that there was no transport of activity from roots during unilateral illumination.

Seedlings of Zea mays were treated with NPA and ^{32}P for 6 hours in a dark cupboard. Seedlings were harvested and roots were removed. Coleoptiles were unilaterally illuminated for 2, 3 and 4 hours. A group of 10 coleoptiles was used for each period of illumination. Each period consisted of 5 replications. A group of 10 coleoptiles was used to establish the initial distribution of radioactivity in the halves.

Analysis of variance of the results is shown in Table 42 (Appendix). The analysis shows that the side (S) effect is not significant.

Fig. 54 shows mean counts on dry and fresh weight. It does not indicate any differential distribution of activity. It shows a general drop in activity in the coleoptiles as compared to the initial (M.S.V. for T is greater than M.S.V. for P + PT). This general drop in activity could probably be attributed to the movement of ^{32}P into water in which coleoptiles were standing vertically during the period of illumination. Coleoptiles did not show any curvature.

The results strongly suggest that there is lack of differential distribution in absence of curvature which is in agreement with the results of the last experiment. Hence, differential distribution is probably caused by curvature or by some other changes which also induce curvature.

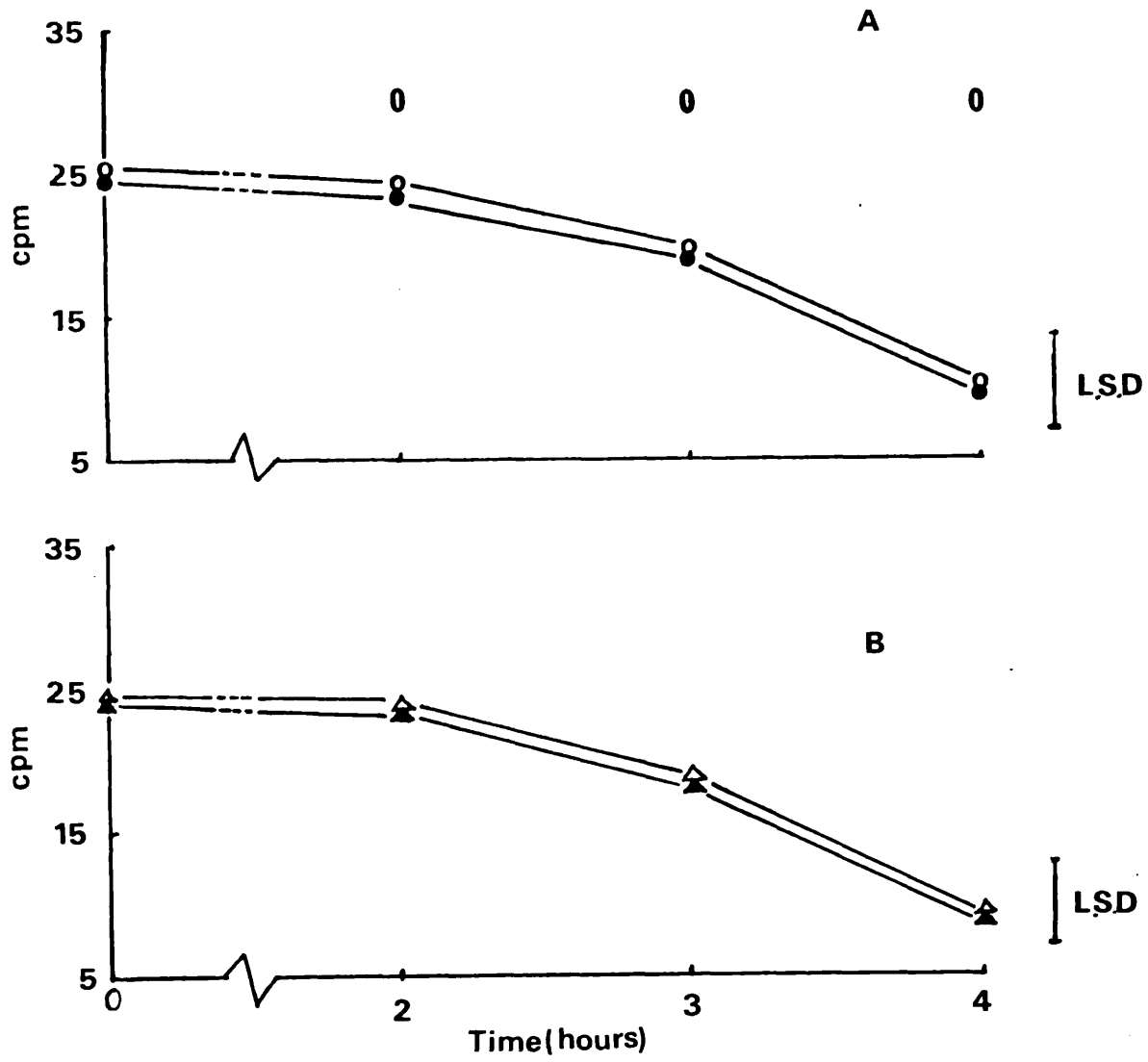


Fig. 54. The effect of NPA on the distribution of ^{32}P in the unilaterally illuminated coleoptiles (without roots) of Zea mays.

Coleoptiles did not produce any curvature and hence, it is shown as 0 at the top.

A :- mean counts/min/mg. dry weight

Full circle :- illuminated side

Open circle :- shaded side

B :- mean counts/min/10 mg. fresh weight

Full triangle :- illuminated side

Open triangle :- shaded side

CHAPTER IX

THE EFFECT OF UNILATERAL APPLICATION OF MERSALYL ON THE DISTRIBUTION OF IONS

In all preceding experiments it has been observed that differential distribution of the three elements (Ca, K, P) was correlated with the curvature. The lack of differential distribution in case of seedlings pre-treated with NPA suggests that differential distribution is probably the result of differential growth reaction because it happens both with geotropism and phototropism. Unilateral application of IAA results in differential distribution of ions which is associated with the curvature. Woodcock and Wilkins' (1970) study of unilateral application of IAA led them to believe that IAA probably brings about changes in membrane permeability or acts through inhibition and enhancement of ion pumps. The generation of surface potential differences resulting from the unilateral application of IAA is quite comparable to the geo-electric effects which develop in horizontally orientated organs. The lower side becomes more electropositive as compared to the upper side. Unilateral IAA application resulted into generation of electro-potential differences even though growth has been checked osmotically by mannitol (Woodcock and Wilkins, 1971). This suggests surface potential differences are the result of IAA concentration gradient. This led them to use mersalyl which could act in the generation of surface potential differences without affecting the growth of the organ. Mersalyl is a sodium salt of O - [(3-hydroxymercurie-2-methoxypropyl) carbonyl] phenoxy-acetic acid. This substance is known to be effective diuretic

and it increases the flow of urine from the kidney. It does effectively stop the reabsorption of sodium in the proximal part of the tubule. As already discussed in the Introduction that unilateral mersalyl application resulted into increase of 14 m V electropositive on the "donor" side with respect to the "other" side 18 minutes after application. This suggests that IAA and mersalyl both have potential inducing effects. In my experiments of unilateral IAA application, there was an increase in accumulation of potassium and phosphorus on the "donor" side and an accumulation of calcium on the "other" side. The object of the following experiments was to study the distribution of ions after application of mersalyl and to see whether any kind of parallelism exists between its effects and those of IAA on ion distribution and on curvature. It was thought necessary to check whether the parallelism in the potential inducing effects of IAA and mersalyl could be extended to ion distribution.

SECTION I DISTRIBUTION OF ^{45}Ca.

(a) Experiment with the seedlings of *Zea mays* (with roots)

Seedlings of *Zea mays* were treated with ^{45}Ca for 6 hours in a dark cupboard. Hydrated paste of mersalyl in lanolin was made up at a concentration of 10^{-4}M. A group of 10 seedlings was used for each period of mersalyl application. There were three different periods (20,40 and 60 minutes). A group of 10 seedlings was used to determine the initial distribution of radioactivity in the coleoptile halves. Each period of application consisted of 5 replications. Coleoptiles were decapitated about 1 mm. from the tip and a tiny drop of mersalyl

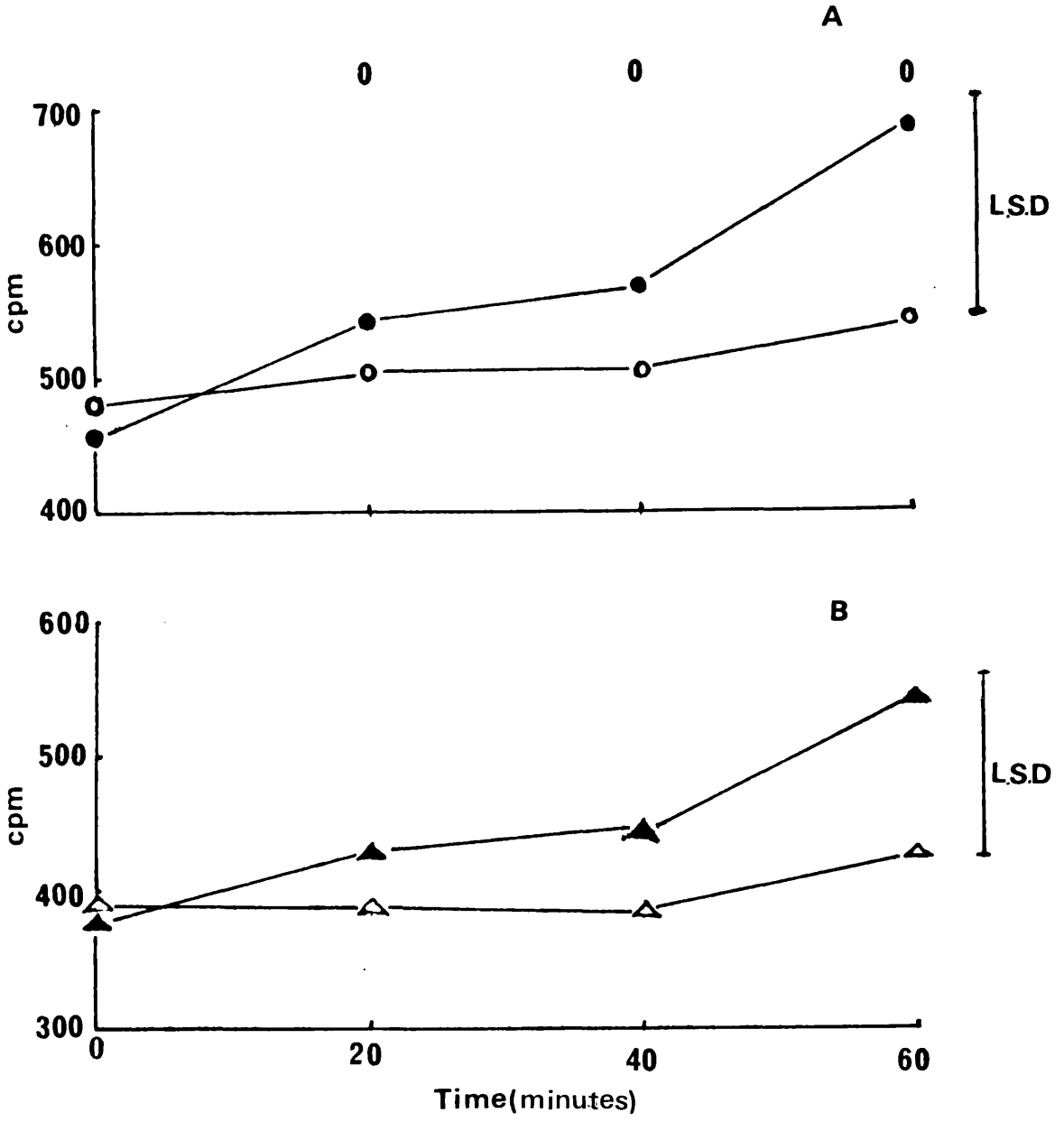


Fig. 55. The effect of unilateral application of mersalyl (10^{-4}M in hydrated lanolin paste) on the distribution of ^{45}Ca in the coleoptiles (with roots) of Zea mays. Coleoptiles did not produce any curvature and hence, it is shown as 0 at the top.

A :- mean counts/min/mg. dry weight

Full circle :- other side

Open circle :- donor side

B :- mean counts/min/10mg. fresh weight

Full triangle :- other side

Open triangle :- donor side

was applied unilaterally to the decapitated end. The "other" side of the coleoptile was marked by ink in order to be able to distinguish from the donor side. After mersalyl application seedlings were put back into a dark cupboard for the periods mentioned above. Radioactive assay and preparation of sample were carried out as described in the chapter Materials and Methods.

Analysis of variance of the results is shown in Table 43 (Appendix). The analysis shows that the side (S) effect is not significant.

