A Thesis Submitted in Two Parts:

1. A Plant Growth Inhibitor and Plant Growth Inhibition.

2. Extensibility of Cell Wall Material in Indole-3-acetic Acid.

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Summary of a Thesis Submitted in Two Parts:

1. A Plant Growth Inhibitor and Plant Growth Inhibition.

2. Extensibility of Cell Wall Material in Indole-3-Acetic Acid.

1. A Plant Growth Inhibitor and Plant Growth Inhibition.

A non-toxic ether extractable substance, capable of inhibiting plant cell elongation growth, was found in the cotyledons and leaves of various herbaceous plants. Radish cotyledons had the highest concentration of this "inhibitor" substance of any of the sources investigated.

When the inhibitor was applied at the top of an Avena plant coleoptile, the inhibition was manifested as a positive courature within 2½ hours. Between 3 and 13 degrees, the curvature was linearly proportional to the inhibitor concentration. Thus by measuring the curvature it was possible to analyze quantitatively for the inhibitor.

The inhibitor was found to be stored in the radish seed, synthesized in the plant, and to disappear with leaf growth.

Chemical investigations indicate that the inhibitor is an ester of indole acetic acid with some as yet undetermined substance. In a purified form this ester is readily hydrolysed in water to form auxin. The amount of hydrolysis is decreased by the addition of various substances or the presence of impurities in the extract.

Growth measurements showed that the positive curvatures could be accounted for by the measured amount of inhibition.

The movement of inhibitor through Avena coleoptiles is non-polar. This fact was used to separate contaminating auxin from crude inhibitor extract by collecting the inhibitor moving through the coleoptile acropetally. Auxin can not be transported in this direction as it is only passed through the coleoptile basipetally. The velocity of inhibitor transport in the coleoptile is approximately 11 mm. / hour.

Diffusion coefficient determinations indicate that hydrolysis of inhibitor could account for the auxin (probably indole acetic acid) diffusing from radish cotyledons. The determinations also indicate that that the molecular weight of the inhibitor may be between 100 and 175

2. Extensibility of Cell Wall Material in Indole-3-acetic Acid.

Artificial silk was found to have an increased extensibility, compared to water controls, in certain organic acids known not to be growth substances as well as in .2% indole acetic acid.

Onion roots were found to have an increased extensibility in .2% indole acetic acid and in .2% acetic acid.

Experiments of Robbins and Jackson were repeated and found to give no conclusive evidence as to the effect of indole acetic acid on the extensibility of stem and root cell wall materials.

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Chapter I. A Plant Growth Inhibitor

A. Introduction.

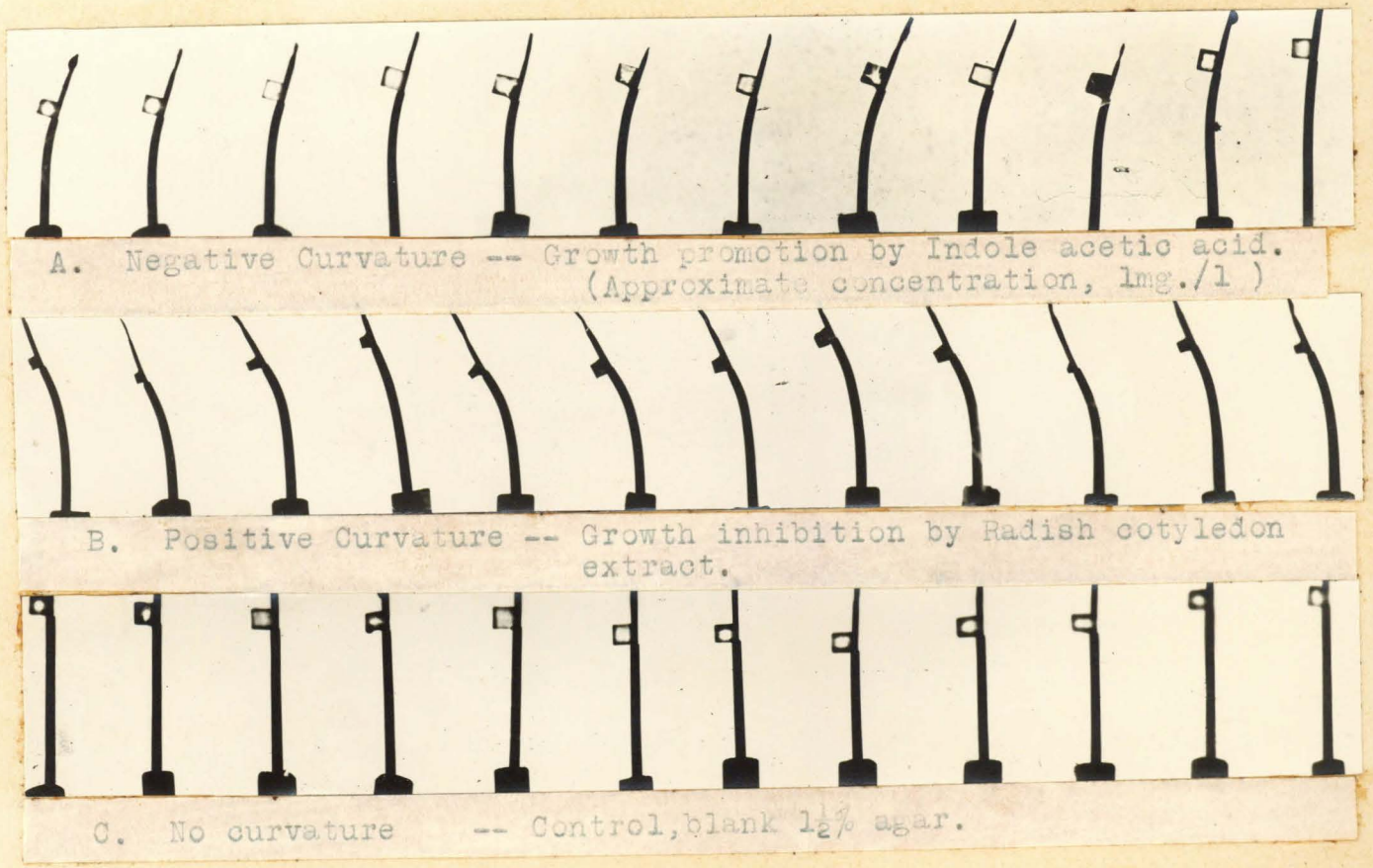
Two types of growth inhibition are generally considered in plant physiology. The first and most widely discussed is that concerned with the maintenance of the dormant state of plant tissues; seed germination, bud inhibition, etc. Bud inhibition is often spoken of as a "correlation" phenomenon in view of the fact that the non-development of the lateral buds is determined by the presence of the terminal bud. It is commonly agreed that the terminal bud maintains its dominance over the lateral buds by its production of auxin; however, the mechanism by which auxin causes this inhibition to take place is still an open question. The numerous theories about this matter are well summarized by Van Overbeek (1938). The second type of growth inhibition, however, is not concerned with the maintenance of a dormant state as in bud inhibition, but is actually concerned with the interference of the elongation growth of the cell. It is this kind of inhibition with which we are concerned.

If a growth promoting substance is applied in a 1½% agar block, size 1 x 2 x 2 mm., unilaterally to the cut surface of the top of decapitated Avena coleoptile,

and if the substance is capable of being transported down the coleoptile, the coleoptile will curve in a direction away from the agar block. This is caused by a greater growth of the treated side than of the untreated. This is known as a negative Avena curvature. If, however, the substance caused an inhibition of growth, then the untreated side will grow more than the treated and the curvature will be toward the agar block. This is known as a positive curvature. (For illustrations of positive and negative curvatures see figure 1.) Thus it is clear that in considering substances causing an inhibition of cell elongation growth, one must consider positive Avena curvatures.

B. Early work on substances causing positive growth curvatures in Avena coleoptiles.

A summary of previous work on positive Avena curvatures is found in Phytohormones, (Went & Thimann, 1937). Here it is pointed out that the positive curvatures obtained by Stark (1921), Nelson (1924), and Seubert (1924), were probably not caused by growth inhibiting substances as they claimed but were caused by the unequal resumption of auxin production of the Avena stump ("physiological tip") after the normal tip had been removed. Also both Stark and Seubert failed to give the magnitude and cur-



A. Negative Curvature -- Growth promotion by Indole acetic acid. (Approximate concentration, 1mg./l)

B. Positive Curvature -- Growth inhibition by Radish cotyledon extract.

C. No curvature -- Control, blank 1½% agar.

Figure 1. Growth curvatures of Avena coleoptiles 90 minutes after application of the material being tested.

vature rate of their test plants for the substances investigated. For these reasons it is difficult to draw any conclusions from their data.

Gorter (1927) first found a relationship to exist between positive curvatures (of rather slight magnitude) and the "physiological tip" regeneration; although she did not realize that the positive curvatures were caused by an unequal auxin production at this regenerated tip, she did find that they always appeared after the two and a half hours which are necessary for regeneration of a "physiological tip". Since positive curvatures never appeared in less than this time, and since substances claimed to be growth inhibitors by previous investigators gave the same positive curvatures as plain agar, she came to the conclusion that the tip regeneration was the cause of the positive curvatures. In concluding her paper she says:-

"Summarizing one can therefore say that at present only growth accelerating substances have been found. Only when one will find a substance that will cause a positive curvature within two and a half hours may one speak of a growth inhibiting substance."

C. Recent work on substances causing positive growth curvatures in Avena coleoptiles.

Recently Skoog (1936) has found that a block of pure agar on the top of a decapitated Avena coleoptile actually inhibits the formation of auxin in the regenerating tip. This has the result that when pure agar alone is placed on one side of the Avena test plants, the production of auxin after two and a half hours will be less on the side of the plant below the agar block than on the side without it. That side will hence have a decreased growth rate and positive curvatures will thus appear. This completely explains the relationship suggested by Gorter between the onset of tip regeneration and the appearance of positive curvatures.

Meyer (1936) has reported positive Avena curvatures from plant extracts prepared by dehydrating the plant material with alcohol, grinding it to a powder in a mortar and extracting it with lanolin. The lanolin extract was then applied to Avena coleoptiles according to the technique of Laibach (1933) and the curvature measured after 24 hours. With this method it is difficult to see how any positive curvatures could be caused by the tip regeneration effect of Gorter as in this method the plant is not decapitated. Except for nasturtium cotyledon extract the substances were only inhibiting at higher concentrations. At lower concentrations a negative curvature resulted. This would indicate a toxic action at the higher concentrations. An ether extract of the Nasturtium cotyledons

according to the method used for radish cotyledons showed the presence of growth inhibitor. (See Chapter III.)

Skoog (1936) found that indole ethyl amine and also tryptophane are capable of causing slight positive curvatures within the first two hours when tested on deseeded plants, but that these positive curvatures soon thereafter change into negative ones;probably upon their transformation in the plant, into growth promoting substances. They are thus certainly not to be considered as growth inhibiting substances.

Czaja (1934) has reported that tissues rich in tannin, for example callus tissue of Populus, are able in some cases to diffuse out a growth inhibiting substance. He also found that tannic acid and gallic acid of a 1% concentration are likewise capable of causing positive curvatures. These curvatures he attributes to the actual shrinkage of the coleoptile cells on the treated side so that they actually become shorter than those on the untreated part. He obtained a maximum curvature of twenty degrees in three hours.

Upon repeating his experiment using 1% solutions of tannic and gallic acids I was unable to obtain any positive curvatures whatsoever. While this may have been due to the fact that Czaja prepared his agar test blocks by soaking them for 24 hours in the solutions and I prepared mine by taking the solutions up in melted agar so that the final

concentration was the same as Czaja's, it seems unlikely that this slight difference in procedure should make any difference in the final action of the substances themselves on the test plants.

Brecht (1936) has shown that acetic acid in lanolin paste when applied directly to intact Avena coleoptiles is capable of causing positive curvatures. These curvatures, however, are preceded by negative curvatures and are themselves acknowledged to be the result of an injury effect of the acetic acid.

Vielman (1938) has recently found that lanolin pastes of acetic and of formic acid in concentrations of from .0625 M to .25M when applied to the top of a 1 cm. long piece of hypocotyl of Helianthus or Lupinus causes a substance to diffuse out which is capable of eliciting positive Avena curvatures. These curvatures are never initiated in less than two hours.

D. Definition and factors causing positive growth curvatures in Avena coleoptiles.

The statement may be safely made that whenever a positive curvature occurs in an Avena plant there has been a relative inhibition of growth. Since this inhibition is the result of a disturbance of the normal increase in cell volume it is desireable to consider for a moment the

factors governing this process that could be affected to give a decreased growth rate.

For our purposes growth may be defined as an irreversible increase in cell volume. This growth may be brought about either by: 1. water uptake; 2. an increase of the cell constituents mainly by cell wall formation; or 3. both processes working together. The last case is the one that occurs but in it water uptake is the major factor. (Phytohormones, 1937.)

The ability of a cell to take up water may be said to be the result of the difference between the osmotic pressure of the cell contents and their tendency to take up water, and the opposite action (turgor pressure) of the cell wall which surrounds the cell contents and exerts a compressing force on them, or tends to cause them to lose water. This difference between the tendency of the cell contents to take up water and the opposing tendency on the part of the cell wall, has been defined by Ursprung and Blum (1924) as the "Suction Force". It is then due to the suction force that a cell is able to increase its water content and hence to grow. A decrease either in suction force or in available water will thus decrease the amount of growth of the cell. The suction force may be decreased by lowering the osmotic pressure, increasing the turgor pressure (compressing force) on the cell contents, injury, or death. The water avail-

able to the cell may be decreased by interrupting the normal water supply either by interfering with the transport of the water in the conducting system or by applying hypertonic solutions. Any one, or combination of these factors would thus be capable of causing a relative growth inhibition and hence a resulting positive curvature.

In view of the above facts, we may now say that substances claimed to be inhibitors of the cell growth process itself must act either by: 1. By reversibly lowering the osmotic pressure, or 2. by increasing the turgor pressure. Substances which cause positive curvatures by: 1. exosmosis, as for example the salts investigated by Kisser and Beer, (1934), or 2. by injuring the cell so that it is no longer capable of growth, as shown by Brecht (1936) for acetic acid are thus not to be considered as "inhibitors" of the growth process.

E. Discovery of a true growth inhibiting substance in radish cotyledons.

An ether extraction of radish cotyledons according to the method of Van Overbeek (1938) for auxin failed to show any auxin but instead showed the presence of an inhibiting substance. This was manifested after 90 minutes as a positive curvature in the standard Avena test

(Phytohormones, 1937). Curvatures obtained with this extract may be seen in figure 1. The extracted cotyledons were from radish plants of the strain "French Breakfast". The plants were seven days old and had been grown in the open in loamy soil. Five grams fresh weight of cotyledons were used. At the same time that these positive curvatures were found Mr. Wm. Bergren of these laboratories, reported similar curvatures from ether extracts of radish cotyledons. A preliminary note describing these properties has been published. (Stewart, Redemann, and Bergren, 1939).

From the foregoing discussion we have seen that a true growth inhibitor must be able to cause positive Avena coleoptile curvatures in less than two hours, and must neither cause the cells to permanently lose their growth ability, nor to become plasmolysed. Preliminary observations showed that the radish cotyledon extract satisfied these requirements. Heretofore no substance had been found which so completely fulfilled the criteria for a true growth inhibitor. It became the object of this work to investigate the properties of this cotyledon extract. In subsequent discussion reference made to growth inhibitor will refer to this substance.

Chapter II. The Bio-assay for Growth Inhibitor.

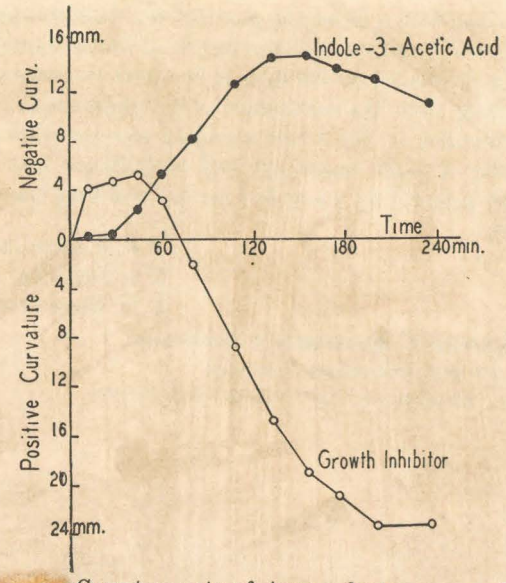
In Chapter I it was seen that it was possible to obtain from radish cotyledons, by the auxin ether extraction method, a substance that caused positive curvatures in Avena plants. It was next of interest to know if the standard Avena test, perhaps with modifications, could be used as a bio-assay for this inhibitor substance. To settle this point it was necessary to know: 1.) The optimal time after application of the inhibitor to measure the positive curvature; 2.) The curvature range which was linearly proportional to the concentration of the inhibitor, and 3.) The most reactive type of test plants to use. The answers to these questions were sought by the experiments described below.

