THE EFFECT OF AUXIN AND MALEIC HYDRAZIDE ON THE WATER UPTAKE OF SEGMENTS OF PISUM STEM

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THESIS

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THE EFFECT OF AUXIN AND MALEIC HYDRAZIDE ON THE WATER UPTAKE OF SEGMENTS OF PISUM STEM

By

Paul On Pong Ts'o

AN ABSTRACT

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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As indicated by the literature, the recent trend of the research in plant growth hormones is to focus attention on the effects of growth hormones on the water uptake process which is a primary step of growth.(27)

The purpose of this paper is to discuss the possibilities of using segments of Pisum stem as the material for research on water uptake, with special attention to the interreleationship of auxin and maleic hydrazide to the process.

Fourteen segments, 26 mm. long, cut from the stem of pea seedling (Pisum sativum L. var. Alaska), were used as the material in each replicate. Water uptake was calculated as the percentage increase in fresh weight over the initial fresh weight of fourteen stem segments, after 12 to 16 hours exposure to the aerated experimental solutions. Each treatment consisted of four replicates, i.e., 64 segments. Differences between treatments were subjected to analysis of variance. Indole-3-acetic acid was used as the auxin in the present investigation.

Data from the preliminary study indicated that the most effective concentration of auxin for rapid uptake was around 10 p.p.m..

The augmentative effect of potassium salts on the auxin-induced water uptake was confirmed in using the pea stem as the test material. The amount of water uptake by pea segments in the auxin solution in the presence of salt was significantly higher than that of the pea segments in the solution with auxin only. However, there were no significant differences among the kinds of potassium salts used. Apparently the anions did not have a determinative effect in the reaction.

Aeration was confirmed as essential for the exertion of auxin effect

in using the pea stem as the test material. In the non-aerated solution, auxin failed to induce any significant increase in the amount of water uptake over the controls. However, the augmentative effect of auxin at different concentration levels showed up significantly when the solutions were aerated.

Data obtained indicate that maleic hydrazide has an inhibitory effect on water uptake of pea segments. In the presence of auxin, a higher concentration level of maleic hydrazide is required in order to be effective, but the inhibition is more pronounced. It shows that the inhibition exerted by the maleic hydrazide to the auxin-induced water uptake is a physiological reaction.

A possible explanation as to the mechanism involved in the augmentative effect of potassium salts and the inhibition of maleic hydrazide has been discussed. Suggestions are also given for future research in the subjects investigated.

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INTRODUCTION

Ever since the discovery of auxin, the relationship between this biochemical substance and the growth process has received most of the attention of plant physiologists. However, a study of the literature indicates that the recent trend is to focus attention on the effect of auxin on the water uptake process which is a primary step of growth.(27).

The plant material which was used most often in the study of the above subject was the storage tissue, i.e., potato slices. An experiment with this material requires a long time, about 7-10 days, and the material has to be in an asceptic condition, which adds to the difficulties of doing the research.(6, 32) Other kinds of material which characteristically elongate, i.e. Avena coleoptile, have been suggested in the literature, (13), but from the writer's own experience, this material is difficult to handle and to obtain reliable results.

The purpose of this paper is to discuss the possibilities of using segments of Pisum stem as the material for research on water uptake, with special attention to the interrelationship of auxin and maleic hydrazide to the process. The latter substance has received considerable attention recently as a growth inhibitor (24, 21). It is the writer's feeling that more detailed physiological studies about the action of this chemical on the plant cells should be made before it is recommended for the commercial purposes.

LITERATURE REVIEW

Effect of Auxin on Water Uptake

Reinders in 1938 (22) was the first to use storage tissue, i.e., potato slices, to study the relation of auxin, aeration and water uptake. In a six-day experiment, Reinders found that water absorption was stimulated in the aerated condition and that a significant additive increase occurred when auxin was added in suitable concentration, while under anaerobic conditions the potato discs might even lose water. The changes of dry weight were also determined, and it was found that an increase of dry weight loss occurred when discs were kept aerated with appropriate auxin concentration. Though Reinders' data were subjected to certain criticisms, (17, 32), great interest had been aroused in the subject.

Commoner et al in 1943, (6), in an attempt to settle the opposing points of view of Heyn (12) and Czaja (10), set the potato discs in a slightly hypertonic medium, and observed that there was an additive effect of auxin when potassium salts were added to the solution. When auxin was absent, the potassium salts had no effect whatsoever. They concluded that since the increase of water uptake stimulated by auxin could occur in the isotonic solution in which the wall pressure is equal to zero, the main causal mechanism was not likely to be the effect of auxin in increasing cell wall plasticity. They suggested that since the presence of salt did exert a significant augmentation of auxin action, this effect on water uptake was probably due to the influence of the auxin system on the absorption of osmotically active substances in the

cells such as salts.

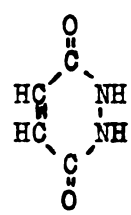
One year later, Van Overbeek (32) criticised Commoner's conclusion on the ground that bacteria might have affected the potato discs in the sucrose solution and also indicated that the increase of water uptake stimulated by auxin can occur in distilled water, in which the accumulation of osmotically active substances was impossible. He observed that the potato discs immersed in distilled water under asceptic conditions, and showing auxin-induced water uptake, instead of having a higher osmotic value, had a decrease of osmotic pressure proportional to the increase of their weight. The data led him to the conclusion that either an auxin-induced increase in non-osmotic water uptake, or an auxin-induced decrease in wall pressure, or both, can be the only cause of the effect. However, in another publication, Commoner (7) stated that he measured the salt absorption in the Avena coleoptiles as well as potato slices and found that the water uptake followed the salt absorption quite closely, and that both were sensitive to the presence of auxin and C_4 acids in a similar way.

Sally Kelly, the first one to use Avena coleoptile as the material to study this phenomena, (13), in 1947 tried to establish a relationship between water uptake and respiration, on the basis of their response to various enzyme inhibitors and auxin. She found that the same concentration range of the inhibitors and of the auxin is effective in affecting both processes of water uptake and respiration. She also found that the auxin-induced water uptake is an aerobic process since the auxin has no effect under anaerobic conditions or in a solution in which the inhibitor was present.

The results and opinions of Levitt, 1948, (17) were quite contradictory to the current trend. He reported that the auxin-induced water uptake at room temperature was not lost by subsequent transfer to 0-2°C. after 24 hours, but might actually increase, and also that 0.001 M KCN had no effect on auxin-induced water uptake. He further found that the respiratory loss of dry matter in the presence of auxin was markedly lower at 21-24°C. than at 25-29°C., though the rate of water uptake at the former temperature was much higher. All these data led him to believe that active absorption was not likely to be the mechanism of auxin stimulation, and the only alternative was a decrease in wall pressure, i.e. an increase in wall plasticity.

Maleic Hydrazide as a Plant Growth Inhibitor.

In 1949, maleic hydrazide was first described by Schoene and Hoffman (24) as a growth regulator, and it began to receive wide attention. Chemically, maleic hydrazide is 1,2 dihydro pyridine 3,6 dione, and has the following structural formula:



Maleic hydrazide is slightly acidic in nature, and with bases it will form soluble or insoluble salts which also possess the growth regulating property, and sometimes may be even more effective (24). Maleic hydrazide does not dissolve completely in 1 percent aqueous solution, but at 5°C. a 0.5 percent solution can be kept at pH 6 indefinitely without crystal formation. It has also been reported that at room temperature,

it can be kept without apparent deterioration at pH 4. Concentrated maleic hydrazide in 30 percent solution can be obtained with diethanolamine as the solvent (21).