Fig. 55 shows mean counts on dry and fresh weight basis in the halves. This indicates that in the "other" side there is first a small rise (not significant) and then a greater rise (this is also not significant), whereas in the "donor" side there is first no detectable change and then a small rise (not significant). The figure indicates that there has been some migration of ^{45}Ca from the "donor" to the "other" side. The figure shows that there has been some transport from the roots during mersalyl application but this is not significant (as M.S.V. for T is of the same order as M.S.V. for P + PT). There were no curvatures in the coleoptiles. Anyhow, the results show a change in the same direction as for IAA.

The results suggest a possible movement of ^{45}Ca away from the "donor" side and the movement is not associated with the curvature.

(b) Experiment with Zea coleoptiles (without roots)

An experiment was carried out with root-less coleoptiles of Zea mays. The object was to see whether any migration occurs from "donor" to the "other" side after mersalyl application.

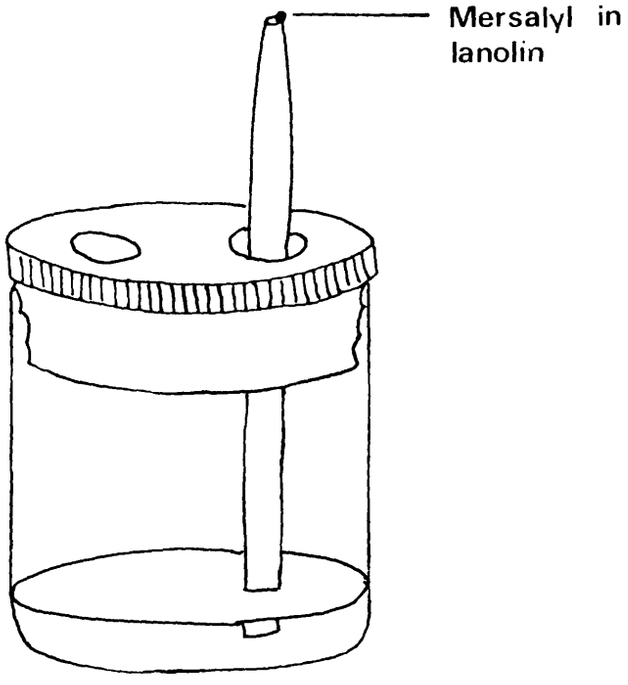


Fig.56. Unilateral application of mersalyl to the apex of a decapitated vertical coleoptile of Zea mays.

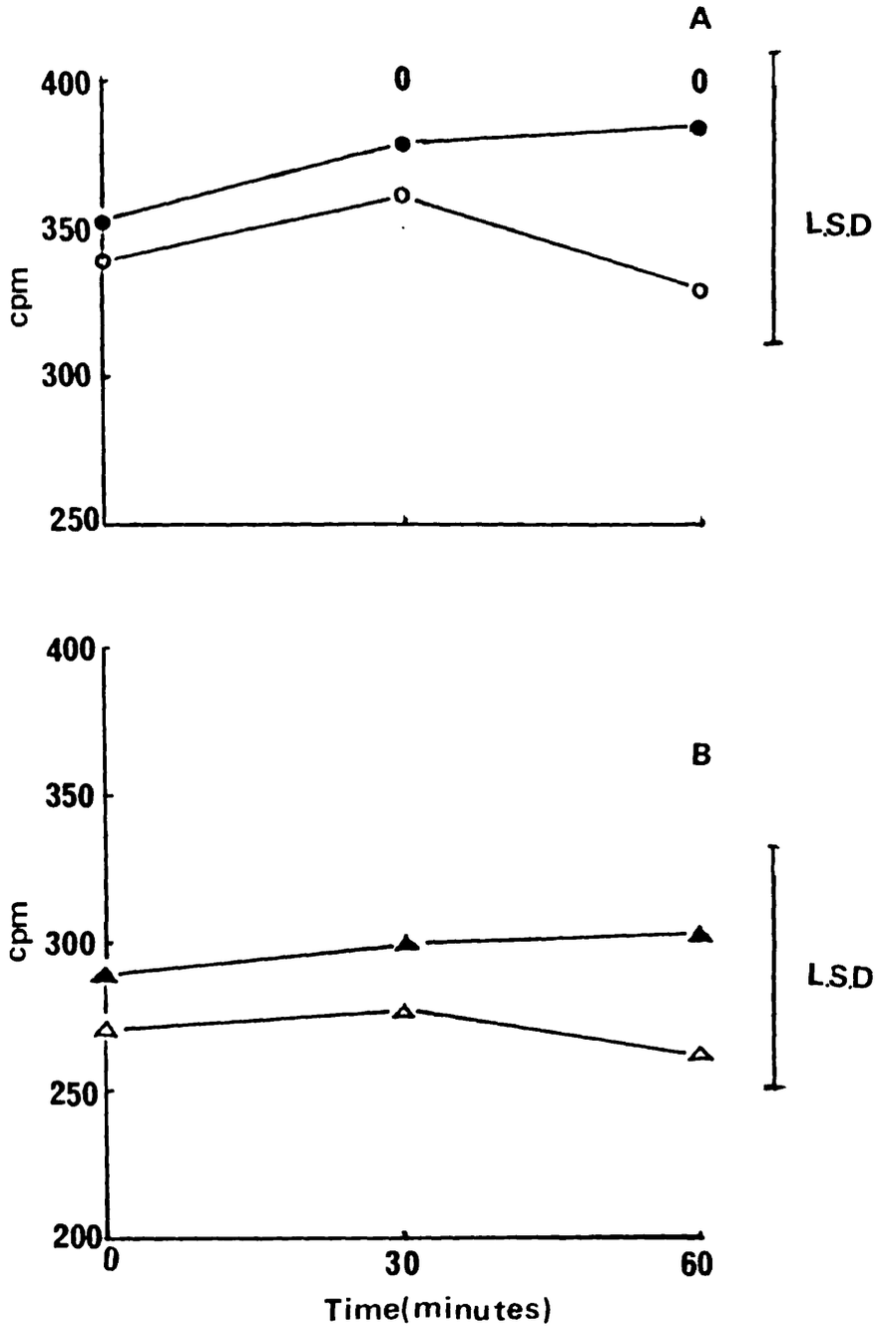


Fig. 57. The effect of unilateral application of mersalyl (10^{-4}M in hydrated lanolin paste) on the distribution of ^{45}Ca in the coleoptiles (without roots) of Zea mays. Coleoptiles did not produce any curvature and hence, it is shown as 0 at the top.

A :- mean counts/min/mg. dry weight

Full circle :- other side

Open circle :- donor side

B :- mean counts/min/10mg. fresh weight

Full triangle :- other side

Open triangle :- donor side

Seedlings of Zea mays pre-treated with ^{45}Ca were harvested and roots were removed. Coleoptiles were placed in vertical position in small glass tubes (size 1" x 1") containing some distilled water (Fig.56). Each glass tube was covered from the top by a plastic cap with two small holes through which coleoptiles could be inserted. A group of 10 coleoptiles was used for each mersalyl application. There were two periods of mersalyl application (30 and 60 minutes). Each consisted of 5 replications. A group of 10 coleoptiles was included to determine the initial distribution of activity in the halves.

Analysis of variance of the results is shown in Table 44 (Appendix). The analysis shows that the side (S) effect is not significant.

Fig.57 shows distribution of radioactivity in the halves. The figure shows a possible movement of ^{45}Ca away from the "donor" side (but this is not significant). There was no curvature in the coleoptiles.

Since the results of the experiments with rooted and rootless plants are consistent and show the same trends we can come to the tentative conclusion that mersalyl probably does effect the distribution of ^{45}Ca without producing a differential growth response.

SECTION 2 DISTRIBUTION OF ^{42}K

Experiments similar to the prrceding were also carried out for ^{42}K.

(a) Experiment with the seedlings of Zea mays (with roots)

Seedlings of Zea mays were treated with ^{42}K for 2 hours

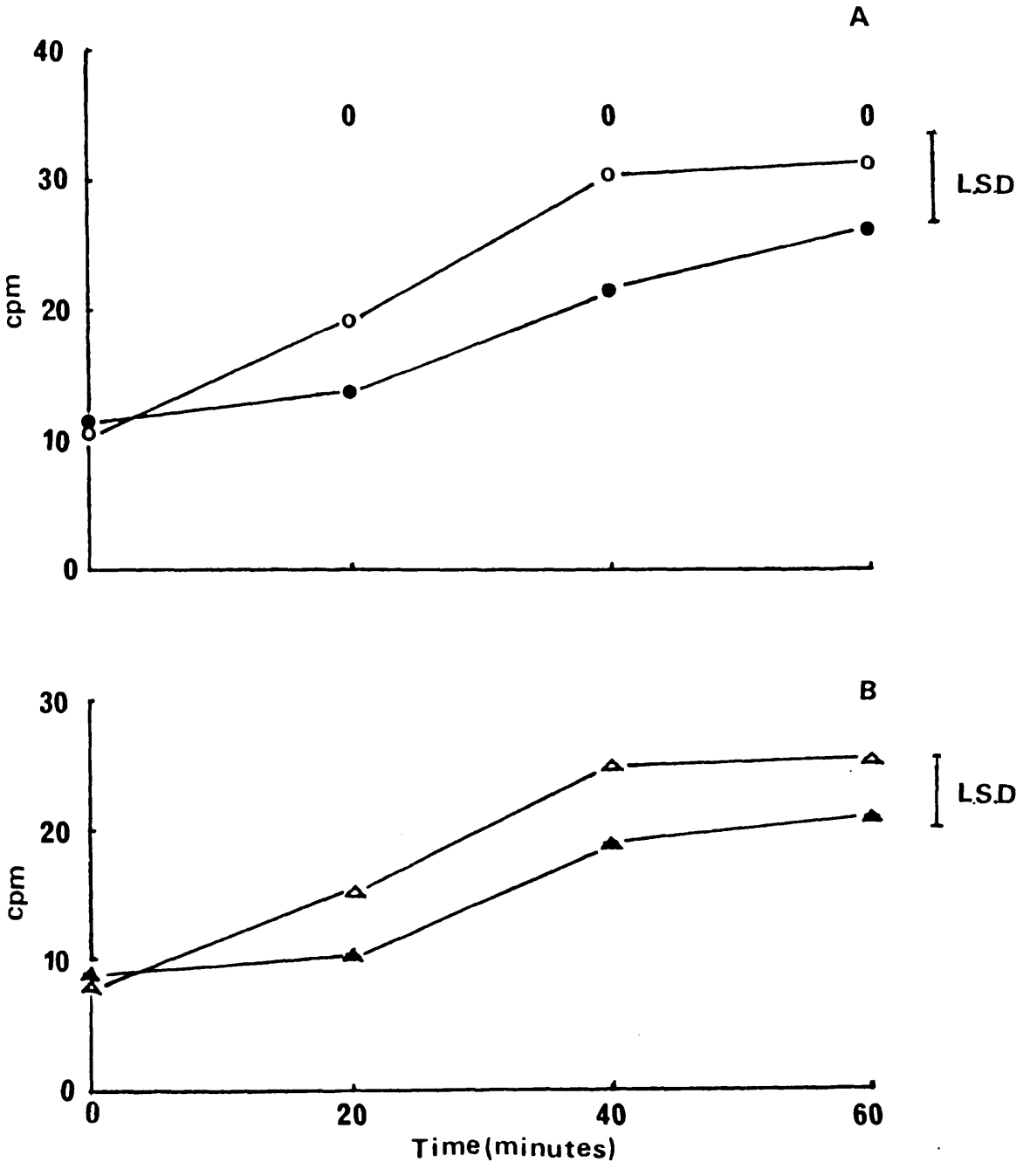


Fig. 58. The effect of unilateral application of mersalyl (10^{-4}M in hydrated lanolin paste) on the distribution of ^{42}K in the coleoptiles (with roots) of Zea mays. Coleoptiles did not rpoduce any curvature and hence, it is shown as 0 at the top.

A :- mean counts/min/mg. dry weight

Full circle :- other side

Open circle :- donor side

B :- mean counts/min/10mg. fresh weight

Full triangle :- other side

Open triangle :- donor side

in a dark cupboard. Hydrated paste of mersalyl (conc.10^{-4}M) was applied to the decapitated coleoptile tips. There were three periods of unilateral application (20, 40 and 60 minutes). A group of 10 seedlings was used for each application. Each application consisted of 5 replications. A group of 10 seedlings was used to establish the initial distribution of radioactivity in the halves. Radioactive assay and preparation of sample were carried out as described in Materials and Methods.

Analysis of variance of the results is shown in Table 45 (Appendix). The analysis shows that the side (S) effect is significant.

