THE EFFECT OF 3-AMINO-1,2,4-TRIAZOLE ON THE UPTAKE, RETENTION, DISTRIBUTION, AND UTILIZATION OF LABELLED PHOSPHORUS BY YOUNG BEAN PLANTS.

by

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We accept this thesis as conforming to the required standard

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ABSTRACT

Bean plants were grown in a phosphate-free nutrient solution to the early trifoliate stage. At this time, they were transferred to a minus phosphate nutrient solution containing 100 p.p.m. 3-amino-1,2,4-triazole for 48 hours, and then placed into a labelled phosphate nutrient solution for another hour. The plants were then returned to a phosphate-free nutrient solution and harvested one, 24, 48, and 96 hours after the period of initial phosphate uptake.

AT-treatment did not affect uptake of P^{32} but did decrease loss of P^{32} to the phosphate-free nutrient solutions after it had been absorbed by the plants. The proportion of absorbed phosphate found in the stems and leaves of AT-treated plants was higher than in these organs in the control plants. This phosphate represented an increase in both acid-soluble activity and acid-insoluble activity. The accumulation of acid-soluble activity in the shoots of AT-treated plants was an accumulation of inorganic phosphates, sugar phosphates, and nucleotides. AT appeared to inhibit downward translocation of acid-soluble and acid-insoluble activity.

The incorporation of P^{32} into esterified compounds (i.e., nucleotides and sugar phosphates) was unaffected by AT indicating that AT does not interfere with oxidative phosphorylation nor with glycolysis. However, AT did inhibit transfer of P^{32} from the acid-soluble fraction

to the acid-insoluble fraction. Therefore, the principal effect of AT is to inhibit the incorporation of phosphate into one or more of the nucleic acid, phospholipid, or phosphoprotein fractions.

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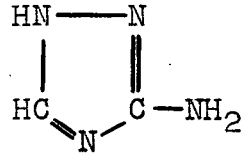
INTRODUCTION.

Formerly used only in small quantities in photographic work (6), 3-amino-1,2,4-triazole underwent field tests in 1952 as a new abscission-promoting and growth-inhibiting chemical (5,21). In 1954, W.W. Allen, chief formulating chemist of the American Paint Co. (now known as Amchem Products Inc.), was granted a patent for the use of 3-amino-1,2,4-triazole as a herbicide (6). Known also as amino triazole, amitrol, AT, or ATA, this herbicide is formulated and sold by Amchem under the trade name of "Amizol." The herbicide is also sold under the trade names of "Weedazol" and "Amino Triazole Weed-killer."

AT is a heterocyclic compound possessing the following structural formula:



AT has a molecular weight of 84.5 and a melting point between 153 - 159°C (21). It is water soluble and will react with acids and bases to form acetate, lactate, hydrochloride, nitrate, phosphate and sodium salts (29). AT can also be diazotized in the presence of nitrite and phenol to form a yellow azo dye (3). Because this reaction appears to be specific for AT, it affords a useful technique for identifying amino triazole on paper chromatograms. Other chromatographic techniques have also been developed for detecting AT (31,33,40). Amino triazole

forms stable complexes with several metals, including iron, nickel, cobalt, copper and magnesium (40). The importance of amino triazole-metal complex formation will be discussed later.

Amino triazole came to a position of national prominence in the United States during the Thanksgiving holiday of 1959 in what has become known as the "Cranberry Crisis." Amchem recommended amino triazole as a post-harvest spray for the control of redroot (Lachnanthes tinctoria) in cranberries, and in April of 1958 applied to the Federal Department of Agriculture (FDA) for a one p.p.m. tolerance for AT on cranberries (17). After investigating toxicity data, FDA notified Amchem in May of 1959 that a tolerance for amino triazole could not be established. The compound caused thyroid adenomas in rats at a level as low as 10 p.p.m. in the diet. Amchem therefore withdrew its petition.

On Nov. 9, 1959, Health, Education and Welfare Secretary Arthur S. Fleming urged that there be no further sales of cranberries or cranberry products produced in Washington and Oregon because of possible contamination with amino triazole. He said that this herbicide caused cancer in the thyroid of rats when it was contained in their diets. He was acting in conjunction with the Delaney clause of the Food Additive Amendment (24) which prohibits the addition to foods of any substance that has been shown to produce cancer in laboratory animals when fed at any dosage. Because all

cranberries became suspect in the eyes of the public, cranberries from Wisconsin, New Jersey, and Massachusetts were implicated and the bottom fell from the cranberry market. In order to clear sufficient cranberries for the Thanksgiving trade, a crash program involving 100 FDA inspectors and 60 chemists was established to analyze the cranberry crop. The cranberry crisis cost the U.S. Federal Government $10 million in indemnity to cranberry growers (25) and in addition, the government bought the unsold cranberries for government use.

The wave of controversy which resulted from this crisis has done much to stimulate interest in the metabolic effects of AT-treatment both in animal and in plant tissues. Numerous articles have appeared recently on several aspects of plant metabolism as affected by amino triazole and these will be discussed briefly in the following text.

Extensive experiments have indicated that many plants readily absorb amino triazole through the roots or through the aerial parts (7,26,30). Surface agents of the alkylaryl sulfonate type (e.g., X-77) and the alkylaryl polyoxyethelene glycol type (e.g., Multifilm C and Multifilm L) were both effective in increasing the amount of AT absorbed through the leaves (19).

Girdling experiments were used to determine how AT is translocated (21). When AT is applied to the soil or is taken up from a nutrient solution, it is translocated upward in the xylem. This is indicated by the chlorosis which occurs in the growing regions above the bark-

girdled regions of cotton plants. However, foliar applications of amino triazole indicate that the phloem is involved in translocation when amino triazole is absorbed through the leaves. When cotton plants are bark-girdled half-way up the main stem, the amino triazole is not translocated past the girdle whether the herbicide is applied to the leaves above the girdled bark or to the leaves below the girdled bark. Thus, plants appear to translocate AT in a manner similar to that used to translocate other herbicides.

Further details regarding the translocation and accumulation of AT by plants have resulted from the use of amino triazole-5-C^{14} and from radioautography (7,16,33,35). When labelled AT was applied to the cotyledons of cotton plants, it was absorbed and translocated to the roots within two hours (16). Analysis of the culture solution showed no indication of leakage from the roots. Amino triazole moved in the direction of food transport and at the same rate.

The efficiency of AT as a herbicide depends upon its ability to penetrate and to translocate readily to all parts of a plant while retaining sufficient toxicity to kill the vital organs. Radioautograms indicate that AT (or some effective metabolite) definitely tends to accumulate in the meristems, such as active buds, root tips, etc. It is scarce or lacking in dormant buds, storage parenchyma and mature tissues in general (7). This accumulation of AT in young growing tissue coincides

with the sites where AT is known to attain its maximum toxic effect.

Further experiments have indicated that movement of AT through the phloem is dependent upon production of photosynthate. Starch-depleted nut grass plants were given one drop of radioactive AT on one leaf (7). When this leaf was exposed to sunlight, AT could move readily from this leaf to all parts of the plants. However, if the leaf was kept in darkness while the remainder of the plant was exposed to light, the AT remained in the darkened leaf. This indicated that photosynthesis was essential to the translocation of AT by nut grass.

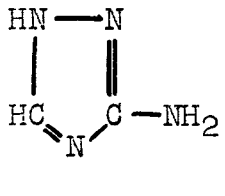
Besides affecting growth, AT is known to cause chlorosis in many plants and in many cases, chlorosis is followed by the death of the plant. Measurements of chlorophyll content in cotton regrowth-leaves have shown that AT reduces the concentrations of both chlorophyll a and b (28). Similar depressions in chlorophyll content have been measured in beans (30), tomatoes (41), barley and potatoes (32). AT-treatment also decreases carotene and xanthophyll content of various plants (1,28). The overall effect of AT is a two-fold increase in the anthocyanin content of cotton leaves (28). Spectral analysis of the composition of the anthocyanins is unaltered by AT (28).

In their early work, Miller et al (21) found that tissues formed at the time of, or subsequent to the absorption of amino triazole were characterized by chlorosis.

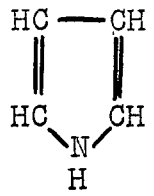
This suggested that chlorophyll synthesis was affected. Further work indicated that AT did not entirely inhibit the change from protochlorophyll to chlorophyll but that AT had its principle effect during the biogenesis of chlorophyll. Laboratory studies have shown that AT possesses the ability to form stable complexes with iron, copper, and magnesium (40). Though Miller et al (21) could not demonstrate that the restriction of chlorophyll synthesis was due to an immobilization of Mg, Fe, Mn, N, P or K, it has been shown recently that ferrous ion can partially reverse the inhibition of chlorophyll synthesis as well as completely reverse the inhibition of carotenoid synthesis and multiplication of several microorganisms (1). This suggests that AT acts as a chelating agent.

There is further evidence which suggests that amino triazole chelates metals. AT inhibited the activity of a phosphorylase preparation from the blue-green alga, Oscillatoria princeps (20). This inhibition appeared to be the result of the chelation by AT of the essential metal required by this enzyme since inhibition could be effectively reversed by the addition of manganese or ferric ions.

It has been suggested (1,21,40) that the similarity of structure of the triazole ring to the pyrrole rings of chlorophyll together with the ability of AT to form stable complexes with metals was such that amino triazole might substitute for at least one pyrrole ring thus blocking chlorophyll synthesis prior to the protochlorophyll stage.



amino triazole



pyrrole

However, Miller and Hall (28) have almost completely excluded the likelihood that AT enters into the synthesis of pseudochlorophyllous porphyrins. Using radioactive AT, they recovered only extremely low C^{14} activity from their pigment fractions.

Cytological data has indicated that chlorosis is due to a lack of chloroplasts, rather than some effect on chlorophyll per se (34). Microscopic examination of the chlorotic tissues of several plants has indicated that the plastids are few in number, shrunken and misshapen. Wort and Loughman (44) have shown that an altered phospholipid metabolism may be responsible for the inhibition by AT of plastid development. Sund (41) found that AT blocked the synthesis of riboflavin, and the need for certain flavin coenzymes might account for a decrease in plastid development. Both groups of investigators also presented evidence which indicated that AT inhibited protein synthesis.

It is interesting to note the effect of AT on various iron-porphyrin enzymes since these enzymes are also pyrrole-containing compounds. Pyfrom et al (32) were among the first investigators to study the effects of AT on catalase activity. Using barley and potato plants with low con-

centrations of AT, they showed that catalase activity is depressed whenever AT is present in the tissues. It has been shown recently that AT inhibits the synthesis of tryptophane peroxidase-oxidase, an iron-porphyrin enzyme found in the liver of rats (8). Whereas the cytochrome oxidase enzyme in chlorotic corn tissue was not affected by AT-treatment (27), the cytochrome oxidase activity of etiolated wheat seedlings is increased by AT-treatment (43). There is no information available to indicate whether or not AT interferes with other cytochrome enzymes which commonly occur in plants.

It has been suggested (1) that the main action of AT in vivo is to interfere with the synthesis of porphyrin-containing enzymes, and only when AT is present in high concentrations does it inhibit existing enzymes. If AT acts primarily as an iron chelator, it is more likely to prevent synthesis by making iron unavailable rather than by making it ineffective when it is already part of the enzyme molecule.

Inhibition of catalase activity results in an increase of H_2O_2 in plant tissues. Racusen (33) has proposed that an increase in H_2O_2 would lower the amount of IAA by action of indoleacetic acid oxidase. This would result in an indirect AT-induced growth inhibition. Russell (36) has found that AT does increase the peroxide level of individual root cells. He recognized the significance of an increased peroxide level with respect to the IAA reduction sequence. He therefore attempted to

establish whether or not IAA would reverse AT-growth inhibition. His experiments indicated that there was no such interaction. However, other workers (21) have shown that AT does antagonize the growth effects of IAA thus lending support to Racusen's hypothesis.

Reduction of growth inhibition has also been obtained by adding purines and pyrimidines to plants simultaneously with AT (4,41,42). Because these compounds are necessary for the production of nucleic acids, it has been proposed that AT interrupts protein synthesis since nucleic acid is necessary for protein production (41,44).

Many workers have noted that AT stimulates respiration (21,27,29,36). Wort and Shrimpton (43) found that foliar applications of 4000 p.p.m. AT resulted in an immediate and continued increase in respiration of both wheat and bean plants. All concentrations of AT up to 840 mg/l increased the respiratory rate of cotton leaf discs (21,29). Application of AT to homogenates of wheat seedlings grown in the dark for 6 days also increased the oxidation of reduced cytochrome c (43) indicating that cytochrome oxidase activity was increased by AT-treatment.

