INFLUENCE OF SOME MONOVALENT AND DIVALENT CATIONS ON 2,4,5-TRICHLOROPHENOXYACETIC ACID ABSORPTION BY EXCISED PLANT PARTS OF BEAN

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CHAPTER I

INTRODUCTION

Many of the papers published to date dealing with the uptake of herbicides characterize those factors which influence the movement of the herbicide through the cell wall. Of equal importance to the herbicidal activity of any compound is the rate and degree of entry into the plant cell. The degree of movement of a herbicide through plant cell membranes determines the amount of herbicide entering the plant tissue from foliar applications and may influence the amount of herbicide reaching a critical site within the cell. The facility with which herbicides traverse membranes may also influence the translocation of herbicides within the plant. Herbicides must move from leaf mesophyll cells to phloem tissue and be contained within the phloem during translocation to the lower portions of the plant. Thus the permeability of membranes to herbicides is an important factor in determining the magnitude of toxicity of herbicides to plants. The rate of absorption and translocation often are responsible for resistance and susceptibility of plants to certain herbicides. Treatments which enhance the absorption or translocation of herbicides will be of importance in improving the usefulness of herbicides in agriculture.

There are many studies dealing with the absorption and translocation of herbicides in plants but very little is known about the involve-

ment of mineral ions in the processes of absorption and translocation of herbicides by plants. Some knowledge relative to these processes may be obtained by studying the effects of certain nutrient ions on the permeability of cells of various plant tissues to the herbicide. The present study was initiated to determine the role of several monovalent and divalent cations on the accumulation of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in excised leaf, stem and root tissue.

CHAPTER II

REVIEW OF LITERATURE

Since the introduction of the phenoxy herbicides, a great deal of research has been conducted to characterize the mechanisms of entry of these herbicides into the cell. Among the early work in the area is that of Audus (5) who studied the relationship between pH and 2,4-D inhibition of root growth. Blackman and Robertson-Cunninghame (7) concluded, from their work with the influence of pH on the toxicity of 2,4-D in Lemna minor, that both the undissociated molecules and dissociated anions possess growth regulating properties. Erickson, et al., (16) and Wedding & Erickson (44) confirmed these results and showed that pH appeared to have an influence on the concentration of undissociated 2,4-D molecules required to give a 50% inhibition of photosynthesis.

Rice (30) showed that the amount of ammonium salt of 2,4-D absorbed by bean leaves was positively correlated with temperature during treatment. He also reported that there was no significant difference between the amounts of the salt absorbed at 100 ft-c of light and at 900 ft-c, however, the amount absorbed in the dark was significantly greater.

In other work with bean plants, Basler, Todd and Meyer (6) reported that 2,4-D absorption was little affected even by rather severe water stress even though translocation of 2,4-D was almost completely blocked below 80% relative turgidity.

Field studies with blackjack oak by Dalrymple and Basler (14)

revealed seasonal variations in rate of absorption of 2,4,5-T.

In a comprehensive study of foliar penetration of 2,4-D by excised bean leaf tissue, Sargent and Blackman (31) showed that the rate of penetration is enhanced when (a) the leaves are young; (b) the temperature is raised (Q_{10}=2.3-2.8); and (c) a surface-active agent is added to the external solution. They (32) were further able to show that 2,4-D uptake was accelerated by light intensities exceeding 1000 ft-c.

Of special pertinence to this paper is the work of Blackman, et al (9) who reported that 2,4-D uptake by Lemna minor is at first very rapid but later decreases and eventually becomes negative. Continued work by Wedding and Blackman (43) revealed a completely different uptake pattern for Chlorella pyrenoidosa. This alga demonstrated a linear uptake pattern over many hours. They showed that this linear accumulation was sensitive to temperature, light and inhibitors, and thus it appeared to be metabolically regulated. Further studies (33, 34, 35) on the uptake of various substituted phenoxyacetic acids by stem tissues of higher plants revealed that, in general, the uptake patterns of the less active auxins were linear with time, whereas the uptake patterns of the highly active auxins (2,4-D and 2,4,5-T) were initially high but decreased progressively with time and ended in a phase of net loss. This led Saunders, Jenner and Blackman (34) to propose the presence of two accumulation mechanisms. They designated these "Type I" accumulation in which the rate of accumulation was reduced by a change with time in the properties of the tissues and was noted only for strong auxins; and "Type II" accumulation, in which the amount accumulated increased continuously with time and the controlling processes were stable. Type II accumulation could be demonstrated for both weak and strong auxins

and appeared to operate simultaneously with Type I accumulation in the case of strong auxins. Type II accumulation (35) was shown to be inhibited by low temperature, anaerobiosis, 2,4-dinitrophenol, and iodacetate and was associated with the metabolic conversion of auxins to products which do not readily diffuse out into the external medium. Work with several regulators of glycolysis and the TCA cycle in Chlorella cells led Swets and Wedding (38) to suggest that the non-diffusible metabolite of 2,4-D was an aspartyl-2,4-D complex. Type I accumulation (35) was also sensitive to inhibitors but did not form any metabolites of the auxin. Type I accumulation could be abolished by pretreatment in buffer (34) or stabilized for longer periods of time by streptomycin or synthalin (40). Further work (41) suggested the involvement of a lipid moiety of the membrane and thereby raised the possibility of a membrane bound transport system.

That a membrane bound transport mechanism such as Skou (36) described may exist in plants has very recently been indicated by a number of investigators. For example, membrane-bound ATPases have been isolated from Arachis hypogaea cotyledons (29, 30), from root tissue of bean (18), carrot (3), and beet (4), from Chara cells (4), and have also been demonstrated in loblolly pine by histochemical methods (25).

CHAPTER III

MATERIALS AND METHODS

General Considerations

Excised plant parts were used to determine the influence of a number of monovalent and divalent ions on the process of absorption of 2,4,5-T in plant tissue. Throughout this investigation, the experimental material was excised leaf, stem and root tissue from Phaseolus vulgaris L. var "Contender". The incubating solutions consisted of 0.01M Tris-maleate buffer, pH adjusted to 5.5 with 1N HCl, plus 2,4,5-T and the cation in question. The incubations were carried out in 10 cm petri dishes in a growth chamber at 30° C. The cations were supplied in the form of their chloride salts, and the initial concentration of the cations was in all cases 10 millimolar.

The absorption of auxin into the cell was determined by using carboxyl ^{14}C labeled 2,4,5-T (2,4,5-T-1-^{14}C) with a specific activity of 31.5 mc/mM as a tracer. The level of 2,4,5-T-1-^{14}C in each treatment was determined prior to incubation by counting a small aliquot in a liquid scintillation counter. The activity of the incubation solution was maintained at 0.002 to 0.05 μc/ml.

The radioactivity of a sample was determined by pipetting a small aliquot of an alcohol extract into a vial containing toluene scintillation solvent (8 gm PPO plus 0.1 gm POPOP/2l toluene) and counting in a liquid scintillation counter. For cases in which it was

necessary to determine the radioactivity of a water sample a p-dioxane scintillion solvent (4 gm PPO, 50 mg POPOP and 120 gm naphthalene per 1000 ml p-dioxane) was used. In an effort to determine the relative concentration of 2,4,5-T in the plant tissue as well as in the incubation solution, the fresh weight and dry weight values for each plant part was used to determine the volume of the cell so that the 2,4,5-T-1-^{14}C concentration in the cell could be calculated. It was assumed that the hydrated portion of the plant tissue represented cellular material and that one gram of water represented one cubic centimeter of cellular space. For those experiments in which two concentrations of 2,4,5-T was used, the 2,4,5-T-1-^{14}C was maintained at a constant level while the concentration of the unlabeled 2,4,5-T was varied. Thus in the figures representing these experiments the abscissa represents only the amount of 2,4,5-T-1-^{14}C taken up by the plant part and not the total level of 2,4,5-T within the cell.

For statistical analysis all treatments were run in duplicate and all treatments were considered to be independent and were analyzed in the form of the completely randomized block design. Least significant difference was determined using the error mean square value and the appropriate degrees of freedom value.

Leaf Tissue

Bean seeds were germinated in the dark at 30° C in vermiculite moistened with Hoaglands solution (24). After the cotyledons had emerged through the vermiculite (about three days) the plants were transferred to the growth chamber at 30° C under continuous light (1200 ft-c). At 8 days 4 leaf disks were obtained from each primary

leaf using a #12 cork borer (18 mm diameter). Care was taken to avoid the midrib. Twelve leaf disks per sample were floated abaxial surface down in a petri dish in 25 ml of incubating solution. The petri dishes were placed in the growth chamber at 30° C with 1800 ft-c light (eight Sylvania lifeline fluorescent bulbs plus four 25 watt incandescent bulbs).