Fig.58 shows mean counts on dry and fresh weight basis in the "donor" and "other" sides. This indicates that there has been accumulation of ^{42}K with the greatest concentration on the mersalyl "donor" side. This figure shows that the activity in both sides increases with time (M.S.V. for T is of the same order as M.S.V. for P + PT), but "donor" increases more than the "other". The differences between the means are higher than L.S.D. after 40 minutes mersalyl application. There were no curvatures in the coleoptiles after mersalyl application.

Hence, the results suggest that mersalyl brings about differential distribution of potassium without producing differential growth. Increased accumulation of ^{42}K after mersalyl application is in the same direction as IAA.

(b) Experiment with Zea coleoptiles (without roots)

An experiment was carried out to study the effect of unilateral application of mersalyl on the distribution of ^{42}K in the root-less coleoptiles. The object was to see whether there is any lateral migration of activity from the "other" side to the "donor" side as a result of mersalyl application.

Seedlings of Zea mays pre-treated with ^{42}K were harvested and roots were removed. Coleoptiles were placed vertical in small glass tubes as shown in Fig.56. A group of 10 coleoptiles was used for each period of mersalyl application. There were three periods of mersalyl application (20, 40 and 60 minutes). Each period of application consisted of 5 replications. A group of 10 coleoptiles was included to establish the initial distribution of ^{42}K in the halves.

Analysis of variance of the results is shown in Table 46 (Appendix). The analysis shows that the side (S) effect is significant.

Fig.59 shows distribution of radioactivity in the sides. This indicates that there has been increase in the "donor" side with corresponding decrease in the "other" side (but this is not significant). Coleoptiles did not produce any curvature. L.S.D. of the means show that the differences between the side means are not significant at any one time. Although the increase in the "donor" side is not significant, the direction of the effects is consistent with the effects seen in the rooted plants.

Hence, the results in this section suggest that increase of ^{42}K in the "donor" side after mersalyl application is in the same direction as IAA but mersalyl does not produce a differential growth response.

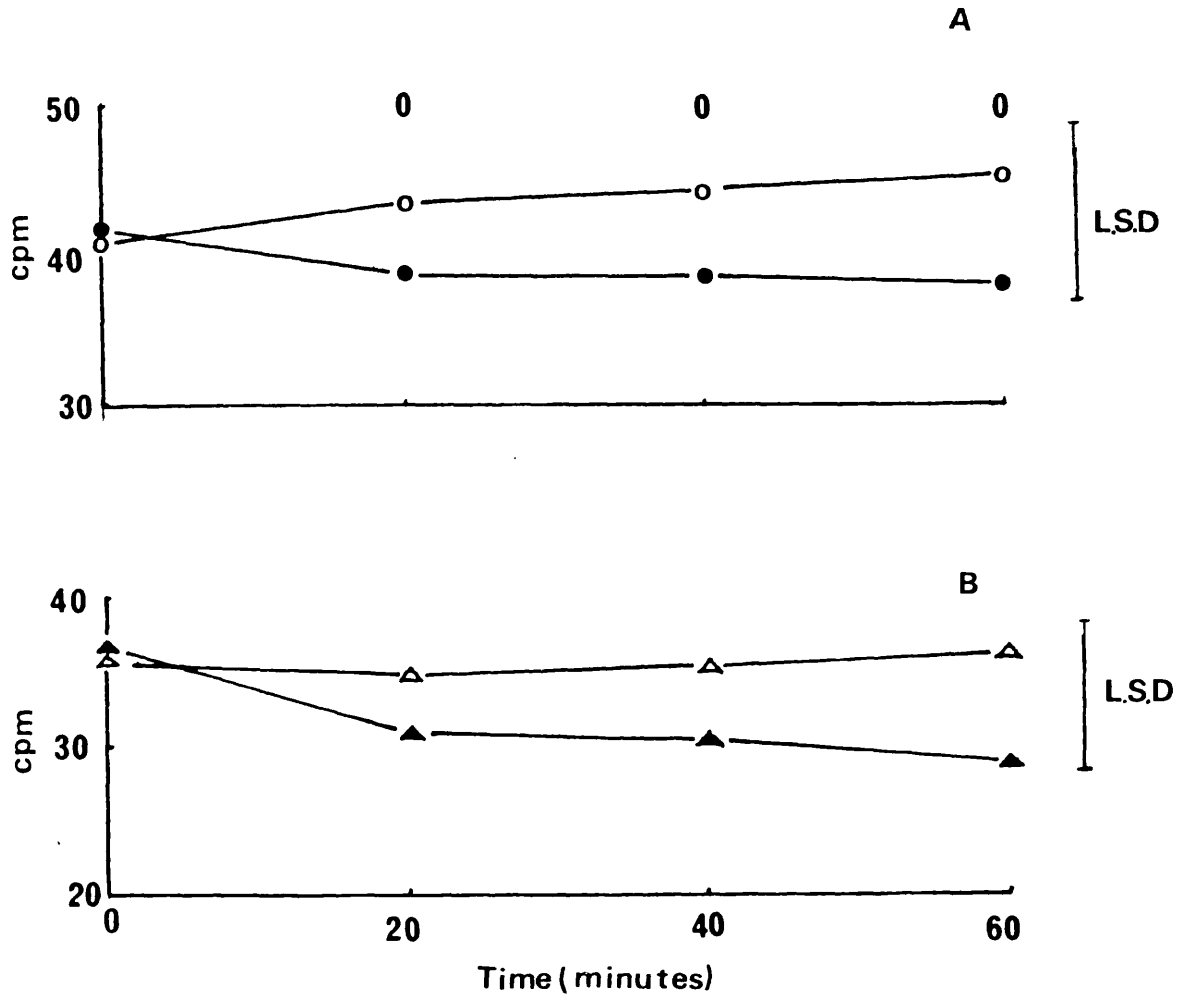


Fig. 59. The effect of unilateral application of mersalyl (10^{-4}M in hydrated lanolin paste) on the distribution of ^{42}K in the coleoptiles (without roots) of Zea mays. Coleoptiles did not produce any curvature and hence, it is shown as 0 at the top.

A :- mean counts/min/mg. dry weight

Full circle :- other side

Open circle :- donor side

B :- mean counts/min/10mg. fresh weight

Full triangle :- other side

Open triangle :- donor side

CHAPTER X

DISCUSSION AND CONCLUSION

It has been shown in earlier chapters that results show a correlation of ion distribution with geotropic response. The earlier workers (Bode 1959b, Arslan-Cerim, 1966) did not study any correlation of ion distribution with geotropic response.

In Chapter III results show that the differential distribution of ions is observed only after the appearance of geotropic curvature. The increased accumulation of calcium in the upper side was correlated with the curvature (Section 1). The results suggest that a migration of calcium from lower to upper side is established at an early stage of geotropic response (Figs. 8 and 12). There is no evidence for a differential longitudinal transport from the roots although the results show increased accumulation of calcium into hypocotyl as a whole during geotropic response (Fig.8). The concentration of calcium in the upper side increases with time which is in agreement with the results of Arslan-Cerim (1966). It cannot be decided from her results whether these differences between the upper and lower side are due to differential longitudinal transport from roots or due to migration from lower to upper side. The reality of lateral migration from lower to upper side is clearly shown in Figs. 11 and 13 for root-less seedlings. The increased accumulation of calcium in the upper side of root-less hypocotyls and coleoptiles again shows a correlation with the curvature. On the basis of the results presented in Section 2 (Chapter III) it may be suggested that there is a strong indication of a concentration gradient of potassium that is

established across the hypocotyl during geotropic curvature. The increase in potassium concentration in the lower side is correlated with the curvature (Fig.14). The figure indicates that lateral migration of potassium is established at an early stage of geotropic response since there is a drop in potassium concentration in the upper side with a corresponding increase in the lower side after 1 hour horizontal orientation. The results (Fig.14) show an increased transport of potassium from the roots into hypocotyl as a whole.(Time effect is significant Table 5 Appendix). The concentration of potassium in the lower side increases with time and also with the curvature. Bode's (1959b) results showed a maximum increase of 5% in potassium concentration in the lower side of the hypocotyl which did not increase with time. He failed to provide an adequate reason for the drop in potassium concentration in the lower side after 120 minutes of geotropical stimulation as compared to the potassium concentration after 90 minutes. Bode explained this drop in terms of a basally directed movement of potassium going to the roots. The idea of lateral migration of potassium from upper to lower side receives confirmation from the results (Fig.16), which clearly show that the increased potassium concentration in the lower side of hypocotyl is at the expense of migration from the upper side. The results discussed in Section 3 (Chapter III) show that the increase of phosphorus in the lower side is correlated with the curvature (Fig.17). The experiment with root-less seedlings (Fig.18) suggests that the redistribution of phosphorus can only be explained in terms of a lateral migration. This can be clearly observed if we compare Figs. 17 and 18. Hence, it

can be summarized that the increase of calcium in the upper side and the increase of potassium and phosphorus in the lower side are occurring simultaneously with geotropic response. The increase in concentration of any particular ion in any side results from its migration from the other side. This increase is the result of greater accumulation of ion in any side since results suggest an increase both on a dry and a fresh weight basis. So this increase is not the result of increase or decrease in cell volume due to differential growth. Hence, the results strongly suggest that a concentration gradient of ion is established which is associated with the curvature.

It has been possible to investigate the distribution of ions during geoinduction. Seedlings were given enough stimulus ($\frac{1}{2}$ hour) at 25°C to show normal geotropic response if kept at that temperature but curvature was prevented by exposing the plants to 4°C. In Chapter IV it was shown that $\frac{1}{2}$ hour horizontal orientation at 25°C did not produce any geotropic curvature. The results (Fig.20) show that the accumulation of calcium in the upper side is stopped at 4°C. Differential distribution can only be established if seedlings show geotropic response. In Section 2 (Chapter IV) it has been shown that the movement of potassium into lower side is completely stopped as soon as seedlings are exposed to 4°C. In the case of phosphorus, differential distribution is not observed after $\frac{1}{2}$ hour stimulus at 25°C (Fig.26), but 1 hour horizontal orientation at 25°C results in a significant increase in the lower side (Fig.25). Thus, differential distribution of ions is not connected to geoinduction but is probably connected to geotropic response.

Further evidence to the above statement is given when the results of the experiments (Chapter V) with NPA are considered. NPA is an inhibitor of auxin transport and so it inhibits geotropic response. Seedlings pre-treated with NPA do not respond to a geotropic stimulus. Differential distribution of ions was not observed even after horizontal exposure for 3 hours. Thus, the movement of calcium into upper side and of potassium and phosphorus into lower side was completely inhibited through the inhibition of geotropic response. This is a strong evidence for the fact that differential distribution of ions is caused by curvature.

As has been seen in Chapter VI, an approach was made to see whether there is any relationship between ion distribution and curvature (due to unilateral IAA application). Unilateral IAA application to the Helianthus hypocotyls produced curvature in the absence of a gravitational stimulus. There has been increased accumulation of calcium in the concave side (Fig.35). This increased accumulation is correlated with the curvature. It has been clearly shown that there has been migration of calcium from convex to concave side. It may be that the cells of the concave side develop a capacity to accumulate more calcium as a result of IAA action on the convex side. The results shown in Section 2 (Chapter VI) suggest that a concentration gradient of potassium is established across the hypocotyl after the unilateral IAA application. The concentration of potassium in the convex side increases with time and also with the curvature. This increase in potassium concentration is mainly due to migration from the concave side. (Figs. 36 and 37).

Tagwa and Bonner (1957) reported that potassium ions probably make the wall soft and elastic which is necessary for auxin-induced growth. The results in Section 3 (Chapter VI) clearly show that the increased accumulation of phosphorus in the convex side is correlated with the curvature (a result of IAA concentration gradient). The results discussed have clearly shown that there is an increase in the concentration of potassium and phosphorus in the convex side and that of calcium in the concave side. This observation is in keeping with earlier experiments demonstrating that the differential distribution of ions is caused by curvature. As indicated previously, Woodcock and Wilkins (1970) reported that unilateral IAA application results in surface potentials arising from IAA concentration gradient on the donor side. Hence, the geo-electric effect has been considered as a result of IAA concentration gradient which develops in the horizontal organ and this effect has nothing to do with the actual perception mechanism. We would conclude, therefore, that the re-distribution of ions is the result of differential growth (a result if IAA concentration gradient). Hence, differential distribution of ions is not connected to geo-electric effects since this effect develops within 10 minutes of horizontal exposure and is independent of differential growth. In a separate paper (1971) the above authors have suggested that IAA probably brings about changes in membrane potentials by affectingthe permeability or the ion pumps of the cell membrane. Other results favour the second idea. It seems very likely, therefore, that IAA-induced differential growth brings about changes in ion pumps. This results in

differential distribution of ions. IAA cannot bring about differential distribution of ions by simply affecting the permeability of the cell membranes since our results have clearly shown an increase in ion concentration both on a dry and fresh weight basis. This suggests an active movement of ion against a concentration gradient.