Thus, it appears that there may be several loci at which AT induces growth inhibition. Briefly, these would seem to include such things as inactivation of certain enzyme systems through chelation of essential metal, oxidation of IAA by H_2O_2, decreased photosynthesis (43) and increased respiration which lower the amount of available sugars, and finally, a decreased phospholipid and

nucleic acid production in AT-treated plants.

Many plants can metabolize AT. The use of paper chromatography for the detection of AT in plant extracts has given an insight into this problem of AT metabolism. Aldrich (4) removed AT from plant tissue by grinding the tissues in 80% ethanol. The eluate was chromatographically separated on Whatman No. 1 paper using ethanol:water: 1-butanol (1:1:4) as the solvent. The paper was sprayed first with 5% KNO_2 and then with phenol in 20% HCl. In the experiments using this technique, two yellow spots appeared on the chromatograms. The assumption was made that the spot with the lower Rf value was "bound" AT, that is, AT bound to protein. The other spot was designated as "free" AT and appeared at a higher Rf value.

While studying the translocation and fate of AT in Canada thistle, Johnson grass and soybeans, Rogers (35) was able to demonstrate that even though soybeans were very susceptible to AT, the plants were able to metabolize all of the AT supplied. In the case of soybeans and Canada thistle, he demonstrated the presence of an unknown entity having a small Rf value. Chromatograms prepared from Johnson grass, which was least susceptible to AT-treatment, did not have this entity, a fact which he felt might be of importance for explaining tolerance of plants to AT.

Herrett (22) has found that the rate of AT-metabolism within bindweed is more rapid than in thistle. He isolated two metabolites which he has designated as Unknown I and

Unknown II. In Canada thistle, he noted that there was a lag in the transport of AT from the treated leaf to the stem. The formation of a transportable form of AT was postulated as the limiting reaction for its distribution through the phloem from the leaf of application to the remainder of the plant. Herrett showed that movement of AT through the xylem did not require synthesis of a transportable form of AT and he supported the claim that the xylem is not the major pathway of AT movement out of leaves. The difference in sensitivity to AT by bindweed and thistle was concluded to be a result of major differences in absorption and metabolism of the herbicide.

In 1957, Racusen (33) undertook some experiments to determine whether AT itself or some product of AT-metabolism was the actual material which was toxic to certain plant activities. He demonstrated that amino triazole was transformed into two new products in the leaves of young bean plants. He designated these AT-metabolites as Compound X and Compound Y. Because it was not certain that X and Y were actually transformation products of AT, proof of their origin was established using C^{14}-labelled AT.

Compound X was the major product of AT metabolism. It was formed at a uniform rate in young bean leaves for at least 4 days. After 5 days, 93% of the AT had been incorporated into Compound X. Racusen eluted Compound X from his chromatograms and determined its toxicity using Lemna minor. Like AT, Compound X produced chlorosis and stunting but its toxicity was lower than that of AT alone. Racusen

also isolated Compound Y which he found to be non-toxic to Lemna cultures. He could not establish whether or not X and Y were merely detoxification by-products of AT metabolism as suggested by their lower toxicity, or whether they were formed as a direct consequence of some toxic reaction of AT. However, both compounds were characterized by the same ring system and free amino group of AT. Because healthy, turgid bean leaves were required for their formation, Racusen has suggested that the transformation of AT into X and Y is probably carried out by some enzyme system in the leaf.

Another approach to the problem of AT metabolism has been investigated (12,15). Guided by the hypothesis that AT interferes with the production of porphyrins which are essential for chlorophyll production, Carter and Naylor (15) studied the metabolism of certain porphyrin precursors in AT-treated plants. When excised tips of bean plants were exposed to glycine-1,2-C^{14} in a solution of AT, large quantities of radioactivity appeared in an unknown, ninhydrin sensitive compound which was not present in the controls. The unknown was designated compound "1". Glycine-1-C^{14}, glycine-2-C^{14}, serine-1,2,3-C^{14} reacted similarily whereas glucose, succinate and bicarbonate labelled with C^{14} were ineffective. When AT-5-C^{14} was used, compound "1" became heavily labelled indicating that a complex between AT and serine or glycine was formed by bean plants.

Massini (26) has recently isolated and purified an AT metabolite which he calls ATX. ATX is a white powder

composed of fine needle-like crystals. It has a melting point of 230-232°C. as compared to AT at approximately 155°C. This compound has been identified as 3-amino-1,2,4-triazolyl alanine. Indications at present are that this compound is not the same as compound "1" described by Carter and Naylor nor is it similar to Compound X described by Racusen. Rather, these latter compounds are thought to be identical (13,14).

Recent studies by Carter and Naylor (13) have shown that compound "1" (i.e. Compound X) is the principal metabolic product formed in beans, alfalfa, silver maple, and honeysuckle when treated with AT. However, they also demonstrated that nine other compounds derived C^{14} from AT thus demonstrating the complexity of AT metabolism in plants.

The present study is an attempt to discover the effect of AT on several aspects of phosphate metabolism in young bean plants, the roots of which were first exposed to 100 p.p.m. AT for 48 hours followed by a one hour exposure to radioactive phosphorus. The influence of AT on the uptake and retention of radioactivity and on the distribution of activity within the plant was determined. Attempts were also made to study the manner in which AT affected the incorporation of inorganic phosphate into organic compounds. A comprehensive study of the effect of AT on the phosphate metabolism of barley has already been made (44) and this work will be discussed later.

EXPERIMENTAL.

Bean seeds (Phaseolus vulgaris, Top Crop, 1960) obtained from Buckerfield Seed Co. were sown in vermiculite saturated with phosphate-free nutrient solution. The plants were grown in a constant environment chamber at a temperature of 72°F., a relative humidity of 62%, and a light intensity of approximately 2500 f.c. The plants were given 14 hours of light daily. After six days, uniform seedlings were transplanted to bottles containing fresh phosphate-free nutrient solution. These solutions were given continuous aeration.

Eleven days after germination, when the plants had grown to the early three-leaf stage, plants were again selected for uniformity so that only eight bottles, each containing four plants, remained. At midday (corresponding to seven hours of light), the plants in four bottles were quickly transferred to bottles containing 100 p.p.m. AT in fresh phosphate-free nutrient solution. The control plants were transferred to fresh phosphate-free nutrient solutions. These solutions were continuously aerated.

At midday, 48 hours later, the roots of all the plants were quickly rinsed with distilled water and then immersed in a fresh culture solution to which had been added $NaH_2P^{32}O_4$. This solution had an activity of 80 uC P^{32} per 3000 mls. of culture solution. After a one hour exposure to P^{32}, the roots of all the plants were quickly rinsed in distilled water and the plants were returned to bottles

containing fresh phosphate-free nutrient solution. Four AT-treated and 4 control plants were removed one, 24, 48, and 96 hours after the start of P^{32} absorption for the extraction of phosphorus compounds.

EXTRACTION OF LABELLED COMPOUNDS.

Labelled compounds were extracted from plant tissues by the procedures of Wort and Loughman (44). The two groups of four plants from the AT-treated and control groups were quickly divided into roots, stems and leaves. Each plant part was cut into smaller pieces and immersed in 12 mls. of ice-cold 16% trichloracetic acid (TCA) contained in chilled mortars. The cold TCA caused an immediate cessation of metabolic activity. After grinding for approximately five minutes, the material was transferred quantitatively to 25 ml. screw cap vials using a minimum amount of ice-cold wash water. The vials were then centrifuged for 10 minutes at 3000 r.p.m. and the supernate was decanted into another vial. The residue was re-extracted with five mls. of ice-cold 8% TCA and centrifuged in a refrigerated centrifuge for 10 minutes at 3750 r.p.m. This supernate was poured into the first supernate solution and the combined supernate was made to 25 mls. with ice-cold water. This solution comprised the acid-soluble phosphate compounds (i.e., inorganic phosphates, nucleotides, and sugar phosphates). To determine the total acid-soluble count, a two ml. aliquot was removed and made to 10 mls. in a volumetric. This solution was counted in a 20th Century

Electronics M6 liquid counter (see below). To reduce changes, the remainder of the acid-soluble phosphate solution was frozen solidly.

The screw cap vial containing the plant residue was inverted on a paper towel to dry. This residue, comprised of acid-insoluble phosphate compounds (i.e., phospholipids, nucleic acids and phosphoproteins) was transferred to 150 ml. Kjeldahl flasks and digested using five mls. of concentrated nitric acid and five mls. of 60% perchloric acid. The liquid was reduced to five mls. with heating. This required from two to three hours. The cooled solutions were then made to 10 mls. with water and counted in the M6 liquid counter. This count represented the total acid-insoluble phosphate compounds.

SEPARATION OF ACID-SOLUBLE PHOSPHATE COMPOUNDS.

The acid-soluble solutions, containing inorganic phosphates, sugar phosphates and nucleotides, were allowed to thaw. The solutions were centrifuged at 3750 r.p.m. in order to remove polysaccharide material formed during freezing. A 10-ml. aliquot was taken from each solution and placed in a continuous liquid-liquid extraction system. A three to four hour extraction with ethyl ether was needed to remove the TCA. After extraction, the solutions were poured into chilled vials and if any ether layer remained on these solutions, it was removed with a capillary pipette. If stored overnight, these solutions were frozen to reduce any chemical changes. When thawed, the solutions were

again centrifuged at 3750 r.p.m. to remove any polysaccharide material.

Two-milliliter samples of these solutions were reduced to approximately one-half ml. using an infra red lamp and a current of warm air from a hair drier. The reduced solutions were spotted on acid-washed 3 MM Whatman papers. (Sheets of 3 MM Whatman paper were placed in a tray, covered with 2N acetic acid, and allowed to stand overnight. The acid was removed next morning and this wash was repeated several times. When the acid solution no longer gave a precipitate when ammonium hydroxide and ammonium oxalate were added, the papers were thoroughly rinsed with distilled water. This washing removed metal impurities, particularly calcium, which interfere with the chromatography of certain phosphate compounds.) The chromatograms were equilibrated in the chromatography chambers in an atmosphere of H_2S. This converted any metals which remained on the papers to sulfides and these sulfides remained at the origin. This gas was generated by dropping crystals of sodium sulfide into 6N HCl. After one hour equilibration, the solvent was added to the trays. The solvent used was tert-butanol: water:picric acid (80 mls:20 mls:2.0 gms). The solvent was allowed to run for 20 hours.

In order to find the areas of activity on each chromatogram, autoradiograms were prepared. Each chromatogram was placed on a piece of plywood covered with filter paper. The chromatogram was covered with a sheet of Saran Wrap and in the darkroom, X-ray film, Du Pont Medical Type 508, was

placed over the chromatogram. A piece of black paper and a second piece of plywood were placed over the X-ray film. The two pieces of plywood, together with their chromatograms, X-ray film, etc., were wrapped with light-proof black paper. These packages were stored in a press for 48 to 72 hours depending on the relative activity of the spots. Before the films were removed for development, a pin was thrust through each film and chromatogram in several spots to insure the correct replacement of the film.

The chromatograms were cut into segments containing inorganic phosphates, nucleotides or sugar phosphates. Each segment was wet ashed in a 150 ml. Kjeldahl flask using two mls. of nitric acid and 2.2 mls. of perchloric acid. The digests were made to 10 mls. and counted in the M6 liquid counter. This count represented the relative activity of the acid-soluble fractions. These fractions were not further characterized.

RETENTION OF P^{32}.

To determine the amount of P^{32} which had been lost from the plants to the nutrient solutions, the solutions which remained after the 24, 48, and 96 hour harvests were each made to one liter in a volumeteric flask. A 10-ml. aliquot of each of these solutions was counted in the M6 liquid counter.

THE M6 LIQUID COUNTER.

The radioactive solutions were counted in a 20th Century Electronics M6 liquid counter. The construction and uses of this counter are fully described by Russell and Martin (37,38). The counter tube was supported in a lead castle in order to shield the tube from all but the most penetrating cosmic rays thereby reducing the background count to below 16 c.p.m. In order to record the number of emissions passing from the radioactive solution and through the counter, the tube was connected to a Nuclear-Chicago scaler (Model 151A) equipped with a Model T1 timer.

The 20th Century Electronics M6 liquid counter is a thin-walled Geiger-Muller counter surrounded by a glass jacket into which a sample of liquid 10 mls. in volume can be placed. The counter is designed to record up to 10% of the emissions from a liquid sample of P^{32}. This type of counter has two distinct advantages over conventional end window counters designed to assay dry samples of plant material. Firstly, the position of the radioactive sample relative to the position of the counter is always the same when a 10-ml. aliquot is added to the jacket surrounding the liquid counter. Small variations in the position of dry samples relative to the end window counter often results in large variations with regard to the number of particles which will pass from the dry sample through the counter. The M6 tube is also designed so that samples of P^{32} slightly

in excess of 10 mls. give no appreciable alteration in the counting rate. Secondly, liquid samples are much easier to prepare than are dry samples and losses which occur during the preparation of liquid samples are usually negligible compared to losses encountered when preparing small samples of dry material. Liquid counting may have one disadvantage when using liquid samples of P^{32}. This isotope tends to adsorb to glass surfaces. This loss may result in appreciable experimental errors. Adsorption losses are prevented by adjusting the sample to pH 3 or by adding carrier phosphate to solutions which contain less than one p.p.m. P^{32}. If these precautions are not taken, then solutions which contain low concentrations of P^{32} may lose as much as 83% of their activity after six days due to adsorption of P^{32} on the walls of glass containers when the solutions are stored.