A variety of morphological and physiological actions of maleic hydrazide on different plants has been reported. Most of them are in the nature of inhibition. The vegetative growth of the sprayed plants has usually been reported as being suppressed temporarily. Upon recovery, the plants showed symptoms of loss of apical dominance with abnormal lateral growth. Heavy applications led to fatal results (8,9,16,19,21,24,37). In addition to the effect on vegetative growth, disturbances of reproductive process, such as delayed blossoming, male sterility, and reduction of number of flower buds were generally observed (1,21,23,35). The leaves of the stunted plants were described as having an intense dark green color with an increased development of anthocyanin (9,19,21). The suggestion that the action of the maleic hydrazide might be related to phosphorus metabolism was made because of the similarity in the appearance caused by the maleic hydrazide applications and the phosphorus deficiency (21). General observations were made that plant susceptibility decreases with age (1,8,21). Maleic hydrazide was reported to be selective in action to the grasses,(8,9). However, that statement is questionable, because of the contradictory reports (21). Naylor and Davis (21) noticed the appearance of viscous droplets of a sweet tasting liquid, probably a sugar solution, on the abaxial surfaces of leaves of corn plants after spraying. Wittwer et al (36) recommended an application of preharvest foliage sprays of maleic hydrazide to carrots and onions to prevent sprouting in storage. In the writer's laboratory, microscopic examination of

sprayed onion buds showed that the development of the buds was greatly retarded. Naylor and Davis (20) have measured the respiration of the root tips in maleic hydrazide solution at pH 4, and pH 6, and the results showed that at pH 4 the respiration was inhibited but that at pH 6 it was normal. Greulach et al (11) reported that in examining the onion root tip, they could not find cytological evidence from mitotic abnormalities to indicate that the maleic hydrazide inhibited cell elongation. However, they suggested that 1 to 100 p.p.m. concentration of the chemical may inhibit mitosis only, whereas 1000 p.p.m. may inhibit cell enlargement also.

Very recently, an interaction of maleic hydrazide and auxin as indicated by pea stem curvature test was reported by Leopold and Klein (18). These workers suggested that maleic hydrazide may be an antiauxin.

Segments of Pisum Stem as Material for Studying Hormone Effects on Plant Cells.

Early in hormone research, Went in 1934 (34) suggested that segments of Pisum stem may be useful for the convenient quantitative test for auxin. The detailed technique was described by Thimann and Went (31).

In an interesting paper, Tang and Bonner (26) reported that the etiolated pea seedling contains an enzyme which inactivates the auxin in vivo, and that a factor is present which inhibits the enzymatic inactivation. The lower content of the auxin inactivating enzyme in plants exposed to light indicates that the inhibitor is more active in these plants than in those which are etiolated.

Bond (2) in 1948 applied lanolin with aspargine, auxin and 2,3,5,-trichlorophenoxyacetic acid to the pea roots and could not find a

distinctive response. However, when tryptophane and 2,4,5,-trichlorophenoxyacetic acid were applied, root diameter was increased. The increase was considered chiefly due to the proliferation of cambium and xylum parenchyma.

In 1949, Thimann and Bonner (30) reported that the growth of pea stem and Avena coleoptile was inhibited by arsenite, parachloromercuribenzoate, and phenylmercuric acid. The inhibitions were not prevented or reversed by malate or other organic acids.

Kelly and Avery Jr. (14) in 1949, reported that the pea stem is a thousand times more sensitive than Avena coleoptile in response to stimulation of respiration by 2,4-D. In a continuation of the work, Kelly and Avery jr. in 1951 found that the age of the stem tissue and the starvation period before treatment had a definite effect on the response of the stem tissue to 2,4-D stimulation (15).

Naylor and Davis (21) in 1950 reported that pea plants sprayed with maleic hydrazide at concentrations from 500 to 4000 p.p.m., flowered very poorly and lost their apical dominance. Those plants which received a 4000 p.p.m. application eventually died.

MATERIALS AND METHODS

Pea seeds (Pisum sativum L. var. Alaska) were soaked in water for 5 hours and then germinated in moist sand in aluminum pans. The germinator was maintained at 24-25°C. temperature with a high relative humidity. At the age of 7 days, the plants attained a length of 12-15 cm., and had two nodes bearing a scale, and a third, bearing a leaf on the top. Plants in which the length of the internode between this top leaf and the terminal bud was less than 6 mm. were selected for experimental test. A segment of the pea stem 26 mm. long was cut off from the third internode 5-10 mm. below the terminal bud. Fourteen segments, representing 0.8-0.95 gram of fresh weight of tissue, were used in each replicate.

The surface moisture on the stem segments was removed immediately by blotting them on filter paper with cheese cloth. The weights of the segments were recorded before placing them into a 250 ml. beaker containing 100 ml. of the test solution. Segments used in the study of the auxin effect were washed with running water for 30 minutes to diminish the amount of natural auxin before immersion in the test solution. Surface moisture was then removed and weights recorded as described above.

The solutions in which the segments were immersed were aerated by passing air through sintered blocks. The rate of aeration was measured by a wet test gas meter and adjusted by a stopcock to pass 2 liters of air per minute into each beaker. Each beaker was covered by a watch glass to prevent spattering and to reduce evaporation to a minimum.

The pea segments were immersed for 12-18 hours in solutions prepared for the different experiments. After the period of immersion, the solution was decanted off and the segments were collected from a piece of cheese cloth on top of a funnel. They were immediately blotted uniformly with the cheese cloth and filter paper as described above. Then they were re-weighed. All the above operations were carried out at room temperature.

Water uptake was calculated as the percent increase in fresh weight over the initial fresh weight of fourteen stem segments, after exposure to the experimental solutions. Each treatment consisted of four replicates, i.e., 64 segments. Differences between treatments were subjected to analysis of variance.

The term auxin used in this investigation refers to indole-3-acetic acid obtained from the Eastman Kodak Company. The maleic hydrazide employed was obtained from the United States Rubber Company as a 30 percent solution of the diethanolamine salt.

EXPERIMENTAL RESULTS

Preliminary Study of the Test Material.

A preliminary study was made to determine the proper concentration of auxin to use and the minimum exposure time necessary for testing water uptake by Pisum stem segments. The data of Table I and Fig. 1 show that the most effective concentration of auxin for rapid water uptake is around 10 p.p.m.. Differences within the range of 5 p.p.m. to 25 p.p.m. were small, however.

The data of Table II and Fig. 2 indicate that the water uptake of the pea segments in the absence of auxin is not significantly different in the aerated or non-aerated solutions. Rates of water absorption follow closely to the general growth curve. After immersion in water for 12 hours, the rate of absorption reaches a steady state, and the curve levels off. When auxin was present in the solution, the segments continued to take in water, even after 16 hours of immersion. The curve for rate of water absorption shows the tendency of leveling off (Table III and Fig. 3), but at a higher value than in the absence of auxin.

TABLE I

THE EFFECT OF DIFFERENT CONCENTRATIONS OF AUXIN ON THE WATER UPTAKE OF PEA SEGMENTS
Calculated as percentage of increase in fresh weight over the original after 14 hours.