After the designated time period each sample of leaf disks was thoroughly washed in a polyethylene Buchner funnel fitted with a side arm. Tap water was forced in through the side arm until the funnel reservoir was full and then the water was allowed to drain through the bottom of the funnel. This operation was repeated approximately 4 times during a two minute period for each sample after which the disks were blotted lightly and placed in a freezer. The disks were then lyophilized.

The leaf disks were then weighed and homogenized in a 40 ml glass hand homogenizer in 10 ml of absolute ethyl alcohol and a 50 µl aliquot of the homogenate was counted in a liquid scintillation counter.

Stem Segment Tissue

Etiolated hypocotyl tissue was obtained from bean seedlings germinated in vermiculite moistened with Hoaglands solution. These were allowed to grow for 6 days in the dark at 30° C, at which time it was possible to obtain 4 or 5 two-cm stem segments starting approximately 2 cm below the hypocotyl hook. Ten stem segments (about 1 gram fresh wt.) were incubated in each petri dish containing 25 ml of incubating solution. The petri dishes were maintained in the dark at 30° C. At the end of the specified time period the segments were rinsed in the manner described for the leaf disks. The segments were then

lyophilyzed, weighed and homogenized in 10 ml absolute ethyl alcohol. A 100 µl aliquot of the homogenate from each sample was counted in a liquid scintillation counter.

Root Segment Tissue

Root tips were obtained from bean seedlings which were germinated by placing seeds between two layers of germination paper rolled into a cylinder and placed in a beaker containing Hoaglands solution. An attempt was made to maintain sterile conditions by autoclaving all materials used in germination and by pre-soaking the seeds for one minute in a solution of 1 part 5.25% NaClO : 1 part 80% EtOH. After a 72 hour germination period the lower 2 cm of the primary root tips were excised with a stainless steel razor blade and 10 root tips (0.1 gm fresh weight) were placed in a petri dish containing 20 ml of incubating solution. The petri dishes were immediately transferred to a growth chamber and maintained in the dark at 30° C.

At the end of the designated time period the root tips were rinsed in the manner described for the leaf tissue. Again the segments were freeze-dried, weighed, and homogenized in a hand homogenizer in 5 ml absolute ethyl alcohol. A 200 µl aliquot of the homogenate was counted in a liquid scintillation counter.

CHAPTER IV

RESULTS AND DISCUSSION

Previous work in this area, although far from conclusive, offers support to the hypothesis that some mono-and divalent ions do influence the uptake of the phenoxyacetic acid herbicides. For instance, Szabo and Bucholtz (39) reported that NH_4^+ ions increased the penetration of 2,4-D through the epidermis of sedum, beans and sunflower. Using stem curvature as an indicator of 2,4-D uptake, Orgell and Weintraub (26) confirmed the stimulatory effects of NH_4^+ ions. More recently Aliev and Al'Tergot (1, 2) have demonstrated an increased toxicity of 2,4-D by K^+ and NH_4^+ ions.

In view of the recent work showing the presence of a cation activated ATPase in plants and other evidence for a possible relationship between a similar system in animals and the uptake of non-electrolytes (12, 13, 17), it seemed relevant to study the influence of the cations which activate this ATPase on the uptake of 2,4,5-T by excised plant tissues. 2,4,5-T was selected as the appropriate auxin molecule because evidence suggested that it was not significantly metabolized in a 24 hour period by herbaceous plants (35, 37) and thus would prevent the interference with or the masking of the "Type I" accumulation by "Type II" accumulation described by Blackman (34)

Leaf Tissue

Sodium was stimulatory to 2,4,5-T-1-^{14}C uptake in leaf disks in both the light and dark as shown in Figure 1. The rate of uptake appears to increase through 4 hours and then levels off. Much the same pattern of uptake exists in the case of K^+ ions (Figure 2). There also appeared to be a small enhancement of uptake by light. Raven (29) and Mac Robbie (23) using algae, and Rains (28) using corn have described a light stimulated uptake of Na^+ and K^+. The apparent additive effect of light and ion stimulation on uptake of 2,4,5-T-1-^{14}C seems indicative of either a single uptake mechanism influenced by both light and ions or separate mechanisms which are responsible for 2,4,5-T uptake.

Calcium also stimulated 2,4,5-T-1-^{14}C uptake (Figure 3). In this case however, light alone stimulated the uptake of 2,4,5-T-1-^{14}C but Ca stimulation was not additive to light. This may indicate that the stimulatory role of Ca^{++} was mediated by a different mechanism from Na^+ and K^+.

These experiments confirm the reports of Sargent and Blackman (31, 32) that light at intensities greater than 1000 ft-c does indeed stimulate the uptake of auxin-like herbicides in leaf tissue.

The role of light possibly could be mediated through the opening of stomata. However, Sargent and Blackman (32) point out that the stomata are fully opened at 500 ft-c while 2,4-D uptake was not enhanced until light intensities exceed 1,000 ft-c. The role of stomata cannot be completely disregarded, however, since the rate of uptake is proportional to stomatal density. Sargent and Blackman attributed the uptake rather to guard cells and/or accessory cells which have been shown to be rich in ectodesmata and may furnish the route of entry for the

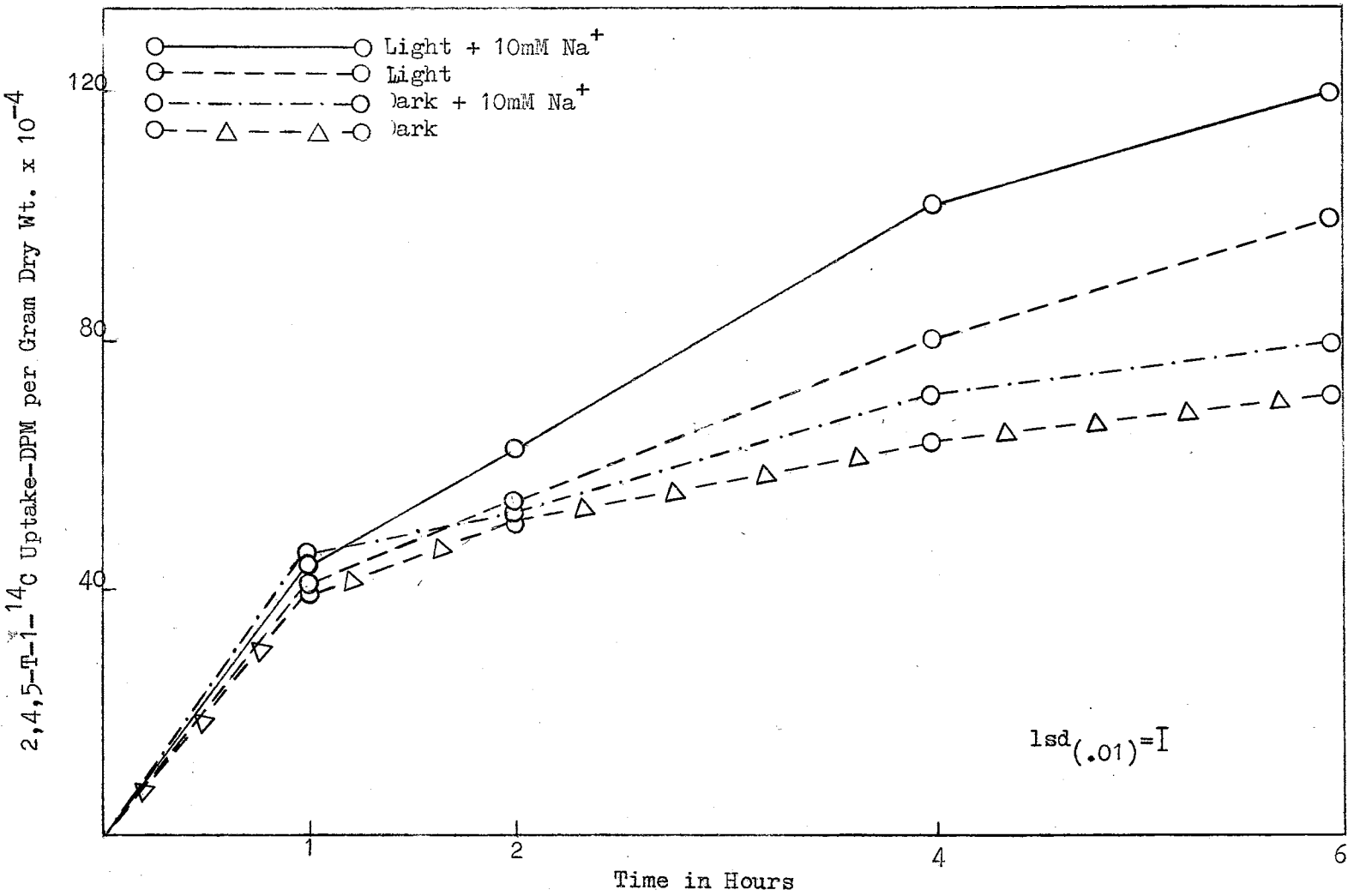

Figure 1. Influence of Light and Na^+ Ions on Uptake of 2,4,5-T-1-^{14}C from 10^{-3} M2,4,5-T Solution by Leaf Disks