COUNTING PROCEDURE AND CORRECTION AND ANALYSIS OF RESULTS.

Because the time interval between consecutive disintegrations in a sample of P^{32} is subject to random variation, greater accuracy was obtained by counting a larger total number of counts. This was done by counting all samples for 20 minutes or for 10,000 counts depending upon which came first. Further, any solutions which were more active than 6800 counts per minute (c.p.m.) were diluted until they counted below this rate. This was done in order to prevent losses of counts which occured when the scaling unit was recording above this maximum counting

rate.

All samples were corrected for background and radioactive decay. The activity of each sample was corrected to a chosen reference time, namely, to the activity of the sample one hour after initial phosphate uptake by the plant.

In order to determine whether or not the difference between sample means was due to treatment or to chance, the means of the small samples were compared using the 'Student's' t-test (9).

$$t = \frac{\bar{X}_1 - \bar{X}_2}{S.D.\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

To determine the significance of the t-value given by the above equation, the one-sided table for 'Student's' t-distribution was used.

RESULTS.

The response of bean plants to AT-treatment is quite striking. For the first 24 hours after treatment with 100 p.p.m. AT, treated plants look very similar to control plants with the exception that some chlorosis of the petioles of the first trifoliate leaves can usually be found. However, after 48 hours, the basal portions of the expanding first trifoliates become partially chlorotic. A concentration of 100 p.p.m. AT does not result in the immediate cessation of growth so that any secondary trifoliates which appear at this time are characteristically white, indicating a complete lack of chlorophyll and carotenoids. Areas within the laminae of primary leaves also begin to lose their normal green color 48 hours after treatment. These leaves are usually quite flaccid as compared to the primary leaves of control plants, with the result that the margins of treated leaves tend to curl upward. Some of the primary leaves may abscize during this period. After 96 hours, many of the treated plants have abscized both of their primary leaves or if not, these leaves are very nearly ready for abscission. If the plant is jarred, these leaves will usually fall. The older trifoliate leaves are characteristically yellow while the young trifoliates are white. Growth appears to have terminated 96 hours after AT-treatment.

INTAKE, RETENTION AND DISTRIBUTION OF P^{32}.

The 48 hour exposure of the roots of young bean plants to 100 p.p.m. AT had very little effect on the total uptake of P^{32}. This is clearly illustrated in Fig. 1. One hour after the beginning of phosphate uptake, the t-value for the P^{32} content of AT-treated plants did not differ significantly from that of the control plants.

Thereafter, however, the roots of control plants lost more P^{32} to the final phosphate-free nutrient solutions than did the roots of AT-treated plants (Table I). This effect was most noticeable 24 hours after the end of AT-treatment by which time the roots of control plants had lost 31.8% of their original activity to the nutrient solution whereas the AT-treated plants lost only 1.7%. All plants continued losing activity to the nutrient solutions for the duration of the experiment. This loss appeared to reach a steady state between the 48 hour and 96 hour harvests. Ninety-six hours after the beginning of phosphate uptake, AT-treated plants had retained 6.9% more of their original activity than had the controls.

There was a marked increase in the amount of P^{32} translocated to the leaves of AT-treated bean plants (Table II). T-values differed significantly one hour after the beginning of phosphate uptake. Ninety-six hours after the beginning of phosphate uptake, the leaves of AT-treated plants had approximately 40,000 c.p.m. more activity than the leaves of control plants.

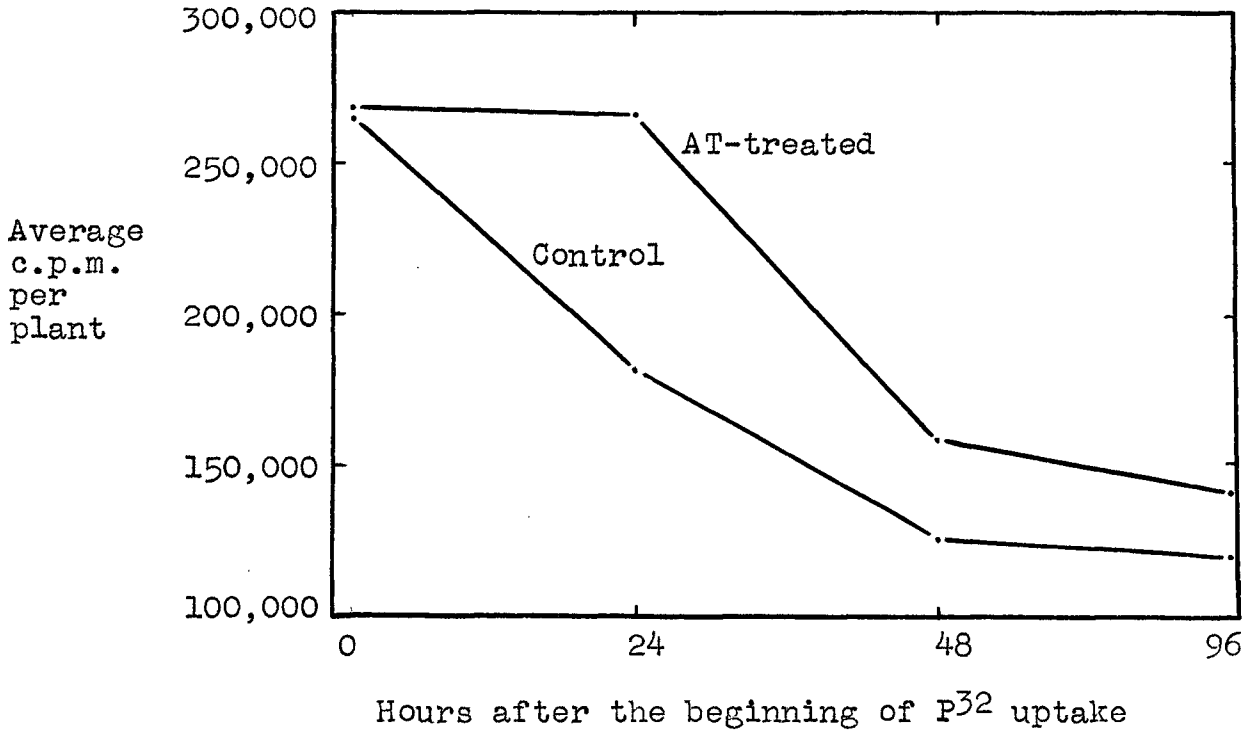


Fig. 1. The effect of AT-treatment on the uptake and retention of phosphate by whole bean plants.

TABLE I

UPTAKE AND RETENTION OF P^{32}

Hours after initial contact with P^{32}	Plant	Total activity per plant (cpm)	Activity lost to the nutrient solution (cpm)	Percentage of total initial activity lost
1	AT	268,880		
	Control	266,675		
24	AT	264,325	4,555	1.7
	Control	182,255	84,420	31.8
48	AT	158,418	110,462	41.0
	Control	125,154	141,521	53.7
96	AT	141,006	127,874	47.5
	Control	122,886	143,789	53.9

TABLE II

DISTRIBUTION OF P^{32} IN LEAVES, STEMS AND ROOTS.

Hours after initial contact with P^{32}	Plant	Organ	Total activity (cpm/organ)	Percentage of total P^{32} found in each organ	t-value
1	Treated	Leaves	1,932	0.71	
		Stem	13,637	5.06	
		Roots	253,311	94.22	32.96 ***
					15.38 **
	Control	Leaves	281	0.10	16.87 **
		Stem	4,564	1.67	
		Roots	261,830	98.22	
24	Treated	Leaves	7,265	2.15	
		Stem	41,905	20.10	
		Roots	215,154	77.74	0.49
					4.21 *
	Control	Leaves	2,950	1.64	3.27 *
		Stem	13,549	7.49	
		Roots	165,756	90.87	
48	Treated	Leaves	33,102	20.85	
		Stem	35,827	22.57	
		Roots	89,489	56.57	37.61 ***
					41.73 ***
	Control	Leaves	5,518	4.11	93.28 ***
		Stem	12,718	10.34	
		Roots	106,918	85.54	
96	Treated	Leaves	44,721	31.96	
		Stem	22,966	16.27	
		Roots	73,319	51.76	4.41 *
					7.70 *
	Control	Leaves	3,940	2.77	5.48 *
		Stem	15,225	12.12	
		Roots	103,721	85.10	

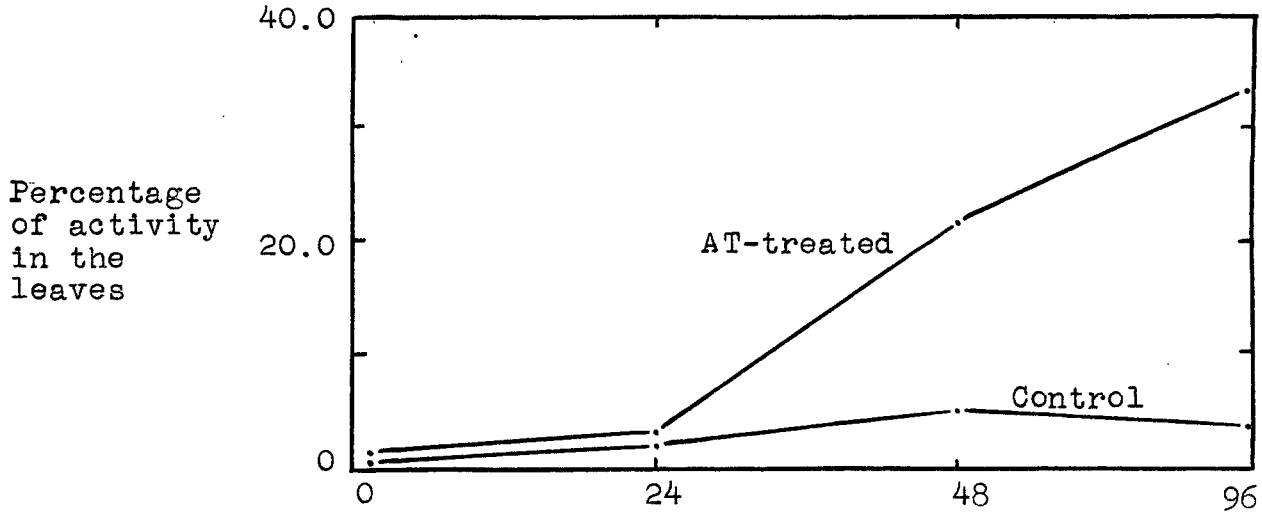
* significant at 0.05 level
** significant at 0.01 level
*** significant at 0.001 level

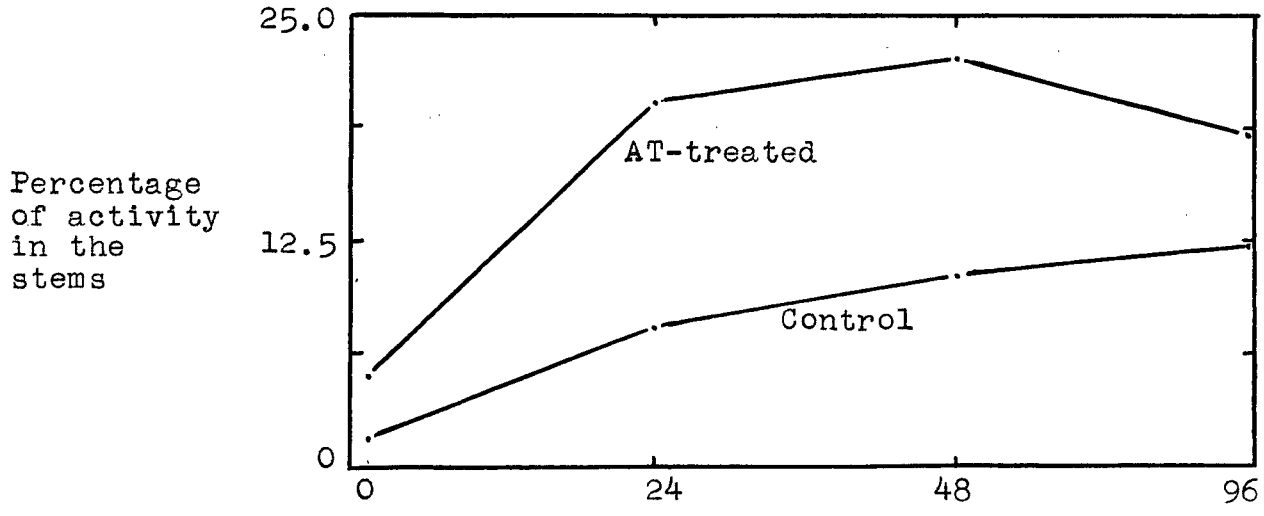
When the activity in the leaves, stems and roots is expressed as a percentage of the total activity in the plant, a much clearer picture of the distribution of P^{32} within the plant is given (Fig. 2). The roots of AT-treated plants lost activity to the shoots steadily for 96 hours, at which time only 51.8% of the activity remained in the roots. For the first 24 hours, the largest amount of the activity translocated from the roots appeared primarily in the stems. Thereafter, the leaves of AT-treated plants rapidly accumulated P^{32} while the activity in the stems decreased slightly. Thus, the over-all picture is a steady loss of activity from the roots mirrored by a steady accumulation of P^{32} in the leaves.