Concentration	0 p.p.m.	5 p.p.m.	10 p.p.m.	20 p.p.m.	25 p.p.m.
	10.8	24.5	27.3	22.4	24.1

TABLE II

THE RATE OF WATER UPTAKE OF PISUM SEGMENTS IN DISTILLED WATER IN RELATION TO AMOUNT OF AERATION
Calculated as percentage of increase in fresh weight over the original.

Time	3 Hours	6 Hours	9 Hours	12 Hours	15 Hours
Non-aerated	5.63	6.31	7.48	7.19	6.73
Aerated	5.01	7.28	7.83	7.82	7.94

TABLE III

THE RATE OF WATER UPTAKE OF PISUM SEGMENTS IN THE AERATED SOLUTION CONTAINING 10 p.p.m. AUXIN
Calculated as percentage of increase in fresh weight over the original.

Time	10 Hours	14 Hours	16 Hours
	22.4	27.3	29.5

Effect of Salts on the Auxin-Induced Water Uptake

Pea segments were immersed for 16 hours in the following aerated solutions; distilled water, 10 p.p.m. of auxin in distilled water, 10 p.p.m. of auxin in 0.002M. KNO_3, 10 p.p.m. of auxin in 0.002 M. KCl, 10 p.p.m. of auxin in 0.002M. KBr, and 10 p.p.m. of auxin in solution containing 0.001 M. KNO_3 and 0.001 M. KH_2PO_4. The results are shown and tabulated in Fig. 4 and Table IV.

These data indicate quite clearly that the presence of salts has a significant augmentative effect on the auxin-induced water uptake. Differences among the kinds of potassium salts used were small and insignificant. These agree with the data of Commoner et al (6).

TABLE IV

THE EFFECTS OF DIFFERENT POTASSIUM SALTS AT 0.002 M. CONCENTRATION ON THE AUXIN-INDUCED WATER UPTAKE OF PEA SEGMENTS IN 10 p.p.m. AUXIN SOLUTION.
Calculated as percentage of increase in fresh weight over the original 16 hours.

Treatment	Treatment means	Difference				
Auxin with KNO_3 and KH_2PO_4	34.3					
Auxin with KBr	33.5	0.8				
Auxin with KCl	32.5	2.11	1.3			
Auxin with KNO_3	31.5	2.80	2.0	0.7		
Auxin	27.5	7.00**	6.2**	4.9**	4.2*	
Water	7.4	26.90**	26.1**	24.8**	24.1**	19.9**

Note: ** Difference exceeds LSD at 1% level : 4.51.
* Difference exceeds LSD at 5% level : 3.26.

Effect of Aeration on the Auxin-Induced Water Uptake

Pea segments were immersed for 16 hours in the following aerated and non-aerated solutions: distilled water, 2p.p.m. of auxin and 10 p.p.m. of auxin. The results are shown in Fig. 5 and are tabulated in Table V.

These data definitely indicate that aeration is essential for the exertion of auxin effect on water uptake. In the non-aerated solution, auxin failed to induce any significant increase in the amount of water uptake over the controls. However, the augmentative effect of auxin at different concentration levels showed up significantly when the solutions were aerated. This agrees with Kelly's data (13).

TABLE V

THE EFFECTS OF THE AERATED AND NON-AERATED AUXIN SOLUTION AT 2 p.p.m. AND 10 p.p.m. CONCENTRATION LEVELS ON THE WATER UPTAKE OF PEA SEGMENTS
Calculated as percentage of increase in fresh weight over the original after 16 hours.

Treatment	Treatment means			Difference		
10 p.p.m. auxin aerated	33.76					
2 p.p.m. auxin aerated	16.89	16.87**				
10 p.p.m. Auxin non-aerated	10.31	23.45**	6.58			
2 p.p.m. auxin non-aerated	9.80	23.95**	7.09*	0.51		
Water non-aerated	6.86	26.90**	10.03*	3.45	2.94	
Water aerated	5.50	28.26**	11.39**	4.81	4.30	1.36

Note: ** Difference exceeds LSD at 1% level : 10.79.
* Difference exceeds LSD at 5% level : 6.86.

Effect of Maleic Hydrazide on Water Uptake

Two separate experiments were performed to study the effect of maleic hydrazide on water uptake. The differences between the control segments and the segments treated with maleic hydrazide were much smaller than those obtained in the study of auxin effect. A small experimental error or variation of materials which is often inevitable may have a serious effect. For this reason two separate experiments, each replicated four times, were conducted to increase the accuracy of the data.

In experiment I, pea stem segments were immersed for 12 hours in

the following aerated solutions: distilled water, and 50, 250, and 500 p.p.m. of maleic hydrazide in distilled water. In the experiment II pea segments were immersed for 12 hours in the following aerated solutions: distilled water, and 250, 500, and 1000 p.p.m. of maleic hydrazide in distilled water. The data of experiment I are shown and tabulated in Fig. 6 and Table VII, and the data of experiment II are in Fig. 6 and Table VII.

These data clearly indicate the inhibitory effect of maleic hydrazide on the water uptake of pea segments. In the maleic hydrazide solution at the concentration levels of 250 p.p.m., 500 p.p.m., and 1000 p.p.m., the water uptake of pea segments was significantly lower than that of the control at 1 percent or 5 percent level for L.S.D.. However, at low concentrations of maleic hydrazide i.e. 50 p.p.m. the water uptake of pea segments was not affected.

TABLE VI

EXPERIMENT I. THE EFFECTS OF DIFFERENT CONCENTRATIONS OF MALEIC HYDRAZIDE ON THE WATER UPTAKE OF PEA SEGMENTS
Calculated as percentage of increase in fresh weight over the original after 12 hours.

Treatment	Treatment means	Difference		
Maleic hydrazide 50 p.p.m.	11.79			
Distilled water	11.07	0.72		
Maleic hydrazide 250 p.p.m.	6.60	5.19**	4.47*	
Maleic hydrazide 500 p.p.m.	5.80	5.99**	5.27**	0.80

Note: ** Difference exceeds LSD at 1% level : 4.87.
* Difference exceeds LSD at 5% level : 2.94.

TABLE VII

EXPERIMENT II. THE EFFECTS OF DIFFERENT CONCENTRATIONS OF MALEIC HYDRAZIDE ON THE WATER UPTAKE OF PEA SEGMENTS
Calculated as percentage of increase in fresh weight over the original after 12 hours.

Treatment	Treatment means		Difference	
Distilled water	9.37			
Maleic hydrazide 250 p.p.m.	6.91	2.46*		
Maleic hydrazide 500 p.p.m.	6.60	2.77*	0.31	
Maleic hydrazide 1000 p.p.m.	4.80	4.57**	2.11*	1.80

Note: ** Difference exceeds LSD at 1% level : 3.91.
* Difference exceeds LSD at 5% level : 2.36.

Interaction of Maleic Hydrazide and Auxin on Water Uptake

Pea segments were immersed for 12 hours in the following aerated solutions: distilled water, 5 p.p.m. of auxin in distilled water, 5 p.p.m. of auxin in 250 p.p.m. maleic hydrazide, 5 p.p.m. of auxin in 500 p.p.m. maleic hydrazide, and 5 p.p.m. of auxin in 800 p.p.m. maleic hydrazide. The data are shown and tabulated in Fig. 7 and Table VIII.