Throughout this thesis the materials and methods used are those which are so completely described in "Phytohormones" (Went and Thimann, 1937) that repeated enumeration of them will not be given here. For example, it is to be understood that the Avena test plants were grown from the standard Siegeshafer strain of seed according to the rigid technique outlined in the aforementioned monograph. Inhibitor was always prepared according to the method described in Chapter I and applied to the Avena plants in 1½% agar blocks unless otherwise specifically noted.

A. Curvature rate of Avena coleoptiles to inhibitor.

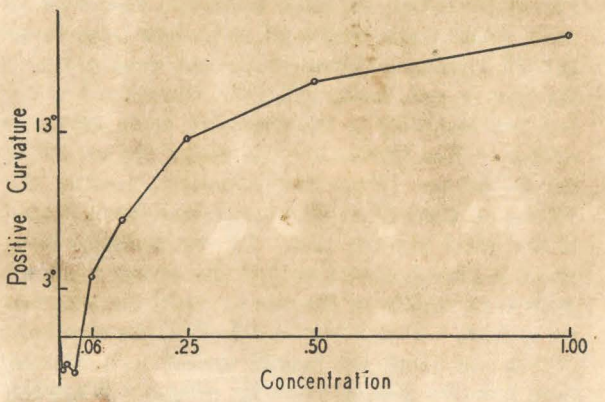
To determine the optimal time for measuring the angle of curvature a "Photokymograph" test was made according to the method given by Schneider and Went (1938) and improved by Went and White (1939). In making this test the growth movement of the Avena test coleoptile, during a given period of time, is recorded photographically by means of a shadow cast on a revolving drum of photographic paper. The shadow cast is not that of the coleoptile itself but is that of a very fine (No. 36, .012 mm. diameter) silver wire inserted in the primary leaf. The primary leaf is previously broken loose as is the procedure in the Avena test. This prevents it from further growth and keeps it from lifting tha agar blocks up from the cut top of the coleoptile. Inserting the silver wire and thus "extending" in effect the coleoptile has two advantages. First, it keeps the coleoptile itself in the dark and allows only the silver wire to intercept the beam of light thus preventing phototropic curvatures of the coleoptile, and secondly it amplifies the curvature. At small angles a curvature of 1° moves the shadow on the drum about 1 mm. This whole machine is kept in the regular Avena testing dark room at constant humidity and temperature.

The results of such a test for the inhibitor are shown in figure 2. (The curvature values, the ordinate



Curvature rate of Avena plants upon application of: (*a*) .05 mg/liter, indole–3–acetic acid, and (*b*) growth inhibitor. (Ordinate values given as mm deviation of the extended coleoptile from the vertical position.)

Figure 2.



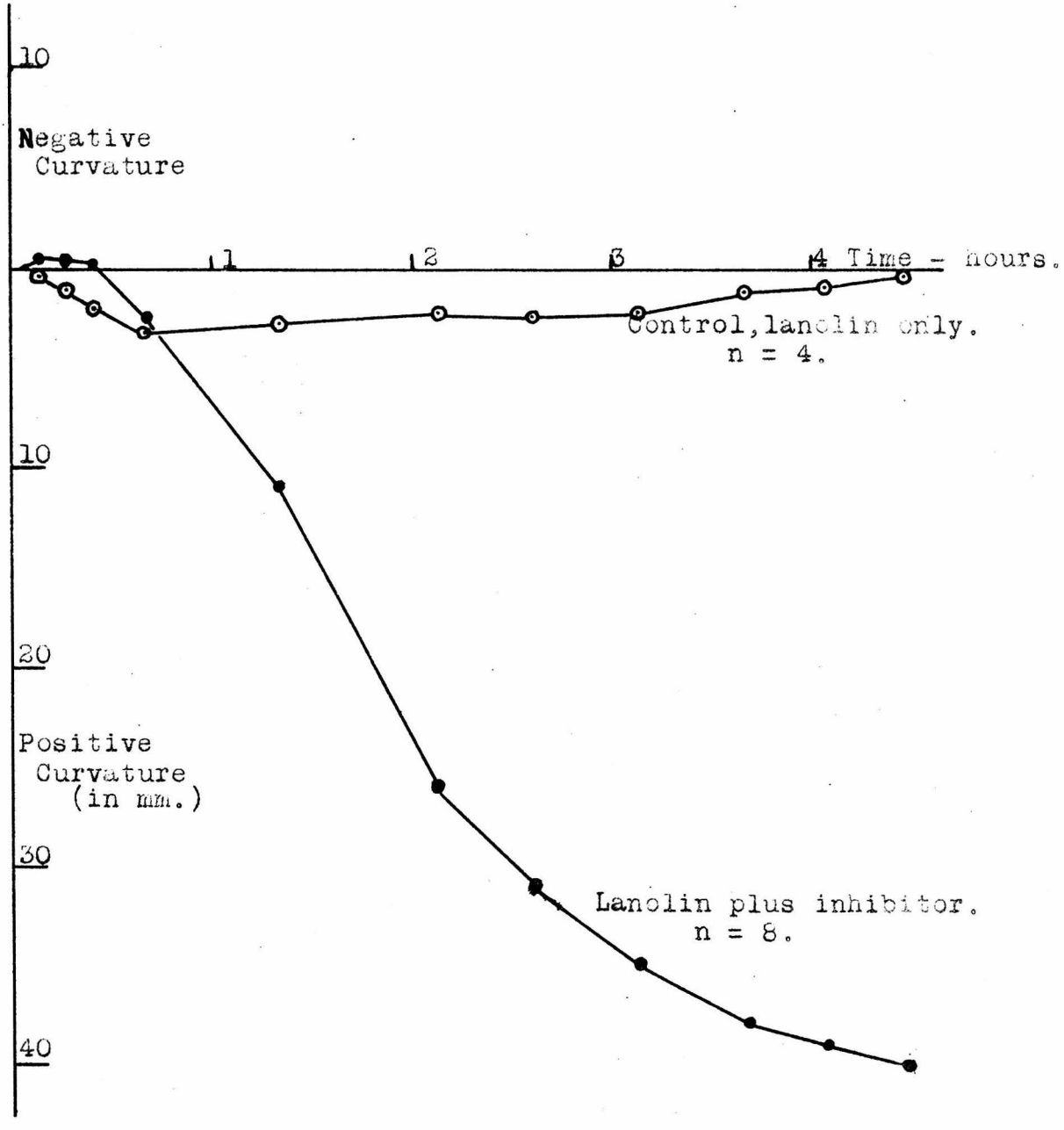
Relation between positive curvature of Avena plants and two-fold dilutions of inhibitor substance.

Figure 4.

axis for this experiment, and all subsequent photokymograph tests, will be given in actual mm. deflection of the silver wire or "extended coleoptile tip".) It is observed from this data that for the inhibitor, a negative curvature is initiated which rapidly changes between the first and second hour into a positive curvature and reaches a maximum in about three hours. It is interesting to note that the reaction rate for the negative curvature preceeding the positive curvature is different from the negative curvature caused by pure auxin alone. This is shown by the control run made at the same time as the inhibitor test but by using a growth promoting substance, indole-3-acetic acid, .05 mg. per liter. Each point on the graph is the average of twelve Avena test plants. This experiment has been repeated many times showing the same "time-positive-curvature" relationship.

Another experiment of the same nature was performed except in this case the inhibitor was applied unilaterally in lanolin paste at the tip of intact plants. The silver wire to "extend" the coleoptile was inserted through the tip of the plant and down into the primary leaf. It is seen in figure 3. That the positive curvature rate is the same here as in the test using inhibitor applied in agar blocks although the initial negative curvature is absent. This point will be discussed in Chapter VI.

Fig. 3 Reaction rate of Avena plants to inhibitor applied in lanolin to intact plants. n equals number of plants averaged for each point.



From both of these experiments it is clearly seen that after a 150 minutes the curvature is still proceding at the maximum rate, but that soon thereafter it begins to decrease rapidly. From this data then the time for measuring the angle of positive curvature was set at 150 minutes from the time of application of the inhibitor.

B. Relation between positive curvature and concentration of inhibitor.

To answer the second question it was necessary to determine the relation between the concentration of the inhibitor and the magnitude of positive curvature. This was done by the following experiment:

A concentration series of an active inhibitor extract was prepared and 48 Avena plants used to determine the degrees of positive curvature at each concentration value. The standard Avena technique was used except that the curvatures were measured after 150 minutes from the time of application of the inhibitor instead of after 90 minutes as is customary when one is testing growth promoting substances. The results are seen in figure 4. This graph shows that positive curvatures between three and thirteen degrees are linearly proportional to the amount of inhibitor. This experiment has been repeated many times with similar results. See Chapter IV.

The "concentration-curvature" relationship was also investigated using a concentration series of inhibitor in lanolin. The lanolin was applied unilaterally on intact plants over a 5 mm. strip, in one case at the tip and in another case at the middle, of the coleoptile. Applying a lanolin paste at the bottom of the coleoptile did not cause any positive curvature. The positive curvatures resulting from the applications at the tip and middle were measured after two hours. Measurements of the curvatures after four hours showed there had been no increase in the sensitivity of the test. At the same time that the lanolin was applied, a similar test using the same amounts of inhibitor in agar blocks was run. All of these results are summarized in figure 5. (Pictures of curved plants may be seem in fig. 22. - Chapt. VI.) it may be seen that the method of applying inhibitor in lanolin at the middle of the coleoptile gives the greatest range over which a linear proportionality exists between curvature and concentration. The same range exists when the lanolin with inhibitor is applied at the tip; however, in this case the magnitude of curvature, is considerably less. It is interesting to see that when the same amount of inhibitor is applied in agar the curvature is linearly proportional over just that range of concentration in which the lanolin with inhibitor is not proportional. It is also significant

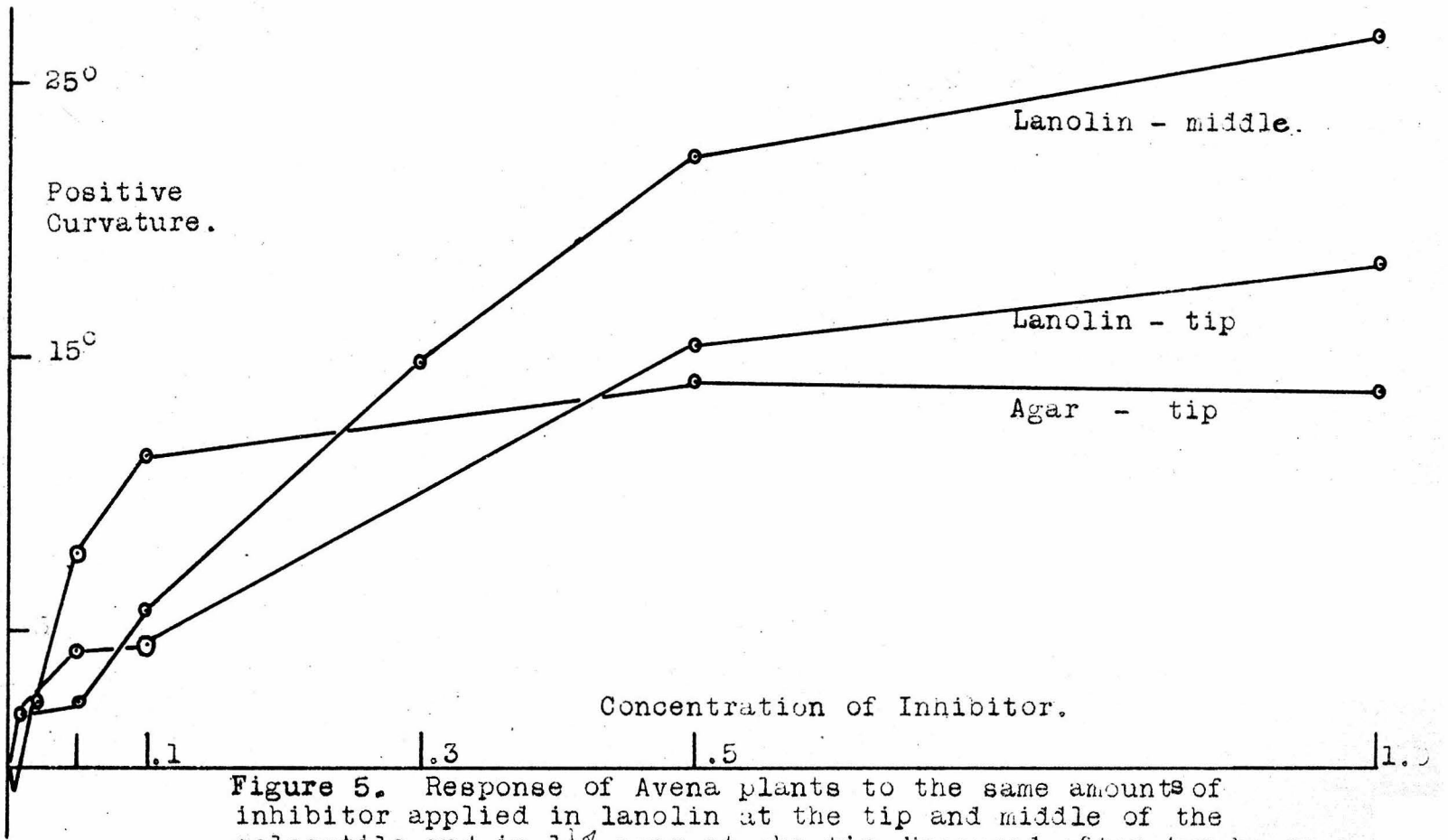


Figure 5. Response of Avena plants to the same amounts of inhibitor applied in lanolin at the tip and middle of the coleoptile, and in 1½% agar at the tip. Measured after two hours. Each point is the average of twelve plants.

that the agar application method is linearly proportional at lower concentrations of inhibitor, thus making it the most sensitive of the three methods for the analysis of inhibitor. The greater curvature of the inhibitor in lanolin applied at the middle of the coleoptile, may be explained in part by the fact that in this case the application is nearer to the region of elongation. Also when the inhibitor is applied on the outside, the distance between the inhibited side of the coleoptile and the growing side is greater and hence gives greater curvature, than when the inhibitor is applied on a part of a transverse section as in the agar block technique.

C. Positive curvature rates of Avena coleoptiles of different auxin sensitivity.

To determine the type of Avena coleoptile showing the highest inhibitor sensitivity the following experiment was performed:

Avena plants were prepared with four different auxin sensitivities. First, "deseeded" (endosperm removed) plants were grown according to the method given by Skoog (1937). This author has shown that such plants are more sensitive to auxin than any other type of Avena plant because by removing the endosperm they are unable to form as much auxin as they otherwise would. Accordingly in the

Avena test for auxin, where auxin is applied, these plants show the greatest response. The second type of plant was obtained by making two decapitations three hours apart as in the usual Avena test. These plants are less sensitive to auxin than the "deseeded" plants, however they are more sensitive than plants which have been decapitated only once. (Phytohormones, 1937). The third group of plants were decapitated just before applying the inhibitor. A fourth group had auxin applied on the tip of the coleoptile in a lanolin paste of 1/10,000 concentration two hours prior to the first decapitation, then after the first decapitation they were used immediately. It was thought that this added auxin would tend to make the plants the least auxin sensitive. These four types of plants were then tested on the photokymograph machine with the same concentration of inhibitor in each case. The technique was the same as that previously described. By this means their reaction rate and magnitude of curvature were determined. The results of this test are given in figure 6. Here it is clearly seen that the plants which were decapitated only once, responded the most while the "deseeded" plants showed the least curvature. The twice decapitated plants were intermediate in their growth inhibition curvature between the once decapitated and the "deseeded" plants. The plants which had auxin added in excess to that naturally present showed even less growth inhibition than