The roots of control plants lost only 14.4% of their initial activity to the shoots during the first 48 hours after the beginning of phosphate uptake. Of this 14.4%, 10.3% was located in the stems and only 4.1% in the leaves. However, after 48 hours, the roots did not change in their P^{32} content whereas the leaves lost some of their activity which reappeared in the stems. This fluctuation indicates that the leaves of control plants accumulate P^{32} for the first 48 hours after which time there is a downward translocation of activity.

EFFECT OF AT-TREATMENT ON THE DISTRIBUTION OF ACID-SOLUBLE AND ACID-INSOLUBLE ACTIVITY.

Grinding plant material with cold 16% TCA separates the acid-soluble phosphate compounds from the acid-insoluble





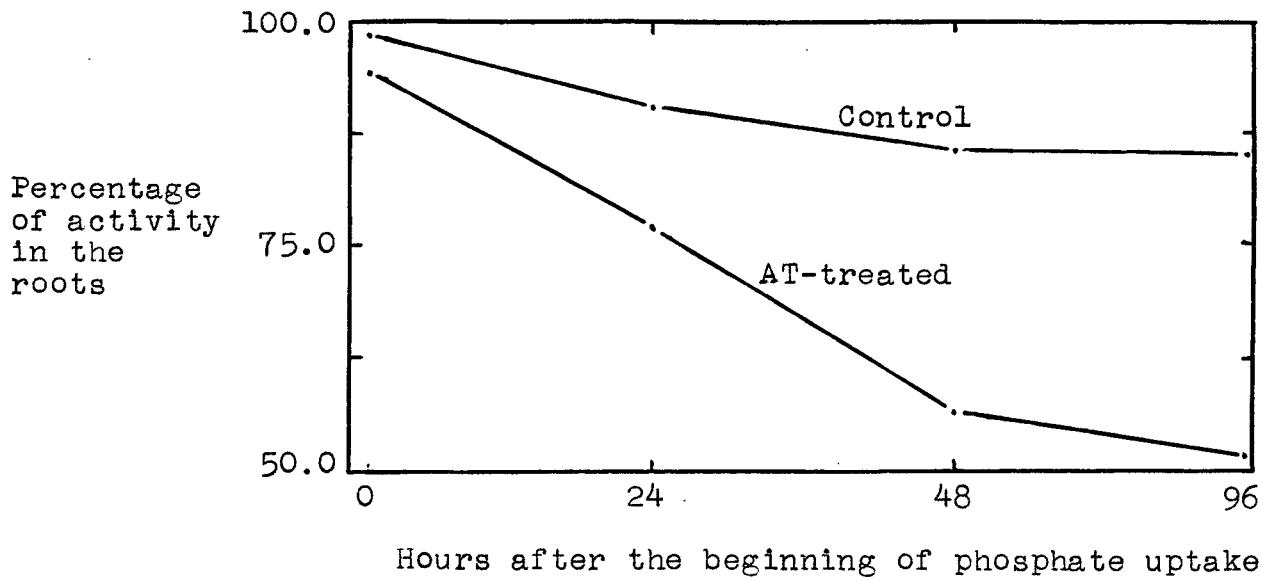


Fig. 2: Distribution of P^{32} in roots, stems, and leaves.

phosphates. The acid-soluble phosphates include inorganic phosphates, sugar phosphates and nucleotides while the nucleic acids, phospholipids, and phosphoproteins are included in the acid-insoluble fraction. The relative activity and the distribution of the acid-soluble and acid-insoluble fractions within bean plants is presented in Tables III and IV.

It was noted in the previous section that treatment of bean plants with 100 p.p.m. AT for 48 hours increased the amount of P^{32} that moved from the roots into the stems and leaves. Figs. 3 and 4 indicate that this increase represents an increase in both the amount of acid-soluble P^{32} and the amount of acid-insoluble P^{32} that moves from the roots into the upper portions of the treated plants.

The roots of AT-treated plants lost acid-soluble P^{32} steadily for 96 hours until only 35.4% of the total activity in the plant remained in the roots. The leaves of treated plants accumulated 43.9% of the total acid-soluble activity present in the plant. The leaves of control plants did not accumulate acid-soluble P^{32} in a similar manner. Though 29% of the total acid-soluble activity of the plant had been translocated to the shoots after 96 hours, only 4% of this activity appeared in the leaves. The fluctuation of acid-soluble P^{32} within the leaves between the 24 hour and 96 hour harvests together with the steady increase of activity in the stems of control plants suggests that acid-soluble P^{32} is being freely translocated from the leaves to the roots as well as in the reverse direction.

TABLE III

DISTRIBUTION OF ACID-SOLUBLE P^{32} IN BEAN PLANTS.

Hours after initial contact with P^{32}	Plant	Organ	Acid-soluble activity (cpm/organ)	Percentage of total acid-sol. activity in organ	t-test
1	Treated	Leaves	1,630	0.9	
		Stem	11,765	6.5	
		Roots	167,281	92.6	4.02 *
			180,676		8.90 **
					12.74 **
	Control	Leaves	155	0.1	
		Stem	3,631	2.2	
		Roots	153,525	97.6	
			157,311		
24	Treated	Leaves	3,375	2.1	
		Stem	27,840	26.0	
		Roots	103,500	71.9	
			134,715		1.11
					2.68
	Control	Leaves	1,043	2.2	2.07
		Stem	6,547	13.7	
		Roots	44,784	84.1	
			52,374		
48	Treated	Leaves	16,865	23.8	
		Stem	18,890	26.7	
		Roots	34,684	49.5	
			70,439		5.71 *
					13.52 **
	Control	Leaves	1,884	5.3	16.85 **
		Stem	6,298	18.9	
		Roots	24,785	75.8	
			32,967		
96	Treated	Leaves	19,434	43.9	
		Stem	9,250	20.7	
		Roots	15,859	35.4	
			44,543		9.50 *
					1.99
	Control	Leaves	1,003	3.9	17.54 *
		Stem	6,259	24.5	
		Roots	18,256	71.5	
			25,518		

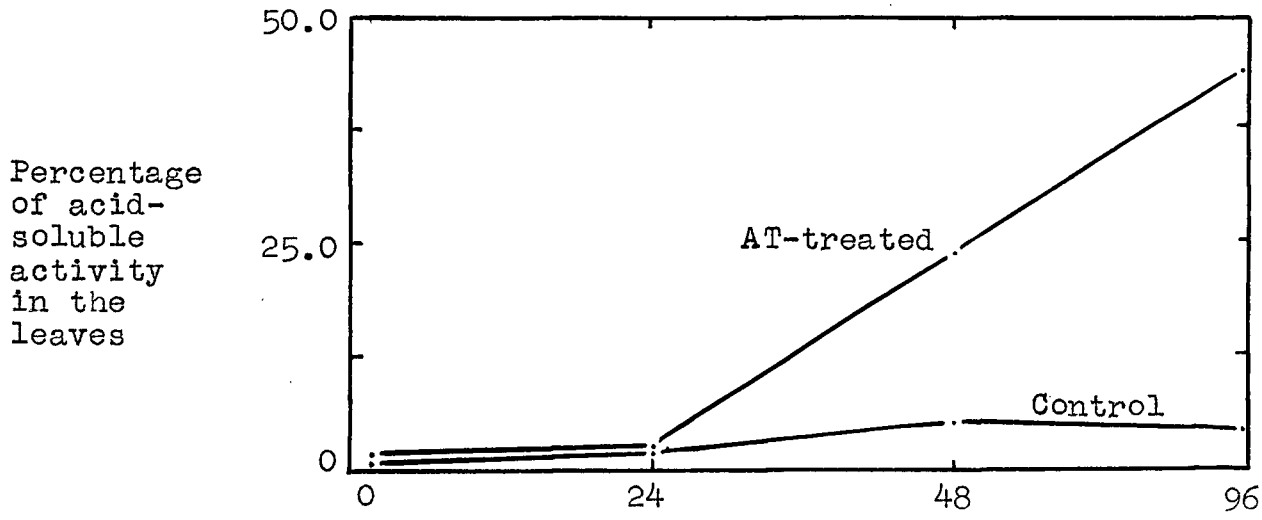
* significant at 0.05 level
** significant at 0.01 level

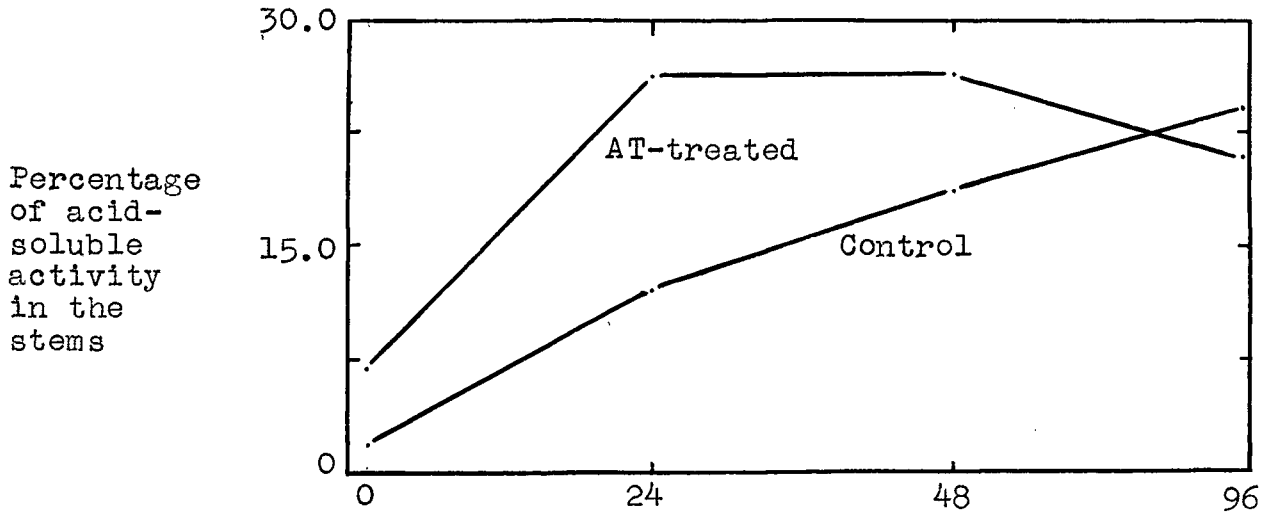
TABLE IV

DISTRIBUTION OF ACID-INSOLUBLE P^{32} IN BEAN PLANTS.