A definite physiological inhibitory effect of maleic hydrazide on auxin-induced water uptake can be observed from these data. The significant differences between the materials treated and untreated by maleic hydrazide in the auxin solution, i.e., 6.61% and 8.45%, are much higher that those of the treated and untreated materials in water which are 1.15% and 1.69% respectively. However, maleic hydrazide at 250 p.p.m.

concentration level, which in the previous experiment significantly lowered the amount of water uptake of pea segments in water, failed to exert its inhibition in the presence of 5 p.p.m. of auxin. A critical concentration of maleic hydrazide for the exertion of its inhibitory effect in a 5 p.p.m. auxin solution might exist within the range of 250 p.p.m. and 500 p.p.m..

TABLE VIII

THE EFFECTS OF DIFFERENT CONCENTRATION LEVELS OF MALEIC HYDRAZIDE ON THE WATER UPTAKE OF PEA SEGMENTS IN DISTILLED WATER AND 5 p.p.m. AUXIN SOLUTION

Calculated as percentage of increase in fresh weight over the original after 12 hours.

Treatment	Treatment means	Difference				
Auxin	31.02					
Auxin with maleic hydrazide 250 p.p.m.	30.10	0.92				
Auxin with maleic hydrazide 500 p.p.m.	24.41	6.61*	5.69*			
Auxin with maleic hydrazide 800 p.p.m.	22.57	8.45*	7.53*	1.84		
Distilled water	8.20	22.82**	21.90**	16.21**	14.37**	
Maleic hydrazide 500 p.p.m.	6.65	24.37**	23.45**	17.76**	15.92**	1.15
Maleic hydrazide 800 p.p.m.	6.21	25.81**	23.89**	18.20**	16.36**	11.69	0.44

Note: ** Difference exceeds LSD at 1% level : 8.43.
* Difference exceeds LSD at 5% level : 5.68.

DISCUSSION

The augmentative effect of potassium salts on the auxin-induced water uptake was confirmed in using the pea stem as the test material. However, the mechanism involved is still unknown. Commoner et al (7) reported that the amount of water uptake closely paralleled the salt absorption curve in the auxin solution and that both can be inhibited by iodoacetic acid. Even so, whether or not this augmentative effect in the auxin solution is solely due to the increase of osmotic value inside the cell obtained by intake of salt still can not be definitely proved. Steward and Preston (25) reported that when potato discs were immersed in 0.05 M. solution of potassium chloride, the amount of water uptake, as well as the rate of respiration, and the amount of protein synthesized, were increased significantly over the control. In the case of calcium chloride solution, the reverse was true. They also found that potassium salts increase and calcium salts decrease the relative utilization of amino acids and the activities of oxidase in the discs. It seems worthy of further investigation to find, whether or not there is any similarity between the effects of potassium salts in the Steward and Preston's experiment, and those observed in the present experiment. It is evident from the present data that the anions do not have a determinative effect. As the writer suggests, the effect of potassium salts may be compared with that of calcium salts which were reported to have a lowering effect (25), together with salts of some other cations, i.e., sodium and magnesium. Respiration of the materials in the solution of

auxin with various salts should be checked for further information. If the increase of the osmotic value in the cell content is the only cause of the augmentative effect of salts, there should not be great differences among different kinds of cation salts used, and the rate of respiration of the material should not be affected greatly in the salt solutions.

The results obtained indicate that in the use of pea stem material for the study of auxin effect, aeration is essential. This conclusion is sufficient to invalidate the view that a change in auxin-induced cell wall plasticity is the only driving force of growth (12,17). It may be true that the cell wall of the material becomes more plastic after the auxin treatment (12), but this auxin action can not occur in the non-aerated condition in which active metabolism is hindered. It is interesting to note that the difference between the amount of water uptake of pea segments in the aerated and non-aerated water solution is insignificantly small, but in the auxin solution the difference is remarkable. It clearly indicates that the exertion of auxin effect requires an abundant supply of oxygen to meet the higher demand of respiration.

The data obtained in the study of the effects of maleic hydrazide, indicate that this chemical compound has an inhibitory effect on the water uptake of pea stem segments. In its presence, a higher concentration level of maleic hydrazide is required in order to be effective, but the inhibition is more profound. Again the difference in amount of water uptake between the material treated with maleic hydrazide and the control water is comparatively small, while in the auxin solution, the difference between the treated and untreated one is considerably larger. The data

obtained show that the inhibition exerted by the maleic hydrazide to the auxin-induced water uptake is a physiological reaction and not a simple subtractive effect. The effective threshold concentration of maleic hydrazide is shifted to a higher level in the presence of auxin.

While this paper was being prepared, Leopold and Klein (18) reported a similar result of interaction of auxin and maleic hydrazide using the split stem test. They claim that the maleic hydrazide is an antiauxin. However, it is the writer's opinion that not enough evidence has been presented for a clear understanding of the mechanism involved.

Recently, profound interest has bee aroused in a search for chemicals which are antagonistic to auxin action (3,4,28,29,33). In the light of these investigations, and guided by the tentative theory of auxin mechanism proposed by Bonner (5), it is possible to classify the different kinds of antagonistic compounds into three general categories, according to their mechanism of action.

The first group of antagonistic compounds are structural isomers of physiologically active chemicals. Being different in the spatial configuration which is specifically required by their isomers, they are physiologically inactive (28). But when they are present in an auxin solution, they become a potent competitive inhibitor, (28,33). The classical example of this group is the cis-trans isomers of cinnamic acid. The cis-cinnamic acid behaves as an auxin, while the trans-isomer acts as an antagonist in the auxin solution (33). Since it is generally believed that auxin has to combine with a protein in order to become active (5,27), a competitive interaction between the auxin and its inactive isomer, i.e., the trans-cinnamic acid, for the occupancy of a specific site on a protein

molecule may be the mechanism involved (33). However, maleic hydrazide evidently can not be classified into this category, because it is physiologically active by itself.

The classical example for the second group of antagonists is cyanide and iodoacetate. These compounds strongly inhibit the respiration and the water uptake to the same extent (13). It has been reported that the inhibitory effect of iodoacetate can be reduced by an increased supply of oxygen (29). It appears that their inhibition to the growth of the material is mainly due to a block in the respiration process.

The typical examples of the third group of compounds are arsenate (5) and 2,4-dinitrophenol (3,4,5). This type of compound inhibits both the growth and the increase in respiration induced by auxin, but is without effect on the basal respiration of the material (3,4,5). This inhibitory effect of the compound can be removed by addition of more phosphate into the media (5). It was suggested by Bonner (5) that the action of this type of antagonist is by blocking the phosphate metabolism in which the auxin may play an important role in the utilization of the respiratory energy.

Whether it is possible or not to classify the maleic hydrazide into the group II and group III discussed above is of great interest and worthy of further investigation. The maleic hydrazide inhibition might be connected with phosphate metabolism as suggested (21). It is the writer's recommendation that a check be made to investigate the effect of maleic hydrazide on the respiration and the water uptake on a comparable basis, in the presence and absence of auxin. More information might be provided by such an investigation.

When the present experiment was still in progress, it was the writer's pleasure to discover that more and more hormone research work was being done with segments of pea stems as the material used in the investigation (33,18,28). It seems to the writer that in the near future, pea stem segments may become the classical material used in general hormone research replacing Avena coleoptile which has certain disadvantages. However, based on the experience of the present experiment, more rigid control of the environmental conditions for the growth of pea seedlings, may provide more satisfactory results.

SUMMARY AND CONCLUSIONS

1. The literature of the effect of auxin on water uptake, maleic hydrazide as a plant growth inhibitor, and of the segments of Pisum stem as the test material used in the investigation are reviewed.