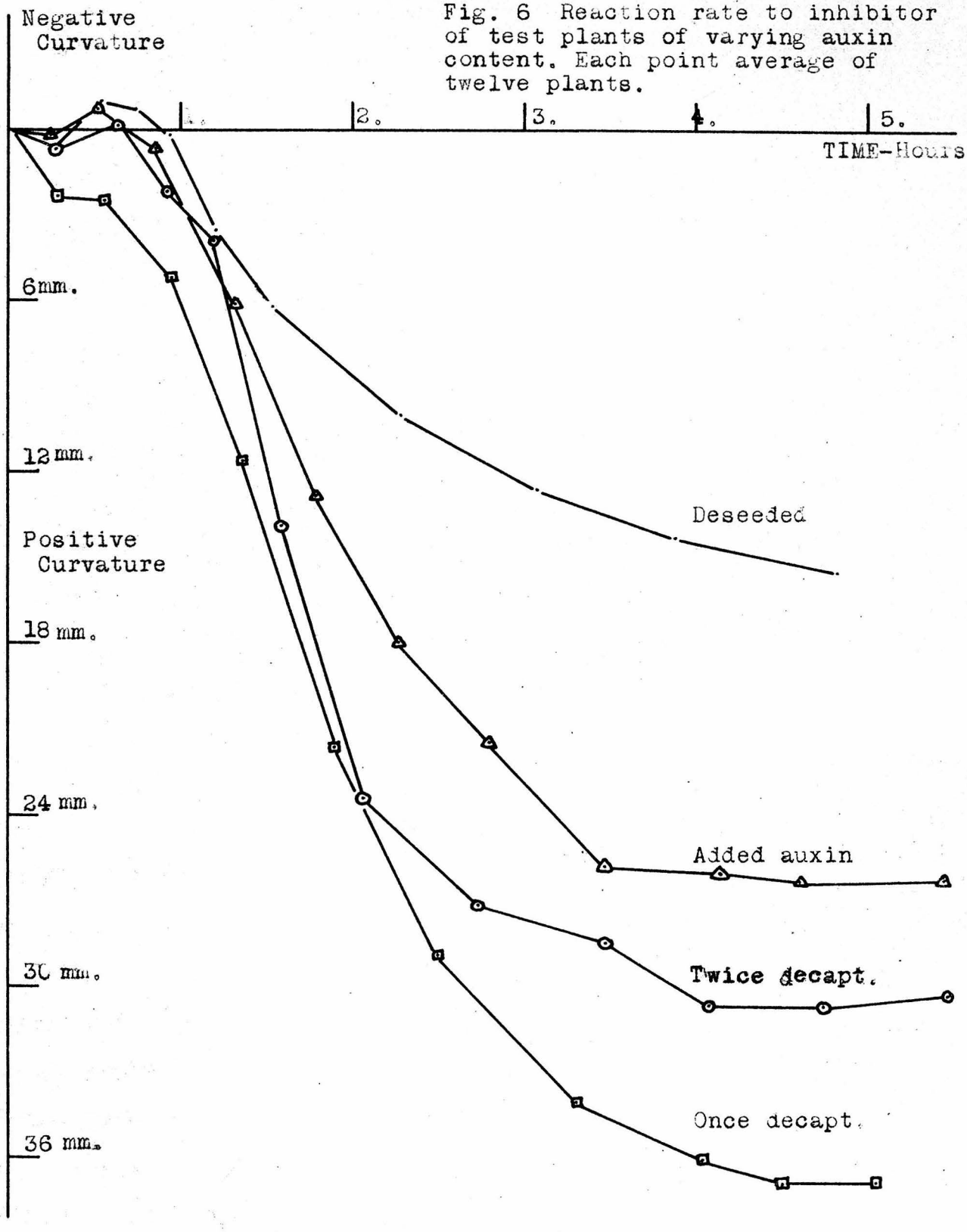


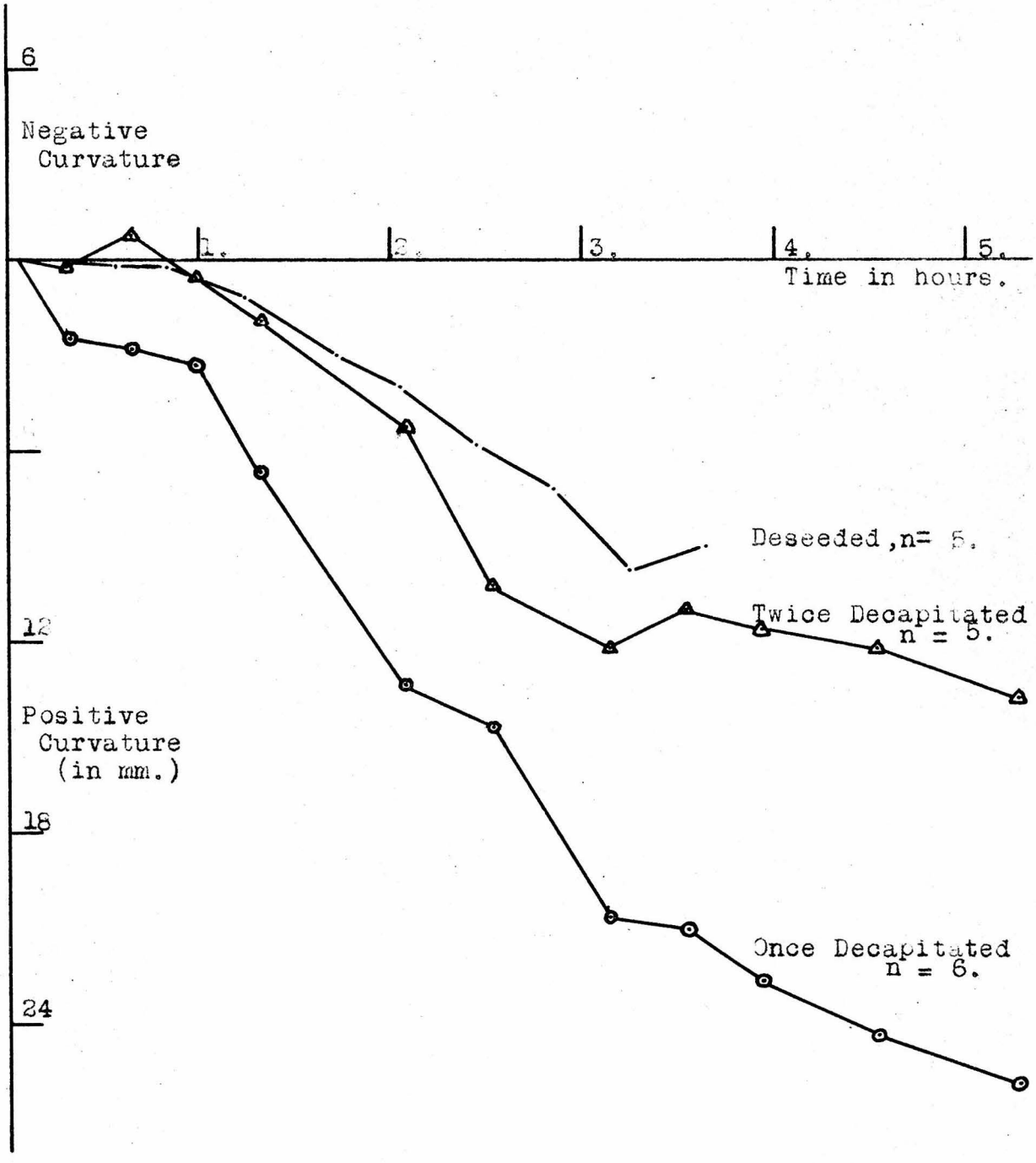
Fig. 6 Reaction rate to inhibitor of test plants of varying auxin content. Each point average of twelve plants.

the once decapitated ones. This may be explained in part by the fact that the applied auxin may have caused a redistribution of the food factor so that there was more food factor present in the discarded decapitated tip of these plants than there was in the decapitated tip of the untreated plants. (Went, 1939). This would decrease the amount of subsequent growth in the auxin treated plants more than in the untreated ones, the relative growth inhibition of the two sides of the coleoptile would be less and accordingly the positive curvature would be smaller.

This experiment has been repeated using purified inhibitor (See Chapter VI.) with similar results, figure 7. Also many comparisons have been made between"deseeded" and once and twice decapitated plants and always with results similar to those reported here.

From the above data it was thought that if the twice decapitated plants responded less to inhibitor than the once decapitated plants a "concentration-positive curvature" graph of the two types of test plants would show that the maximum positive curvature possible was greater with the once decapitated, and also that the range of linear proportionality would be greater with them, than that found for the twice decapitated plants. To test this idea a dilution series of purified inhibitor was made and the same concentrations of solutions tested on both the once and the twice decapitated plants. In figure 8a are shown the

Fig. 7 Reaction rate to purified inhibitor of test plants of varying auxin content ."n" equals number of plants used at given auxin content.



expected results and in figure 8b the results that were actually obtained. It can be seen that they were quite different from those anticipated. This difference may be partially explained by the fact that while the inhibitor was thought to be auxin free it actually was not. This is known to be the case as the test plants were observed an hour and a half after the application of the agar blocks and with the twice decapitated plants, at the lower concentrations of inhibitor, there were clearly negative curvatures. This made it evident why after $2\frac{1}{2}$ hours with the lower concentrations of inhibitor, the twice decapitated test plants showed a smaller positive curvature than the once decapitated. The reason was due to the fact that they were more sensitive to auxin in the inhibitor than were the once decapitated plants, thus they curved negatively and then had to regain that amount of negative curvature before they began to show any positive curvature; hence they finally showed less positive curvature. This negative curvature preceding the positive curvature is further discussed in Chapter VI. As stated, however, it was expected that the maximum angle of the two kinds of plants would be different. That this was not the case may be ascribed to the fact that the experiment was done on too small a scale, only twelve test plants being used for each point. A repetition of it would probably show that the maximum angle was greater for the once decapitated

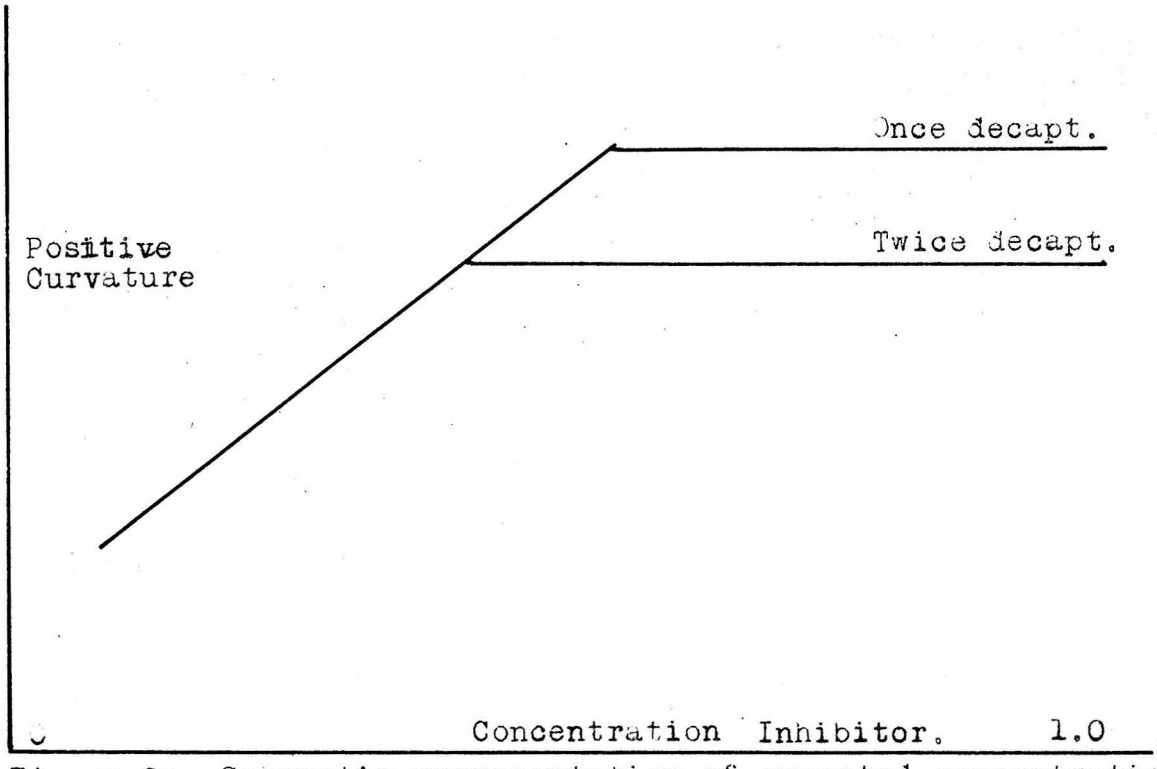


Figure 8a. Schematic representation of expected concentration-curvature relationship with once and twice decapitated plants.

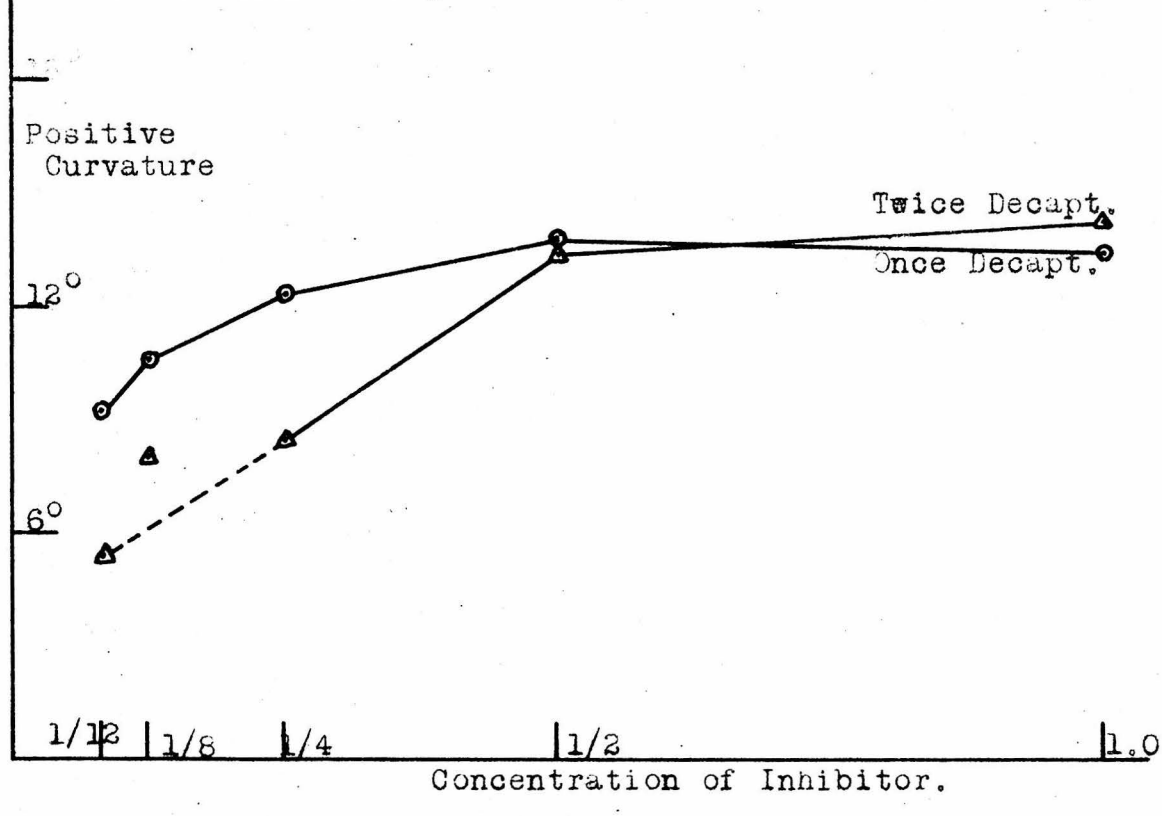


Figure 8b. Results obtained from applying purified inhibitor on once and twice decapitated plants.

plants. This has already been shown by the aforementioned kymograph test. Here the difference in the curvature - 2½ hours after the application of the inhibitor is however not much greater than 4°.

From the foregoing experiments it may be said that the plants that were growing the slowest ("de-seeded") responded the least to inhibitor while those that were growing the fastest responded the most. This made it clear that the best type of plant to use in the bio-assay for inhibitor were those that were only decapitated once. As mentioned these were obtained by using them immediatly after the first decapitation. This is not always desireable, however, because: first, the time between the first and second decapitations allows an irregular set of plants a chance to become more uniform in size, and, secondly, it is necessary to shift the seed planting to the evening in order to have the plants the proper size about noon 36 hours later. Furthermore the differences in curvature between the once and twice decapitated plants are for most experiments not great enough to justify the change in the regular routine of plant preparation for the standard auxin test. For these reasons it became the practice in the inhibitor test, except in special experiments, to use Avena test plants as they are grown for the auxin test.

From the preceeding experiments we may define the standard inhibitor test as being exactly the same as the standard Avena test except;1. That positive curvatures

are measured after 150 minutes instead of negative curvatures after 90 minutes, and; 2., That the positive curvature is only linearly proportional to the concentration of the inhibitor between 3 and 13 degrees.

Chapter III. Distribution of Inhibitor

A. Sources of inhibitor.

Having developed a standardized method of quantitatively analyzing for inhibitor substance it was next of interest to determine its occurrence both in other species and as to its actual distribution within the radish plant itself.

It was first desireable to see if it was present in strains of radish other than the one in which it was originally found, i. e. "French Breakfast." In Table I are listed the results of an inhibitor analysis of the cotyledons of six strains of radish. These were eight days old at the time of extraction and were all extracted and tested for inhibitor according to the standard method set forth in Chapter II. These plants were grown in the open, in loamy soil. It is seen from this data that all of the strains of radish investigated contained the growth inhibitor, but that the strain "French Breakfast" had the most.

Since theoccurrence of the inhibitor in the genus Raphanus was well established the next question was as to its presence in other genera of the same family, i.e. the Cruciferae. The results of a survey as described above for the radish strains, is also shown in Table I. It is to be noted that growth inhibitor can occur in other genera

Table I.