Hours after initial contact with P^{32}	Plant	Organ	Acid-insoluble activity (cpm/organ)	Percentage of total acid-insol. activity in organ	t-test
1	Treated	Leaves	301	0.2	
		Stem	1,871	2.1	
		Roots	86,030	97.5	9.18 **
			88,202		12.16 **
					4.10 *
	Control	Leaves	126	0.1	
		Stem	933	0.8	
		Roots	108,305	99.0	
			109,364		
24	Treated	Leaves	3,890	2.2	
		Stem	14,065	13.7	
		Roots	111,655	84.0	0.96
			129,610		3.61
					2.85
	Control	Leaves	1,906	1.5	
		Stem	7,002	5.4	
		Roots	120,972	91.1	
			129,880		
48	Treated	Leaves	16,236	18.5	
		Stem	16,936	19.3	
		Roots	54,805	62.2	39.83 ***
			87,977		35.22 ***
					60.49 ***
	Control	Leaves	3,634	3.7	
		Stem	6,421	7.2	
		Roots	82,133	89.1	
			92,188		
96	Treated	Leaves	25,287	26.2	
		Stem	13,716	14.2	
		Roots	57,460	59.6	6.50 *
			98,963		1.89
					15.90 *
	Control	Leaves	2,937	2.4	
		Stem	8,966	8.7	
		Roots	85,465	88.9	
			97,368		

* significant at 0.05 level
** significant at 0.01 level
*** significant at 0.001 level





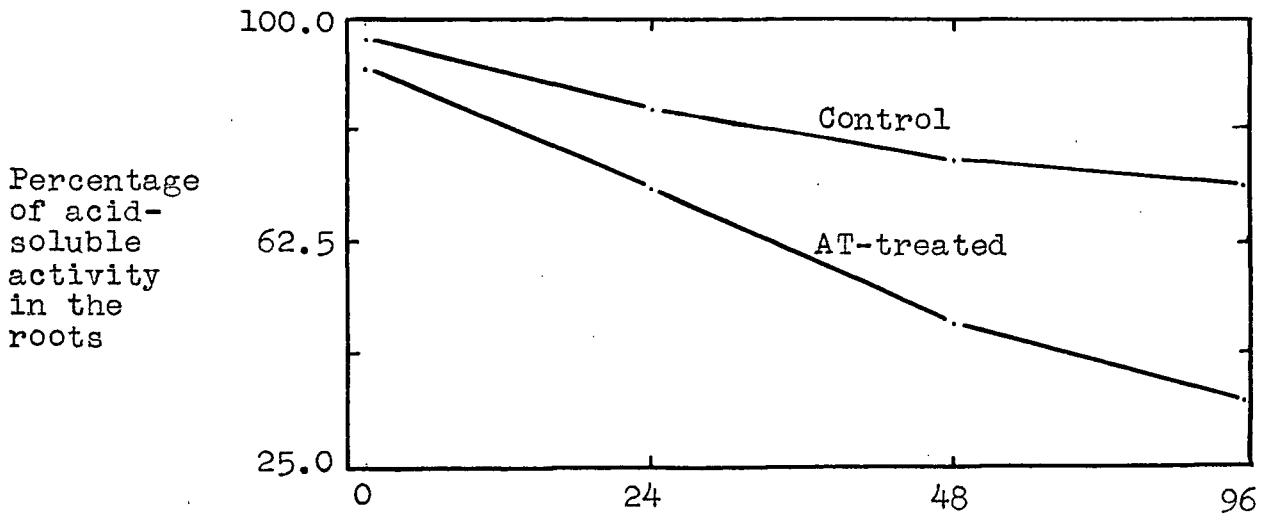
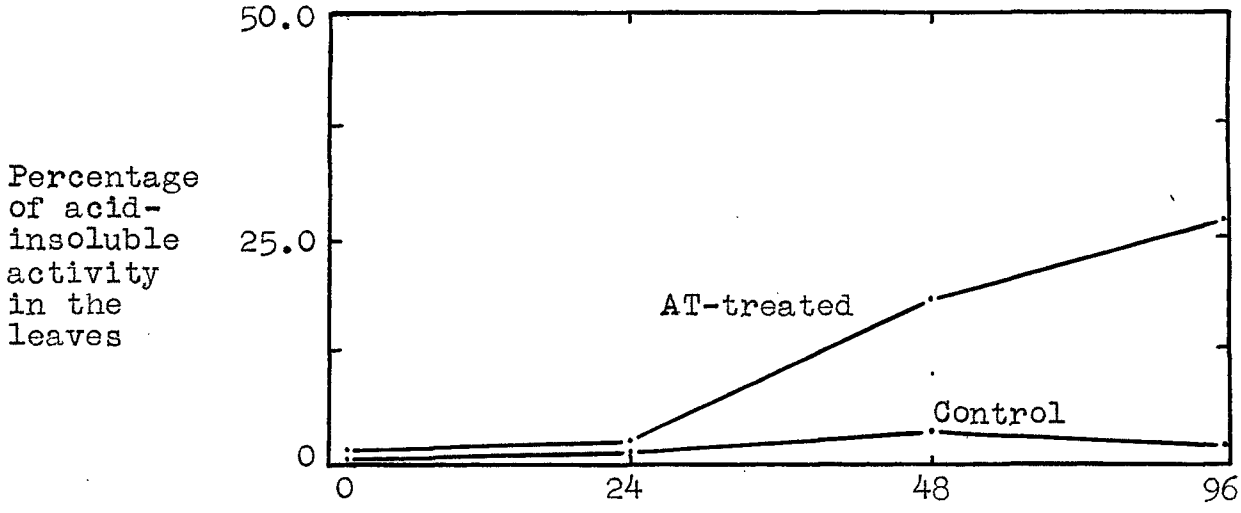
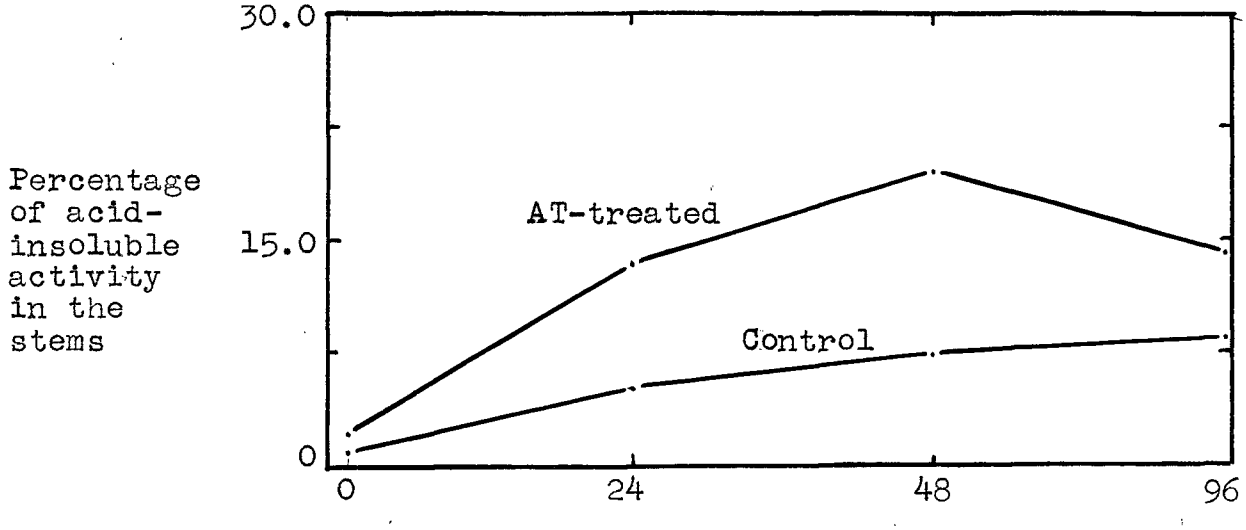


Fig. 3: Distribution of acid-soluble P^{32} in leaves, stems, and roots of young bean plants.





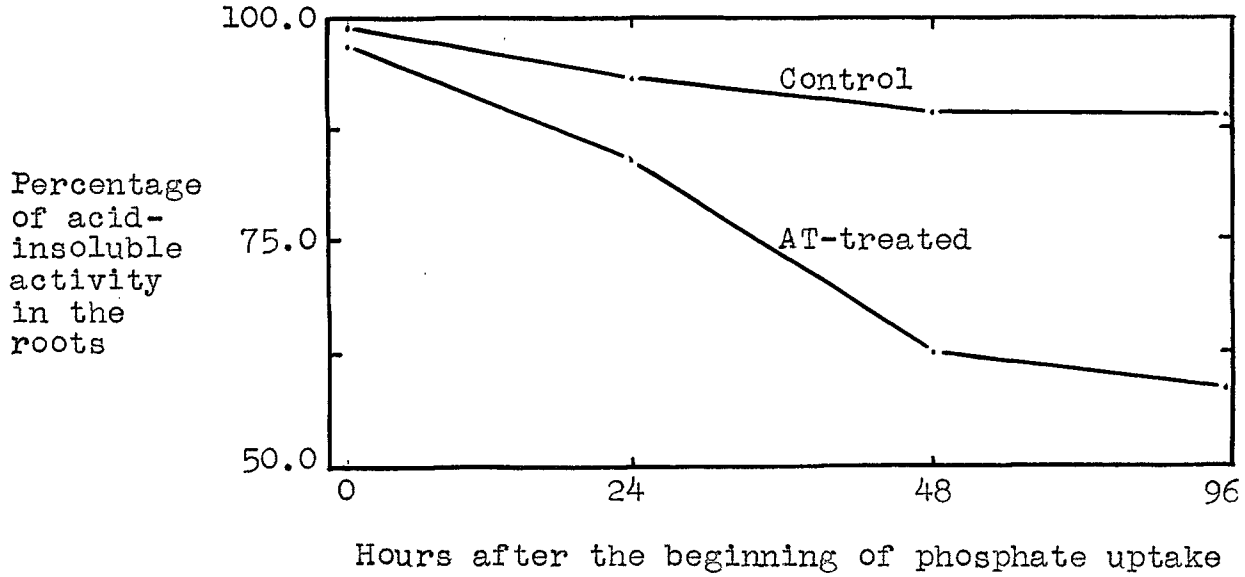


Fig. 4: Distribution of acid-insoluble P^{32} in the leaves, stems, and roots of young bean plants.

The distribution of acid-insoluble P^{32} within treated and untreated bean plants also differed. In all cases, the percentage of P^{32} in the leaves of treated plants, relative to the activity in the entire plant, increased over that of control plants. After 96 hours, only 59.5% of the acid-insoluble activity remained in the roots of AT-treated plants. During this period, 27% of the activity in the plant had accumulated in the leaves. After 96 hours, the roots of control plants had 88.9% of the total acid-insoluble activity whereas the leaves had only 2.4% of the acid-insoluble P^{32}. The results indicate that rather than accumulating P^{32} in the acid-insoluble form within the leaves, control plants tend to translocate activity in either direction.

INCORPORATION OF P^{32} INTO ACID-INSOLUBLE FRACTIONS.

When the P^{32} content of acid-soluble and acid-insoluble fractions in the roots, stems and leaves was expressed as a percentage of the total P^{32} content in these organs (Table V), the results indicated that AT-treatment affects the distribution of P^{32} between these two fractions. Though all plants continued to incorporate P^{32} into acid-insoluble fractions resulting in a corresponding decrease in acid-soluble activity, AT-treated plants incorporated less of the total P^{32} into this fraction. After 96 hours, 56.6% of the P^{32} in the leaves was present in the acid-insoluble fraction whereas 73.9% of the activity appeared in this fraction in the leaves of the control plants (Fig. 5). Though the

TABLE V

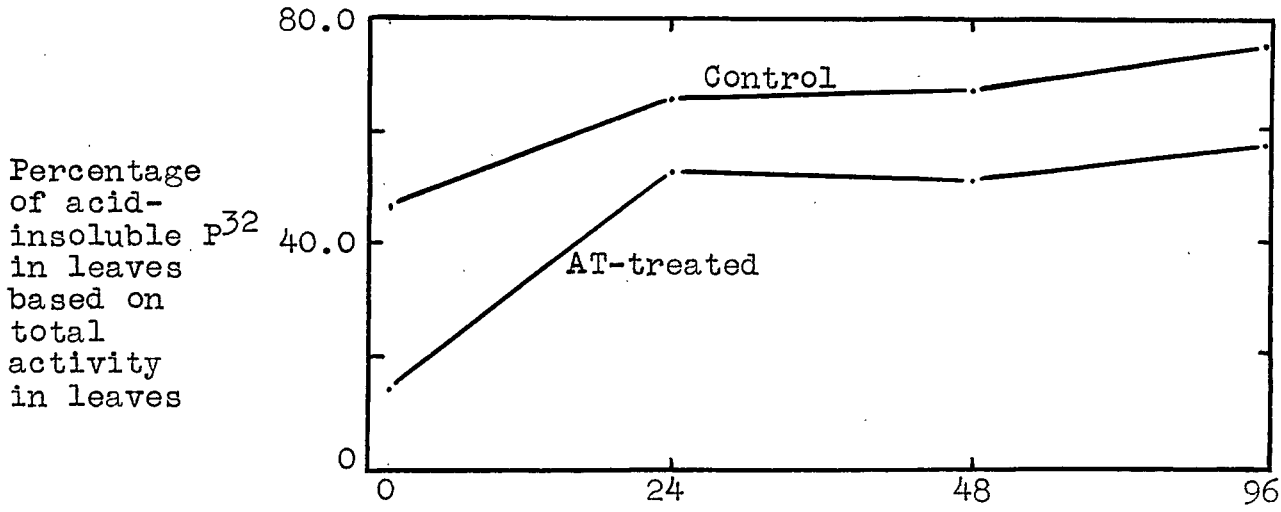
DISTRIBUTION OF P^{32} BETWEEN ACID-SOLUBLE AND ACID-INSOLUBLE FRACTIONS IN EACH PLANT ORGAN