Experiments for the study of effect of unilateral illumination were designed to see whether there is any relationship between ion distribution and phototropic curvature. In geotropism it was clearly shown that differential distribution of ions is correlated with geotropic response. The results discussed in Section 1 (Chapter VII) show that there is increase in calcium in the illuminated (concave) side which is associated with the curvature. It was possible that differential distribution of calcium was due entirely to a lateral migration (Fig.43) from the shaded (convex) side to illuminated (concave) side.

The effect of orientation of vascular bundle was studied with the object of seeing whether there is any differential longitudinal transport of calcium in the vascular bundles i.e. increase in transport from the roots in the vascular bundle of the illuminated (concave) side. There is no evidence of vascular transport affected by unilateral illumination.

The results of the experiments (Section 2, Chapter VII) show an increase in potassium concentration in the shaded (convex) side which results from the lateral migration from the illuminated (concave) side. Phosphorus also follows similar pattern of distribution (Section 3 Chapter VII). Hence, the increased accumulation of calcium in the concave (illuminated) side and that of potassium and phosphorus in the convex

(shaded) side follows same pattern as that in geotropic curvatures.

There was no evidence that NPA inhibits phototropic curvature. The results (Chapter VIII) show that there has not been any differential distribution of ion in absence of phototropic curvature. Hence, the results are in agreement with those for geotropic stimulation where differential distribution was completely inhibited due to inhibition of curvature.

After unilateral mersalyl application a possible movement of calcium away from the side of mersalyl application is observed (Section 2, Chapter IX). Mersalyl brings about a lateral migration of calcium from the donor side. This presents a close similarity with the pattern of distribution which results from IAA application. IAA brings about a differential distribution by producing curvature but mersalyl affects the distribution of calcium without producing differential growth response. But both probably affect the ion pumps. Hence, the mechanism by which they affect the ion pumps may not be the same. Woodcock and Wilkins (1971) reported that mersalyl results 14 mV electropositive on the donor side. This increase probably results from changes in specific ion pumps. In my results it was indicated that any changes in ion pumps could only be established via differential growth. The effect of diuretic in changing calcium levels has been established in animal tissue. In this connection work of Wesson (1962) may be mentioned. The excretion of Ca^{++} and Mg^{++} increases in kidney slices after the injection of mercurin (a diuretic). It is not too fanciful to think that there occurs an extrusion of calcium from the side of mersalyl application. A possibility for the operation of

"calcium pump" has been demonstrated in red cells (Schatzmann, 1970). Cells extruded calcium at a rapid rate against a concentration gradient provided they contained ATP. It has been suggested by the above worker that ATP maintains an uphill calcium transport and it does so by supplying the necessary energy. How differential growth could operate the pumps will be discussed later.

In this connection, the work of Toryama and Jaffe (1972) may be mentioned. They reported that during the bending process of pulvinus of Mimosa pudica L. a small amount of calcium moves rapidly out of the adaxial half of the young pulvinus. This calcium migrates from the tannin vacuole and from the cell wall. The loss of calcium from the cell wall of the adaxial half facilitates the bending process. This change in calcium level in the wall and in the wall of the tannin vacuole controls the turgor movement. This phenomenon is brought about by changes in permeability and is completely different from geotropic response. But the possibility of ion pump operation may still exist.

In Section 2 (Chapter IX), it was clearly shown that there is an increase in potassium concentration in the mersalyl donor side. Hence it is clear from the results that both mersalyl and IAA affect the distribution of potassium and in both experiments a similar pattern of distribution is observed. IAA action on ion distribution is not direct like mersalyl but is indirect, i.e., via curvature. Both affect the ion pump, but the mechanism of ion pump operation must be different. It will be important and interesting extension of this study to investigate the distribution of ions after IAA application on the one side when curvature is stopped either by pre-treatment with NPA

or at $4^{o}C$.

It has been demonstrated (Raven, 1967) that giant algal cells have sodium extrusion pump and inwardly directed potassium and chloride pumps at the plasmalemma. The cation pumps would seem to be powered by ATP (Raven, 1971). The existence of such a distinct pump has not been demonstrated in the cell of higher plants.

It looks probable that there is upward movement of calcium from the lower side of the curved organ. This is possible by cell to cell movement through the plasmadesmata and finally calcium is accumulated in the cells of the upper side (concave side). There is probably downward movement of potassium and phosphorus into the cells of the lower (convex side) side. The movement of calcium is in opposite direction to that of potassium or phosphorus. The existence of a Ca^{++} activated adenosene triphosphatase has been demonstrated in animal cells (Schatzmann, 1970). It might be that auxin brings about the synthesis of this particular enzyme.

It looks probable that as growth proceeds "pumps" develop which tend to increase calcium concentration in the upper side. Calcium increases rigidity and reduces extensibility which probably accentuates the asymmetrical growth of geotropically stimulated organs. The results have clearly indicated that the differential distribution of ions is the result of auxin redistribution which is in contrary to Brauner's (1958) suggestion. In an analysis of geotropic stimulation process he gave the following suggestion. "Also other displaceable ions like potassium and calcium may have a function in geotropic response and their presence can influence the effectiveness of growth hormones directly or indirectly (Thimann and Schneider,

1938; Cooil and Bonner, 1957)". In my experiments the movement of potassium and phosphorus into lower side and that of calcium into upper side has taken place after the start of activity (appearance of curvature) of growth substance.

The need of phosphorus is primarily for the synthesis of protein in auxin-induced growth. Geotropic and phototropic stimulation results in lateral movement of endogenous auxin in stimulated coleoptiles (Went and Thimann, 1937; Briggs et al, 1957; Gillespie and Briggs, 1961). Auxin is redistributed in geotropic response by polar and lateral transport. A concentration gradient of auxin is established across the horizontally placed organ. This increase in auxin concentration in the lower side results into curvature responses. Because of the well-known role of nucleic acids on cellular metabolism it seems that growth regulator-nucleic acid interactions are much more important than any other interaction. It has been suggested by Key et al (1967) and Nooden (1968) that the rate of elongation is dependent on the synthesis of new RNA and protein which is evidenced by the incorporation of ^{32}P into RNA. IAA was found to stimulate the incorporation of ^{32}P into RNA (Nooden, 1968; Nooden and Thimann, 1966; Masuda et al, 1967). It seems likely that during the process of curvature there will be more and more synthesis of RNA in the lower side and phosphorus will be incorporated into RNA. But it remains to be seen whether this increased phosphorus in the lower side is actually incorporated into RNA or it remains in the form of inorganic phosphate.

If all the lines of evidence discussed in earlier

paragraphs are brought together, the general picture would be that the redistribution of ions is the result of processes taking place during curvature.

SUMMARY

The distribution of ^{45}Ca, ^{42}K and ^{32}P was studied in Helianthus annus hypocotyls and Zea mays coleoptiles. Seedlings of Helianthus annus (5 day old) and Zea mays (4 day old) were fed with radioactive solution through the roots. The distribution of radioactivity was studied in the upper and lower, concave and convex, illuminated and shaded, ether and donor halves as necessary.

The distribution of potassium ion was also studied in the hypocotyls of Helianthus annus. The estimation of potassium was carried out by a flame photometer.

1. Seedings were horizontally orientated for 1, 2 and 3 hours. The results have shown that there was higher concentration of calcium in the upper side. The concentration of potassium and phosphorus was higher in the lower side. The difference between the upper and lower sides increased with time and also with the curvature. This increased concentration was both on a dry and fresh weight basis, i.e. active movement against a concentration gradient. The differential distribution was mainly due to lateral migration, Experiments with rootless plants have confirmed the reality of lateral migration.
   Hence, differential distribution was correlated with the curvature.
2. Seedlings were placed horizontal for ½ hour at 25°C and subsequently placed at 4°C to stop the curvature.

Seedlings received enough stimulus at 25°C to show geotropic response. No differential distribution of ion was observed after the curvature had been stopped at 4°C. It was concluded, therefore, that differential distribution of ion is not connected to geotropic perception.

3. Seedlings were pre-treated with NPA to stop the curvature of horizontally orientated seedlings. Differential distribution of ion was not observed due to absence of curvature. It was concluded that differential distribution of ion is caused by curvature.

4. Unilateral IAA application (0.1% IAA W/W in lanolin) resulted into differential distribution of ions in absence of a gravitational stimulus. The concave side showed a higher concentration of calcium, whereas convex side showed a higher concentration of potassium and phosphorus. This was correlated with the curvature. It was suggested that IAA probably changes the ion pumps via differential growth. Hence, IAA action on ion pumps is dependent on differential growth.

5. The effect of unilateral illumination was studied with an object to see whether there is any relationship between ion redistribution and phototropic curvature. The concentration of calcium was higher in the illuminated (concave) side. The orientation of vascular bundles does not have any effect on the distribution of calcium. The concentration of

potassium and phosphorus was higher in the convex (shaded) side. The results have clearly shown the reality of lateral migration. The results follow the same pattern as geotropic curvature.

6. NPA completely inhibited the phototropic curvature. There has not been any differential distribution of ions in absence of phototropic curvature.

7. After unilateral application of mersalyl (10^{-4}M in hydrated lanolin paste) to the decapitated coleoptile tips, a possible movement of calcium was observed from the side of mersalyl application. The concentration of potassium increased in the mersalyl donor side. Mersalyl affects the distribution of ions by altering the ion pumps, but does not produce a differential growth response. Both IAA and mersalyl change the ion pumps but the action of mersalyl is direct. IAA action is dependent on differential growth. Hence, they differ in their mechanism of ion pump operation.

The results have clearly shown that differential distribution of ion is correlated with the curvature. It was concluded that the differential distribution of ions is the result of all processes taking place during curvature.

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A P P E N D I X

Table I

The effect of horizontal orientation on the distribution of ^{45}Ca in the hypocotyls of *Helianthus annus*.

Analysis of variance of the data the means of which have been shown in the fig.8 is given below. There were three horizontal exposures (1,2 and 3 hours) and 1 vertical (initial as 0 hour). Each exposure had 4 replications.

	Source of variance	Sum of Squares	Degrees of freedom	Mean Square variance	Variance ratio	Probability
Dry Wt. basis	S	2803.13281	1	2803.13281	37.73077	<0.1%
	T	2834.92344	3	944.97448	3.7048	<5%
	ST	1552.56594	3	517.52198	6.96596	<1%
	P + PT	3060.78625	12	255.06552	3.43324	
	Residual error	891.51625	12	74.29300		
Fresh Wt. basis	S	550.29031	1	550.29031	34.58521	<0.1%
	T	383.05094	3	127.68365	3.6305	<5%
	ST	224.44094	3	74.81365	4.70196	<2.5%
	P + PT	422.03375	12	35.16947	2.21037	
	Residual error	190.93375	12	15.91110		

L.S.D. on dry weight basis = 15·1001 (P = 2·5%)

L.S.D. on fresh weight basis = 6·9882 (P = 2·5%)

S = Side effect

T = Time effect

ST = Interaction between Side and Time

P + PT = Plant effects

N.S. = Non-significant

Table 2

The effect of horizontal on the distribution of ^{45}Ca in the hycotyls(without roots).

Analysis of variance of the data the means of which have been shown in the fig.11 is given below. There were three horizontal exposures and one vertical (initial as 0 hour). Each exposure had 5 replications.

	Source of Variance	Sum of Squares	Degree of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	500.55625	1	500.55625	6.02430	<5%
	T	9128.14275	3	3042.71425	0.5839	N.S
	ST	359.79675	3	119.93225	1.44341	N.S.
	P + PT	83381.10000	16	5211.31875	62.71934	
	Residual error	1329.43200	16	83.08950		
Fresh wt. basis	S	495.61600	1	495.61600	12.28951	<1%
	T	3696.01400	3	1232.00467	0.6316	N.S
	ST	258.69000	3	86.23000	2.13820	N.S.
	P + PT	31210.63000	16	1950.66437	48.36953	
	Residual error	645.25400	16	40.32837		

L.S.D. on dry weight basis = 15.9691

L.S.D. on fresh weight basis = 11.1264

Table 3

The effect of horizontal orientation on the distribution of ^{45}Ca in the coleoptiles of *Zea mays*.

Analysis of variance of the data the means of which have been shown in the fig.12 is given below. There were three horizontal exposures (1, 2 and 3 hours) and one vertical (initial as 0 hour). Each exposure had 5 replications.