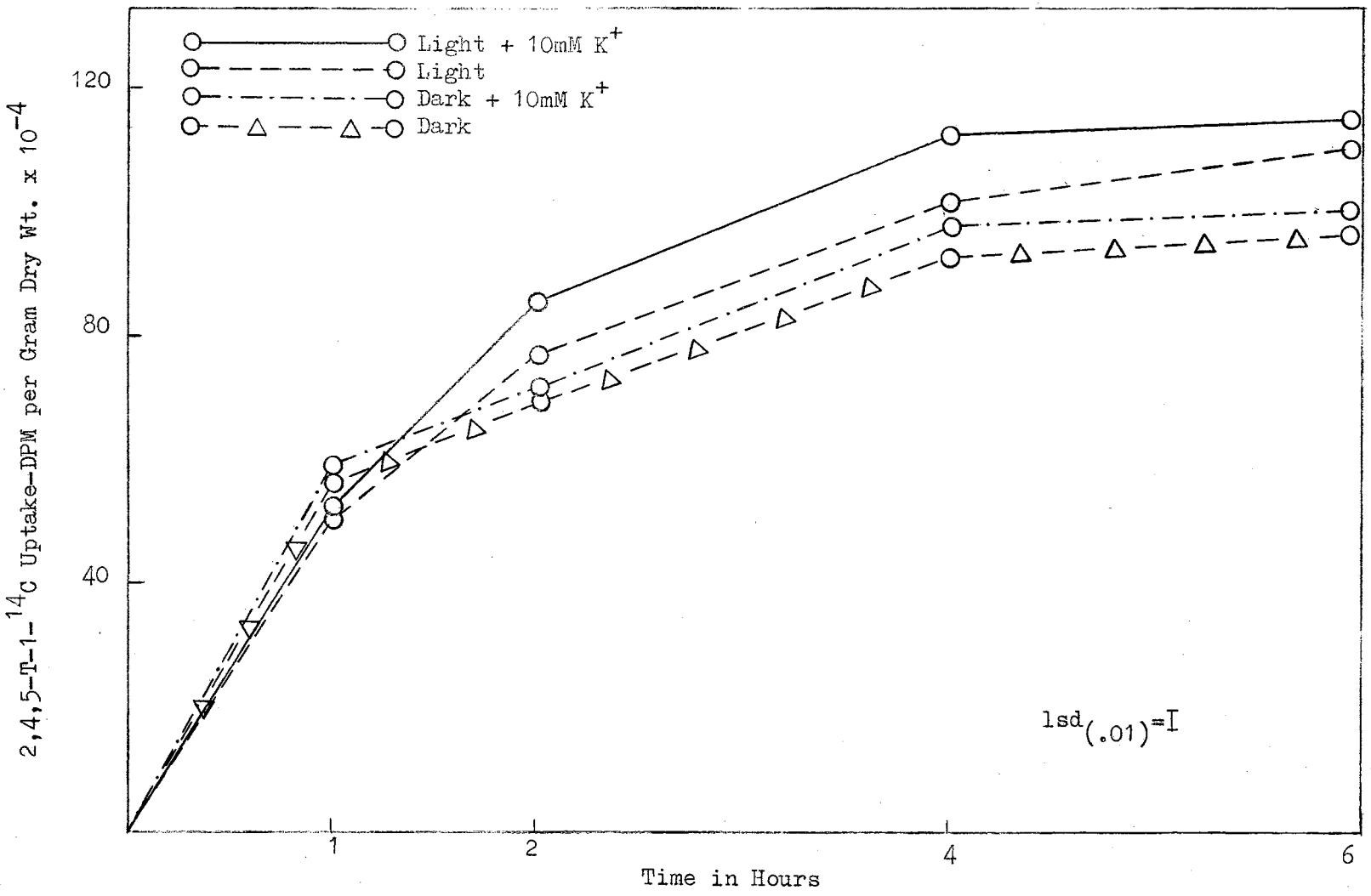

Figure 2. Influence of Light and K^+ Ions on Uptake of 2,4,5-T-1-^{14}C from 10^{-3} M 2,4,5-T Solution by Leaf Disks

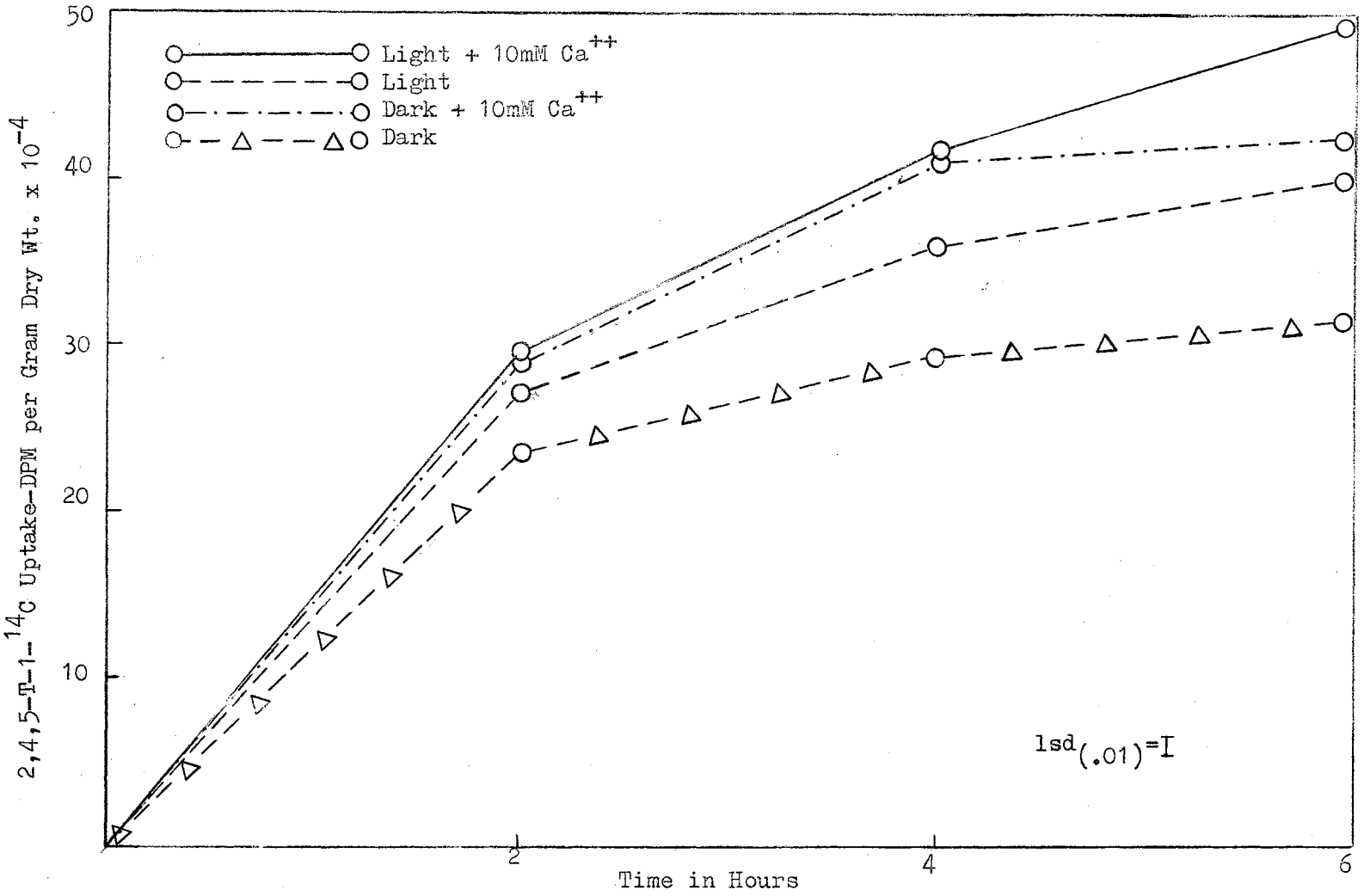

Figure 3. Influence of Light and Ca^{++} Ions on Uptake of 2,4,5-T-1-^{14}C from 10^{-3} M 2,4,5-T Solution by leaf Disks

herbicide and thus increase uptake.