Hours after initial contact with P^{32}	Plant	Organ	Acid-soluble as percent total activity in organ	Acid-insoluble as percent total activity in organ	t-test
1	T	Leaves	84.78	15.22	
		Stem	86.49	13.51	
		Roots	66.37	33.62	7.79 **
					6.18 *
	C	Leaves	52.27	47.73	3.51 *
		Stem	79.07	21.38	
		Roots	58.62	41.37	
24	T	Leaves	47.10	52.90	
		Stem	66.05	33.94	
		Roots	48.10	51.89	6.28 *
					18.51 **
	C	Leaves	34.84	65.16	10.74 **
		Stem	48.64	51.36	
		Roots	26.94	73.05	
48	T	Leaves	49.91	50.09	
		Stem	51.77	48.22	
		Roots	38.24	61.75	5.63 *
					0.73
	C	Leaves	33.91	66.09	5.20 *
		Stem	49.92	50.08	
		Roots	24.61	75.39	
96	T	Leaves	43.42	56.58	
		Stem	40.19	59.81	
		Roots	22.52	77.47	7.16 **

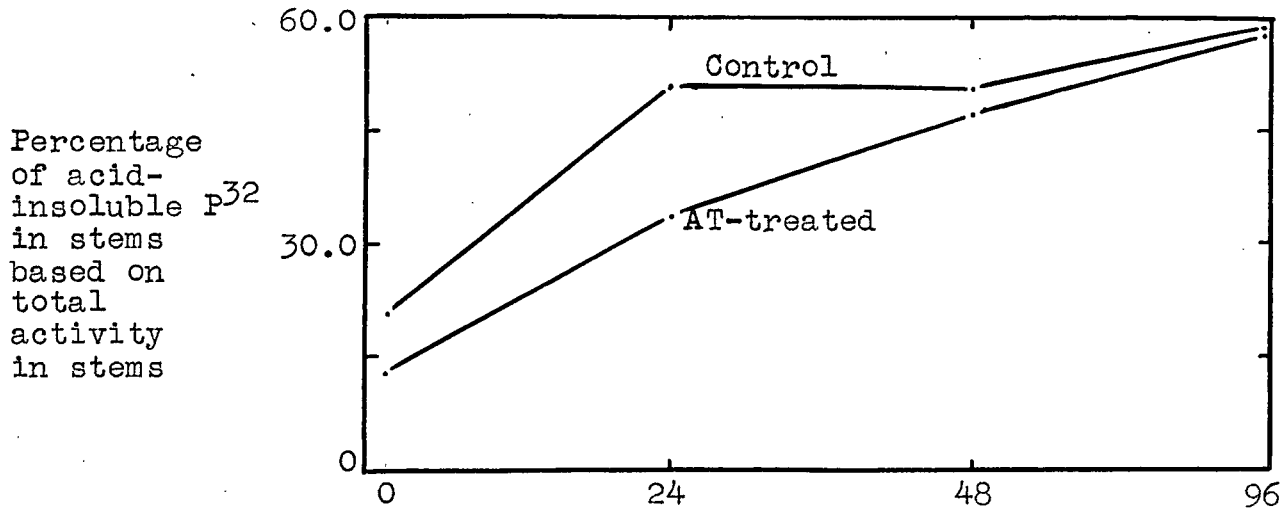
	C	Leaves	26.13	73.87	3.20 *
		Stem	41.17	58.82	
		Roots	17.60	82.40	

T = Treated
C = Control

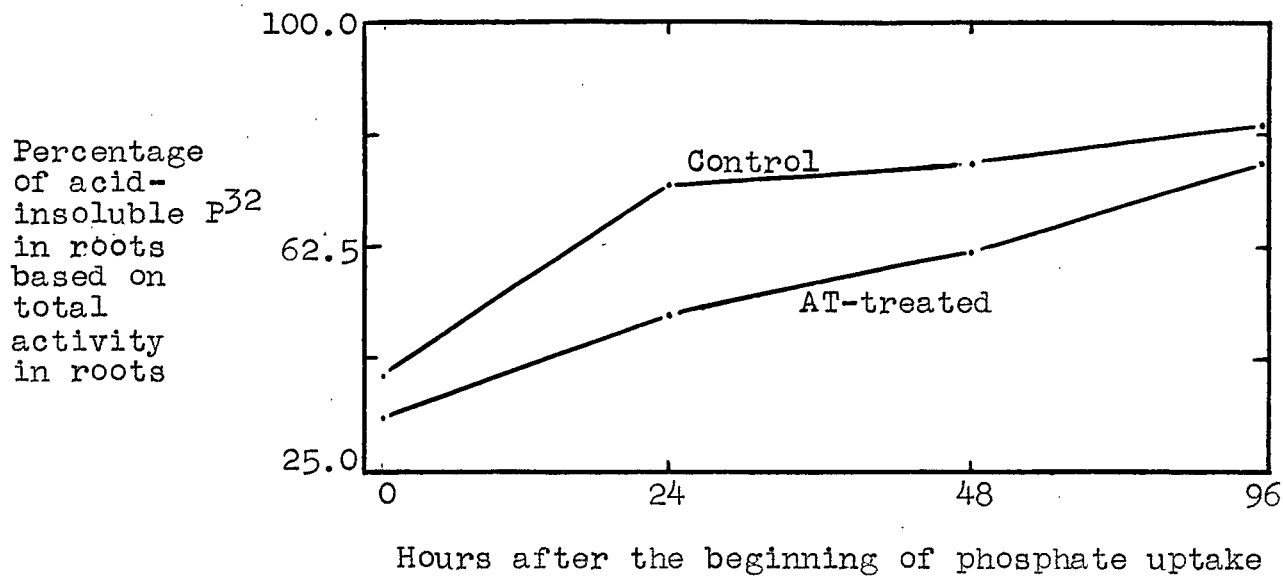
* significant at 0.05 level
** significant at 0.01 level



Percentage of acid-insoluble P^{32} in leaves based on total activity in leaves



Percentage of acid-insoluble P^{32} in stems based on total activity in stems



Percentage of acid-insoluble P^{32} in roots based on total activity in roots

Fig. 5: Incorporation of P^{32} into Acid-Insoluble Fractions.

percentage of P^{32} in the acid-insoluble fraction in the stems of AT-treated plants differed significantly from that in the controls for the first 24 hours, this difference was not significant after 48 hours. AT-treatment also inhibited the incorporation of P^{32} into acid-insoluble fractions in the root.

EFFECT OF AT-TREATMENT ON THE ESTERIFICATION OF PHOSPHATE AND ON THE DISTRIBUTION OF THE VARIOUS ACID-SOLUBLE COMPONENTS.

When the P^{32} content of inorganic phosphates, sugar phosphates and nucleotides in the roots, stems, and leaves was expressed as a percentage of the total acid-soluble P^{32} content of these organs, the results indicated that treatment resulted in no change in esterification of P^{32} for 96 hours (Table VI). Thus, AT-treated plants and control plants esterified approximately the same percentage of the inorganic phosphates present in each organ for the formation of nucleotides and sugar phosphates.

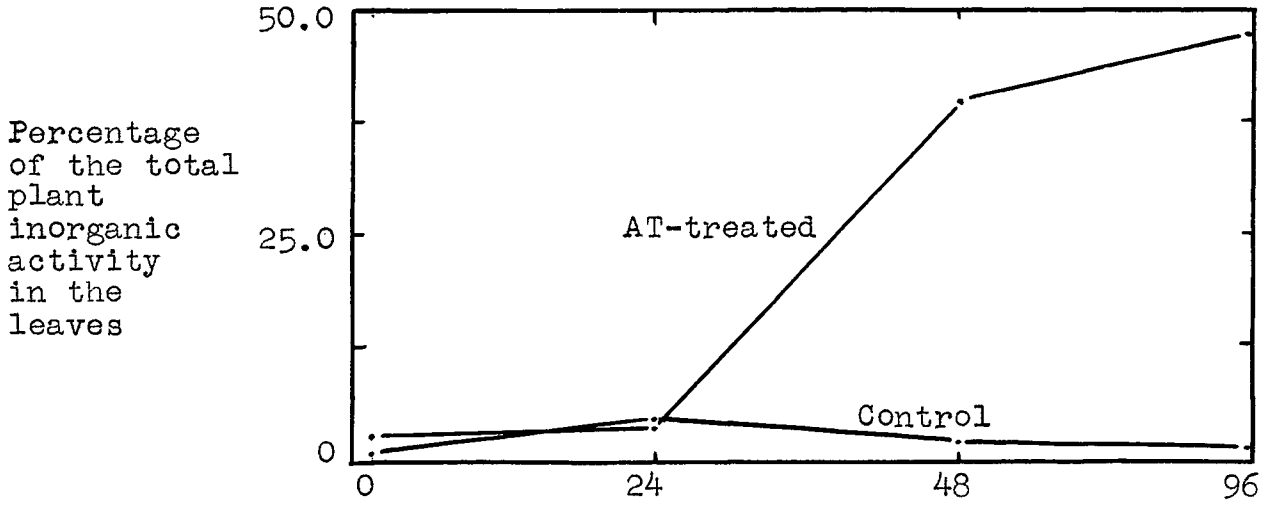
When the inorganic P^{32} content of each organ was expressed as a percentage of the total inorganic P^{32} content of the plant, the results indicated that AT-treatment does affect the distribution of inorganic P^{32} within the plant (Fig. 6). The leaves of AT-treated and control plants have about the same percentage of the inorganic activity present in each plant for the first 24 hours after AT-treatment. Thereafter, however, the leaves of treated plants rapidly accumulate inorganic P^{32} for the duration of the experiment whereas the leaves of control plants

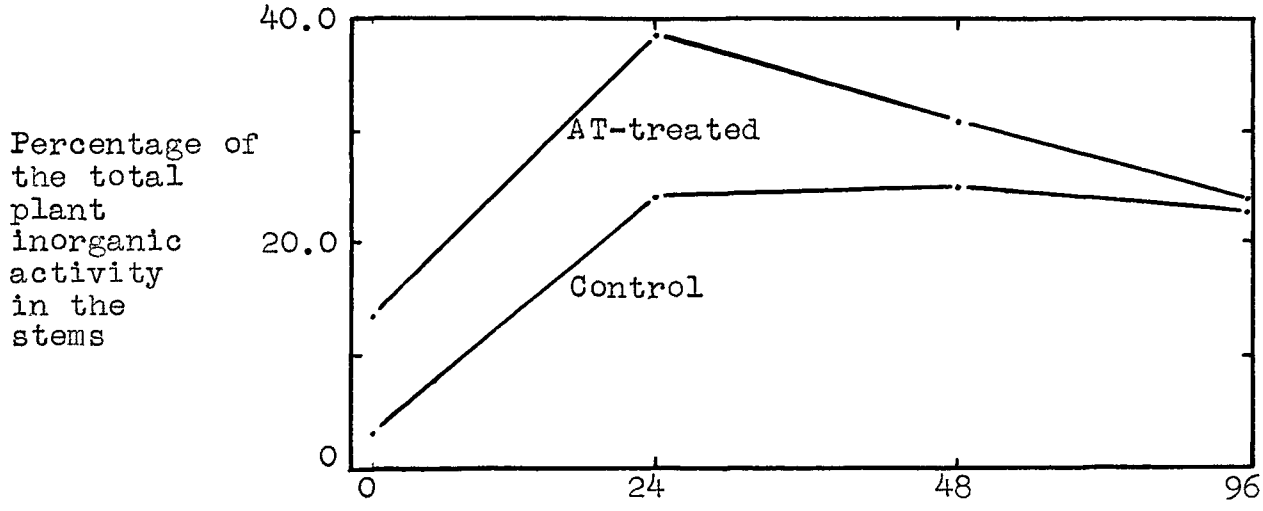
TABLE VI

EFFECT OF AT ON THE ESTERIFICATION OF PHOSPHATE.