	Source of variance	Sum of squares	Degrees of freedom	Mean square Variance	Variance ratio	Probability
Dry wt. basis	S	1271.25625	1	1271.25625	12.14672	<0.5%
	T	2027.83675	3	675.94558	1.1101	N.S
	ST	359.46475	3	119.82158	1.14488	N.S.
	P +PT	9742.12600	16	608.88287	5.81781	
	Residual error	1674.53400	16	104.65837		
Fresh wt. basis	S	901.55025	1	901.55025	13.53283	<0.5%
	T	1175.65275	3	391.88425	1.0198	N.S
	ST	272.35275	3	90.78425	1.36273	N.S.
	P +PT	6148.53200	16	384.28325	5.76833	
	Residual error	1065.91200	16	66.61950		

L.S.D. on dry weight basis = 17·9225

L.S.D. on fresh weight basis = 14·2993

Table 4

The effect of horizontal orientation on the distribution of ^{45}Ca in the coleoptiles (without roots) of Zea mays.

Analysis of variance of the data the means of which have been shown in the fig.13 is given below. There were two horizontal exposures (2 and 3 hours) and one vertical (initial as 0 hour). Each exposure had 5 replications.

	Source of variance	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	20.00833	1	20.00833	6.84475	<2.5%
	T	1.49267	2	0.74633	0.0245	N.S
	ST	5.74867	2	2.87433	0.98329	N.S.
	P + PT	365.48200	12	30.45683	10.41912	
	Residual error	35.07800	12	2.92316		
Fresh wt. basis	S	23.58533	1	23.58533	7.98285	<2.5%
	T	2.21867	2	1.10933	0.0386	N.S
	ST	7.33067	2	3.66533	1.24059	N.S.
	P + PT	344.65800	12	28.72150	9.72127	
	Residual error	35.45400	12	2.95450		

L.S.D. on dry weight basis = 2·9952

L.S.D. on fresh weight basis = 3·0113

Table 5

The effect of horizontal orientation on the distribution of potassium ions in the hypocotyls of Helianthus annus.

Analysis of variance of the data the means of which have been shown in the fig.14 is given below. There were three horizontal exposures (1,2 and 3 hours) and one vertical (initial as 0 hour). Each exposure had 4 replications.

	Source of variance	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
Ash wt. basis	S	115.90031	1	115.90031	218.20808	<0.1%
	T	38.98094	3	12.99365	6.3349	<1%
	ST	46.70094	3	15.56698	29.30830	<0.1%
	P + PT	24.61375	12	2.051145	3.86174	
	Residual error	6.37375	12	0.53114		
Fresh wt. basis	S	11.64031	1	11.64031	400.52688	<0.1%
	T	13.33594	3	4.44531	6.9796	<1%
	ST	5.22594	3	1.74198	59.93907	<0.1%
	P + PT	7.64375	12	0.63698	21.91756	
	Residual error	0.34875	12	0.02906		
Dry wt. basis	S	172.98000	1	172.98000	334.66505	<0.1%
	T	200.18125	3	66.72708	8.6897	<1%
	ST	67.84750	3	22.61583	43.75494	<0.1%
	P + PT	92.14750	12	7.67895	14.85651	
	Residual error	6.20250	12	0.51687		

L.S.D. on ash weight basis = 1·5514

L.S.D. on fresh weight basis = 0·3624

L.S.D. on dry weight basis = 1·3683

Table 6

The effect of horizontal orientation on the distribution of ^{42}K in the hypocotyls of Helianthus annus.

Analysis of variance of the data the means of which have been shown in the fig.15 is given below. There were three horizontal exposures (1,2 and 3 hours) and one vertical (initial as 0 hour). Each exposure had 5 replications.

	Source of Variance	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	698.06025	1	698.06025	15.75268	$\ll$1%
	T	1717.51275	3	572.50425	0.8983	N.S.
	ST	285.11475	3	95.03825	2.14467	N.S.
	P + PT	10197.23200	16	637.32700	14.38215	
	Residual error	709.02000	16	44.31375		
Fresh wt. basis	S	225.62500	1	225.62500	14.61243	$\ll$1%
	T	605.71600	3	201.90533	0.8847	N.S.
	ST	87.47500	3	29.15833	1.88842	N.S.
	P + PT	3651.57800	16	228.22362	14.78072	
	Residual error	247.05000	16	15.44062		

L.S.D. on dry weight basis = 11·6623

L.S.D. on fresh weight basis = 6·8854

Table 7

The effect of horizontal orientation on the distribution of potassium ions in the hypocotyls (without roots) of *Helianthus annus*. Analysis of variance of the data the means of which have been shown in the fig.16 is given below. There were three horizontal exposures (1,2 and 3 hours) and one vertical (initial as 0 hour). Each exposure had 4 replications.

	Source of variation	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
Ash wt. basis	S	22.61281	1	22.61281	75.08924	<0.1%
	T	14.69094	3	4.89698	0.3620	N.S
	ST	12.69844	3	4.23281	14.05569	<0.1%
	P + PT	162.33375	12	13.52781	44.92113	
	Residual error	3.61375	12	0.30114		
Fresh wt. basis	S	4.57531	1	4.57531	58.17616	<0.1%
	T	5.81094	3	1.93698	1.4592	N.S
	ST	2.42594	3	0.80865	10.28212	<0.5%
	P + PT	15.92875	12	1.32739	16.87815	
	Residual error	0.94375	12	0.07864		
Dry wt. basis	S	66.99031	1	66.99031	62.65046	<0.1%
	T	60.39344	3	20.13115	1.0889	N.S
	ST	32.42344	3	10.80781	10.10765	<0.5%
	P + PT	221.85125	12	18.48760	17.28992	
	Residual error	12.83125	12	1.06927		

L.S.D. on ash weight basis = 1·1682

L.S.D. on fresh weight basis = 0·5966

L.S.D. on dry weight basis = 2·2009

Table 8

The effect of horizontal orientation on the distribution of ^{32}P in the hypocotyls of *Helianthus annus*.

Analysis of variance of the data the means of which have been shown in the fig.17 is given below. There were three horizontal exposures (1,2 and 3 hours) and one vertical (initial as 0 hour). Each exposure had 5 replications.

	Source of variance	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	4824.61225	1	4824.61225	14.86904	<1%
	T	48894.77075	3	16298.25692	4.7605	<5%
	ST	4050.18275	3	1350.06092	4.16077	<5%
	P + PT	54778.13200	16	3423.63325	10.55134	
	Residual error	5191.5800	16	324.47375		
Fresh wt. basis	S	1464.10000	1	1464.10000	13.82878	<1%
	T	18255.06900	3	6085.02300	5.0167	<5%
	ST	1383.64600	3	461.21533	4.35629	<5%
	P + PT	19407.25000	16	1212.95312	11.45664	
	Residual error	1693.97400	16	105.87337		

L.S.D. on dry weight basis = 31·5572.

L.S.D. on fresh weight basis = 18·0261.

Table 9

The effect of horizontal orientation on the distribution of ^{32}P in the hypocotyls (without roots) of Helianthus annus. Analysis of variance of the data the means of which have been shown in the fig.18 is given below. There were three horizontal exposures (1,2 and 3 hours) and one vertical (initial as 0 hour). Each exposure had 5 replications.

	Source of variance	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	4999.69600	1	4999.69600	8.31117	$\ll$5%
	T	564.78600	3	188.26200	0.0416	N.S.
	ST	2232.03800	3	744.01267	1.23680	N.S.
	P + PT	7240286400	16	4525.17900	7.52236	
	Residual error	9625.01600	16	601.56350		
Fresh wt. basis	S	1459.26400	1	1459.26400	7.11901	$\ll$5%
	T	128.51000	3	42.83667	0.0265	N.S.
	ST	690.43400	3	230.14467	1.12276	N.S.
	P + PT	25872.54600	16	1617.03412	7.88869	
	Residual error	3279.70200	16	204.98138		

L.S.D. on dry weight basis = 42·9685

L.S.D. on fresh weight basis = 25·0824

Table 10

The effect of $4^{o}C$ on the distribution of ^{45}Ca in the hypocotyls of horizontally placed seedlings of Helianthus annus.

Analysis of variance of the data the means of which have been shown in the fig.19 is given below. There were two horizontal exposures (1 hour at $25^{o}C$ and 1 hour at $25^{o}C$ + 1 hour at $4^{o}C$) and one vertical (initial as 0 hour). Each exposure had 4 replications.

	Source of variance	Sum of square	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	610.04167	1	610.04167	1.72408	N.S
	T	5336.84083	2	2668.42042	2.0530	N.S
	ST	415.08583	2	207.54292	0.58655	N.S.
	P + PT	11697.66250	9	1299.74027	3.67329	
	Residual error	3184.52250	9	353.83583		
fresh wt. basis	S	125.58375	1	125.58375	0.83873	N.S
	T	1952.43583	2	976.21792	2.2003	N.S
	ST	134.12250	2	67.06125	0.44788	N.S.
	P + PT	3993.14875	9	443.68319	2.96320	.
	Residual error	1347.57875	9	149.73097		

L.S.D. on dry weight basis = 40.0366

L.S.D. on fresh weight basis = 26.0440

Table 11

The effect of $4^{o}C$ on the distribution of ^{45}Ca in the hypocotyls of horizontally placed seedlings of Helianthus annus.

Analysis of variance of the data the means of which have been shown in the fig.20 is given below. There were two horizontal exposures ($\frac{1}{2}$ hour at $25^{o}C$ and $\frac{1}{2}$ hour at $25^{o}C$ + $2\frac{1}{2}$ hours at $4^{o}C$) and one vertical (initial as 0 hour). Each exposure had 5 replications.

	Source of variation	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	11.28533	1	11.28533	0.22370	N.S.
	T	770.18067	2	385.09033	0.1597	N.S.
	ST	14.87267	2	7.43633	0.14741	N.S.
	P + PT	28942.89600	12	2411.90800	47.81010	
	Residual error	605.37200	12	50.44766		
Fresh wt. basis	S	3.60533	1	3.60533	0.19785	N.S.
	T	260.73600	2	130.36800	0.1558	N.S
	ST	5.41867	2	2.70933	0.14868	N.S.
	P + PT	10043.14400	12	836.92866	45.92705	
	Residual error	218.67600	12	18.22300		

L.S.D. on dry weight basis = 12·4431

L.S.D. on fresh weight basis = 7·4787

Table 12

The effect of $4^{o}C$ on the distribution of ^{45}Ca in the coleoptiles of horizontally placed seedlings of *Zea mays*.

Analysis of variance of the data the means of which have been shown in the fig.21 is given below. There were two horizontal exposures ($\frac{1}{2}$ hour at $25^{o}C$ + $\frac{1}{2}$ hour at $4^{o}C$ and $\frac{1}{2}$ hour at $25^{o}C$ + $2\frac{1}{2}$ hours at $4^{o}C$) and one vertical (initial as 0 hour). Each exposure had 5 replications.

Source of variance	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
S	1.92533	1	1.92533	0.19194	N.S.
T	49.67467	2	24.83733	0.1376	N.S
ST	2.57067	2	1.28533	0.12813	N.S.
P + PT	2166.69400	12	180.55783	17.99968	
Residual error	120.37400	12	10.03116		
S	1.68033	1	1.68033	0.29897	N.S.
T	27.72067	2	13.86033	0.1252	N.S
ST	1.50867	2	0.75433	0.13421	N.S.
P + PT	1328.47800	12	110.70650	19.69691	
Residual error	67.44600	12	5.62050		

L.S.D. on dry weight basis = 5.5486

L.S.D. on fresh weight basis = 4.1533

Table 13

The effect of $4^{o}C$ on the distribution of ^{45}Ca in the coleoptiles of horizontally placed seedlings of Zea mays.

Analysis of variance of the data the means of which have been shown in the fig.22 is given below. There was one horizontal exposure (1 hourat $25^{o}C$ + 1 hour at $4^{o}C$) and one vertical (initial as 0 hour). Each exposure had 5 replications.

	Source of variation	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Proba-
Dry wt. basis	S	21.63200	1	21.63200	1.28114	N.S.
	T	21.63200	1	21.63200	0.1559	N.S
	ST	3.52800	1	3.52800	0.20894	N.S.
	P + PT	1110.19600	8	138.77450	8.21880	
	Residual error	135.08000	8	16.88500		
Fresh wt. basis	S	14.62050	1	14.62050	1.39120	N.S.
	T	12.32450	1	12.32450	0.1506	N.S
	ST	2.38050	1	2.38050	0.22651	N.S.
	P + PT	654.88600	8	81.86075	7.78940	
	Residual error	84.07400	8	10.50925		

L.S.D. on dry weight basis = 7·1987

L.S.D. on fresh weight basis = 5·6793

Table 14

The effect of $4^{o}C$ on the distribution of ^{42}K in the hypocotyls of horizontally placed seedlings of Helianthus annus.