Stem Tissue

In contrast to the data from leaf disks, Na^+ and K^+ had no influence on 2,4,5-T-1-^{14}C uptake by stem segments (Figures 4 and 5).

Ammonium ions were shown to be highly stimulatory to the transport ATPase enzyme activity (36, 15) and NH_4^+ also increased penetration (39) and toxicity of 2,4-D (26). These findings led to the experiments on the effects of NH_4^+ on 2,4,5-T-1-^{14}C uptake in stem tissue (Figure 6). Ammonium ions stimulated uptake throughout the first 12 hours of treatment at the high concentration (1mM) of 2,4,5-T. At the low concentration (0.05 mM) there was no significant stimulation by NH_4^+.

The apparent stimulation of 2,4,5-T-1-^{14}C uptake by Ca^{++} (Figure 7) appears to be real but was not significant at the 0.01 level. This slight increase might again by attributed to a selective binding to the cell wall.

Undoubtedly, the most outstanding feature of this series of experiments was the divergent 2,4,5-T-1-^{14}C uptake patterns found at high and low concentrations of 2,4,5-T. At the high concentration of 2,4,5-T uptake remained positive throughout the duration of the experiment and reached a maximum at 24 hours. On the other hand, in the presence of low concentration of 2,4,5-T uptake reached a maximum at 6 to 12 hours, then became negative and remained negative for the remainder of the experiment. This phenomenon has been observed previously. Blackman and Sargent (8) reported a similar uptake pattern of 2,3,5-triiodobenzoic acid by root and frond tissue of Lemna minor. They postulated that the processes of entry and egress occurred similtane-