Hours after initial contact with P^{32}	Plant	Organ	Percent acid-soluble activity in the form of: Nucleotides	Sugar phosphates	Inorganic phosphates	Percent esterification
1	T	Leaves	27.4	13.2	59.4	40.6
		Stem	18.6	25.0	56.4	43.6
		Roots	22.4	21.2	56.4	43.6
	C	Leaves	26.1	16.0	57.8	42.1
		Stem	31.4	16.6	51.9	48.0
		Roots	29.6	24.9	45.4	54.5
24	T	Leaves	26.6	19.5	53.9	46.1
		Stem	21.0	22.2	56.8	43.2
		Roots	28.6	18.7	52.6	47.3
	C	Leaves	20.3	22.5	57.2	42.8
		Stem	27.4	18.2	54.4	45.6
		Roots	30.8	25.5	43.7	56.3
48	T	Leaves	25.5	24.0	50.4	49.5
		Stem	17.3	23.0	59.7	40.3
		Roots	24.8	34.6	40.5	59.4
	C	Leaves	32.3	----	----	----
		Stem	27.9	17.1	54.9	45.0
		Roots	25.3	23.9	50.8	49.2
96	T	Leaves	26.9	18.9	54.2	45.8
		Stem	24.1	20.8	55.0	44.9
		Roots	32.4	14.6	53.0	47.0
	C	Leaves	19.8	22.9	57.3	42.7
		Stem	18.9	25.5	55.6	44.4
		Roots	20.7	22.6	56.7	43.3

T = AT-treated
C = Controls





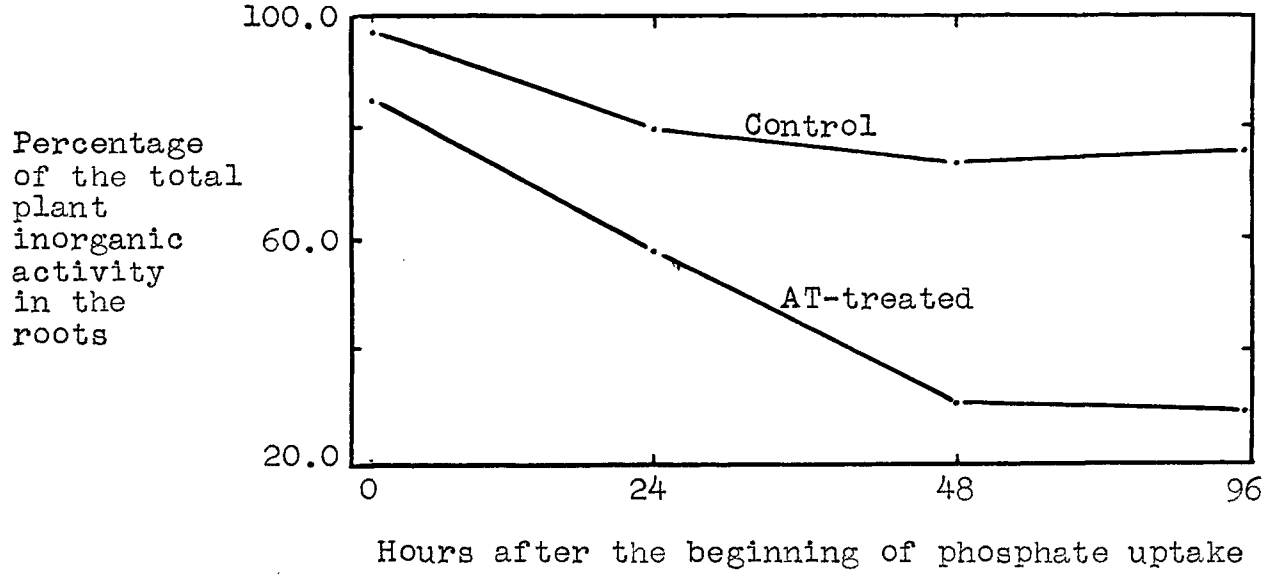


Fig. 6: Distribution of Inorganic P^{32} within the Plant.

actually decrease slightly in their inorganic activity. Ninety-six hours after AT-treatment, the leaves of AT-treated plants contain 47.3% of the total inorganic P^{32} while the leaves of the control plants contain only 1.5% of the total inorganic P^{32} present in the plant. The steady decrease of inorganic P^{32} in the roots of AT-treated plants reflects the accumulation of activity in the leaves. Ninety-six hours after AT-treatment, the AT-treated roots had 75.6% of the inorganic activity. The distribution of inorganic activity in the stems is also worthy of note. The stems of AT-treated plants have much more of the total inorganic P^{32} than do the stems of control plants during the first 48 hours after initial phosphate uptake. However, 96 hours after initial phosphate uptake, the activity in the stems of each set of plants is the same. In spite of this, the leaves of control plants do not accumulate as much inorganic P^{32} as do the leaves of AT-treated plants. This suggests that control plants redistribute inorganic P^{32} in both directions whereas the inorganic P^{32} in AT-treated plants is accumulated in the leaves.

Sugar phosphates also accumulated in the leaves of AT-treated plants (Fig. 7) whereas the leaves of control plants fluctuated markedly in their sugar phosphate activity. Again, control plants seem to freely transport sugar phosphate from the leaves to the roots as well as in the reverse direction whereas AT-treatment results in only upward movement of activity.

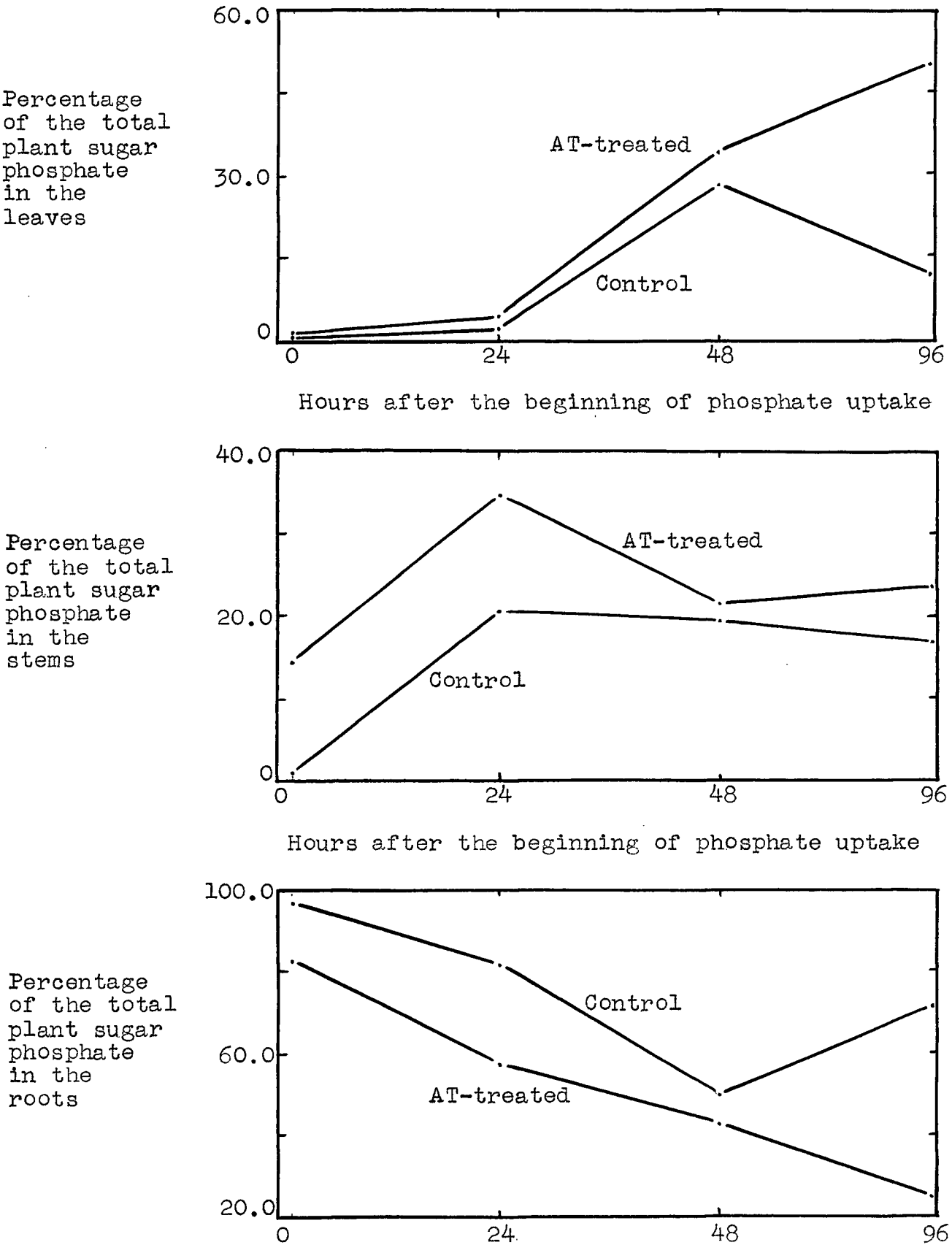
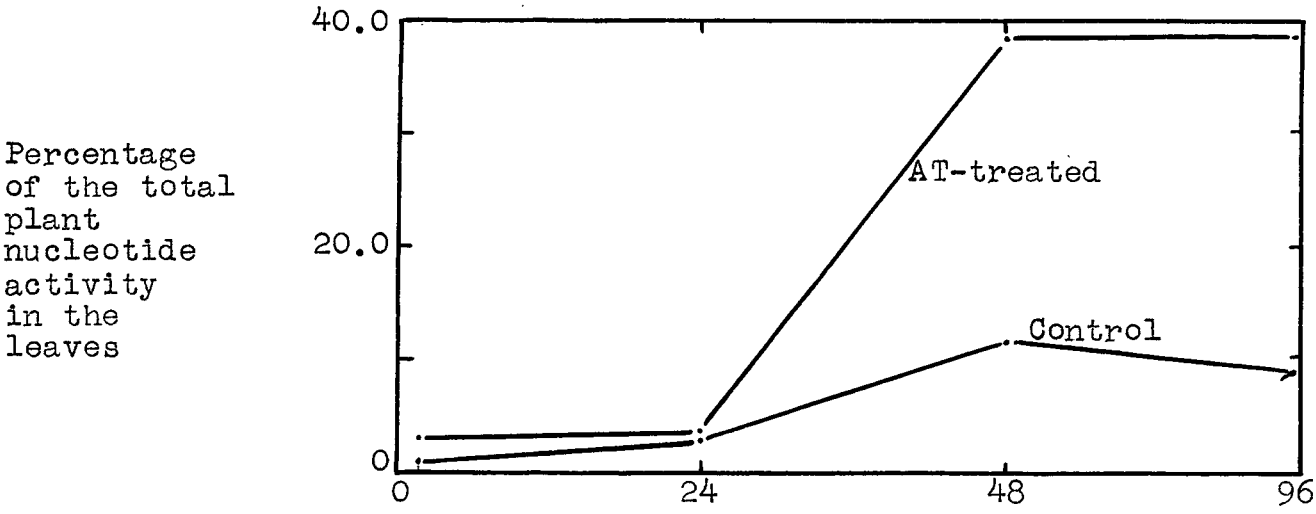
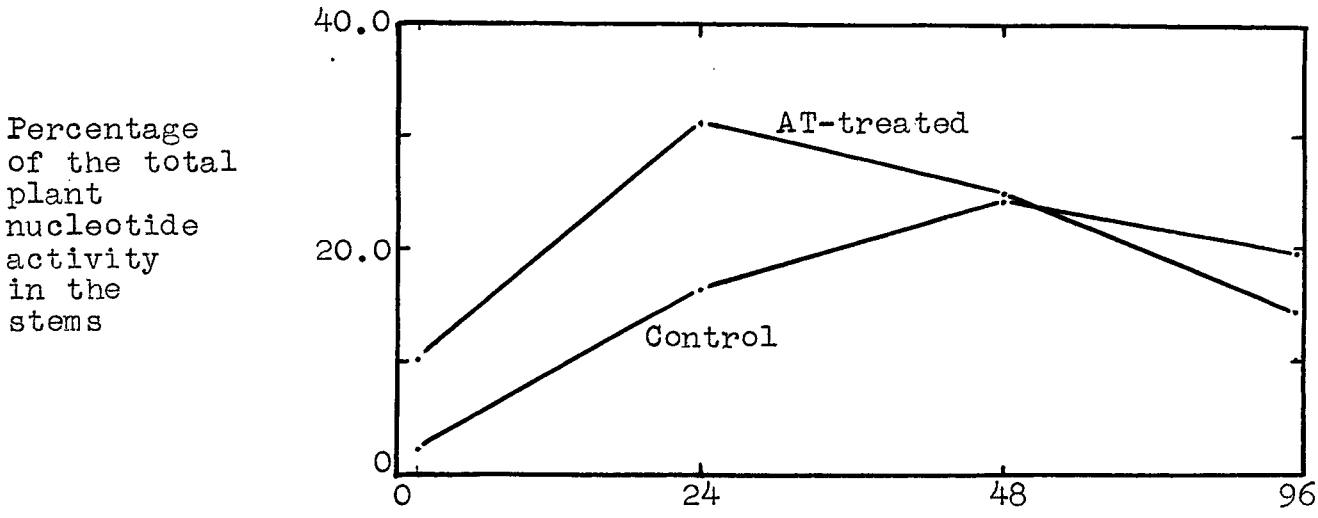


Fig. 7: Distribution of sugar phosphate in young bean plants

The distribution of nucleotide activity within each set of plants is given in Fig. 8. As in the case of inorganic phosphate activity and sugar phosphate activity, the nucleotide activity is also accumulated in the leaves of AT-treated plants. The level of nucleotide activity reaches a steady state in these leaves 48 hours after AT-treatment (38.7%). On the other hand, the nucleotide activity of the leaves of control plants fluctuates with only 22.4% of this fraction occuring in the leaves 96 hours after AT-treatment. The roots of AT-treated plants lost nucleotide activity steadily for 48 hours and thereafter increased slightly in their nucleotide activity. Though this suggests that some of the nucleotides may have been transported from the stem to the roots during this period, nucleotides may have been manufactured within the roots during this period since there was a corresponding decrease in inorganic P^{32} and sugar P^{32} during this same period.