Analysis of Cotyledons for Inhibitor Substance.

Material	No. of Test Plants	Degrees Pos. Curv. per 5 gr. per cc. 1½% agar.
Raphanus sativa:		
Black Spanish	24	18.3
Early Scarlet	24	17.3
Japanese All Season	24	7.2
French Breakfast	24	19.3
Long Scarlet	24	11.0
White Icicle	24	3.2
Brassica rapa:		
Early white flat Dutch	24	3.6
Brassica napobrassica:		
Swedish-American purple top rutabaga	24	2.5
Brassic nigra:		
Ostrich plume mustard	12	6.7
Tropaeolum majus:		
Double golden yellow nasturtium.	8	4.5*

* Given as positive curvature substance in 8, ten day old cotyledons. Extract taken up in .5 cc., 1½% agar.

besides Raphanus.

At this juncture it may be well to point out that if both auxin and inhibitor were extracted simultaneously from the same material the net curvature might be neither a measure of auxin, nor of growth inhibitor. Only if the inhibitor were in high enough concentration to cause a greater positive curvature than the negative curvature caused by the auxin might its presence become apparent. The same conditions would be effective in making extractions for auxin determinations. The same idea has recently been advanced by Goodwin (1939) who presents evidence from diffusion constant determinations (see Chapt. IV.) which indicates the presence of growth inhibiting substances that mask the effect of auxin.

Outside of the Cruciferae, inhibitor has been found in the Tropæolaceae and in the Anacardiaceae. In the nasturtium,--family Tropæolaceae, it was found in the cotyledons nine days after germination. This positive curvature substance from the nasturtium cotyledons was first observed by Meyer (1936). As his method of extraction differed from the ether extraction used for the radish cotyledons, it was considered desireable to make the standard extraction. The results of this test are recorded in Table I. Dr. Haagen-Smit (unpublished) has reported the presence of inhibitor in the fruit of the pepper tree, -- Schinus molle, a member

of the family Anacardiaceae.

From the above data it seems quite likely that the occurrence of inhibitor may be rather widespread. Particularly might this be the case if one believes that its presence could be obscured by the presence of auxin. Indeed while many ether extractions of plant material have shown the presence of auxin and not of inhibitor, perhaps for the reason just mentioned the inhibitor may also be present.

B. Distribution of inhibitor in the radish plant.

It was next of interest to investigate the distribution of inhibitor in the radish plant itself. To do this radish seed, strain "French Breakfast", which was used throughout this work, was sown in flat boxes containing loamy soil, at intervals of three days during the course of two and a half months. During the last two weeks it was planted at one day intervals. At the end of this time the amounts of inhibitor were determined in the cotyledons and the leaves of the plant. The leaves were extracted in the order of their appearance in twos. The smallest size extracted was about 6 mm. long. Except for the first two leaves, the terminal bud was considered indicative of the amount of inhibitor in the next two leaves developing from it. Extractions of the hypocotyl showed no curvatures. These determinations are given in figure 9 as degrees positive curvature per five grams fresh weight of material when the

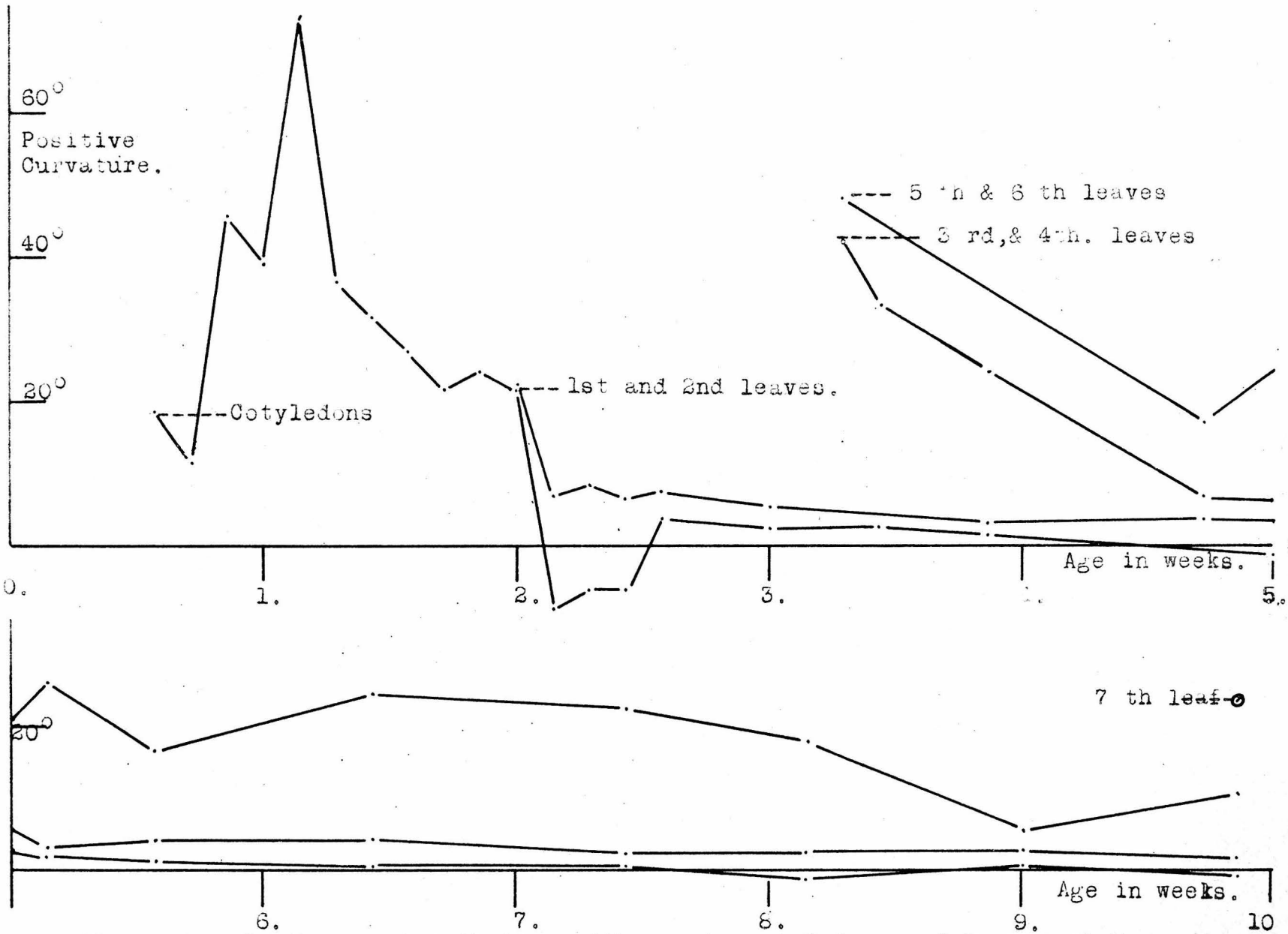


Figure 9. Inhibitor distribution with age, in cotyledons and leaves of the Radish plant. Each point average of twelve test plants. Inhibitor calculated per 5 gr. fresh weight, extract taken up in 1 cc 1½% agar. Leaves numbered in the order of their appearance.

extract is taken up in one cc. of 1½% agar. Each point is the average of twelve test plants. A few determinations were repeated at a later time and found to agree with those given here.

In figure 9 it may also be seen that the amount of inhibitor in the cotyledons reaches a sharp maximum on the eighth day and from that time on the amount declines until they finally contain no inhibitor but actually have auxin. The developing leaves from the terminal primordia to the mature leaf show a similar continual decrease in the amount of inhibitor but with no indication at any time of an increase in inhibitor as in the cotyledons. The age - inhibitor relationship in the cotyledons was first determined by Mr. Redemann. His results were approximately the same as those presented here.

While the graph, figure 9, shows that the concentration per gram fresh weight of the leaves decreased with growth it does not necessarily follow that the total amount of inhibitor per leaf decreases accordingly. A comparison, however, of the amount of inhibitor per leaf shows that the first two leaves of 52 day old plants had enough inhibitor per five grams fresh weight extract taken up in 1 cc. of agar, to cause 3½ degrees positive curvature per leaf, while comparable 69 day old leaves showed a 2½ degree negative curvature. The second two leaves of 52 day old plants had sufficient inhibitor to cause 25 degrees

positive curvature per leaf while comparable 69 day old leaves only showed 5 degrees positive curvature per leaf. Each value is the average of twelve inhibitor test plants.

It is thus apparent that with growth the total amount of inhibitor per leaf does actually decrease until it is no longer detectable and only auxin curvatures result from the ether extractions.

From the fact that the amount of inhibitor decreases as the leaf grows the conclusion might be suggested that the inhibitor is related to the leaf growth process. It is well to bear in mind that in view of the recent work on leaf growth factors, (Bonner, Haagen-Smit, & Went, 1939;) leaf growth must be considered the result of both vein growth and mesophyll growth. If inhibitor were to play a role in leaf growth (see Chapt. VII) it might be active in either part.

Chapter IV. Chemical Properties of the Inhibitor.

A. Solvents for inhibitor extraction.

Mr. C. Ernst Redemann working with Dr. Haagen-Smit began an investigation of the chemical properties of the inhibitor substance (unpublished).

From investigations of numerous solvents, for example, -- benzene, methanol. ethanol, etc., he found that appreciable amounts of inhibitor were extracted by only two of them; di-ethyl-ether, and ethyl acetate. As noted it was di-ethyl-ether that was used in the original extraction. The ethyl acetate was found to extract nearly twice as much inhibitor as the ether and also it did not extract as much chlorophyll. It has recently been reported by Schmauss (unpublished), that the ethyl acetate is nevertheless a less desireable solvent than the ether. The reasons for this are: 1.) The solubility of water is greater in ethyl acetate than in ether, 2.) The ethyl acetate hydrolyzes to form acetic acid, and 3.) That both the water that has been taken up by the ethyl acetate, and even more so the acetic acid, cause the inhibitor to undergo a hydrolysis as will be explained in the next paragraph.

B. Hydrolysis of inhibitor to yield auxin.

When an active extract of the inhibitor was tested for its stability in hot hydrochloric acid and in hot potassium hydroxide Redemann found a very surprising and unexpected reaction. With the alkaline hydrolysis the positive curvature activity was completely lost and replaced by a strong negative curvature activity. This made it likely that the substance causing the positive curvature was a derivative of one of the auxins. It also made it necessary to postulate for the structure of the inhibitor a compound, part of which was this auxin, but without its free carboxyl group as it had been found that the inhibitor contained no acidic or basic groups. From the pronounced fat solubility of the inhibitor it also seemed likely that the linkage between the auxin part of the molecule and the remaining portion was an ester linkage.

C. Identification of the auxin from inhibitor.

An attempt was made to identify the nature of the auxin produced by the alkaline hydrolysis. This was done by determining its stability upon refluxing in 5% potassium hydroxide and in 5% hydrochloric acid. Kogl and Haagen Smit (1934) have shown that under these conditions of pH auxin-a is stable to acid but not to alkali; auxin b

is not stable to either of the reagents; while indole-3-acetic acid (hetero-auxin) is stable to alkali but not to acid. From this "differential destruction" test the evidence was in favor of considering the growth promoting fraction to be indole-3-acetic acid. It should be noted, however, that this test is not considered reliable when there are many impurities present as in this case.

To gain further indication as to the chemical nature of the inhibitor, molecular weight determinations were attempted. The estimation of molecular weights is based on the determination of the diffusion coefficient which is made in the following manner: Three plain 1½% agar plates are stacked one above the other, a fourth agar plate of the same size, in this case 1 x 6 x 8 mm., and containing the substance being investigated is then placed on top of the stack of three. After a given time the distribution of the substance in all four plates is determined; in this case it was measured by the inhibitor test but with only a 120 minutes instead of the regular 150 minutes between the application of the test blocks and determination of the curvature. (This 30 minutes difference would tend to make a less accurate determination than otherwise.) The distribution found in terms of relative concentration is then calculated on the basis that the total amount of curvature of the four plates equals 10,000. From these values one may refer to Diffusion Tables by Scheffer and Kawalki

(1923) and find an "x" value. This "x" may then be substituted in the following formulae given by Bruins (1922) and the diffusion coefficient thus obtained,:

$$D = \frac{h^2}{4\ t\ x}$$

in which h is the thickness of the agar plates in cm.

t is the time in days that the diffusion is allowed to continue.

x is the value from the Diffusion Tables mentioned above.

Having obtained the diffusion coefficient D. one can then calculate the molecular weight from the following relationship as suggested by Öholm (1912):

$$M.\ W. = \left[\frac{7.0(1 + aT)}{D}\right]^2$$

Where:

M.W. is the molecular weight

" " temperature coefficient (1/273)

" " centigrad temperature

" " diffusion coefficient

This method was first used for plant hormone molecular weight determinations by Went (1927).

The data obtained by Mr. Redemann may be calculated to give the following values: 174, 212, and 222. It should be noted that he states that all known errors would tend to make these values too large. The values I later determined were, as predicted, actually lower. See Chapter VII.

From the molecular weight determinations it was concluded that the auxin derived from the positive curvature substance cannot be either auxin a nor b as both of these auxins have molecular weights higher than the highest value found for positive curvature substance.

In summary, the chemical properties of the inhibitor are:

1. That it can be extracted from radish cotyledons with either di ethyl ether or ethyl acetate.
2. That it contains neither acidic nor basic groups.
3. That it can be hydrolysed with 5% potassium hydroxide to yield a growth promoting fraction.
4. That a differential acid-alkali destruction test of this growth promoting fraction indicates that it could be indole acetic acid.
5. That the molecular weight determinations indicate that the growth promoting fraction could be neither auxin a nor b, and
6. That indications are that an ester linkage exists between the growth promoting part of the molecule and the unknown part.

Chapter V. Growth Inhibitor and Growth.

A. Growth effect of inhibitor on Avena coleoptiles.

As mentioned in the introduction, Avena growth curvatures can only be caused by a relative difference in growth rate on two sides of the plant. A rate which was relatively lower on the treated side would cause a positive curvature. This relative difference of growth rate could be brought about either by; 1. An actual inhibition of the growth on the treated side, or 2. By an increase of the growth rate on the untreated side. To settle this question and to investigate the direct effect of inhibitor on growth, growth rate measurements were made as follows:

The upper 15mm. of intact Avena coleoptiles were marked into 3 mm. zones with India ink. The growth of these zones was then followed by measuring the increase in the distance between them at intervals of an hour and a half. To one set of six plants inhibitor was applied symmetrically over the tip millimeter of the coleoptile in a small drop of lanolin paste. To another set of six plants pure lanolin paste alone was applied in a comparable fashion. The growth increase of these two sets of plants was measured accurately by means of a horizontal microscope which could be read to a .01 mm. vertical difference. The plants, throughout the course of the experiment, were kept

in the regular Avena testing dark rooms where humidity and temperature were kept constant at 85%, and 24°C. They were illuminated only with the light transmitted through a Corning orange light filter No. 243.

The results of this determination may be seen in figures 10 and 11. From figure 11. it may be seen that the effect of inhibitor is to decrease the growth rate of all zones of the treated plants by about 20%. From figure 10 it is clearly seen that this decreased growth rate is uniformly distributed over the entire length of the plant.

This experiment was repeated using "purified" or auxin free inhibitor in lanolin. (See Chapter VI for purification method). This test differed from the former only in that here the plants were older at the beginning of the experiment being 30 mm. tall as compared to 25 mm. in the other case. The results of this experiment are seen in figures 12 and 13. While they are essentially the same as those obtained with the unpurified inhibitor they show a greater amount of inhibition as in this instance the growth is completely stopped in six hours. Figure 12 shows that for the first two hours after the application of the purified inhibitor the growth rate of all of the zones is like that found for the unpurified inhibitor but from that time on it decreases more rapidly. Comparing figure 13 with figure 11 one sees that there is roughly a 20% greater growth inhibition effect of the purified inhibitor

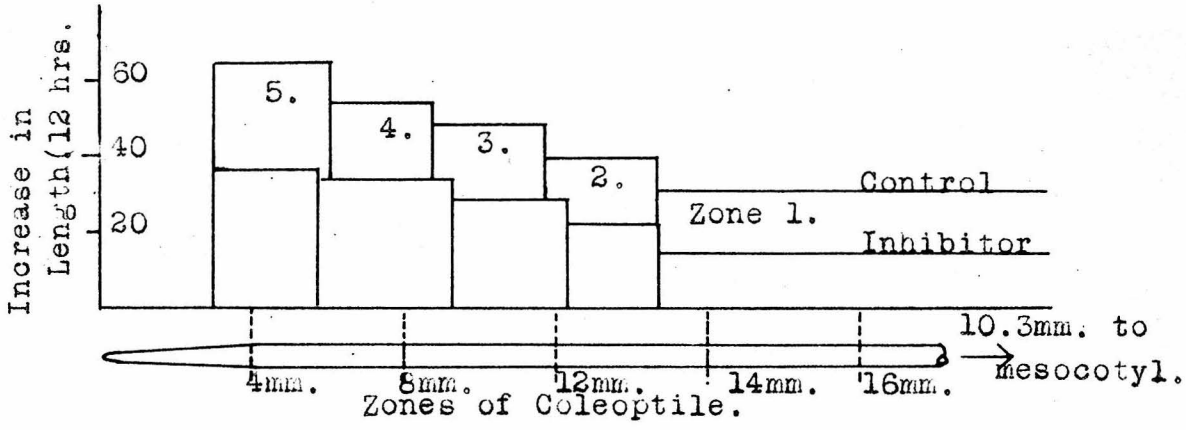


Fig. 10. Growth Distribution of the Avena Coleoptile when Treated with Inhibitor .