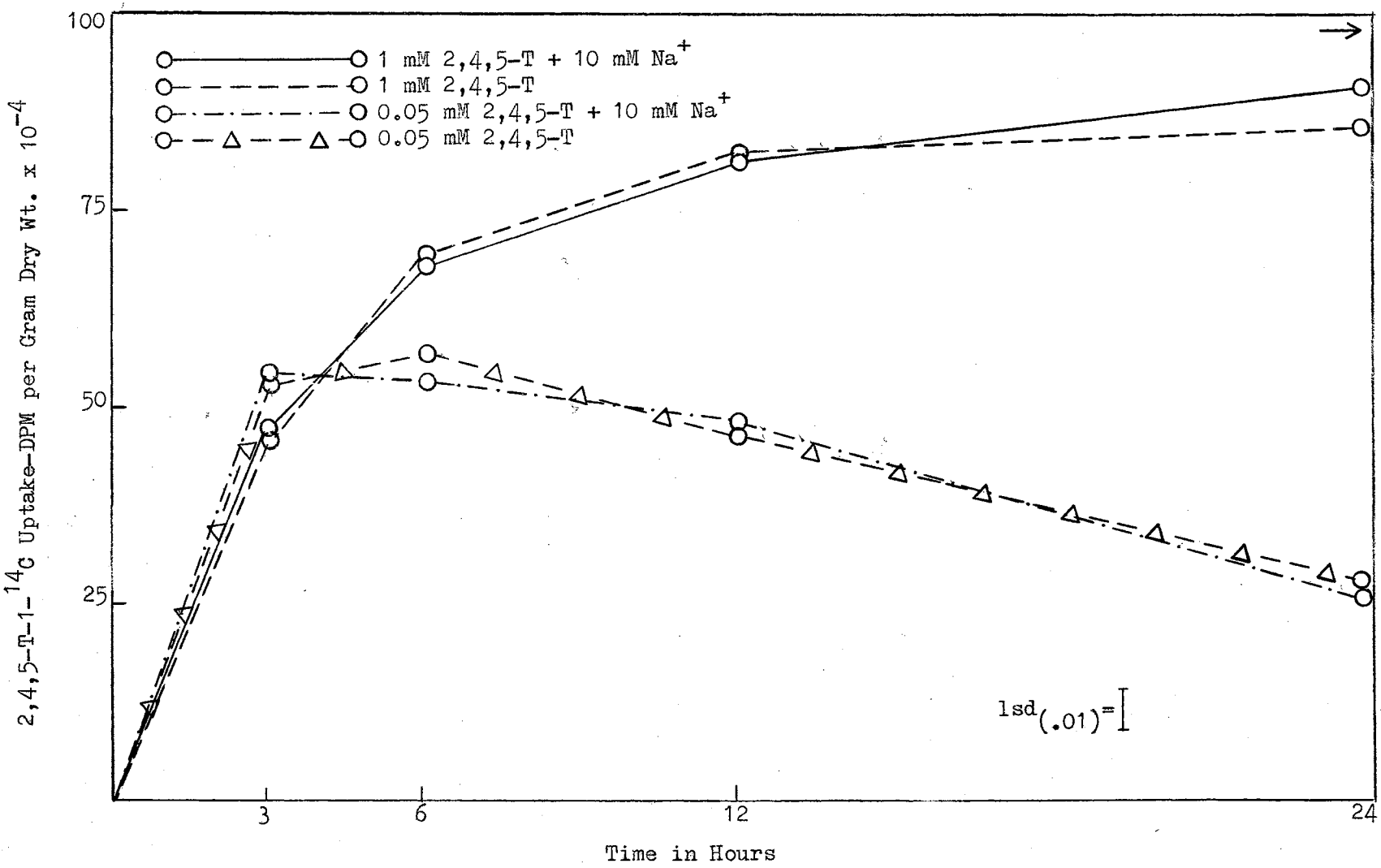

Figure 4. Influence of Na^+ Ions on 2,4,5-T Uptake by Stem Segments

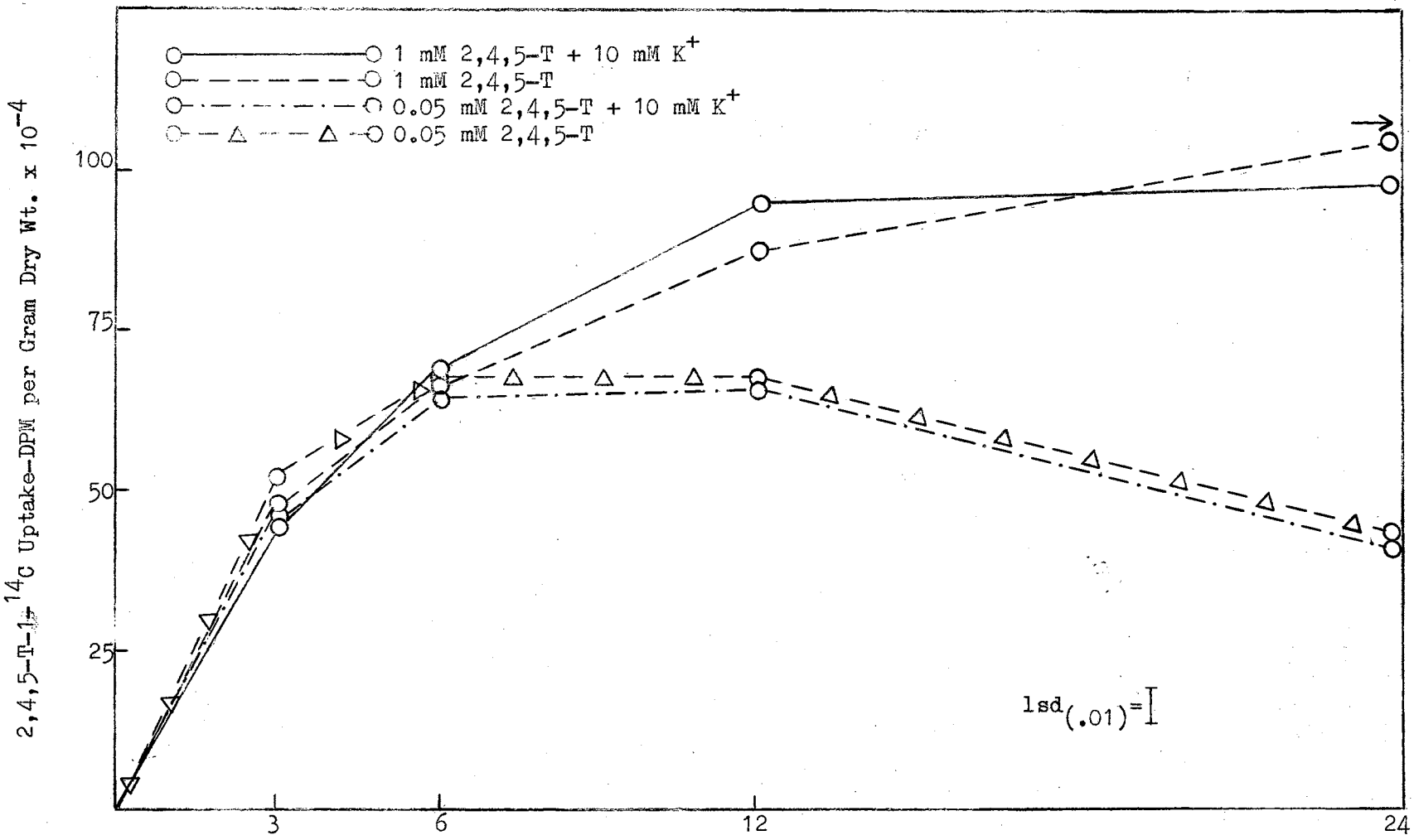

Figure 5. Influence of K^+ Ions on 2,4,5-T Uptake by Stem Segments

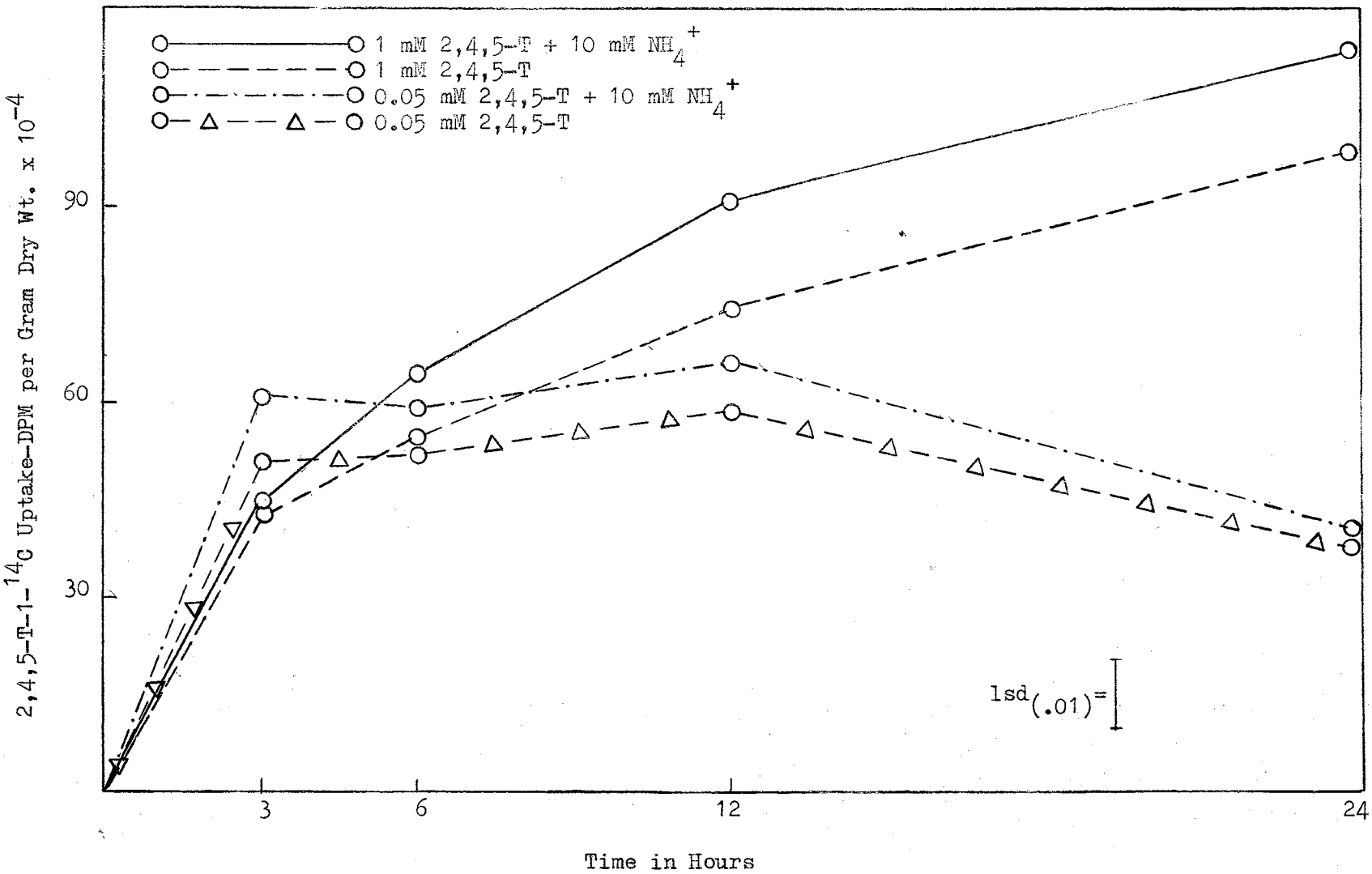

Figure 6. Influence of NH_4^+ Ions on 2,4,5-T Uptake by Stem Segments

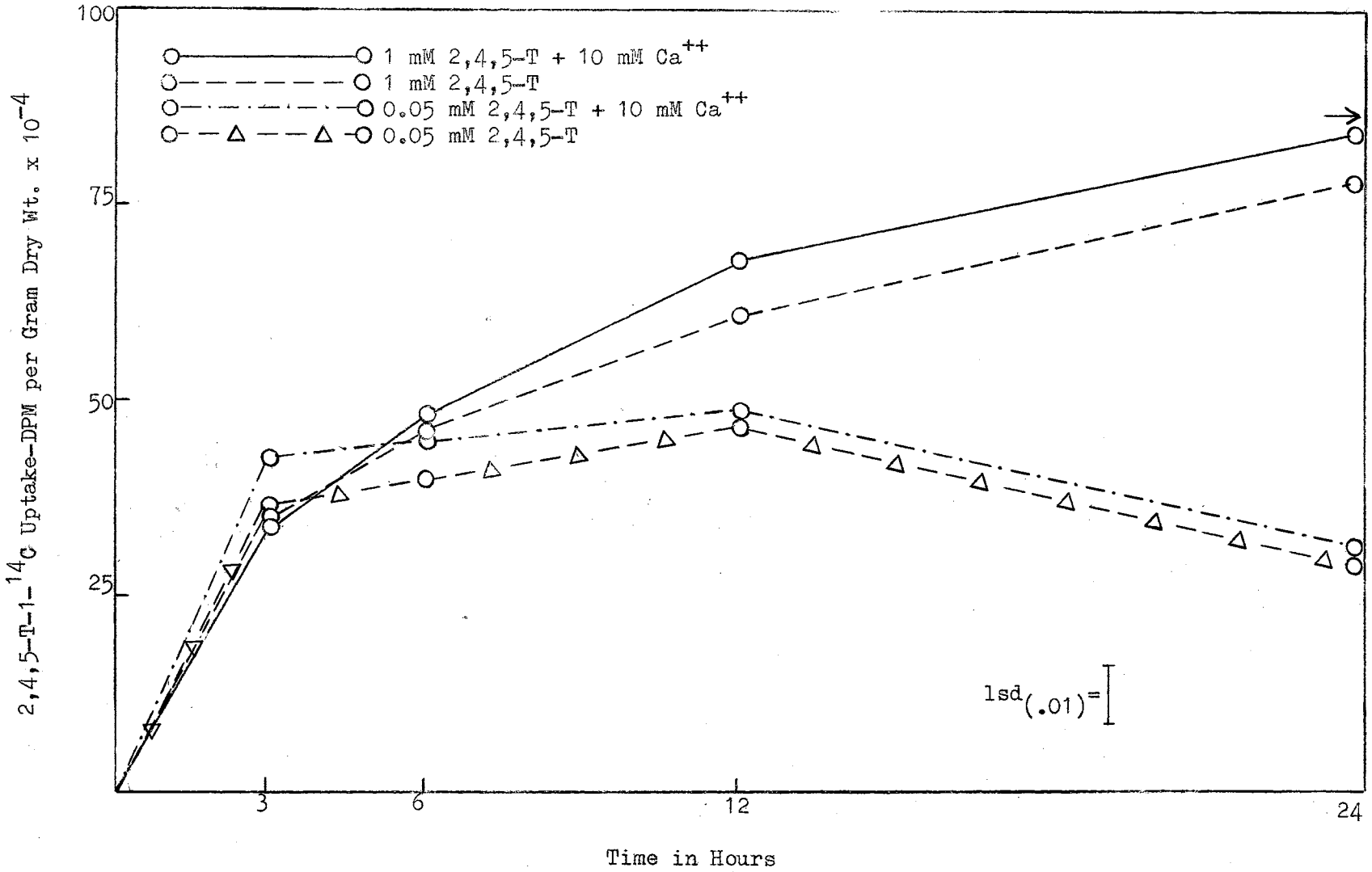

Figure 7. Influence of Ca^{++} Ions on 2,4,5-T Uptake by Stem Segments

ously and that the balance of the two processes were regulated by some physico-chemical mechanism.