2. Fourteen segments, 26 mm. long, cut from the stem of the pea seedling (Pisum sativum L. var. Alaska), were used as the material in each replicate. Water uptake was calculated as the precent increase in fresh weight over the initial fresh weight of fourteen stem segments, after 12 to 16 hours exposure to the aerated experimental solutions. Each treatment consisted of four replicates, i.e., 64 segments. Differences between treatments were subjected to analysis of variance. Indole-3-acetic acid was used as the auxin in the present investigation.

3. Data from the preliminary study indicated that the most effective concentration of auxin for rapid water uptake was around 10 p.p.m..

4. The augmentative effect of potassium salts on the auxin-induced water uptake was confirmed in using the pea stem as the test material. The amount of water uptake by pea segments in the auxin solution in the presence of salt was significantly higher than that of the pea segments in the solution with auxin only. However, there were no significant differences among the kinds of potassium salts used. Apparently the anions did not have a determinative effect in the reaction.

5. Aeration was confirmed as essential for the exertion of auxin effect in using the pea stem as the test material. In the non-aerated solution, auxin failed to induce any significant increase in the amount

of water uptake over the controls. However, the augmentative effect of auxin at different concentration levels showed up significantly when the solutions were aerated.

6. Data obtained indicates that maleic hydrazide has an inhibitory effect on water uptake of pea segments. In the presence of auxin, a higher concentration of maleic hydrazide is required in order to be effective than in its absence, but the inhibition is more pronounced. It shows that the inhibition exerted by the maleic hydrazide to the auxin-induced water uptake is a physiological reaction.

7. A possible explanation of the mechanism involved in the augmentative effect of potassium salts and the inhibition of maleic hydrazide has been discussed. Suggestions are also given for future research in the subjects investigated.

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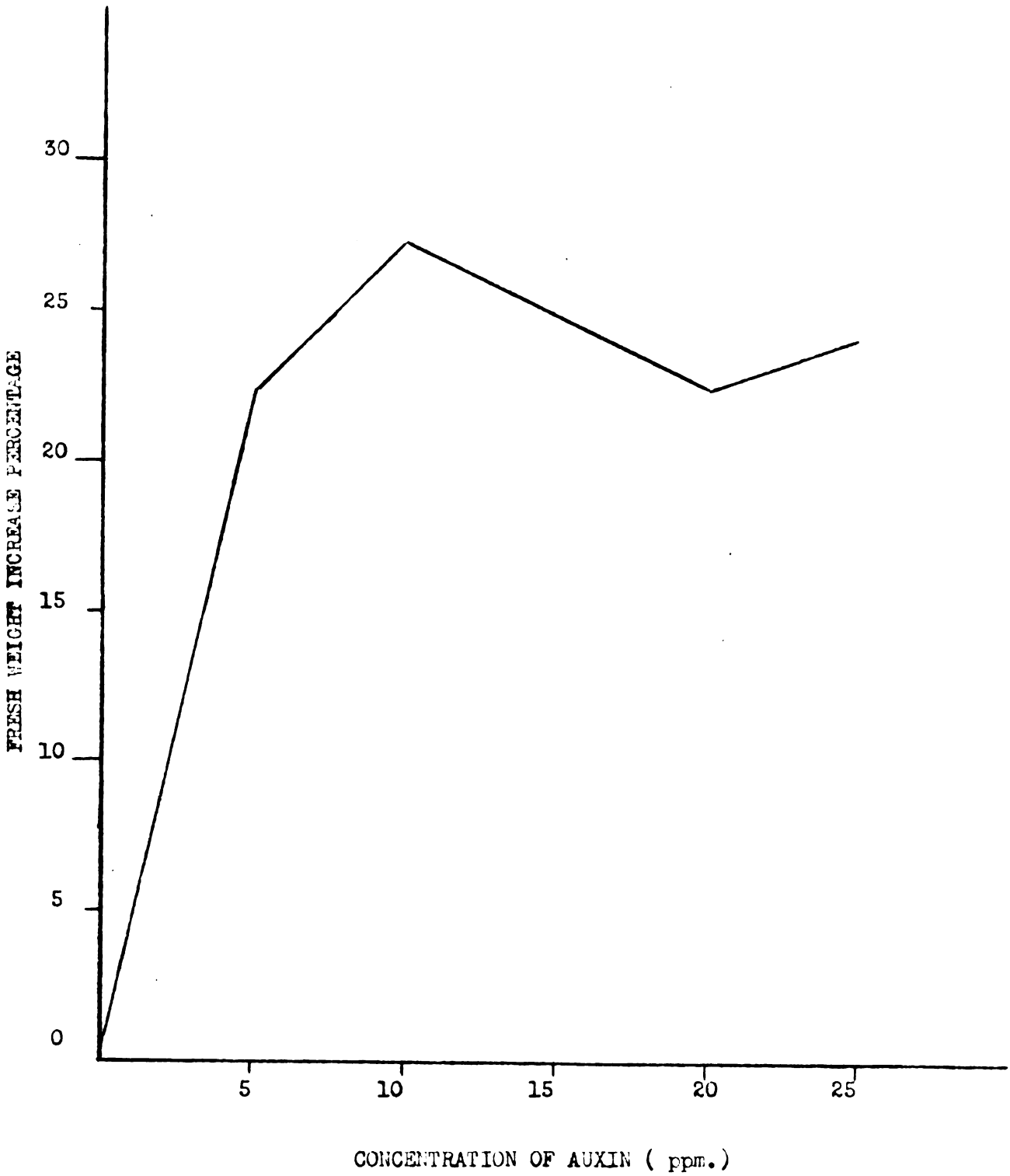


Fig. 1. Effect of different concentrations of auxin on water uptake of pea segments.