Analysis of variance of the data the means of which have been shown in the fig.23 is given below. There were two horizontal exposures ($\frac{1}{2}$ hour at $25^{o}C$ and $\frac{1}{2}$ hour at $25^{o}C$ + $2\frac{1}{2}$ hours at $4^{o}C$) one vertical (initial as 0 hour). Each exposure had 5 replications.

	Source of variation	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	8.74800	1	8.74800	0.19666	N.S
	T	147.90467	2	73.95233	0.0576	N.S
	ST	5.00600	2	2.50300	0.05627	N.S.
	P + PT	15399.03400	12	1283.25283	28.84870	
	Residual error	533.78600	12	44.48210		
Fresh wt. basis	S	2.02800	1	2.02800	0.12419	N.S.
	T	67.42400	2	33.71200	0.0719	N.S
	ST	1.02800	2	0.51600	0.03160	N.S.
	P + PT	5627.64400	12	468.97033	28.71833	
	Residual error	195.96000	12	16.33000		

L.S.D. on dry weight basis = 11·6844

L.S.D. on fresh weight basis = 7·0796

Table 15

The effect of $4^{o}C$ on the distribution of ^{42}K in the hypocotyls of horizontally placed seedlings of Helianthus annus.

Analysis of variance of the data the means of which have been shown in the fig.24 is given below. There was one horizontal exposure (1 hour at $25^{o}C$ + 1 hour at $4^{o}C$) and one vertical (initial as 0 hour). Each exposure had 5 replications.

	Source of variance	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	14.11200	1	14.11200	0.49547	N.S.
	T	20.40200	1	20.40200	0.2232	NS
	ST	34.32200	1	34.32200	1.20504	N.S.
	P + PT	779.33600	8	91.41700	3.42030	
	Residual error	227.85600	8	28.48200		
fresh wt. basis	S	3.78450	1	3.78450	0.36094	N.S.
	T	2.81250	1	2.81250	0.0800	N.S.
	ST	9.94050	1	9.94050	0.94807	N.S.
	P + PT	281.32800	8	35.16600	3.35393	
	Residual error	83.88000	8	10.48500		

L.S.D. on dry weight basis = 9.3496

L.S.D. on fresh weight basis = 5.6727

Table 16

The effect of $4^{o}C$ on the distribution of ^{32}P in the hypocotyls of horizontally placed seedlings of Helianthus annus.

Analysis of variance of the data the means of which have been shown in the fig.25 is given below. There were two horizontal exposures (1 hour at $25^{o}C$ and 1 hour at $25^{o}C$ + 1 hour at $4^{o}C$) and one vertical (initial as 0 hour). Each exposure had 5 replications.

	Source of variation	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	395.30700	1	395.30700	9.07132	<2.5%
	T	475.84867	2	237.92433	0.3146	N.S
	ST	247.40600	2	123.70300	2.83868	N.S
	P + PT	9076.68000	12	756.3900	17.35729	
	Residual error	522.93200	12	43.57766		
Fresh wt. basis	S	114.85633	1	114.85633	7.24585	<2.5%
	T	192.49867	2	96.24933	0.3263	N.S
	ST	76.40267	2	38.20133	2.40998	N.S
	P + PT	3539.38000	12	294.94833	18.60716	
	Residual error	190.21600	12	15.85133		

L.S.D. on dry weight basis = 11.5648

L.S.D. on fresh weight basis = 6.9749

Table 17

The effect of $4^{o}C$ on the distribution of ^{32}P in the hypocotyls of horizontally placed seedlings of Helianthus annus.

Analysis of variance of the data the means of which have been shown in the fig. 26 is given below. There were three horizontal exposures ($\frac{1}{2}$ hour at $25^{o}C$, $\frac{1}{2}$ hour at $25^{o}C$ + $1\frac{1}{2}$ hours at $4^{o}C$) and one vertical (initial as 0 hour). Each exposure had 5 replications.

	Source of variance	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	47.96100	1	47.96100	0.37684	N.S.
	T	3057.32300	3	1019. 10767	0.3257	N.S
	ST	39.98700	3	13.32900	0.10473	N.S.
	P + PT	50064.54600	16	3129.03412	24.58553	
	Residual error	2036.34200	16	127.27137		
Fresh wt. basis	S	19.60000	1	19.60000	0.38055	N.S.
	T	982.43000	3	327.47667	0.2773	N.S
	ST	21.96600	3	7.32200	0.14216	N.S.
	P + PT	18895.02400	16	1180.93900	22.92907	
	Residual error	824.06400	16	51.50400		

L.S.D. on dry weight basis = 19·7640

L.S.D. on fresh weight basis = 12·5728

Table 18

The effect NPA on the distribution of ^{45}Ca in the hypocotyls of horizontally placed seedlings of Halianthus annus.

Analysis of variance of the data the means of which have been shown in the fig.27 is given below. There were three horizontal exposures (1, 2, and 3 hours) and one vertical (initial as 0 hour). Each exposure had 5 replications.

Source of variance	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
S	129.60000	1	129.60000	0.15718	N.S.
T	50539.72100	3	16846.57367	3.7585	<5%
ST	41.89400	3	13.96467	0.01694	N.S.
P + PT	71716.05000	16	4482.25312	5.43617	
Residual error	13192.38600	16	824.52412		
S	133.22500	1	133.22500	0.40633	N.S.
T	18396.02900	3	6132.00967	3.7491	<5%
ST	7.38500	3	2.46167	0.00751	N.S.
P + PT	26169.28600	16	1635.58037	4.98847	
Residual error	5245.95000	16	327. 87187		

L.S.D. on dry weight basis = 50·3051

L.S.D. on fresh weight basis = 31·7220

Table 19

The effect of horizontal orientation on the distribution of ^{45}Ca in the hypocotyls of Helianthus annus.

This experiment was a control for the experiment with NPA. Analysis of various of the data the means of which have been shown in the fig.28 is given below. There were three horizontal exposures (1, 2 and 3 hours) and one vertical (initial as 0 hour). Each exposure had 5 replications.

	Source of Variance	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	53363.02500	1	53363.02500	14.77653	<0.5%
	T	73209.93700	3	24403.31233	1.4686	N.S
	ST	38106.16100	3	12702.05367	3.51727	<5%
	P + PT	265870.54200	16	16616.90887	4.60132	
	Residual error	57781.39400	16	3611.33712		
Fresh wt. basis	S	22586.25625	1	22586.25625	16.88626	<0.1%
	T	26910.43875	3	8970.14625	1.4363	N.S
	ST	16890.06675	3	5630.02225	4.20920	<2.5%
	P + PT	99922.89400	16	6245.18087	4.66911	
	Residual error	21400.84200	16	1337.55262		

L.S.D. on dry weight basis = 105·2794

L.S.D. on fresh weight basis = 64·0715

Table 20

The effect of NPA on the distribution of ^{45}Ca in the hypocotyls of horizontally placed seedlings of *Zea mays*.

Analysis of variance of the data the means of which have been shown in the fig.29 is given below. There were three horizontal exposures (1, 2 and 3 hours) and one vertical (initial as 0 hour). Each exposure had 5 replications.

	Source of variance	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry Wt. basis	S	0.28900	1	0.28900	0.10196	N.S.
	T	111.81400	3	37.27133	0.6111	N.S
	ST	0.70100	3	0.23367	0.08244	N.S.
	P + PT	975.90200	16	60.99387	21.51934	
	Residual error	45.35000	16	2.83437		
Fresh Wt. basis	S	0.01225	1	0.01225	0.00687	N.S.
	T	63.95875	3	21.31958	0.6075	N.S
	ST	0.44275	3	0. 14758	0.08277	N.S.
	P + PT	561.52600	16	35.09537	19.68195	
	Residual error	28.53000	16	1.78312		

L.S.D. on dry weight basis = 2·9495

L.S.D. on fresh weight basis = 2·3393

Table 21

The effect of NPA on the distribution potassium ions in the hypocotyls of horizontally placed seedlings of Helianthus annus. Analysis of variance of the data the means of which have been shown in the fig.30 is given below. There were three horizontal exposures (1, 2 and 3 hours) and one vertical (initial as 0 hour). Each exposure had 4 replications.

	Source of variation	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
Ash wt. basis	S	1.32031	1	1.32031	1.47059	N.S.
	T	51.60844	3	17.20281	4.4820	<5%
	ST	0.07094	3	0.02365	0.02634	N.S.
	P + PT	46.05875	12	3.83823	4.27509	
	Residual	10.77375	12	0.89781		
Fresh wt. basis	S	0.11281	1	0.11281	1.55827	N.S.
	T	4.08844	3	1.36281	3.5675	<5%
	ST	0.93344	3	0.01115	0.15396	N.S.
	P + PT	4.58375	12	0.38197	5.27626	
	Residual error	0.86875	12	0.07239		
Dry wt. basis	S	1.20125	1	1.20125	1.78018	N.S.
	T	44.99250	3	14.99750	3.5022	<5%
	ST	0.26125	3	0.08708	0.12905	N.S.
	P + PT	51.38750	12	4.28229	6.34609	
	Residual error	8.09750	12	0.67479		

L.S.D. on ash weight basis = 2·0167

L.S.D. on fresh weight basis = 0·5728

L.S.D. on dry weight basis = 1·7485

Table 22

The effect of NPA on the distribution of ^{42}K in the hypocotyls of horizontally placed seedlings of Helianthus annus. Analysis of variance of the data the means of which have been shown in the fig.31 is given below. There were three horizontal exposures (1,2 and 3 hours) and one vertical (initial as 0 hour). Each exposure had 5 replications.

	Source of variance	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	0.01600	1	0.01600	0.00570	N.S.
	T	150.92100	3	50.30700	1.0330	N.S
	ST	2.28200	3	0.76067	0.27105	N.S.
	P + PT	779.23000	16	48.70187	17.35402	
	Residual error	44.90200	16	2.80637		
Fresh wt. basis	S	0.00625	1	0.00625	0.00614	N.S.
	T	54.84675	3	18.28225	1.0236	N.S
	ST	0.75675	3	0.25225	0.24773	N.S.
	P + PT	285.77600	16	17.86100	17.54088	
	Residual error	16.29200	16	1.01825		

L.S.D. on dry weight basis = 2.9348

L.S.D. on fresh weight basis = 1.7678

Table 23

The effect of NPA on the distribution ^{32}P in the hypocotyls of horizontally placed seedlings of Helianthus annus.

Analysis of variance of the data the means of which have been shown in the fig.32 is given below. There were three horizontal exposures (1, 2 and 3) and one vertical (initial as 0 hour). Each exposure had 5 replications.

	Source of variance	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	108.24100	1	108.24100	0.16325	N.S.
	T	106162.93100	3	35387.64367	2.8275	N.S
	ST	178.97100	3	59.65700	0.08997	N.S.
	P + PT	200245.71000	16	12515.35687	18.87530	
	Residual error	10608.87800	16	663.05487		
Fresh wt. basis	S	33.48900	1	33.48900	0.13527	N.S.
	T	39341.82600	3	13113.94200	2.7130	N.S
	ST	73.68500	3	24.56167	0.09921	N.S.
	P + PT	77339.20000	16	4833.70000	19.52381	
	Residual error	3961.27600	16	247.57975		

L.S.D. on dry weight basis = 45·1111

L.S.D. on fresh weight basis = 27·5657

Table 24

The effect of NPA on the distribution of ^{32}P in the hypocotyls (without roots) of horizontally placed seedlings of Helianthus annus.

Analysis of variance of the data the means of which have been shown in the fig.33 is given below. There were three horizontal exposures (1,2 and 3 hours) and one vertical (initial as 0 hour). Each exposure had 5 replications.

	Source of variance	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	12.21025	1	12.21025	0.11951	N.S.
	T	28977.20075	3	9659.06692	1.4680	N.S
	ST	36.41675	3	12.13892	0.11882	N.S.
	P + PT	105272.60200	16	6579.53762	64.40038	
	Residual error	1634.65800	16	102.16612		
Fresh wt. basis	S	7.48225	1	7.48225	0.20150	N.S.
	T	11076.06275	3	3692.02092	1.4868	N.S
	ST	11.02275	3	3.67425	0.09895	N.S.
	P + PT	39730.32200	16	2483.14512	66.87143	
	Residual	594.13000	16	37.13312		

L.S.D. on dry weight basis = 17·7078

L.S.D. on fresh weight basis = 10·6756

Table 25

The effect of unilateral application of IAA (0.1% W/W in lanolin) on the distribution of ^{45}Ca in the hypocotyls of vertically placed seedlings of Helianthus annus.