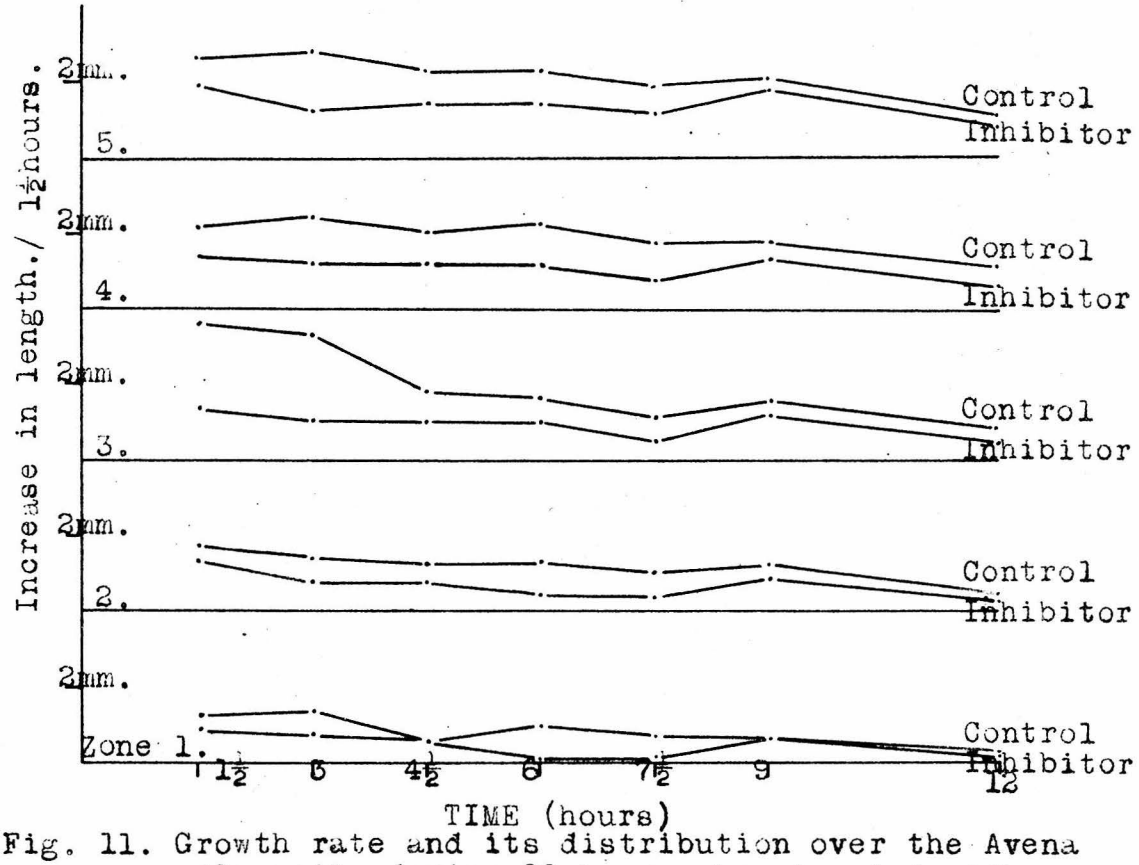


Fig. 11. Growth rate and its distribution over the Avena coleoptile during 12 hours when treated with inhibitor.

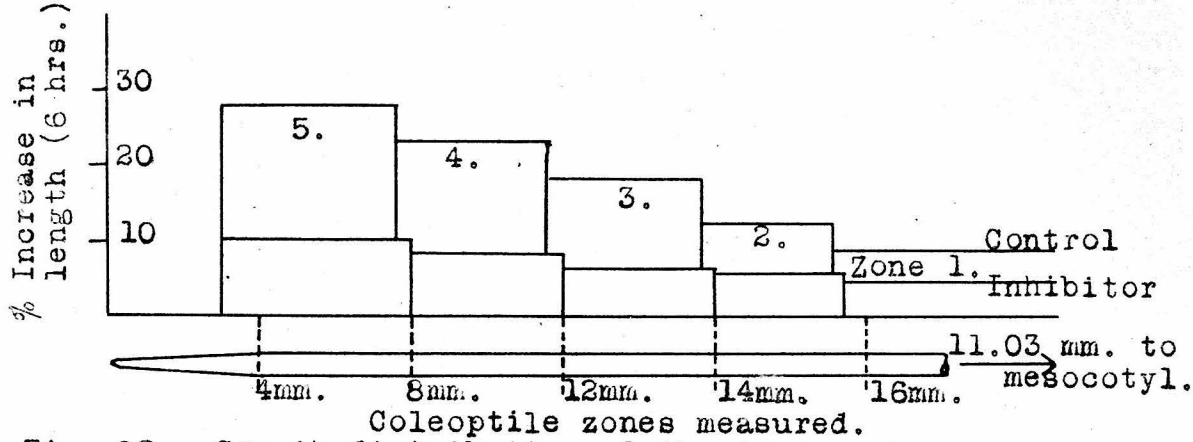


Fig. 12. Growth distribution of the Avena coleoptile during six hours when treated with purified inhibitor.

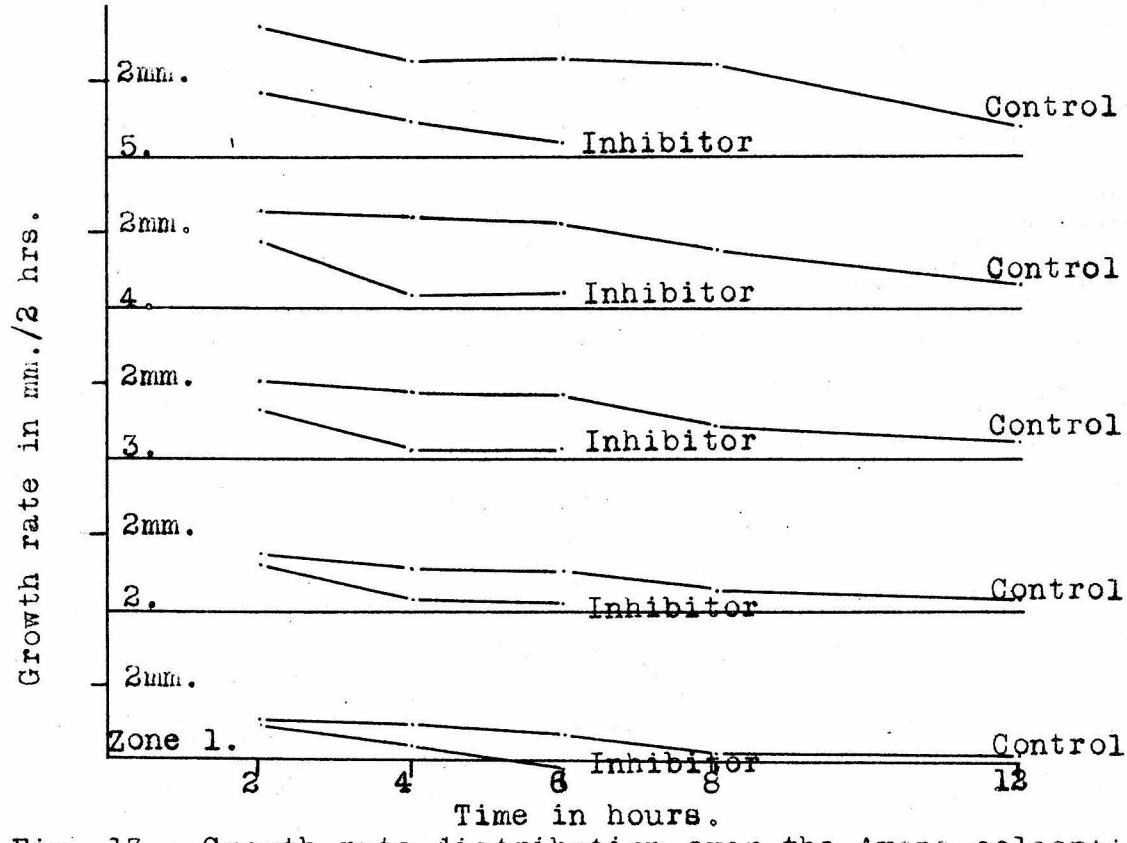


Fig. 13. Growth rate distribution over the Avena coleoptile when treated with purified inhibitor.

over its controls than with the unpurified inhibitor. This is apparently due to the fact that the unpurified inhibitor contained auxin together with other impurities.

B. Observed positive curvature explained on a basis of measured growth inhibition.

Since these results indicated that the inhibitor acted on the Avena coleoptile solely by causing a growth inhibition it was interesting to see if this conclusion could be substantiated by using it to predict the amount of positive curvature obtained by the unilateral application of the inhibitor. This could be done by calculating the amount of positive curvature to be expected if the decreased growth rate determined above were to act by causing the same growth rate decrease but only on one side of an Avena coleoptile.

To answer this point the same concentration of unpurified inhibitor in lanolin, whose effect in decreasing the growth rate is given in figure 11, was applied unilaterally over a 5 mm. strip, 15.6 mm. below the tip of 25 mm. Avena coleoptiles. This meant that the inhibitor was applied where zone 1. would be on the plants measured for the growth rate response. For this reason it was necessary to use the growth rate of zone 1 in calculating the amount of positive curvature to be expected from such an inhibition. This difference in growth rate was found to

be .5 mm. for the first two hours. By substituting in the formulae:

$$\frac{2\pi d\, a}{360^\circ} = l_x - l_v$$

where:

d is the diameter of the plants
a is the angle of the curvature
l_x is the growth rate of the convex side
l_v is the growth rate of the concave side

it is possible to calculate the expected curvature resulting from such growth rate difference on two sides of a growing organ.

The average diameter of the plants which were unilaterally treated was found to be 1.4 mm. by direct measurement. The growth rate difference as mentioned, was .5 mm. for the first two hours, hence by substituting in the above equation and solving for the angle of curvature, "a", we find that it is supposed to equal 20.4° positive curvature. By actual measurement of the plants treated unilaterally with the same concentration of inhibitor in lanolin, after two hours the positive curvature was found to be 23.8° .

From the above data, then, it is apparent that the positive curvatures resulting from the relative growth inhibition effect of the inhibitor are actually the result of a growth inhibition on the treated side. It is also seen that the growth rate measurements from plants treated with inhibitor can explain the positive curvatures obtained by the

unilateral application of inhibitor.

C. Growth effect of inhibitor on tissue sections of Avena and radish.

It was interesting to determine the effect of inhibitor on the growth of 4.0 mm. sections cut from Avena coleoptiles. It is known that when such sections are put in auxin solutions their growth increases over water controls.(Phytohormones, 1937) It is also known that the amount of this growth is proportional within limits to the concentration of the auxin solution. For these reasons a concentration series of an active inhibitor extract was made. The cotyledon extract was taken up in 4 cc. of water and successive tenfold dilutions were made down to 10 of the highest concentration. Two cc. of each of these concentrations was then put in a Syracuse dish and thirty 4.0 mm. sections cut from the upper half of Avena coleoptiles were added. These dishes were kept in the dark room until the time for the growth measurements 24 hours later. At this time they were taken into the light and measured accurately to a .1 mm. by means of an ocular micrometer. The results of this experiment are seen in figure 14. Here it is noted that the highest concentration of inhibitor caused a shrinkage in the original size of the cell. This was due to the fact that the solution was toxic as the sections were flaccid.. The

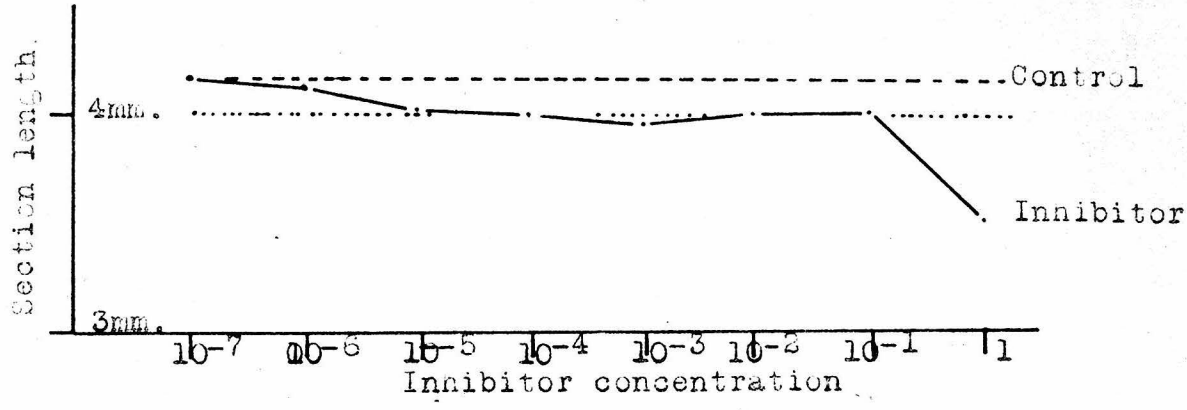


Fig. 14. Growth of 4 mm. sections of Avena coleoptile in inhibitor solutions for 24 hours.

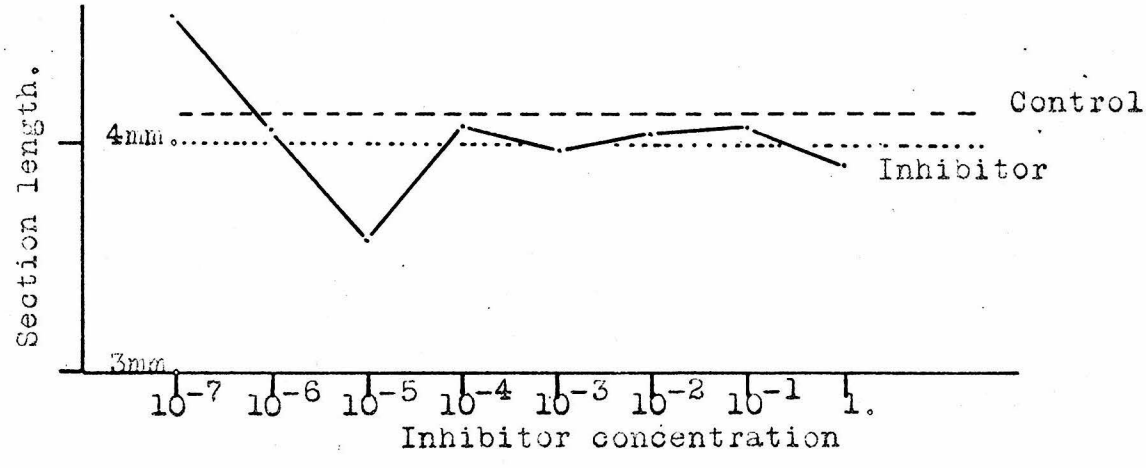


Fig. 15. Growth of 4 mm light grown sections of Radish hypocotyl in inhibitor solution for 24 hours.

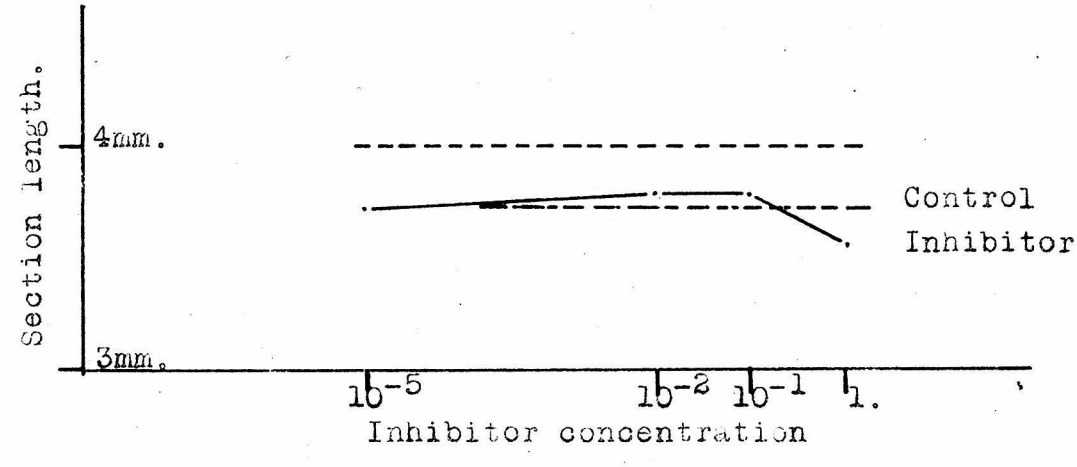


Fig. 16. Growth of 4 mm. dark grown section of Radish hypocotyl in inhibitor solution for 24 hours.

other concentrations inhibited the growth of the sections when compared to the growth of the water controls except at the very lowest concentration. In that case the growth of the sections in the inhibitor and in the water were the same. There was no relation found between the concentration of inhibitor and the amount of inhibition. This experiment has been repeated twice with essentially the same results.