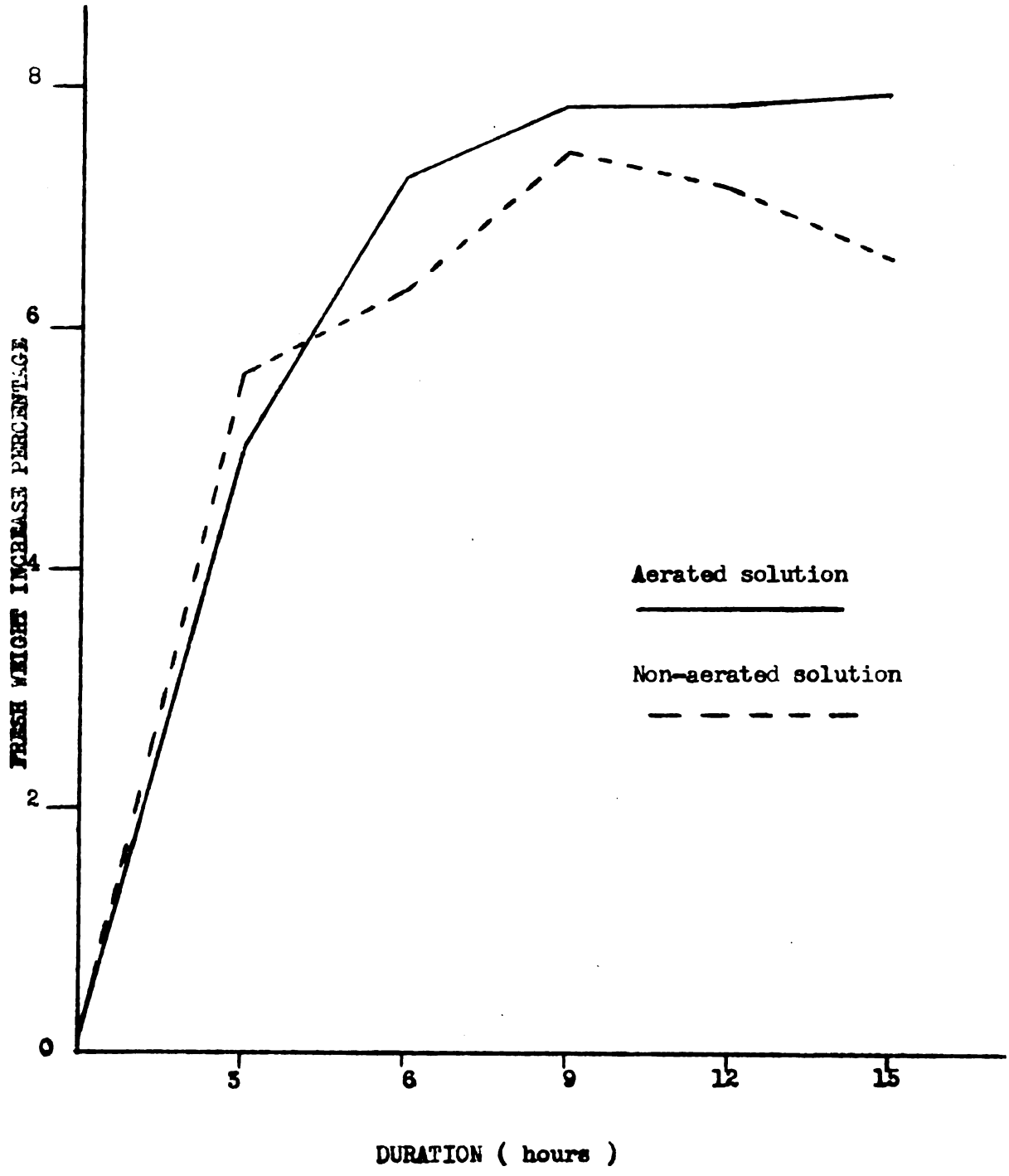


Fig. 2. The rate of water uptake of pea segments in distilled water in relation to amount of aeration.

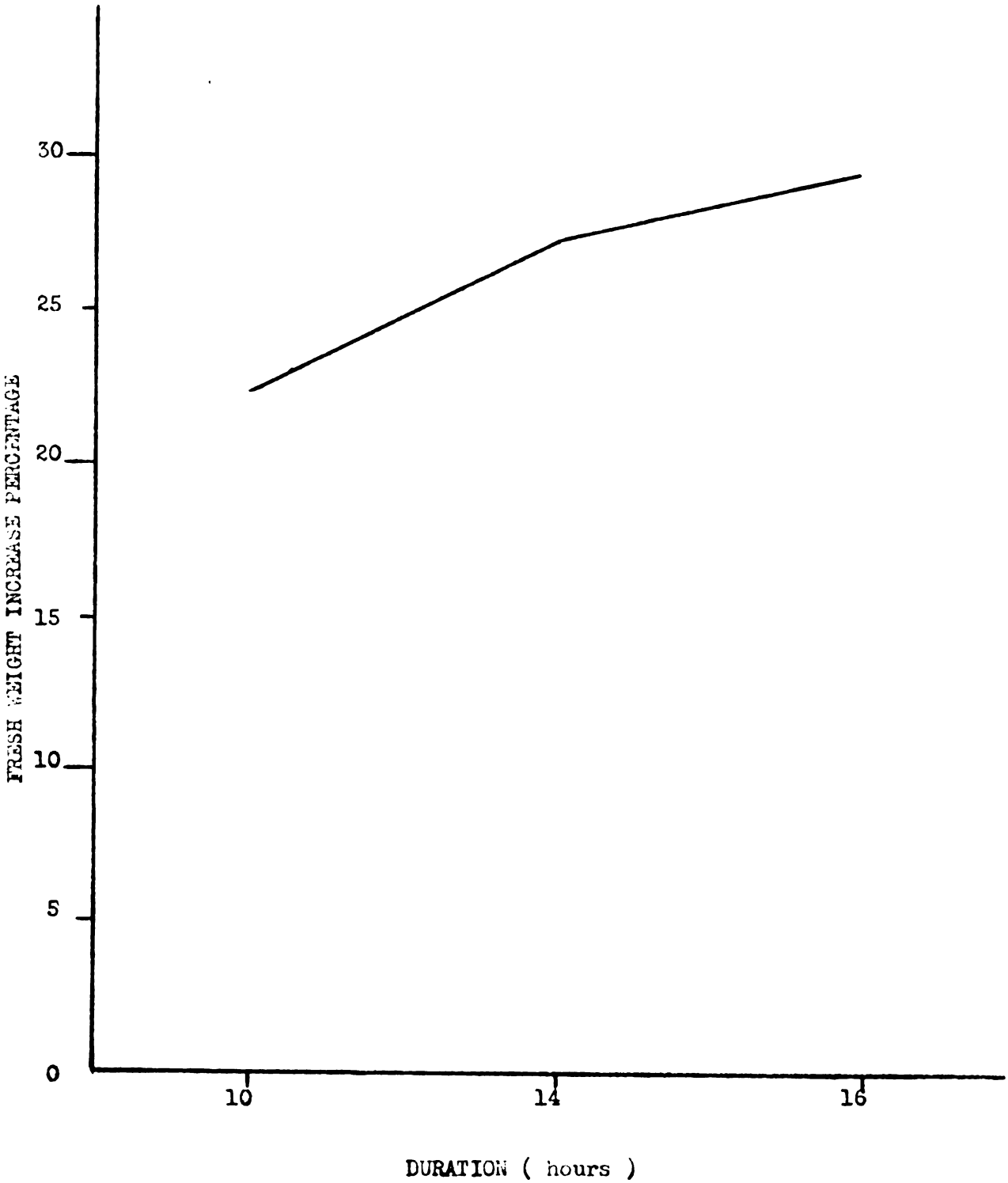


Fig. 3. The rate of water uptake of pea segments in aerated solution containing 10 ppm. auxin.