In an effort to further elucidate the mechanisms of divergent uptake, the point at which there were equimolar concentrations of 2,4,5-T in the tissue and in the incubation solution was determined as described previously. The arrow along the right margin of Figures 4 through 7 reflect this point in stem tissues. At the high concentration 2,4,5-T-1-^{14}C was taken up until the concentration in the cell reached an apparent equilibrium with the bathing medium. At the low concentration, 2,4,5-T-1-^{14}C was actively exuded from the cell to maintain a concentration below that of the bathing medium.

Root Tissue

Considering the fact that the Na^+-K^+ ATPase enzymes isolated from plant tissues so far have been from root tissue it seemed logical to look to these tissues for evidence of this mechanism in uptake.

Since root tissues are notably more sensitive to the auxin-like herbicides than are other plant parts, it seemed advisable to run an initial experiment to determine the concentration optimum for 2,4,5-T relative to uptake. When uptake of 2,4,5-T was plotted against molar concentration of 2,4,5-T in the incubation solution, uptake was proportional to concentration over the entire range tested (Figure 8). Sodium ions caused a slight increase in 2,4,5-T uptake at all concentrations of 2,4,5-T. Sodium was stimulatory at 24 hours when uptake was plotted against time (Figure 9), but only at the lower concentration of 2,4,5-T and after 12 hours of treatment.

Potassium caused a small but significant stimulation of uptake at

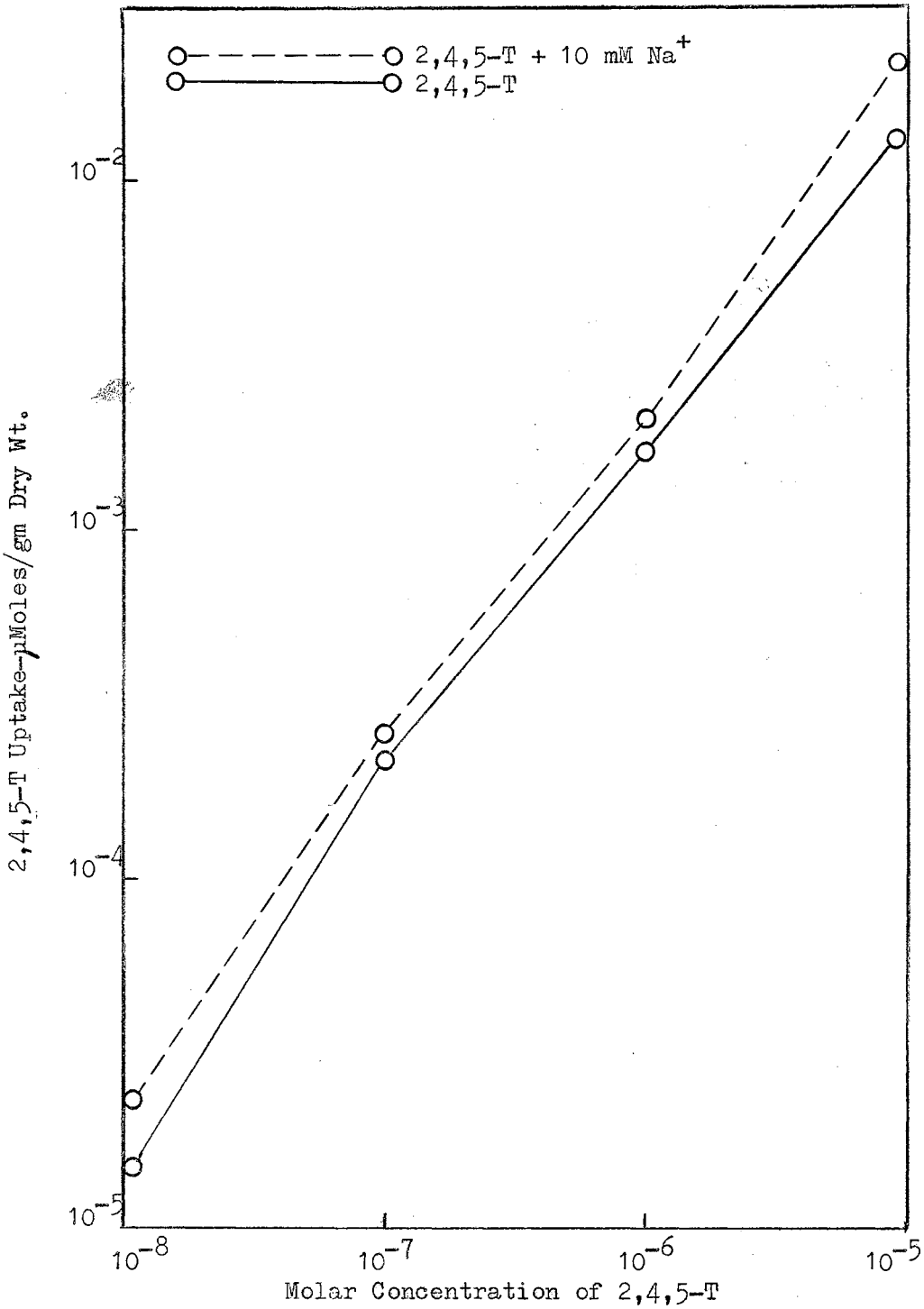

Figure 8. Uptake of 2,4,5-T by Root Tips During a 24 Hour Treatment Period

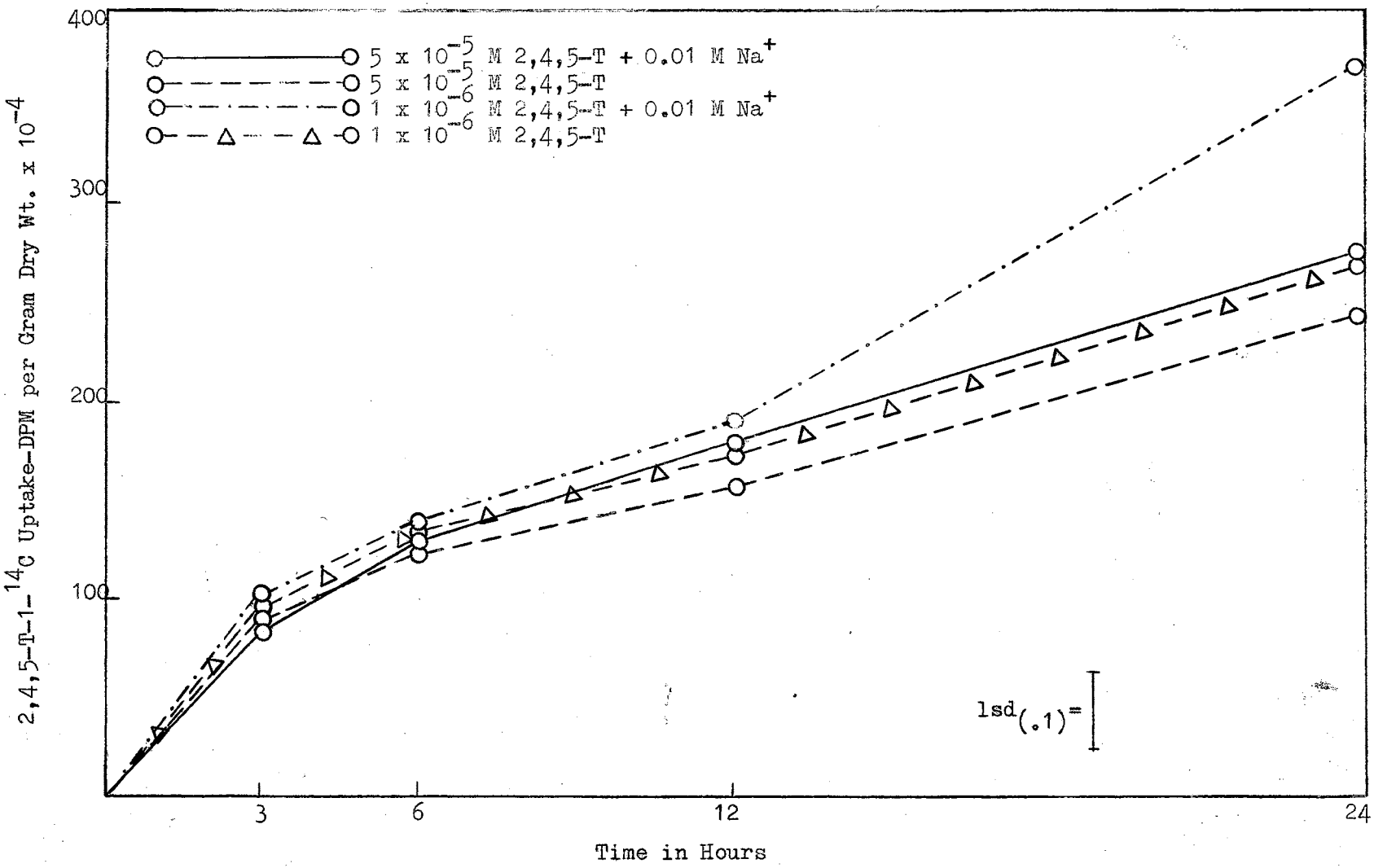

Figure 9. Influence of Na^+ Ions on 2,4,5-T Uptake by Root Tips

the higher concentration of 2,4,5-T and again after 12 hours of treatment (Figure 10).