A similar experiment was done using ten 4.0 mm. sections from radish hypocotyls at the same inhibitor concentrations as above. The radish plants had been grown in the greenhouse in the usual manner and were 5 days old at the time of the experiment. Another set of radish plants was grown which were etiolated by keeping them in the dark rooms used for Avena testing. The results for both the light and dark grown radish sections were similar to those obtained with the Avena coleoptile sections. This data is presented in figures 15. and 16.

In conclusion it may be said that the inhibitor acts on the growth of the Avena coleoptile to cause positive curvatures by actually causing a growth inhibition. The amount of inhibition is sufficient to explain the positive curvatures obtained from the unilateral application of inhibitor at the middle of the coleoptile.

Inhibitor likewise inhibits the growth of isolated sections of Avena coleoptiles and radish hypocotyls except

at the lowest dilutions where, in the case of the radish, it caused a growth promotion. There was no linear proportionality between the concentration of the inhibitor and the amount of section growth inhibition.

Chapter VI. Inhibitor Transport Through Avena and Radish Sections.

A. Non-polar movement of inhibitor in Avena coleoptiles and radish hypocotyls.

The inhibitor substance has been shown to be of neutral chemical character, i. e. it contains neither acidic nor basic groups, hence in regard to the polarity of its transport through plant tissues on the basis of Went's (1932) potential gradient theory for polarity of plants, one would expect it to be transported acropetally as well as basipetally. According to this theory only certain organic acids for example auxin, should move basipetally while bases should move only acropetally. A substance of neutral character should be transported equally well in either direction. The following experiments showed this to be the case with inhibitor substance.

Two plates of blank 1½% agar, 1x6x8 mm. were placed each on a separate microscope slide. On top of the plates were placed on end, twelve 4.2 mm. long sections of Avena coleoptiles. On one of the plates these were left in the normal position (i.e. in the same position with respect to gravity as in the plant) while on the other plate they were inverted. On top of both sets of sections another agar plate was placed similar to the lower one but containing

the inhibitor ("donor plate"). Both slides were then put in a Petri dish in which there was a small piece of moistened filter paper. The whole procedure was carried out in the regular Avena dark room.

After two hours the plates were cut into 1 x 2 x 2 mm. blocks and analyzed for inhibitor. The results were as follows:

Coleoptile Position (Upper plate, inhibitor donor)	Curvature, Avg. 9-12 test plants with Stand. Error.
Normal, lower plate	4.4 ± .5
Normal, upper plate	24.0 ± 1.7
Inverted, lower plate	4.5 ± .8
Inverted, upper plate	24.0 ± 1.7

From these results it is concluded that with the concentration used in this experiment, there is no polarity of inhibitor transport in the Avena coleoptile. Other experiments to be discussed soon verify this conclusion. It should be mentioned that the polar transport of auxin is unaffected over a very large concentration range, accordingly we may expect the same to be the case with the non-polar transport of inhibitor.

The polarity of inhibitor transport was next investigated in 4.2 mm. sections of radish hypocotyls. The experiment was performed in the same manner as described above for the Avena coleoptiles except that radish hypocotyl

sections were used. Duplicate determinations were made. The results are given below:

Position of Hypocotyl (Upper plate, inhibitor donor)	Curvature. (Avg. of 12 test plants.) I	II
Normal, lower plate	5.0°	3.1°
Normal, upper plate	13.6°	11.6°
Inverted, lower plate	3.6°	3.8°
Inverted, upper plate	14.1°	9.0°

These hypocotyls were from 6 day old plants that had been grown in the green house under the regular culture conditions. Before placing the hypocotyl sections on the agar plates they were allowed to stand on wet filter paper for 30 minutes to tend to empty them of auxin and to remove destructive enzymes from the cut surfaces. It may be seen from the above data that radish hypocotyls like Avena coleoptiles had no transport polarity for inhibitor.

In one experiment, performed under conditions which were presumably the same as those above, auxin was found to be present in the receptor agar plates of both the normal and inverted sections. The actual data for these surprising results, was:

Hypocotyl Position (Upper plate inhibitor donor)	Curvature, (Avg. of 12 test plants with Stand. Error)
Normal, lower plate	-7.5° ± .5

Normal, upper plate	$+13.0^{\circ} \pm 1.1$
Inverted, lower plate	$-8.8^{\circ} \pm 1.0$
Inverted, upper plate	$+11.8^{\circ} \pm 1.3$

Attempts to repeat this experiment showed only inhibitor passing through the normal and inverted sections.

B. Chronological record of movement of inhibitor through sections of Avena coleoptiles and radish hypocotyls.

Since it was not possible by the above method to get a chronological determination of the effect of passing inhibitor through radish hypocotyls and in view of the surprising results obtained in one instance, it was found desirable to make such a determination. This was done by mounting a 4.2 mm. section of radish hypocotyl on the shoulder of a decapitated Avena coleoptile. A very small spot of Vaseline was applied to the primary leaf to hold the section upright on the top of the coleoptile. On top of the radish section was then placed an agar block (1 x 2 x 2 mm.) containing the inhibitor. A silver wire was inserted into the primary leaf so that the whole set-up could be placed in front of the kymograph drum as described in Chapter II. By this method any growth promoting or inhibiting substances diffusing from the section would pass on into the

Avena coleoptile and cause a growth reaction which would then be recorded. Four attempts were made with this technique before consistent data were obtained. The reason for not obtaining good results was probably due to lack of skill with the method.

It was decided that before investigating the effects of inhibitor transport through inverse sections of Avena and radish with this method, the transport in the normal direction should be well understood. Toward this end 2.1 mm. Avena coleoptile sections were cut from near the tip and near the base. These were then tested, by the kymograph technique described above, for the effect of inhibitor transport. A similar set of 2.1 mm. radish hypocotyl sections were also investigated in the same way, at the same time. The results of these experiments are shown in figure 17. Here it is clearly seen that in both instances the action of the inhibitor extract was to cause at first a strong negative curvature which then curved positively. Only in the case of the sections cut from the base of the radish hypocotyl did the curvatures actually become positive, however. When the inhibitor used in this experiment was tested directly on the coleoptile it gave a positive curvature of 6.3 degrees in a 1:4 dilution. This indicates that there was sufficient inhibitor in the top blocks on the sections to cause 25.2 degrees positive curvature.

These results are, however, open to criticism as there

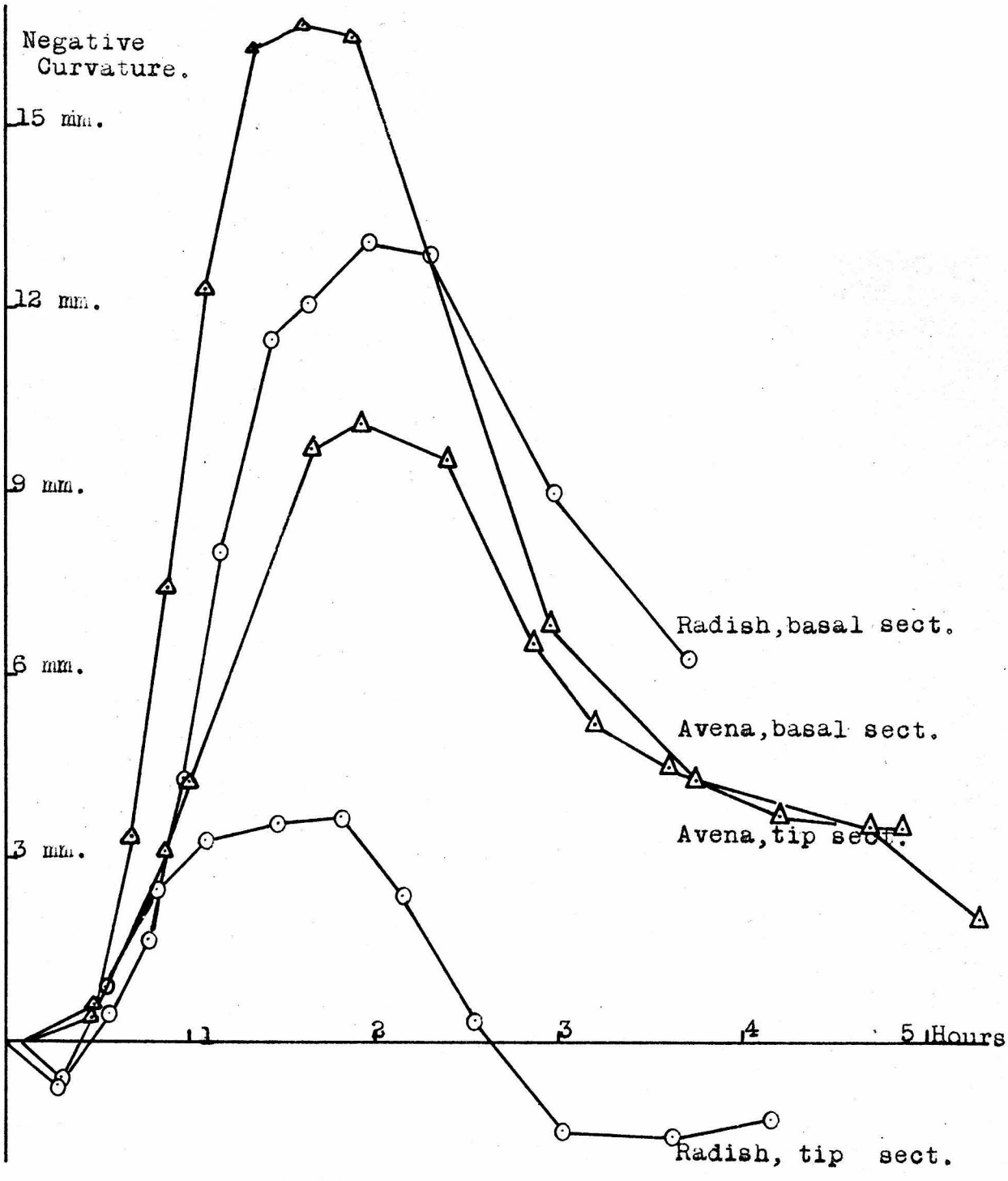


Fig. 17. Reaction rate to inhibitor transported through Avena coleoptile and Radish hypocotyl sections. (normal position, 2.1 mm. long.)

may have been auxin in the crude extract which could be transported through the sections and cause the observed negative curvature. To overcome the objection the following method of inhibitor purification was devised.

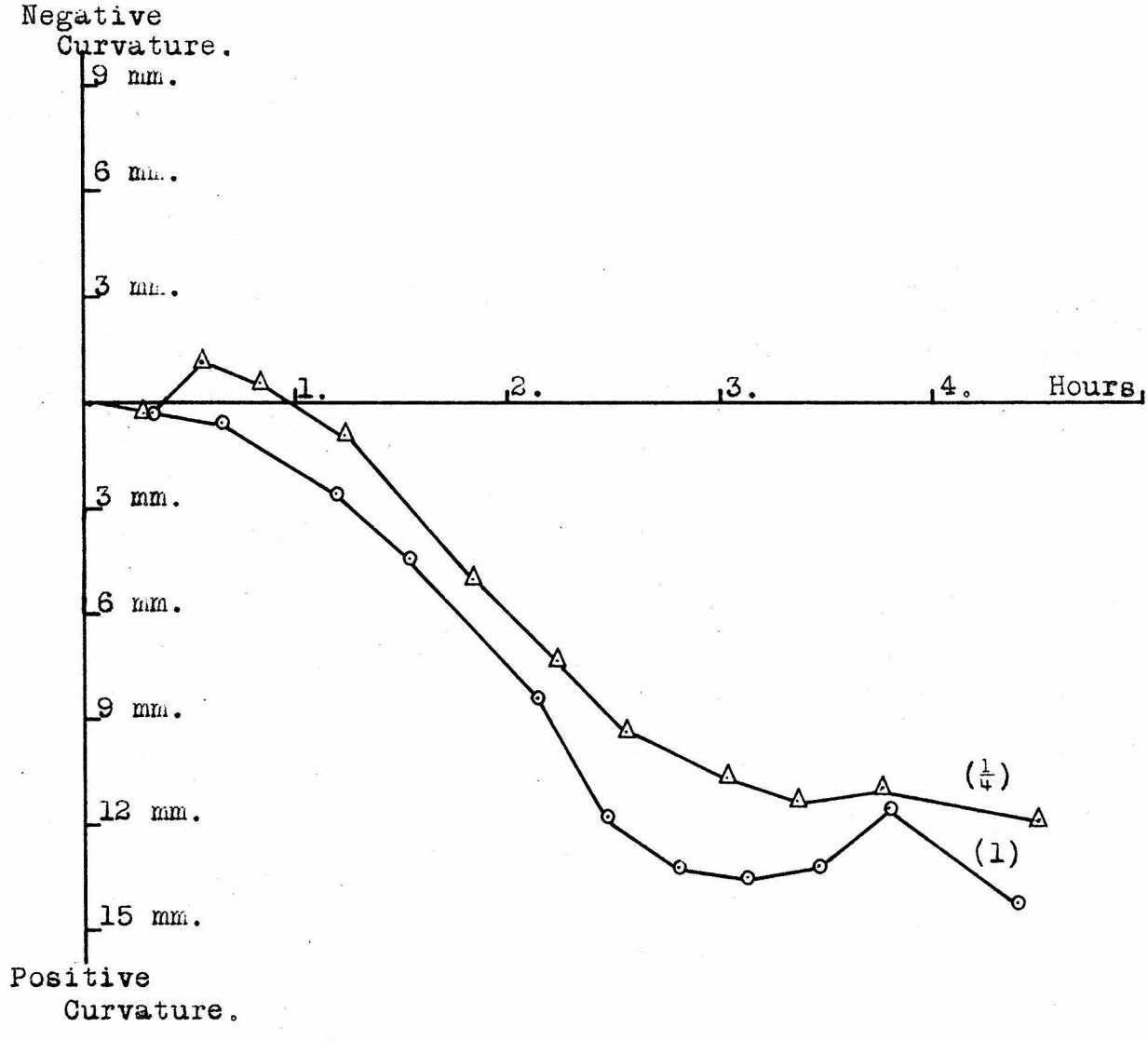
C. Auxin contamination of inhibitor extract and its removal by the "Inverse Transport" purification method.

Since it had been shown by Redemann that any of the usual chemical treatments used to remove auxin from the extract resulted, not in the removal, but in the hydrolysis of inhibitor to yield even more auxin, chemical treatments were not considered. It was also necessary to decide how to determine when all of the auxin was removed. It was assumed that if no initial negative curvature preceded the positive curvature then there was no auxin present, or that if it were present it was below the sensitivity of the plants and so for the purpose of subsequently detecting auxin effects it could be regarded as negligible. Since it was impossible to purify the inhibitor by known chemical means without running the risk of hydrolysing it into auxin, a physiological purification method was developed.

It is well established that Avena coleoptiles will only transport auxin basipetally. Only when the auxin

concentrations are extremely high, -- far higher than those dealt with here--, only then could the auxin pass through Avena sections acropetally. Inhibitor substance on the other hand as we have already seen can be transported in both directions of the Avena coleoptile. It has no polarity of transport. By using these two facts: 1. Auxin transported only toward the base of the coleoptile, and: 2. Inhibitor transported toward the tip or base, it was thought that it might be possible to separate the inhibitor from contaminating auxin by passing it through Avena coleoptile sections from base to tip and thus keep the auxin from passing through. This was tried in the following manner: Twenty 4.2 mm. sections of Avena coleoptiles were inverted on a blank agar plate as earlier described. On top of these inverted sections was then placed another agar plate which contained both inhibitor and any contaminating auxin from the crude extraction. This set up was allowed to remain this way for ten hours. At the end of this time the inhibitor in the bottom agar plate was tested on the kymograph, in two concentrations, 1 and $\frac{1}{4}$. As can be seen from figure 18. (Each point average of 11 test plants on full strength concentration curve, average of 9 plants on $\frac{1}{4}$ concentration curve.) no negative curvatures were present when the inhibitor was purified in this way. (Compare with unpurified inhibitor, figure 2.) This experiment was repeated three times with the

Fig. 18. Reaction rate to purified inhibitor. Two Concentrations tested, 1 and $\frac{1}{4}$. Twice Decapitated Avena test plants used.