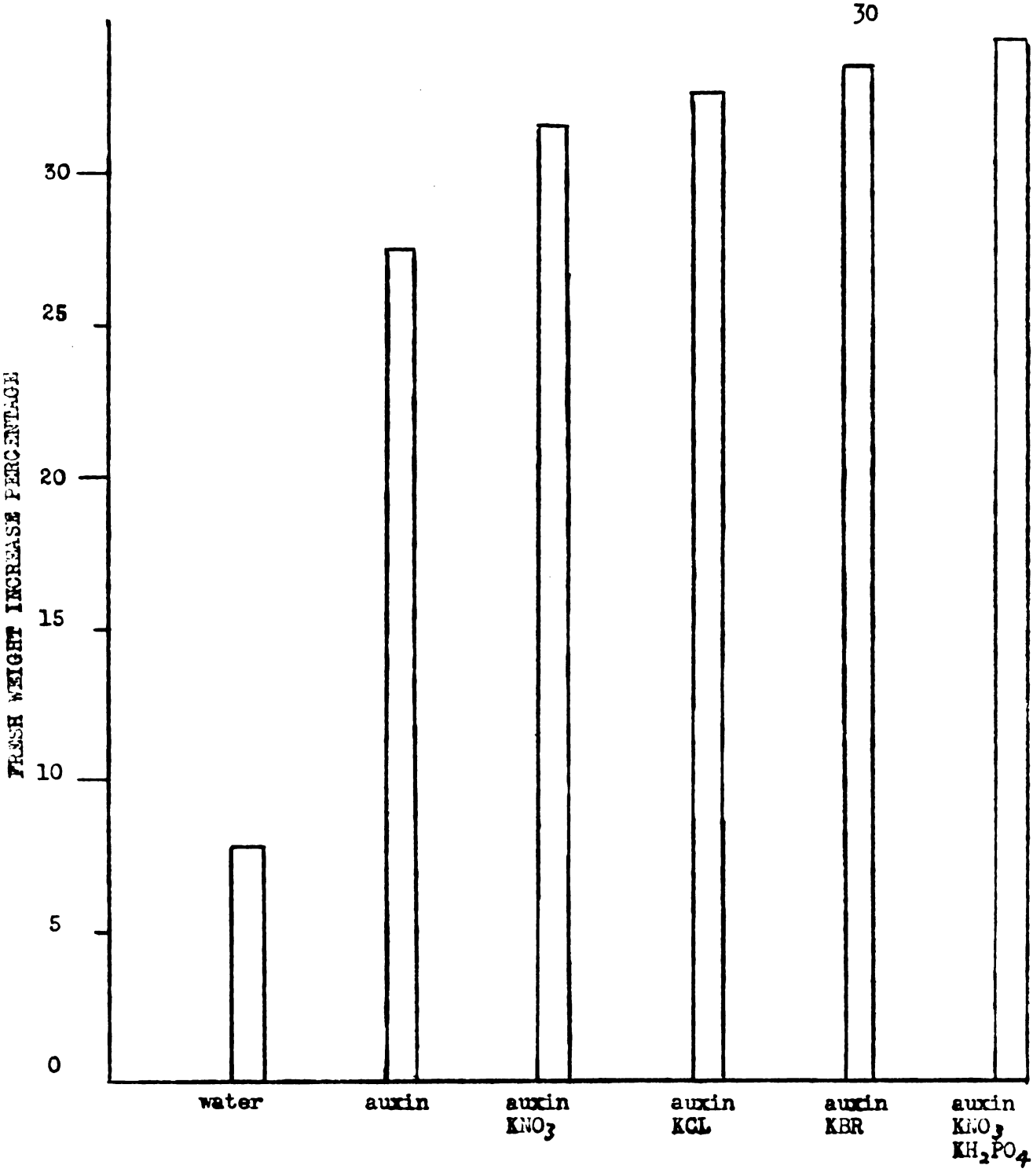


Fig. 4. Effects of different potassium salts at 0.002 M. concentration on the auxin-induced water uptake of pea segments in 10 ppm. auxin solution.

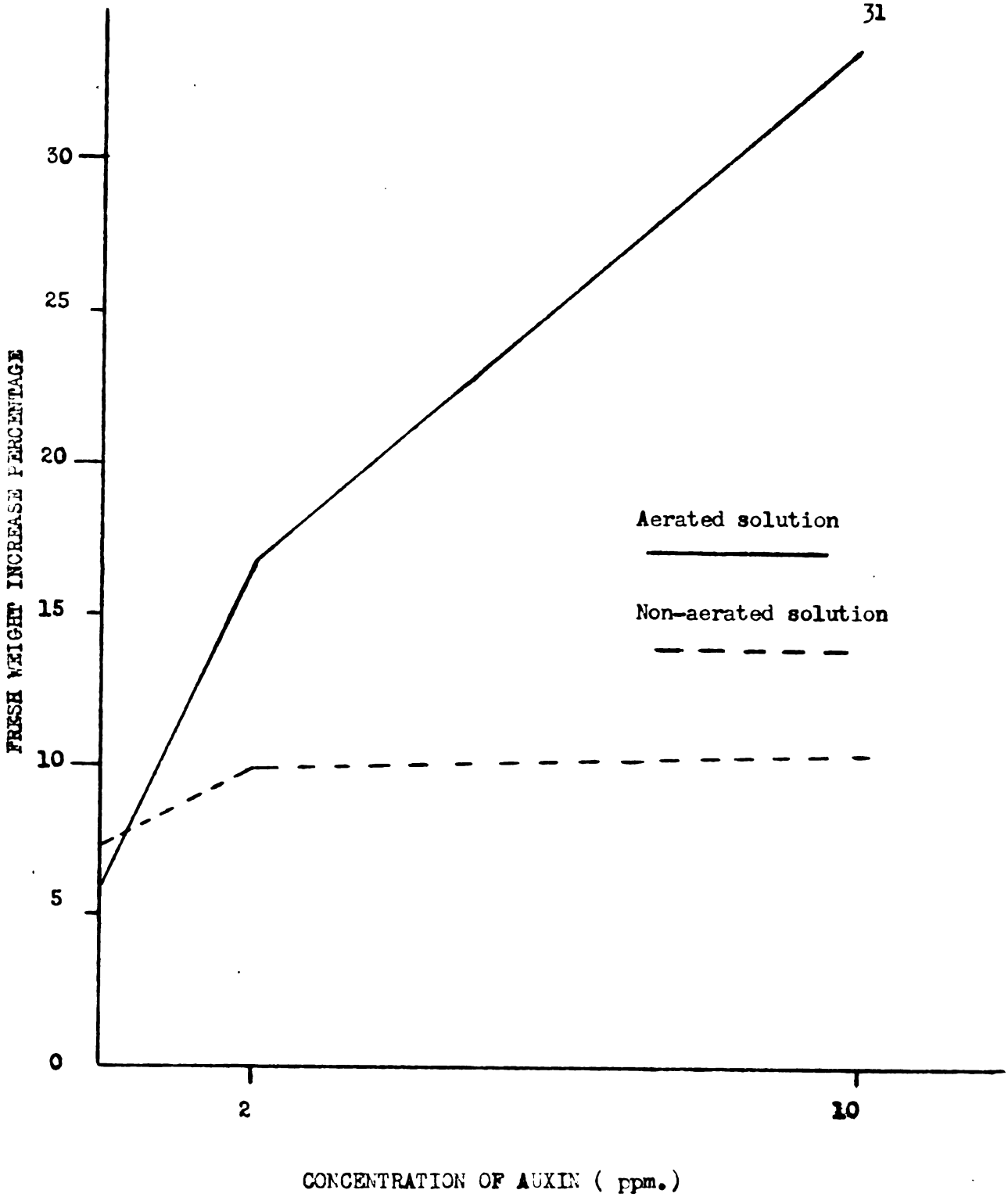
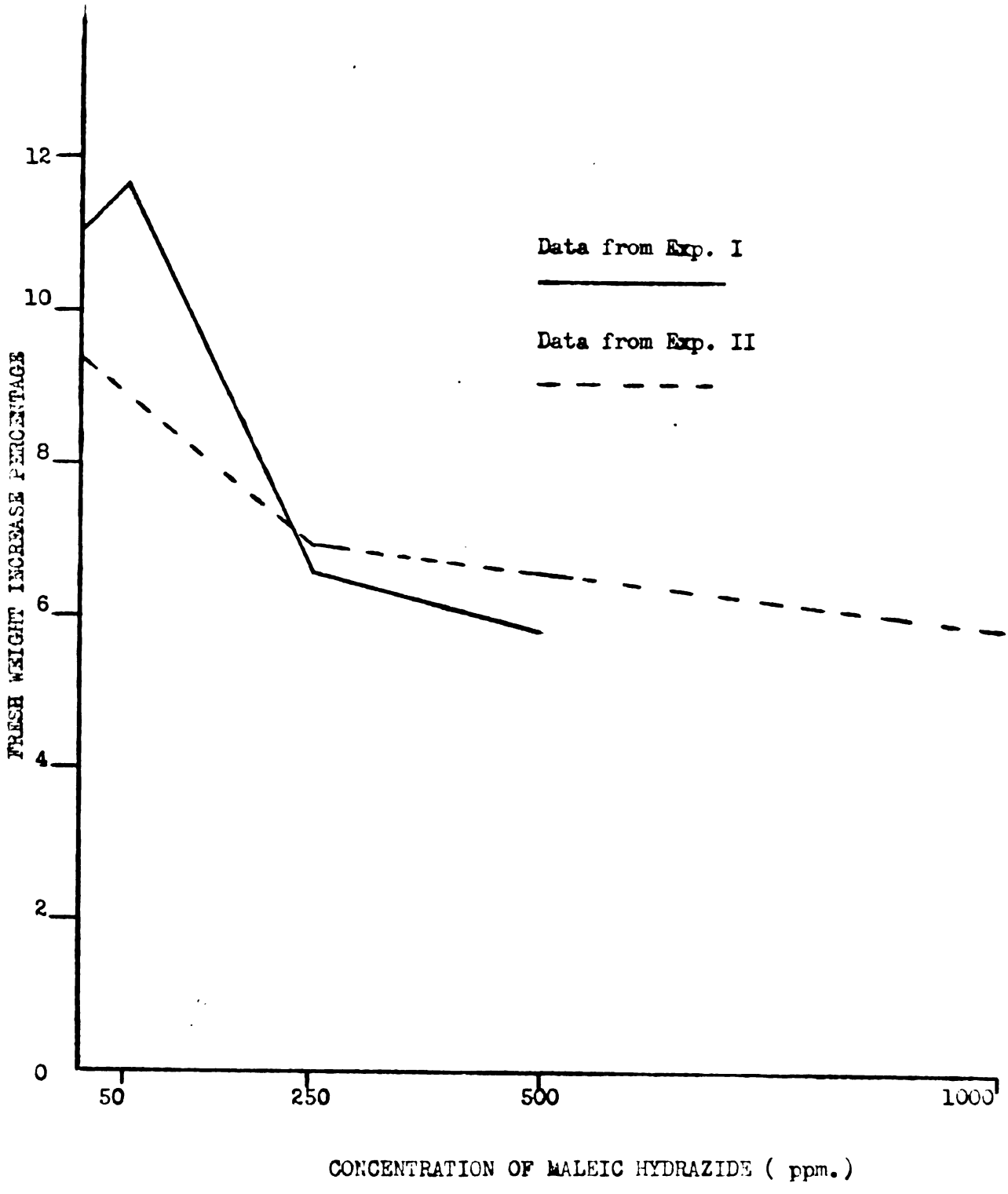