Ammonium ions were stimulatory (Figure 11) and the degree of stimulation was striking, particularly in comparison to Na^+ and K^+. The rate of uptake was increased at 6 hours by NH_4^+ treatment at low concentration of 2,4,5-T, whereas the stimulation was delayed until 12 hours at the higher 2,4,5-T concentration.

The role of Ca in roots has been studied very intensively and it has been shown to have both metabolic and non-metabolic roles in membrane permeability. For instance, Handley, et al (19) reported that Ca effected an inhibition of uptake and loss of Na and K by primary root tissue of Zea mays. This led them to suggest that Ca caused a reduction in the equivalent pore radius in the plasmalemma. Such an effect by Ca could effectively reduce the uptake of 2,4,5-T-1-^{14}C by root segments of bean.

The data of the present experiment (Figure 12) showed a rather general inhibition of 2,4,5-T-1-^{14}C uptake by Ca^{++} except for the very marked increase in rate of uptake after 12 hours at the higher concentration of 2,4,5-T.

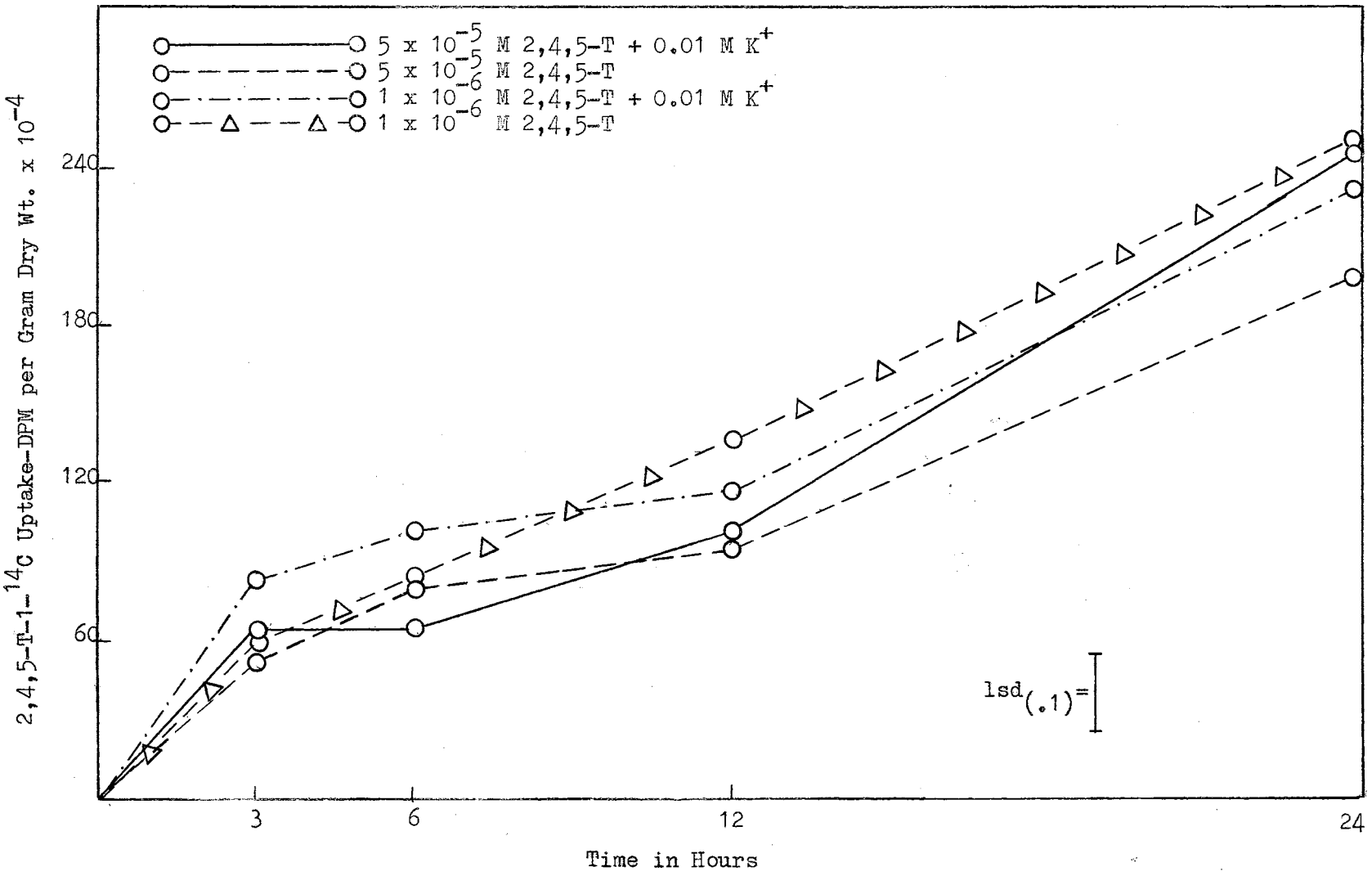

Figure 10. Influence of K^+ Ions on 2,4,5-T Uptake by Root Tips

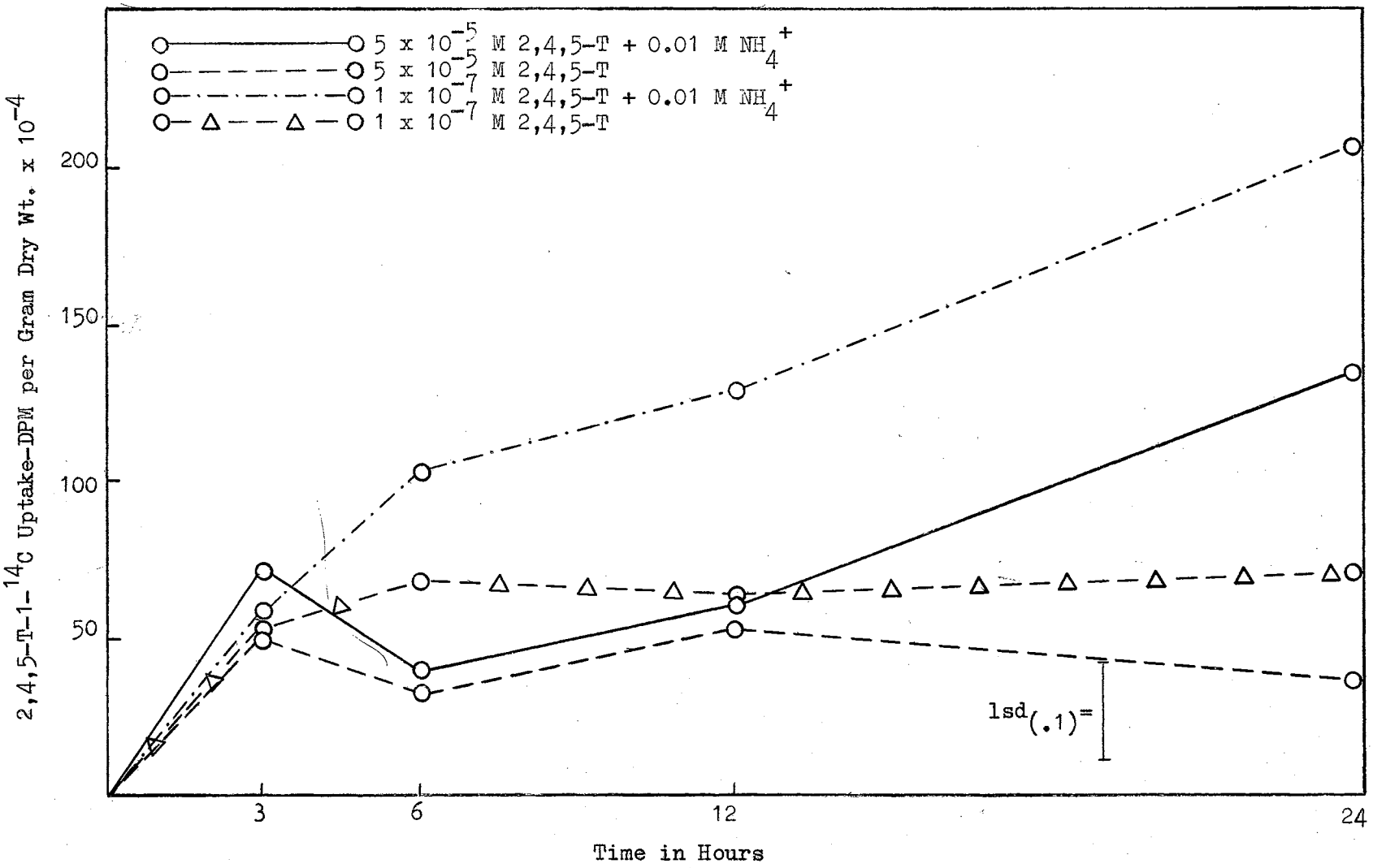

Figure 11. Influence of NH_4^+ Ions on 2,4,5-T Uptake by Root Tips

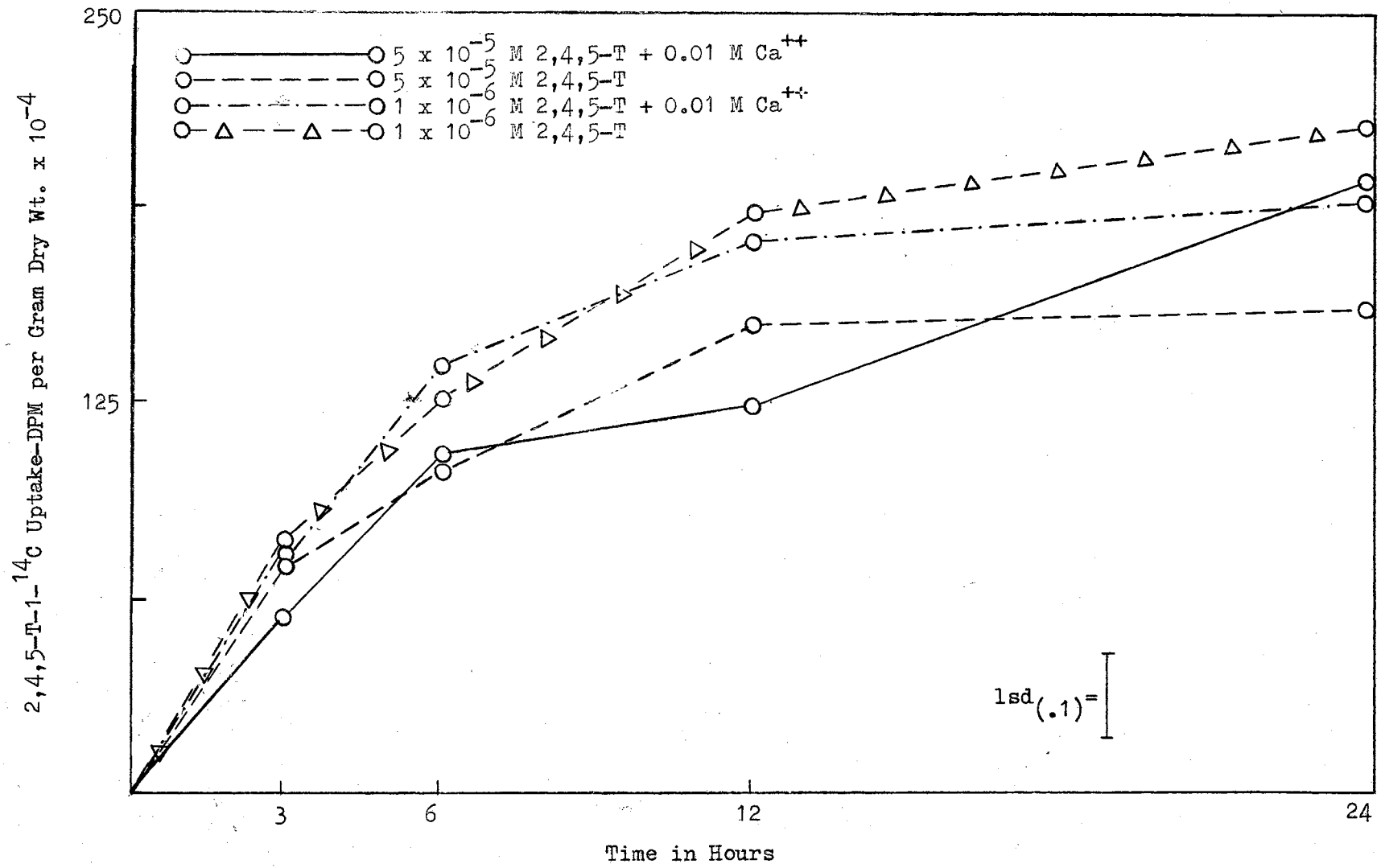

Figure 12. Influence of Ca^{++} Ions on 2,4,5-T Uptake by Root Tips

CHAPTER V

CONCLUSION

The uptake patterns of leaves and roots are similar but are markedly different from stem segments. Both leaves and roots take up 2,4,5-T against a concentration gradient (at least double the bathing concentrations). Uptake of 2,4,5-T is stimulated by Na^+ and K^+ in both types of tissues, the major difference being in time elasped before a response is initiated.

Higinbotham, et al (21) have shown that Na and K both depressed the cell transmembrane electropotential of Avena coleoptile so that the relative charge on the inside of the cell becomes more positive. Ammonium ions depressed the transmembrane potential even more than did Na and K whereas Ca increased the transmembrane potential. This suggests the possibility that Na^+, K^+ and NH_4^+ stimulated 2,4,5-T-1-^{14}C uptake could be in response to changes in transmembrane potential in leaf and root tissue since there would be relatively more positive charges to bind the negative charge of the carboxyl group of 2,4,5-T. However, Ca was not uniformly inhibitory to 2,4,5-T-1-^{14}C uptake so the comparison broke down at that point.