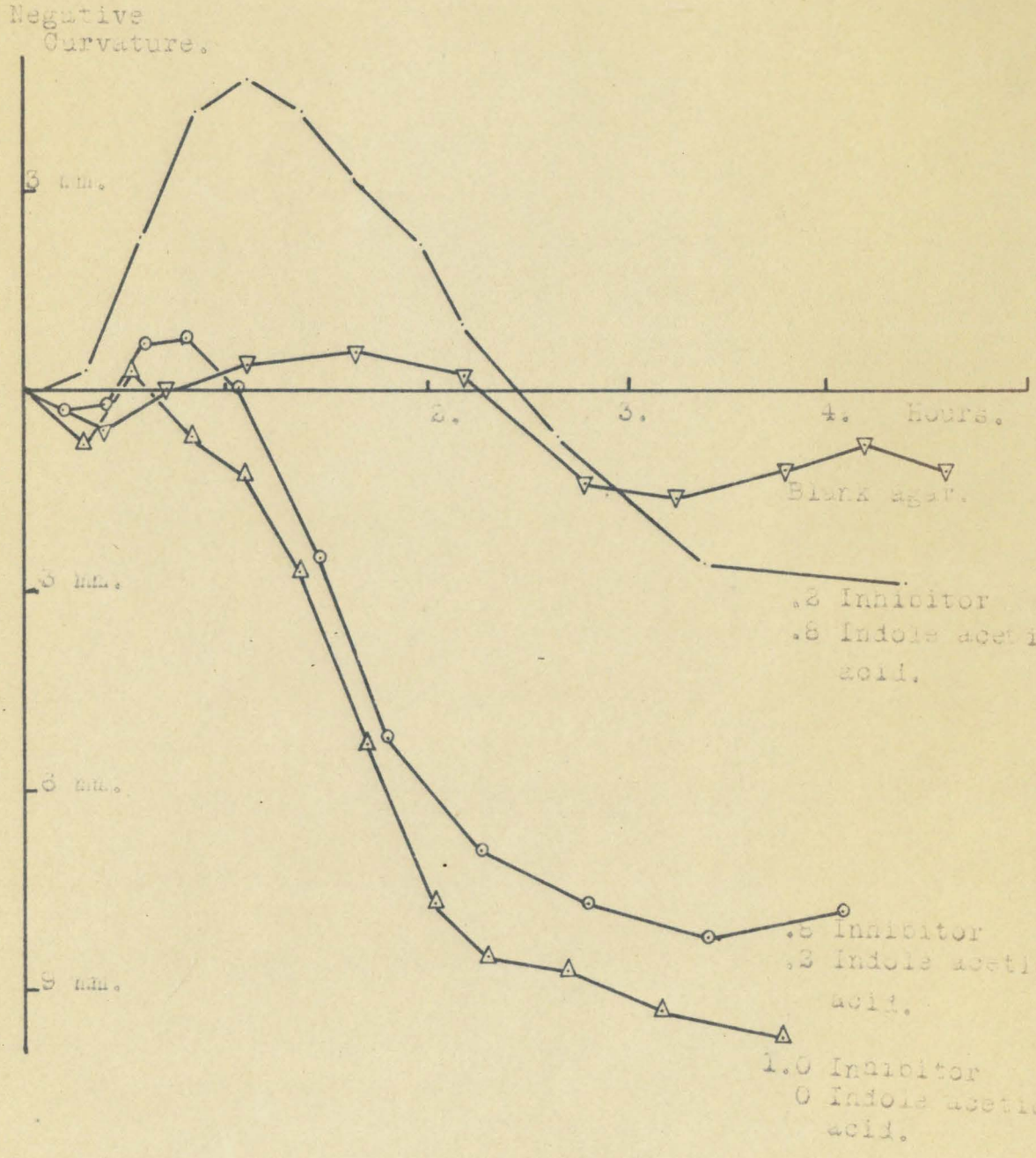


same results in every case. It was concluded that contaminating auxin was removed. In subsequent work when reference is made to purified inhibitor it will be understood that it was purified by this "Inverse Transport" method.

D. Action of Avena test plants to inhibitor contaminated with auxin.

It was next interesting to see if the action of the crude inhibitor extract,--a negative curvature followed by a positive one, could be duplicated by using purified inhibitor and mixing with it indole acetic acid, a growth promoting substance. This experiment was done by testing on the kymograph: 1. purified inhibitor alone; 2. 4/5 purified inhibitor plus 1/5 of an indole acetic acid solution of a concentration of 45 gamma per liter, and; 3. 1/5 inhibitor plus 4/5 of the indole acetic acid solution. The results are seen in figure 19. Each point is the average of 10-12, twice decapitated plants. It is clear that the same type of curvature is found as that obtained with the crude inhibitor extract. It may then be concluded that the negative curvatures preceding the positive curvatures and resulting from the application of the crude unpurified inhibitor extract, are due to auxin which was extracted from the cotyledons together with the inhibitor.

Fig. 18. Reaction rate of twice decapitated Avena coleoptiles to purified inhibitor mixed with indole-3-acetic acid.



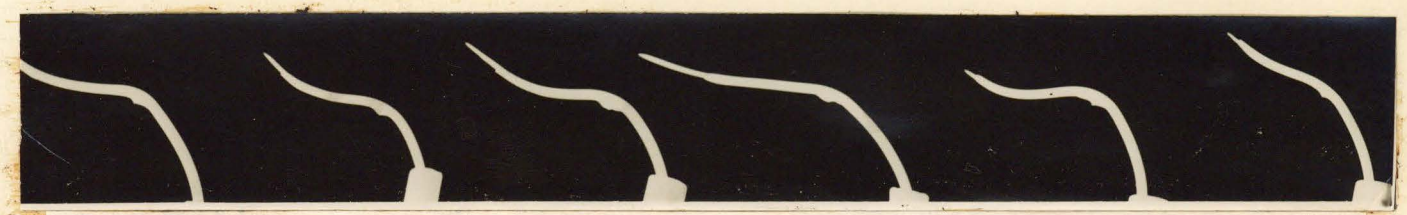
It should be pointed out that when it was found that inhibitor was present in the vegetative buds and young leaves as well as in the cotyledons, many of the crude extracts were made by using the whole radish plant except for the roots. As auxin is present in the hypocotyl this proceedure allowed ample opportunity to extract auxin along with inhibitor. Moreover, it has been shown by Van Overbeek (1933), that it is even possible to obtain auxin from the radish cotyledons themselves by the diffusion method of auxin extraction. According to this method the cotyledons are cut from the plant near the hypocotyl and allowed to diffuse out any substances into 1 x 2 x 2 mm. 1½ agar blocks placed on the cut end. These agar blocks cause very strong auxin curvatures in the Avena test. This indicates that auxin is coming from the cotyledons. The point to be made is that this auxin is probably present in the crude extract but that in the standard inhibitor test its presence would not be noticed because of the obscuring effect of the larger amounts of inhibitor; however a kymograph test, would record the presence of auxin. By direct observation of the test plants it is possible to see the negative curvatures recorded by the kymograph. This is particularly possible in cases where the amount of inhibitor is not especially high. Under such conditions if a measurement were taken of the amount of negative curvature it would serve to answer some of the same questions that are answered

by the kymograph test; i.e. namely, how much auxin and how much inhibitor are present in the extract.

When inhibitor extract contains auxin in the proper amount it is possible to obtain test plants which show S shaped curvatures after an hour and a half. These curvatures may be interpreted as being caused by the auxin manifesting itself first to give a growth promoting effect hence a negative curvature which will move downward into the lower zones of the coleoptile while the inhibitor acts to give a positive curvature in the upper zones. Examples of this type of curvature are seen in figure 20. At the end of $2\frac{1}{2}$ hours when there is a large concentration of inhibitor, the S shaped curvature will be entirely replaced by a completely positive curvature.

E. Curvature rates of Avena coleoptiles to dilutions of inhibitor.

The greater the dilution of the inhibitor the less is the net amount of positive curvature and also the greater is the negative curvature if there is auxin present. This is seen in figure 21. Nine to twelve test plants were averaged for each point. In this experiment, a dilution series (1, 1/2, 1/16, & 1/32) of crude inhibitor extract was tested directly on the coleoptile by the standard kymograph technique. From the results it is clearly seen that after



Positive curvature of Avena coleoptiles 22 hours after unilateral application of inhibitor in lanolin at the middle of the coleoptile. Note also the geotropic tip response.

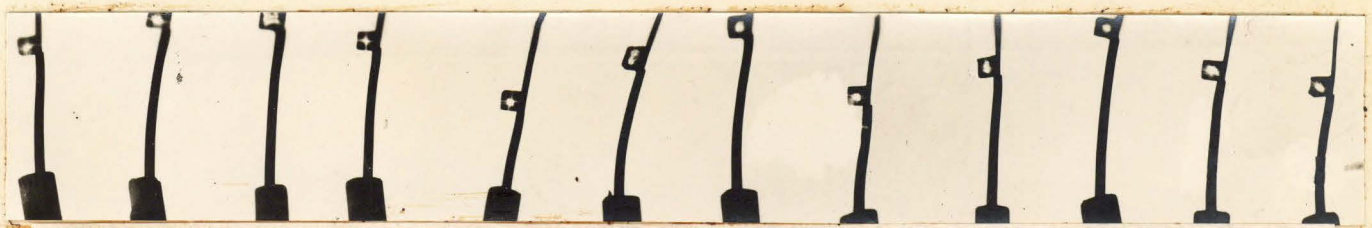
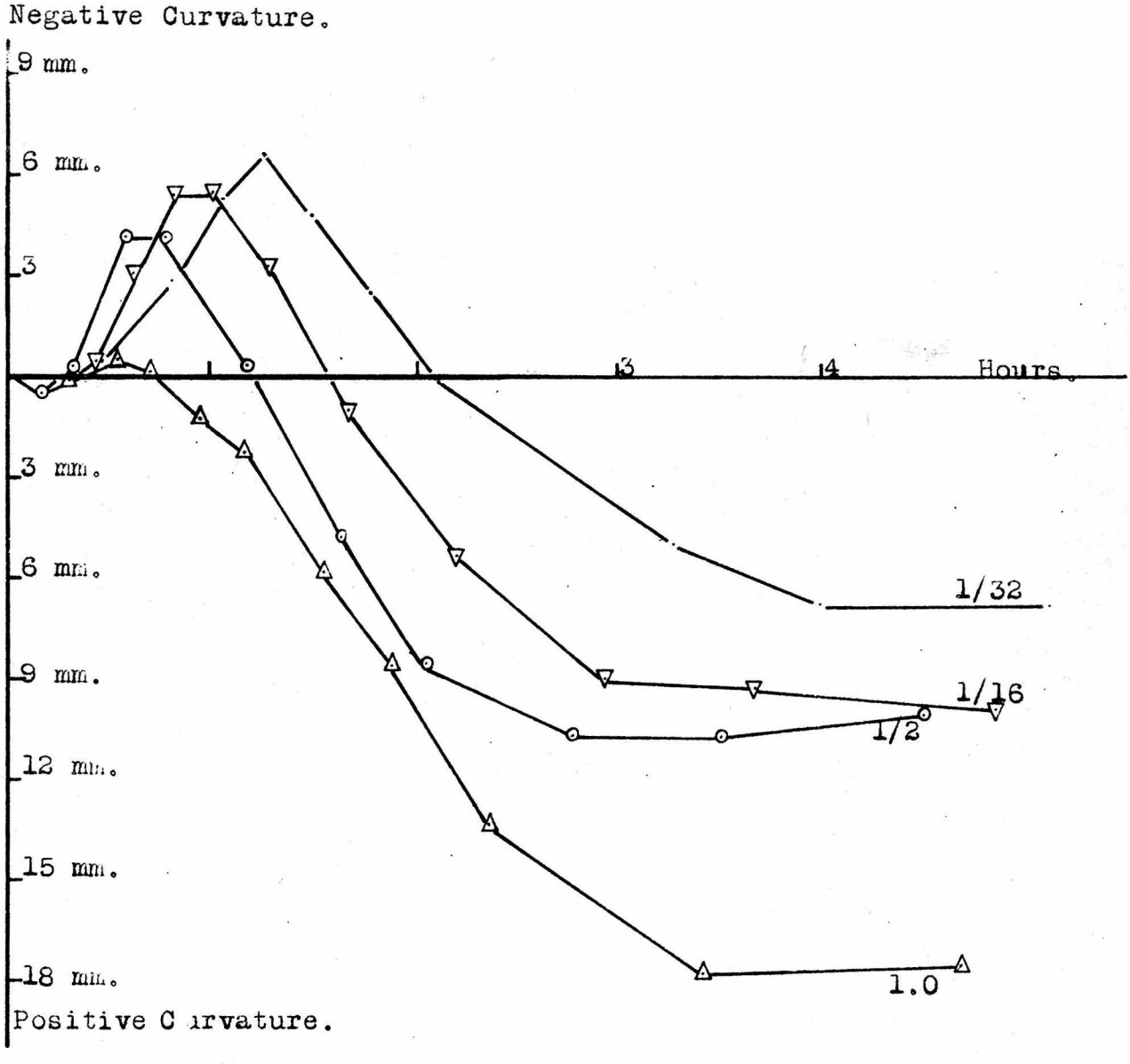


Fig. 20. "S" curvatures an hour and a half after application of inhibitor containing auxin. Plants twice decapitated.

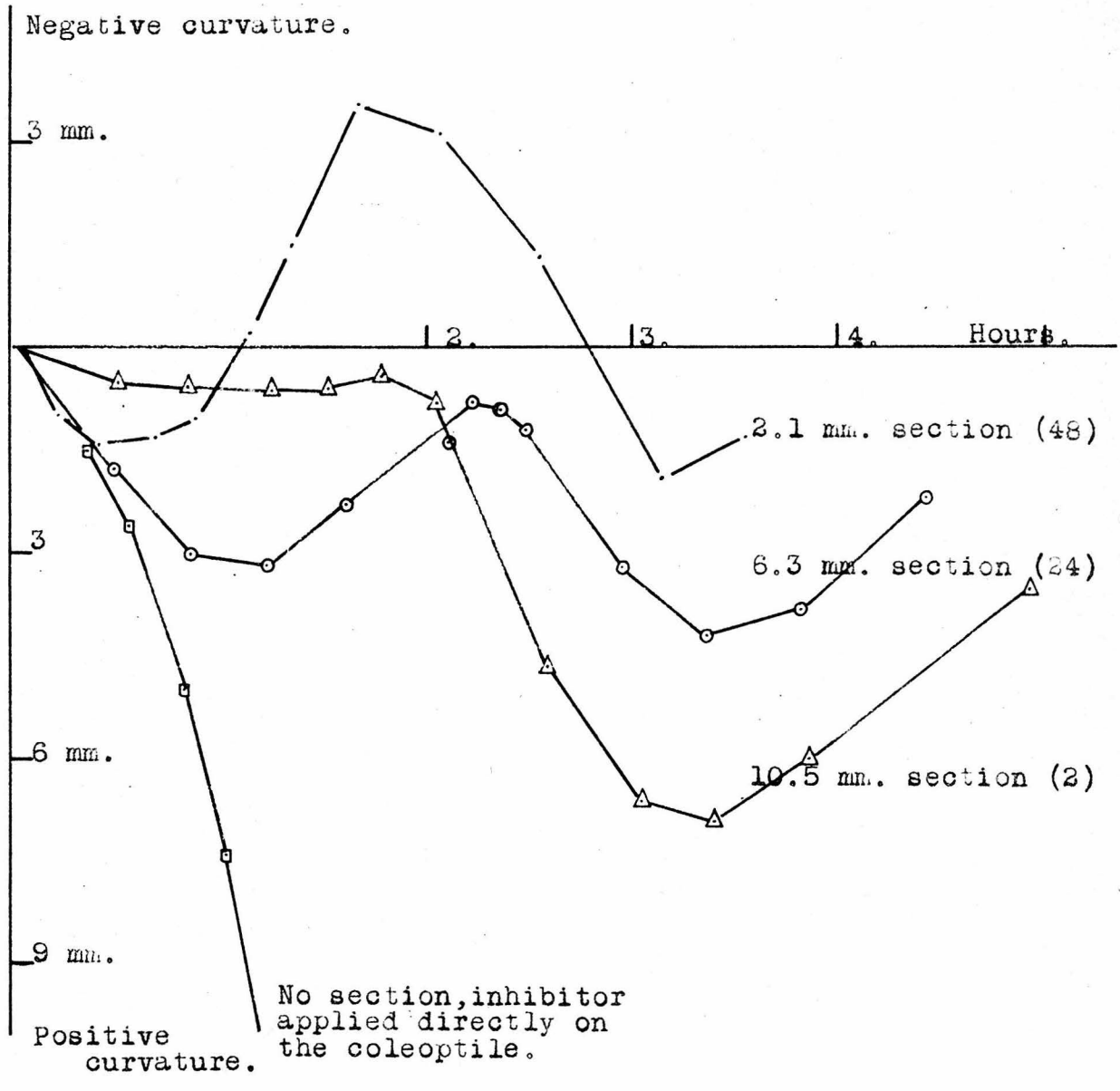
Fig. 21. Curvature rate of once decapitated Avena test plants to different concentrations of unpurified inhibitor.



an hour and a half the plants with the 1/32 inhibitor showed a negative curvature of about -5 degrees while the plants with the inhibitor full strength showed a positive curvature of about 6 degrees. After the next 2½ hours, however, the 1/32 concentration plants had become positively curved to about 7 degrees and the full strength plants to about 18 degrees. This data thus agrees with the explanation for the occurence of S shaped curvatures at low concentrations of inhibitor containing auxin.