Fig. 5. Effects of aerated and non-aerated auxin solutions at 2 ppm. and 10 ppm. concentration levels on the water uptake of pea segments after 16 hours.



Fig, 6. Effects of different concentrations of maleic hydrazide on the water uptake of pea segments.

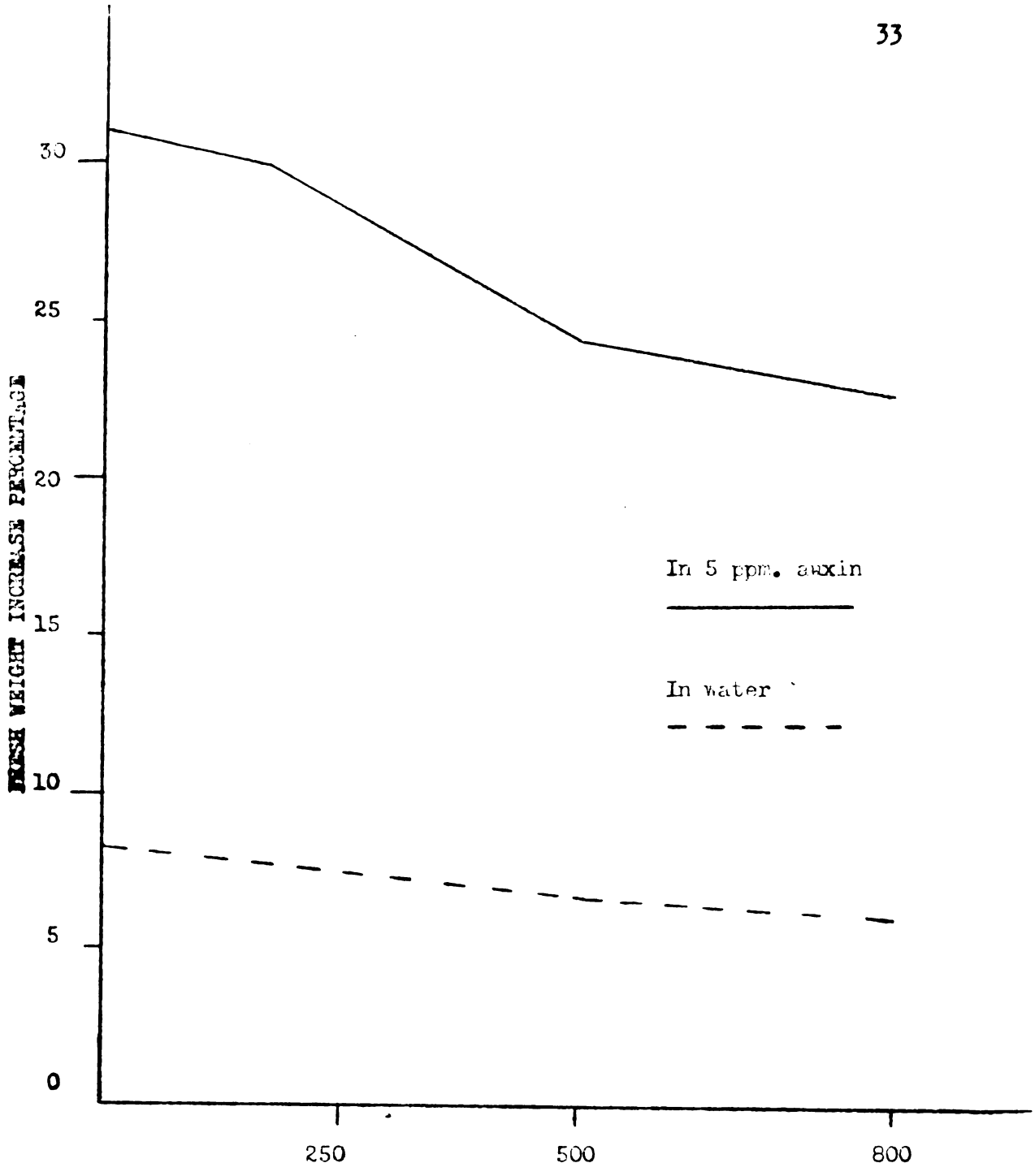


Fig. 7. Effects of different concentration levels of maleic hydrazide on water uptake of pea segments in distilled water and 5 ppm. auxin solution after 12 hours.