The relatively long lag phase between the start of treatment and the initiation of a response in root tissue by Na^+ and K^+ may indicate an inducement mechanism which could result in the synthesis of a new transport enzyme or factors affecting permeability. Johnston and Jack-

son (22) showed that Ca and Sr would induce a Ca dependent ATPase in wheat root tissue and this enzyme may be active in the transport of other ions in plant tissue.

This research has also demonstrated a possible dual uptake pattern of 2,4,5-T by stem segments. Possibly uptake at the low concentration of 2,4,5-T is through the "Type I" accumulation of Saunders et al (34) and uptake at the high concentration represents "Type II" accumulation. There are 2 criticisms of this hypothesis: (1) As mentioned earlier there are no reported instances in which metabolites of 2,4,5-T have been found in herbaceous plants; and (2) it is obvious that movement out of the cell was against a concentration gradient rather than a simple leakage. Qualitative analysis of the 2,4,5-T metabolites found in the excised plant parts could shed some light on this problem.

It seems reasonable to postulate that since the exudation of 2,4,5-T is an active process (against a concentration gradient) it is an energy requiring process. Thus it is possible that high levels of 2,4,5-T in some way reduce the amount of energy available to the mechanism that pumps 2,4,5-T out of the cell, possibly through the ability of the auxin-like herbicides to block some NAD^+ oxidizing enzymes as described by Wedding and Black (42).

This paper has amply demonstrated the need for more research in this area. This study has made it obvious that the possibility of the operation of a cation-dependent pump for the transport of electrolytes cannot be ruled out. There are several other possibilities that remain somewhat unexplored. For instance, the stimulation of 2,4,5-T uptake by Na^+, K^+ and NH_4^+ may reflect the exchange of Ca^{++} by these monovalent cations in the membrane which in turn would make the membrane more

permeable (19). Hiatt (20) and Osmond and Laties (27) have very recently suggested that uptake of cations is in response to the number of counter ions (organic acids and amino acids) in the cytoplasm and vacuole. In this study 2,4,5-T may act as the counter ions to the cations with which they were incubated.

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