Analysis of variance of the data the means of which have been shown in the fig.35 is given below. There were three unilateral applications (2,3 and 4 hours) and one without IAA (initial as 0 hour). Each period of unilateral application had 5 replications.

	Source of variance	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	9650.34225	1	9650.34225	5.40889	<5%
	T	122296.53075	3	40765.51025	3.83577	<5%
	ST	6886.14275	3	2295.38092	1.28653	N.S.
	P + PT	170045.75200	16	10627.85950	5.95677	
	Residual error	28546.62000	16	1784.16375		
Fresh wt. basis	S	5189.28400	1	5189.28400	8.47592	<2.5%
	T	41640.71600	3	13880.23867	3.05771	N.S.
	ST	3337.92400	3	1112.64133	1.81733	N.S.
	P + PT	72630.60000	16	4539.41250	7.41445	
	Residual error	9795.81200	16	612.23825		

L.S.D. on dry weight basis = 73.9992

L.S.D. on fresh weight basis = 43.3483

Table 26

The effect of unilateral application of IAA (0.1% W/W in lanolin) on the distribution of potassium ions in the hypocotyls of vertically placed seedlings of Helianthus annus.

Analysis of variance of the data the means of which have been shown in the fig. 36 is given below. There were three unilateral applications (2, 3 and 4 hours) and one without IAA (initial as 0 hour). Each period of application had 4 replications.

	Source of variance	Sum of square	Degrees of freedom	Mean square variance	Variance ratio	Probability
Ash wt. basis	S	68.44500	1	68.44500	155.92596	≪0.1%
	T	35.00250	3	11.66750	3.9183	<5%
	ST	22.86750	3	7.62250	17.36497	<0.1%
	P + PT	35.73250	12	2.97771	6.78358	
	Residual error	5.26750	12	0.43895		
Fresh wt. basis	S	5.20031	1	5.20031	208.88285	≪0.1%
	T	5.07844	3	1.69281	1.3086	N.S
	ST	1.25594	3	0.41865	16.81590	<0.1%
	P + PT	15.52375	12	1.29364	51.96234	
	Residual error	0.29875	12	0.02489		
Dry wt. basis	S	90.45125	1	90.45125	283.58328	≪0.1%
	T	39.87625	3	13.29208	1.0270	N.S
	ST	26.75125	3	8.91708	27.95689	≪0.1%
	P + PT	155.31250	12	12.94270	40.57805	
	Residual error	3.82750	12	0.31892		

L.S.D. on ash wt. basis = 1·4102

L.S.D. on fresh wt. basis = 0·3553

L.S.D. on dry wt.basis = 1·2022

Table 27

The effect of unilateral application of IAA (0.1% W/W in lanolin) on the distribution of ^{42}K in the hypocotyls of vertically placed seedlings of Helianthus annus.

Analysis of variance of the data the means of which have been shown in the fig.37 is given below. There were two unilateral applications (2 and 3 hours) and one without IAA (initial as 0 hour). Each period of application had 5 replications.

	Source variance	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	638.48533	1	638.48533	8.74917	<2.5%
	T	121.92267	2	60.96133	0.2252	N.S.
	ST	290.35467	2	145.17733	1.98937	N.S.
	P + PT	3248.13200	12	270.67766	3.70910	
	Residual error	875.72000	12	72.97666		
Fresh wt. basis	S	168.98133	1	168.98133	6.69374	<2.5%
	T	41.84600	2	20.92300	0.2193	N.S
	ST	85.51267	2	42.75633	1.69368	N.S.
	P + PT	1144.80400	12	95.40033	3.77903	
	Residual error	302.93600	12	25.24466		

L.S.D. on dry weight basis = 16.2624

L.S.D. on fresh weight basis = 8.8022

Table 28

The effect of unilateral application of IAA (0.1% W/W in lanolin) on the distribution of ^{32}P in the hypocotyls of vertically placed seedlings of Helianthus annus.

Analysis of variance of the data the means of which have been shown in the fig.38 is given below. There were two unilateral applications (1 and 2 hours) and one without IAA (initial as 0 hour). Each period of unilateral application had 5 replications

	Source of variance	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	2590.98133	1	2590.98133	5.87631	<5%
	T	28356.84467	2	14178.42233	1.4077	N.S
	ST	2268.00467	2	1134.00233	2.57190	N.S.
	P + PT	120866.59000	12	10072.21583	22.84366	
	Residual error	5291.03400	12	440.91950		
fresh wt. basis	S	490.45633	1	490.45633	2.91046	N.S.
	T	11232.08467	2	5616.04233	1.5027	N.S
	ST	655.42867	2	327.71433	1.94472	N.S.
	P + PT	44847.64000	12	3737.30333	22.17787	
	Residual error	2022.18000	12	168.51500		

L.S.D. on dry weight basis = 36.7867

L.S.D. on fresh weight basis = 22.7420

Table 29

The effect of unilateral illumination (800 lux) on the distribution of ^{45}Ca in the coleoptiles (with roots, orientation of the vascular bundles was transverse).

Analysis of variance of the data the means of which have been shown in the fig. 40 is given below. There were three unilateral illuminations (2, 3 and 4 hours) and one initial as 0 hour (not illuminated). Each period of unilateral illumination had 5 replications

	Source of variance	Sum of square	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	101.12400	1	101.12400	7.80632	<2.5%
	T	1792.45400	3	597.48467	5.4014	<5%
	ST	62.65000	3	20.88333	1.61210	N.S.
	P + PT	1769.86200	16	110.61637	8.53909	
	Residual error	207.26600	16	12.95412		
Fresh wt. basis	S	186.19225	1	186.19225	17.87817	<0.1%
	T	1380.85475	3	460.28492	5.0529	<5%
	ST	112.62075	3	37.54025	3.60461	<5%
	P + PT	1457.48800	16	91.09300	8.74675	
	Residual error	166.63200	16	10.41450		

L.S.D. on dry weight basis = 6.3054

L.S.D. on fresh weight basis = 5.6536

Table 30

The effect of unilateral illumination (800 lux) on the distribution of ^{45}Ca in the coleoptiles (without roots, orientation of the vascular bundles was transverse) of Zea mays.

Analysis of variance of the data the means of which have been shown in the fig.41 is given below. There was one unilateral illumination (4 hours) and one initial 0 hour (not illuminated). Each had 5 repoications.

Source of variance	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
S	17.67200	1	17.67200	0.22610	N.S.
T	1.68200	1	1.68200	0.0015	N.S.
ST	39.20000	1	39.20000	0.50154	N.S.
P + PT	8841.46800	8	1105.18350	14.14029	
Residual	625.26800	8	78.15850		
S	29.04050	1	29.04050	0.37677	N.S.
T	0.02450	1	0.02450	0.00	N.S
S T	61.60050	1	61.60050	0.79920	N.S.
P + PT	8428.23600	8	1053.52950	13.66836	
Residual error	616.62400	8	77.07800		

L.S.D. on dry weight basis = 15·4882

L.S.D. on fresh weight basis = 15·3807

Table 31

The effect of unilateral illumination (800 lux) on the distribution of ^{45}Ca in the coleoptiles (with roots, orientation of the vascular bundles was parallel) of *Zea mays*.

Analysis of variance of the data the means of which have been shown in the fig.43 is given below. There was one unilateral illumination (4 hours) and one initial as 0 hour (not illuminated). Each had 5 replications.

	Source of variance	sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Proba-
Dry wt. basis	S	98.12450	1	98.12450	10.46382	<2.5%
	T	0.08450	1	0.08450	0.0004	N.S.
	ST	92.88050	1	92.88050	9.90461	<2.5%
	P + PT	18659.15600	8	2332.39450	248.72242	
	Residual error	75.02000	8	9.37750		
Fresh wt. basis	S	126.00200	1	126.00200	9.43588	<2.5%
	T	0.72200	1	0.72200	0.0003	N.S
	ST	120.05000	1	120.05000	8.99015	<2.5%
	P + PT	17127.44200	8	2140.93100	160.32733	
	Residual error	106.82800	8	13.35350		

L.S.D. on dry weight basis = 5.3647

L.S.D. on fresh weight basis = 6.4017

Table 32

The effect of unilateral illumination (800 lux) on the distribution of ^{45}Ca in the coleoptiles (without roots, orientation of the vascular bundles was parallel) of *Zea mays*.

Analysis of variance of the data the means of which have been shown in the fig.44 is given below. There was unilateral illumination (4 hours) and one initial as 0 hour (not illuminated). Each had 5 replications.

	Source of variance	Sum of square	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	9.52200	1	9.52200	2.81300	N.S.
	T	197.19200	1	197.19200	0.2708	N.S.
	ST	0.64800	1	0.64800	0.19143	N.S.
	P + PT	5824.54000	8	728.06250	215.08641	
	Residual error	27.08000	8	3.38500		
Fresh wt. basis	S	18.81800	1	18.81800	5.91761	<5%
	T	203.52200	1	203.52200	0.2927	N.S.
	ST	2.59200	1	2.59200	0.81509	N.S.
	P + PT	5562.39600	8	695.29950	218.64764	
	Residual error	25.44000	8	3.18000		

L.S.D. on dry weight basis = 3·2232

L.S.D. on fresh weight basis = 3·1243

Table 33

The effect of unilateral illumination (800 lux) on the distribution of ^{42}K in the coleoptile (with roots, orientation of the vascular bundles was transverse) of Zea mays. Analysis of variance of the data the means of which have been shown in the fig.45 is given below. There were two unilateral illuminations and one initial as 0 hour (not illuminated). Each period of unilateral illumination had 5 replications.

	Source of variance	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	20.83333	1	20.83333	5.61520	<5%
	T	552.78067	2	276.39033	3.7058	N.S
	ST	20.90467	2	10.45233	2.81721	N.S.
	P + PT	894.99400	12	74.58283	20.10229	
	Residual error	44.52200	12	3.71016		
Fresh wt. basis	S	35.20833	1	35.20833	9.77014	<1%
	T	445.12467	2	222.56233	2.6468	N.S
	ST	38.01267	2	19.00633	5.27417	<5%
	P + PT	1009.04400	12	84.08700	23.33373	
	Residual error	43.24400	12	3.60366		

L.S.D. on dry weight basis = 3·3744

L.S.D. on fresh weight basis = 3·3257

Table 34

The effect of unilateral illumination (800 lux) on the distribution of ^{42}K in the coleoptiles (without roots, orientation of the vascular bundles, was transverse) of *Zea mays*.

Analysis of variance of the data the means of which have been in the fig.46 is given below. There were two unilateral illuminations (3 and 4 hours) and one initial as 0 hour (not illuminated). Each period of illumination had 5 replications.

	Source of variance	Sum of squares	Degrees of freedom	Mean Square variance	Variance ratio	Proba-
Dry wt. basis	S	9.74700	1	9.74700	1.89213	N.S.
	T	31.66467	2	15.83233	0.4011	N.S
	ST	11.10200	2	5.55100	1.07759	N.S.
	P + PT	473.66000	12	39.47166	7.66242	
	Residual error	61.81600	12	5.15133		
Fresh wt. basis	S	5.63333	1	5.63333	1.12002	N.S.
	T	36.20600	2	18.10300	0.4611	N.S.
	ST	6.16027	2	3.08033	0.61243	N.S.
	P + PT	471.13600	12	39.26133	7.805975	
	Residual error	60.35600	12	5.02966		

L.S.D. on dry weight basis = 3.9761

L.S.D. on fresh weight basis = 3.9290

Table 35

The effect of unilateral illumination (800 lux) on the distribution of ^{32}P in the coleoptiles (with roots, orientation of the vascular bundles was transverse) of Zea mays.

Analysis of variance of the data the means of which have been shown in the fig.47 is given below. There were three unilateral illuminations (2,3 and 4 hours) and one initial as 0 hour (not illuminated). Each period of illumination had 5 replications.

	Source of variance	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	1605.28900	1	1605.28900	13.05840	<0.5%
	T	10996.46900	3	3665.48967	1.9527	N.S
	ST	628.93700	3	209.64567	1.70539	N.S.
	P + PT	30033.58000	16	1877.09875	15.26947	
	Residual error	1966.90400	16	122.93150		
Fresh wt. basis	S	553.53600	1	533.53600	9.24017	<1%
	T	7716.00200	3	2572.00067	1.7356	N.S
	ST	134.53800	3	44.84600	0.74861	N.S.
	P + PT	23710.61400	16	1481.91337	24.75757	
	Residual error	958.48600	16	59.90537		

L.S.D. on dry weight basis = 19.4241

L.S.D. on fresh weight basis = 13.5594

Table 36

The effect of unilateral illumination (800 lux) on the distribution of ^{32}P in the coleoptiles (without roots, orientation of the vascular bundles was transverse) of *Zea mays*.