Thus, the accumulation of acid-soluble activity in the leaves of AT-treated plants does not appear to be confined to any of the fractions which comprise the acid-soluble activity, but rather, the accumulation represents an accumulation of inorganic phosphates, sugar phosphates and nucleotides. The leaves of control plants do not accumulate any of these fractions. Furthermore, the incorporation of P^{32} into esterified compounds (i.e., nucleotides and sugar phosphates), as revealed by the percentage esterification in each plant organ, is unaffected by AT-treatment.





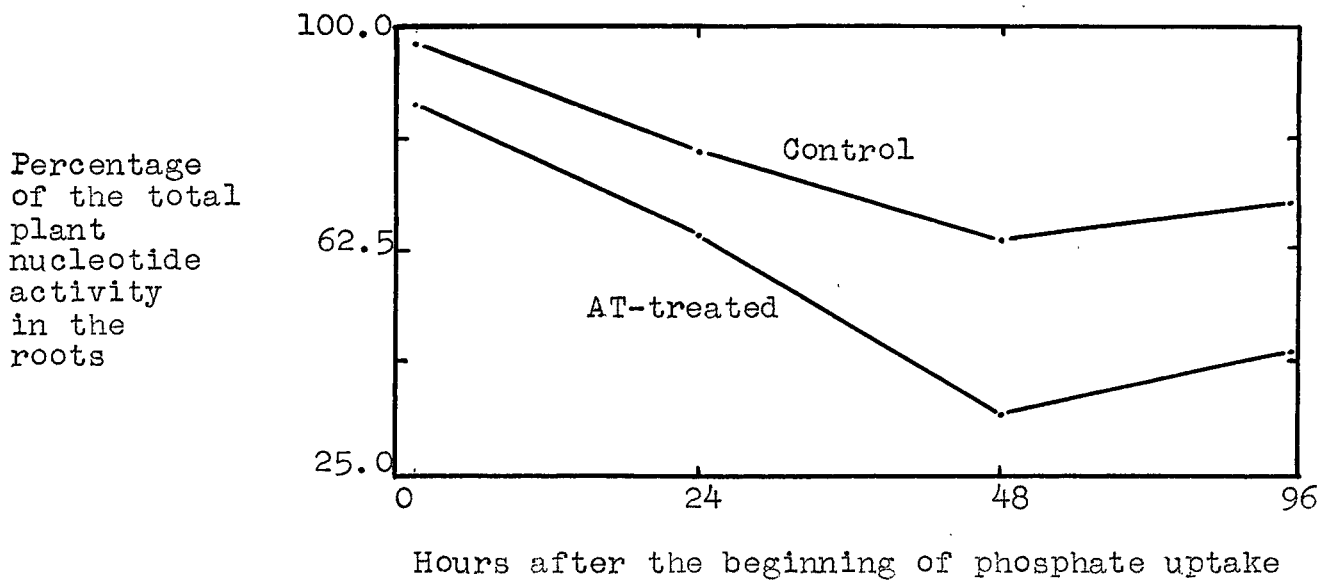


Fig. 8: Distribution of nucleotide P^{32} in young bean plants.

DISCUSSION

A 48 hour exposure of the roots of young bean plants to 100 p.p.m. amino triazole did not affect their ability to take up phosphate from the nutrient solution. However, Wort and Loughman (44) found that AT reduced the ability of young barley plants to absorb P^{32} and furthermore, this reduction in absorption increased with an increase of the length of exposure to AT. The decrease in absorption of P^{32} was greatest in barley plants treated with 100 p.p.m. AT for 96 hours prior to the absorption period. Herrett and Linck (23) found that the treatment of P-deficient Canada thistle plants with AT resulted in a marked reduction in the uptake of foliar applied P^{32}. These workers found that the activity of AT was greatly reduced in phosphorus-deficient plants as compared to plants supplied with an adequate amount of phosphate. If this were also the case in bean plants, a phosphate deficiency might tend to antagonize the AT-inhibition of P^{32} uptake by the roots of these plants. However, the bean plants had no visible signs of phosphorus deficiency prior to phosphate uptake, thus, this latter possibility seems unlikely.

AT-treated bean plants retain more P^{32} when returned to a phosphate-free environment than do control plants. This is analogous to the effect of AT-treatment on the retention of P^{32} by barley plants (44) in which it was found that plants lost up to one-third of the activity during 3 hours after the beginning of phosphate uptake. Bean plants lost

activity steadily for 48 hours and reached a relatively stable state thereafter. At this time, control plants had lost 53.7% of their initial activity whereas treated plants lost only 41%. As reported by Wort and Loughman, the rapid initial loss of P^{32} may be a diffusion process whereas the slower loss occuring after 48 hours is probably associated with metabolic processes occuring within the plant.

AT-treated plants transport much more P^{32} from the roots to the shoots than do control plants. The steady rise of P^{32} in the leaves mirrored by the steady loss of activity from the roots indicates that the leaves of AT-treated plants accumulate P^{32}. On the other hand, the P^{32} content of the leaves of control plants increases but little and fluctuations of activity appear to occur during the 96 hour period. This fluctuation together with the steady loss of activity from the roots and the steady increase in P^{32} activity in the stems suggests that control plants do not accumulate P^{32} in the leaves. These results indicate that AT inhibits downward translocation of phosphate compounds. Wort and Loughman (44) have found that barley plants react in a similar manner to AT-treatment. They found that barley plants required an exposure to 100 p.p.m. AT of over 4 hours before increased transport of phosphate compounds from the roots to the leaves occured.

When the phosphate compounds were separated into acid-soluble P^{32} (inorganic phosphates, sugar phosphates, and nucleotides) and acid-insoluble P^{32} (phosphoproteins, nucleic acids and phospholipids), the results indicated that

both of these fractions are accumulated in the leaves of AT-treated plants. The acid-soluble and acid-insoluble content of the roots of these plants decreased steadily. Thus, the pronounced increase in translocation of P^{32} due to AT-treatment is an increase in the amount of acid-soluble P^{32} and acid-insoluble P^{32} which is translocated rather than an increased transport of just one of these fractions.

Further characterization of the acid-soluble fraction reveals the interesting fact that the accumulation of acid-soluble activity in the leaves of AT-treated plants is not confined to any of the fractions which comprise the acid-soluble activity. Instead, the accumulation represents an accumulation of inorganic phosphates, nucleotides, and sugar phosphates.

Synthesis of certain organic phosphate compounds (e.g., phospholipids, ATP, ribonucleic acid, deoxyribonucleic acid) is essential for normal growth in plants (2). Esterification of inorganic phosphate via reduced pyridine nucleotides and the cytochrome system (oxidative phosphorylation) or via the photosynthetic phosphorylative mechanism results in the generation of ATP, a high-energy phosphate, which is then utilized for mediating vital reactions requiring a supply of energy. It has been shown that growth is dependent on the continuous synthesis of ATP (2). Certain growth inhibitors directly or indirectly effect the maintenance of the ATP pool. Iodoacetate, for example, blocks sugar utilization by inhibition of glycolysis. This results in a reduction of ATP. Arsenate substitutes for inorganic phosphate in the

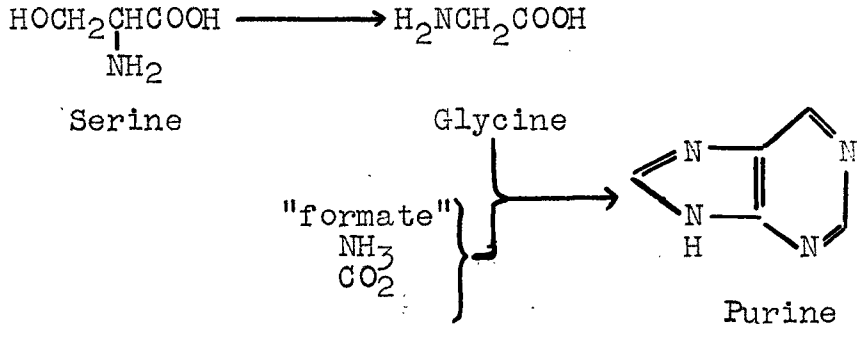
oxidation of triose phosphate thereby uncoupling oxidation from ATP synthesis (10). Arsenate inhibits the uptake, incorporation and turnover of radiophosphorus in mung bean seedlings (2). DNP (2,4-dinitophenol) also uncouples phosphorylation from oxidative metabolism and thereby reduces the incorporation of phosphate into ATP and other nucleotides (44). However, DNP has no effect on the proportion of phosphate entering the nucleic acid fraction.

Unlike these growth inhibitors, AT does not affect the incorporation of inorganic phosphate into sugar phosphates and nucleotides. This suggests that AT-treatment does not affect oxidative or photosynthetic phosphorylation which are responsible for the production of ATP. AT-treatment does not seem to affect those glycolytic reactions whereby sugars are phosphorylated. However, AT-treatment does inhibit transfer of phosphate from the acid-soluble to the acid-insoluble fraction. Thus, the principle effect of AT is on the incorporation of phosphate into one or more of the nucleic acid, phospholipid, or phosphoprotein fractions. These findings are in agreement with those of Wort and Loughman (44) and their work with barley plants. They have suggested that inhibition of phosphate incorporation into the acid-insoluble fraction by AT-treatment may account for the diversion of phosphate to the system responsible for upward translocation of activity.

Though the actual mechanism of protein synthesis is not known, there is much evidence to suggest that both a soluble ribonucleic acid (RNA) and a ribonucleoprotein are

involved in the sequence of reactions whereby amino acids are incorporated into proteins (18,39). Because AT may inhibit the incorporation of phosphate into either one or both of these compounds, then the inhibition of growth due to AT-treatment may actually be an inhibition of protein synthesis. Sund (41) and Wort and Loughman (44) have also postulated that AT interferes with protein synthesis. Sund has found that certain purine precursors, purines or purine ribosides when added to tomato plants simultaneously with low concentrations of AT will partially alleviate growth inhibition due to AT-treatment. Of the purines, adenine, guanine and hypoxanthine were effective. Growth inhibition of Chlorella pyrenoidosa due to AT-treatment is also reversed by the addition of purines (42). Aldrich (4) has found that one of the pyrimidines, uracil, is also effective for reversing AT-growth inhibition.

These results imply that AT interferes with normal purine and pyrimidine metabolism. Carter and Naylor (15) have shown that there is a sharp reduction in the free glycine and serine pools in AT-treated bean plants. Both these amino acids can participate in the synthesis of the purine ring (11).



AT-inhibition of incorporation of P^{32} into acid-insoluble fractions could also be due to an altered phospholipid metabolism. Sund (41) has also investigated this problem. Knowing that purines not only stimulated riboflavin production but also became incorporated into the riboflavin molecule, Sund felt that an effect comparable to that of purines on AT-growth inhibition might be produced by riboflavin. Therefore, he added riboflavin to tomato plants simultaneously with AT. Because flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) act as the prosthetic group (coenzyme) of several flavoprotein enzymes and are riboflavin derivatives, these compounds were also investigated.

Whereas the purines and related compounds had been effective in partially reducing the growth inhibition due to AT-treatment, riboflavin, FMN, and FAD brought about a marked reduction of the inhibition of plastid formation. These also reduced AT-growth inhibition. Sund concluded that the inhibition of plastid formation and consequently of chlorophyll synthesis by AT resulted from the effectiveness with which AT blocked the synthesis of certain metabolites, particularily riboflavin, which are necessary for normal chloroplast development. Riboflavin and its derivatives are necessary for normal growth. Sund has also found that albinistic corn and peas treated with AT have much less riboflavin than untreated tissues.

Aaronson (1) has also provided some evidence which indicates that AT interferes with phospholipid metabolism. He found that crude soybean lecithin could reverse AT-inhibition of chlorophyll synthesis in Ochromonas danica, a phytoflagellate. In the presence of iron, lecithin was even more effective in reversing AT-inhibition.

In summary, there is mounting evidence which suggests that the inhibition by AT of plastid formation and growth is actually due to an interference by AT of normal protein and phospholipid synthesis. AT-growth inhibition can be reversed by certain purine precursors, purines, or purine ribosides whereas both inhibition of plastid formation and of growth due to AT-treatment can be reversed by riboflavin and riboflavin derivatives.

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