Analysis of variance of the data the means of which have been shown in the fig.48 is given below. There were three unilateral illuminations (2,3 and 4 hours) and one initial as 0 hour (not illuminated). Each period of illumination had 5 replications.

	Source of variance	Sum of square	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	1836.02500	1	1836.02500	16.77946	<0.1%
	T	4921.50100	3	1640.50003	0.6430	N.S
	ST	740.52900	3	246.84300	2.25590	N.S.
	P + PT	40821.14400	16	2551.32150	23.31656	
	Residual	1750.73600	16	109.42100		
Fresh wt. basis	S	413.44900	1	413.44900	6.18850	<2.5%
	T	4062.03400	3	1354.01133	0.6029	N.S
	ST	206.39300	3	58.79767	1.02976	N.S.
	P + PT	35932.69200	16	2245.79325	33.61500	
	Residual error	1068.94800	16	66.80925		

L.S.D. on dry weight basis = 18.3258

L.S.D. on fresh weight basis = 14.3195

Table 37

The effect of NPA on the distribution of ^{45}Ca in the unilaterally illuminated coleoptile (with roots, orientation of the vascular bundles was transverse) of *Zea mays*.

Analysis of variance of the data the means of which have been shown in the fig.49 is given below. There were two unilateral illuminations (2 and 3 hours) and one initial as0 hour (not illuminated). Each period of illumination had 5 replications.

	Source of variance	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	0.61633	1	0.61633	0.02684	N.S.
	T	882.35000	2	441.17500	2.5123	N.S
	ST	3.24467	2	1.62233	0.07065	N.S.
	P + PT	2107.28800	12	175.60733	7.64774	
	Residual error	275.54400	12	22.96166		
Fresh wt. basis	S	0.97200	1	0.97200	0.05001	N.S
	T	785.12600	2	392.56300	2.5306	N.S
	ST	2.95400	2	1.47700	0.07600	N.S.
	P + PT	1861.53200	12	155.12766	7.98173	
	Residual	233.22400	12	19.435.33		

L.S.D. on dry weight basis = 8·3948

L.S.D. on fresh weight basis = 7·7233

Table 38

The effect of NPA on the distribution of ^{45}Ca in the unilaterally illuminated coleoptiles (without roots, orientation of the vacular bundles was transverse) of Zea mays.

Analysis of variance of the data the means of which have been shown in the fig.50 is given below. There were three unilateral illuminations (2, 3 and 4 hours) and one initial as 0 hour (not illuminated). Each period of illumination had 5 replications.

	Source of variance	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Proba-bility
Dry wt. basis	S	2.30400	1	2.30400	0.48372	N.S
	T	132.77700	3	44.25900	0.7044	N.S
	ST	6.78600	3	2.26200	0.47490	N.S.
	P + PT	1005.31800	16	62.83270	13.19142	
	Residual error	76.21000	16	4.76312		
Fresh wt. basis	S	1.29600	1	1.29600	0.33513	N.S.
	T	119.10800	3	39.70267	0.6863	N.S
	ST	4.50000	3	1.50000	0.38789	N.S.
	P + PT	925.60600	16	57.85037	14.95953	
	Residual error	61.87400	16	3.86712		

L.S.D. on dry weight basis = 3·8234

L.S.D. on fresh weight basis = 3·4450

Table 39

The effect of NPA on the distribution ^{42}K in the unilaterally illuminated coleoptiles (with roots, orientation of the vascular bundles was transverse) of Zea mays.

Analysis of variance of the data the means of which have been shown in the fig.51 is given below. There were three unilateral illuminations (2,3 and 4 hours) and one initial as 0 hour (not illuminated). Each period of illumination had 5 replications.

	Source of variance	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	61.50400	1	61.50400	0.20579	N.S.
	T	31466.50700	3	10 488.83567	0.48698	N.S
	ST	916.37600	3	305.45867	1.02205	N.S.
	P + PT	344681.14800	16	21542.57175	72.08007	
	Residual error	4781.92000	16	298.87000		
Fresh wt. basis	S	56.88225	1	56.88225	0.26470	N.S.
	T	18815.07275	3	6271.69092	0.4325	N.S
	ST	487.53475	3	162.51158	0.75625	N.S.
	P + PT	232007.04200	16	14500.40120	67.47808	
	Residual error	3438.25800	16	214.89112		

L.S.D. on dry weight basis = 30·2866

L.S.D. on fresh weight basis = 25·6812

Table 40

The effect of NPA on the distribution of ^{42}K in the unilaterally illuminated coleoptiles (without roots, orientation of the vascular bundles was transverse) of Zea mays.

Analysis of variance of the data the means of which have been shown in the fig.52 is given below. There were three unilateral illuminations (2,3 and 4 hours) and one initial as 0 hour (not illuminated). Each period of illumination had 5 replications.

	Source of variance	Sum of squares	Degrees of freedom	Mean Square variance	Variance ratio	Probability
Dry wt. basis	S	8.46400	1	8.46400	0.07772	N.S.
	T	3827.62500	3	1275.87500	0.1181	N.S
	ST	143.45400	3	47.81800	0.43906	N.S.
	P + PT	172843.64400	16	10802.72775	99.18881	
	Residual error	1742.57200	16	108.91075		
Fresh wt. basis	S	0.00900	1	0.00900	0.00011	N.S.
	T	2966.84100	3	988.94700	0.1244	N.S
	ST	116.97300	3	38.99100	0.47823	N.S.
	P + PT	127219.57800	16	7951.22362	97.52229	
	Residual error	1304.51800	16	81.53237		

L.S.D. on dry weight basis = 18·2831

L.S.D. on fresh weight basis = 15·8186

Table 41

The effect of NPA on the distribution of ^{32}P in the unilaterally illuminated coleoptiles (with roots, orientation of the vascular bundle was transverse) of Zea mays.

Analysis of variance of the data the means of which have been shown in the fig.53 is given below. There were three unilateral illuminations (2,3 and 4 hours) and one initial as 0 hour (not illuminated). Each period of unilateral illumination had 5 replications.

	Source of variance	Sum of squares	Degrees of freedom	Mean Square variance	Variance ratio	Probability
Dry wt. basis	S	0.36100	1	0.36100	0.01115	N.S.
	T	117.05900	3	39.01967	0.0392	N.S
	ST	9.85100	3	3.28367	0.01115	N.S.
	P + PT	15906.24600	16	994.14037	30.71546	
	Residual error	517.85800	16	32.36612		
Fresh wt. basis	S	0.96100	1	0.96100	0.02956	N.S.
	T	111.61400	3	37.20467	0.0392	N.S
	ST	12.44900	3	4.14967	0.12764	N.S.
	P + PT	15204.13000	16	950.25812	29.22803	
	Residual error	520.19000	16	32.51187		

L.S.D. on dry weight basis = 9·9667

L.S.D. on fresh weight basis = 9·9892

Table 42

The effect of NPA on the distribution of ^{32}P in the unilaterally illuminated coleoptiles (without root, orientation of the vascular bundles was transverse) of Zea mays.

Analysis of variance of the data the means of which have been shown in the fig.54 is given below. There were three unilateral illuminations (2, 3 and 4 hours) and one initial as 0 hour (not illuminated). Each period of unilateral illumination had 5 replications.

	Source of variance	Sum of squares	Degrees of variance	Mean Square ratio	Variance	Probability
Dry wt. basis	S	6.24100	1	6.24100	0.36550	N.S.
	T	1663.44100	3	554.48033	2.3627	N.S
	ST	1.57700	3	0.52567	0.03079	N.S.
	P + PT	3754.93000	16	234.68312	13.74415	
	Residual error	273.20200	16	17.075125		
Fresh wt. basis	S	3.13600	1	3.13600	0.22182	N.S.
	T	1489.87300	3	496.62433	2.3167	N.S
	ST	0.24200	3	0.08067	0.00571	N.S.
	P + PT	3429.94200	16	214.37137	15.16318	
	Residual error	226.20200	16	14.13762		

L.S.D. on dry weight basis = 7.2391

L.S.D. on fresh weight basis = 6.5871

Table 43

The effect of unilateral application of mersalyl (10^{-4}M in the hydrated paste of lanolin) on the distribution of ^{45}Ca in the coleoptiles (with roots) of Zea mays.

Analysis of variance of the data the means of which have been shown in the fig. 55 is given below. There were three unilateral applications (20, 40 and 60 minutes) and one without mersalyl (0 minute). Each period of application had 5 replications.

Source of variance	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
S	29154.60025	1	29154.60025	3.45208	N.S.
T	88184.09675	3	29394.69892	0.49421	N.S
ST	30749.44675	3	10249.81558	1.21364	N.S.
P + PT	915690.48800	16	59480.65550	7.04286	
Residual error	135128.42800	16	8445.52675		
S	20958.08400	1	20958.08400	4.16119	N.S.
T	60782.75300	3	20260.91767	0.5288	N.S
S T	17746.19000	16	5915.39667	1.17449	N.S.
P + PT	612988.63800	16	38311.78987	7.60673	
Residual error	80585.02600	16	5036.56412		

L.S.D. on dry weight basis = 160.9990

L.S.D. on fresh weight basis = 124.3303

Table 44

The effect of unilateral application of mersalyl (10^{-4}M in hydrated paste of lanolin) on the distribution of ^{45}Ca in the coleoptiles (without roots) of Zea mays.

Analysis of variance of the data the means of which have been shown in the fig. 57 is given below. There were two unilateral applications (30 and 60 minutes) and one without mersalyl (0 minute). Each period of application had 5 replications.

	Source of variance	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	5137.82533	1	5137.82533	1.69502	N.S.
	T	3105.83267	2	1552.91633	0.0132	N.S
	ST	2236.16467	2	1118.08233	0.36887	N.S.
	P + PT	1407125.11400	12	117260.42610	38.68548	
	Residual error	36373.47000	12	3031.12250		
Fresh wt. basis	S	4438.40033	1	4438.40033	2.12374	N.S.
	T	550.66867	2	275.33433	0.0037	N.S
	ST	989.71667	2	494.85833	0.23679	N.S.
	P + PT	876021.08000	12	73001.75666	34.93081	
	Residual	25078.74800	12	2089.89566		

L.S.D. on dry weight basis = 96·4520

L.S.D. on fresh weight basis = 80·0877

Table 45

The effect of unilateral application of mersalyl (10^{-4}M in hydrated paste of lanolin) on the distribution of ^{42}K in the coleoptile (with roots) of Zea mays.

Analysis of variancw of the data the means of which have been shown in the fig. 58 is given below. There were three unilateral applications (20, 40 and 60 minutes) and one without mersalyl (0 minute). Each period of application had 5 replications.

	Source of variance	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	186.62400	1	186.62400	11.61681	<1%
	T	2229.16200	3	743.05400	1.8339	N.S
	ST	86.06600	3	28.68867	1.78579	N.S.
	P + PT	6486.18800	16	405.38675	25.23416	
	Residual error	257.04000	16	16.06500		
Fresh wt. basis	S	119.02500	1	119.02500	12.91767	<1%
	T	1342.30600	3	447.43533	1.7955	N.S
	ST	56.46900	3	18.82300	2.04284	N.S.
	P + PT	3987.07800	16	249.19237	27.04461	
	Residual error	147.42600	16	9.21412		

L.S.D. on dry weight basis = 7·0220

L.S.D. on fresh weight basis = 5·3178

Table 46

The effect of unilateral application of mersalyl (10^{-4}M in hydrated paste of lanolin) on the distribution of ^{42}K in the coleoptiles (without roots) of Zea mays.

Analysis of variance of the data the means of which have been shown in the fig. 59 is given below. There were three unilateral applications (20, 40 and 60 minutes) and one without mersalyl (0 minute). Each period of application had 5 replications.

	Source of variance	Sum of squares	Degrees of freedom	Mean square variance	Variance ratio	Probability
Dry wt. basis	S	212.06025	1	212.06025	4.37982	N.S.
	T	2.46875	3	0.82292	0.0093	N.S
	ST	98.44275	3	32.81425	0.67773	N.S.
	P + PT	41582.55400	16	2598.90962	53.67693	
	Residual error	774.68200	16	48.41762		
Fresh wt. basis	S	161.20225	1	161.20225	5.17088	<5%
	T	110.05875	3	36.68625	0.0204	N.S.
	ST	80.34275	3	26.78092	0.85905	N.S.
	P + PT	28808.32000	16	1800.52000	57.75525	
	Residual error	498.8000^0	16	31.17500		

L.S.D. on dry weight basis = 12·1962

L.S.D. on fresh weight basis = 9·7817