Since the 2.1 mm. Avena sections tested on the kymograph for inhibitor reaction diffused out auxin to a large extent, as was seen in a previous experiment, it was thought worthwhile to investigate the effect of treating sections longer than 2.1 mm. with inhibitor. This was done in the same manner as described for the 2.1 mm. sections except that the sections (of different lengths) were all cut from the upper half of the Avena coleoptile. The results, of this experiment are seen in figure 22. (Blank controls for all sections were run but are not given on this graph as they would not affect any of the conclusions drawn.) It is to be noted that the longer the section the less auxin it transmits, (indicated by the mm. of negative curvature given in parenthesis). This can be understood if one assumes with Van der Wey (1933) and Van Overbeek (1939) that it is possible for the plant to use or destroy the auxin as it tends to pass through the plant section.

Fig. 22. Reaction rate of oncedecapitated Avena coleoptiles to inhibitor. transported through coleoptile sections in the normal position. Each point is the average of seven test plants.



Thus a short section would use less auxin than a longer one, while the longest section would be capable of using the most auxin and hence would give a record of transmitting the least. This assumption would also explain the difference in the reaction to unpurified inhibitor of the Avena and radish sections cut from the rapidly growing zones, apical sections, and from the slow growing zones, (basal sections). The rapidly growing zones would be capable of using more of the auxin and hence would transmit less than the basal zones. This was actually found to be the case as was seen in figure 19.

The effect of transport of unpurified inhibitor through Avena sections in the normal, basipetalous direction seemed now well established. The presence of the auxin curvatures could be ascribed to auxin in the crude extract. Likewise could the presence of a negative curvature preceding the positive curvature, when the agar test block was applied directly, be ascribed to contaminating auxin for when the auxin was removed by the Inverse Transport purification these negative curvatures were no longer present. There were still two points unsettled, however, concerning the transport of purified inhibitor. The first point was to determine the transport of purified inhibitor in the normal direction. The second point was to conclusively answer the question as to whether or not auxin diffused out of the inverted sections as indicated by one exceptional radish

polarity test.

F. Transport of purified inhibitor in Avena coleoptile sections.

These questions were answered with respect to the Avena coleoptile sections by the following two experiments. In the first experiment both, 2.1. mm. normal position sections and 4.2 mm. inverted sections were tested for transport of purified inhibitor by the kymograph technique. The results are seen in figure 23 where each point is the average of 9 or 10 plants. It is noted that in both instances there is an auxin curvature present. Since auxin free inhibitor was used, this negative curvature must have been the result of the transport process. It is easy to believe that in the transport through the sections in the normal position some of the inhibitor could have been hydrolysed to auxin which then would diffuse on out, but in the case of the inverted section it is difficult to see how any auxin could diffuse out, as it would be going against the well-established polarity of auxin transport. To explain this negative curvature one may assume that inhibitor was transported through the inverted section as inhibitor, (which we have seen is possible), and that actually no auxin was transported against the polarity, but that at the bottom cut surface of the section and the top out

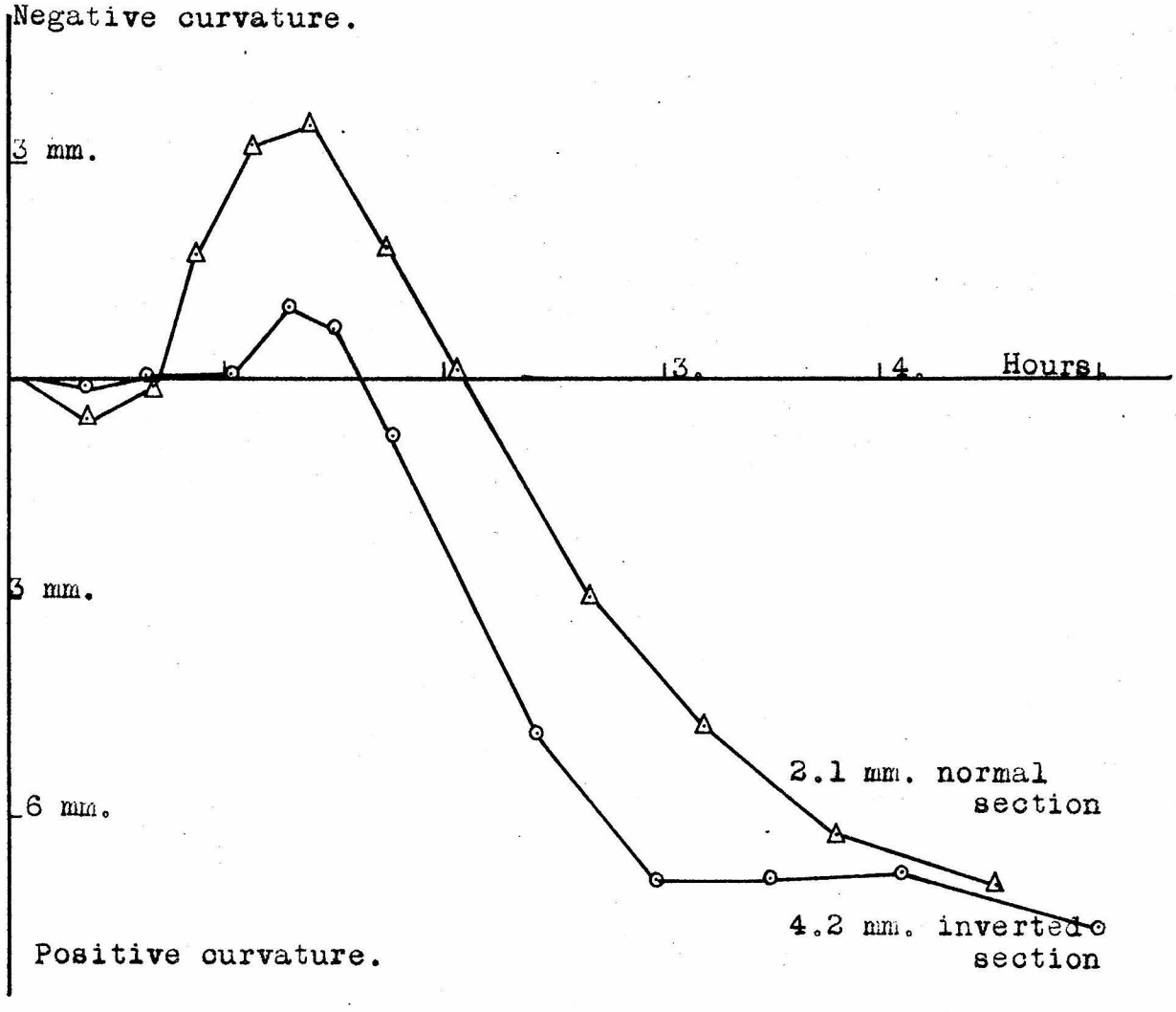


Fig. 23. Reaction rate of twice decapitated Avena coleoptiles to purified inhibitor transported through 2.1 mm. coleoptile sections in normal position, and 4.2 mm. sections in the inverted position.

surface of the coleoptile there was a hydrolysis of inhibitor to auxin. This might have come about by the presence of hydrolytic enzymes as it has been shown by Van Overbeek (1936) that at cut surfaces of Avena coleoptiles there are enzymes liberated. (He found the presence of peroxidase).

To verify this experiment it was repeated in the following manner: 4.2 mm. Avena coleoptile sections were cut from the upper half of coleoptiles and were mounted in the inverted position on test plants in the manner previously described. To one set of twelve such plants was applied an unpurified inhibitor made from the extraction of the cotyledons alone. To a similar set of plants blank 1½% agar was applied. To a third set of six plants with inverted 4.2 mm. sections, agar containing an indole acetic acid solution of 500 gamma per liter was applied, while to a fourth set,also consisting of six plants,the same concentration of indole acetic acid was applied directly to the Avena coleoptile without any intervening inverted coleoptile section. Finally inhibitor of the same concentration as that used above and blank agar, were each tested directly on sets of six plants. These were all run on the kymograph machine at the same time so that any factors of varying sensitivity of the test plants to inhibitor or auxin were ruled out. The results are seen in figure 24. From this figure the following points are evident: 1. That in agreement with Gorter's (1927) statement blank agar can

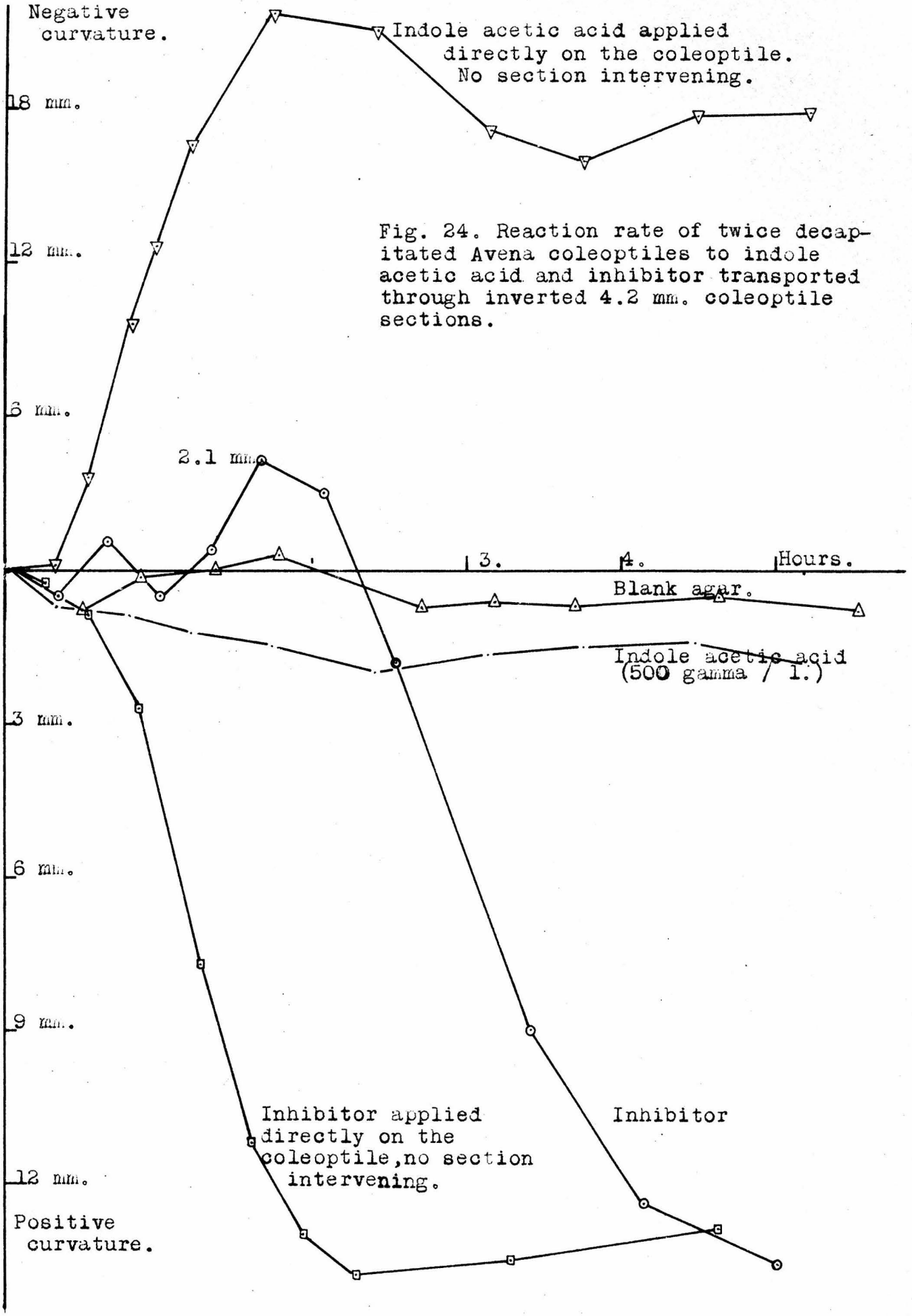


Fig. 24. Reaction rate of twice decapitated Avena coleoptiles to indole acetic acid and inhibitor transported through inverted 4.2 mm. coleoptile sections.

cause positive curvatures after two hours and a half; 2. That there is no transport of pure auxin alone through the inverted section; 3. That the inhibitor extract had little auxin in it itself,--shown by the test of inhibitor directly on the coleoptile.;4. That in agreement with the former experiment, inhibitor transported through the inverted Avena sections caused a negative curvature to precede the positive one, and; 5. That this positive curvature occurred at the same rate as the positive curvature resulting from the direct application of the inhibitor thus indicating that the transported inhibitor still acted the same as the non-transported inhibitor.

G. Transport rate of purified inhibitor in Avena coleoptiles.

From these two experiments it is possible to determine the rate of transport of inhibitor through inverted section of Avena coleoptile. The beginning of the eumotoric phase (see Went and White, 1939) is taken as the moment of the beginning of the negative curvature preceding the positive curvature. This is based on the assumption that the first inhibitor to pass through the section becomes hydrolysed, as explained, at the cut surfaces and hence continues on into the coleoptile as auxin alone. The subsequent positive curvature of the Avena coleoptile may be explained as

resulting from more inhibitor being transported through the section than can be changed into auxin at the cut surfaces.

It has been found by Went and White (1939), and can also be determined from figure 1., that the time required for the beginning of negative curvatures in Avena plants in response to indole-acetic acid is 37 minutes. Accordingly the 37 minutes required for the initiation of this curvature must be subtracted from tha total time necessary for the initiation of the curvature in order to find the time required for the inhibitor to pass through the section and arrive at the top of the coleoptile. From figure 22 the total time required from the moment of application of inhibitor to the beginning of the response in the coleoptile is 62 minutes. Subtracting the 37 minutes, as explained, indicates that the inhibitor was transported through this 4.2 mm. section in 25 minutes. This is at the rate of 10.1 mm. per hour. Calculating the velocity from the second experiment one finds that it is 10.9 mm. per hour. The rate of auxin transport in Avena coleoptiles is between 10 - 12 mm. per hour so that the value found in these two instances for inhibitor is the same as that of auxin.

From figure 22 an indication of the transport rate of inhibitor through coleoptile sections in the normal position may be determined. The 2.1 mm. and the 6.3 mm. show

a definite time for the beginning of the eumotoric phase. This time is seen to be 62 minutes for the 6.3 mm. sections while for the 2.1 mm. sections it is 40 minutes. The difference between these two times, 22 minutes, is the time required for the inhibitor to be transported through the additional 4.2 mm. of the 6.3 mm. sections. This is at the rate of 11.5 mm. per hour or approximately the same as the transport rate for the inverted sections. As this value is based on only one experiment it should be borne in mind that it is only an indication of the transport velocity of inhibitor in the normal direction.

H. Transport of purified inhibitor in radish hypocotyl sections.

An experiment similar to the one just described for Avena sections was carried out using radish hypocotyl sections. These were 4.2 mm. long and were out from the upper half of the hypocotyls of plants that had been grown in the greenhouse in the usual way. Five different sets of twelve plants each were tested on the kymograph by the technique previously described. The sets tested were: 1. Sections in the normal position with inhibitor applied on top; 2. Control sections in the normal position with blank agar; 3. Sections in the inverted position with inhibitor applied; 5. Inhibitor of the same concentration

as in the 1st and 3rd sets tested directly on the coleoptile. The results of this experiment are given in figure 25. Here it is seen that as with the Avena sections, the transport of the inhibitor through the inverted sections shows at first a definite auxin curvature followed by an inhibitor curvature. In this case, as in the case of the Avena, hydrolysis of inhibitor may take place at the cut surface. From this experiment it is also clear that in agreement with the earlier experiments it is found that inhibitor has no transport polarity in radish.

In the one exceptional experiment on polarity of radish hypocotyls for inhibitor it will be recalled that it was found that during the two hours allowed for transport nothing but auxin had come through the inverted section. As may now be understood from figure 25, the two hours allowed for the inhibitor transport was insufficient time for inhibitor to come through the section in large enough amounts to escape hydrolysis, accordingly only auxin was found in the lower plate. From figure 25, the rate for inhibitor transport through the 4.2 mm. inverted radish sections is found to be 63 minutes. This is at the rate of 4.0 m. hour, or only about a third as fast as in the Avena coleoptile.

From a similar experiment where indole - 3-acetic acid (850 gamma per liter) was transported through 4.2 mm. radish hypocotyl sections in the normal position, the velo-

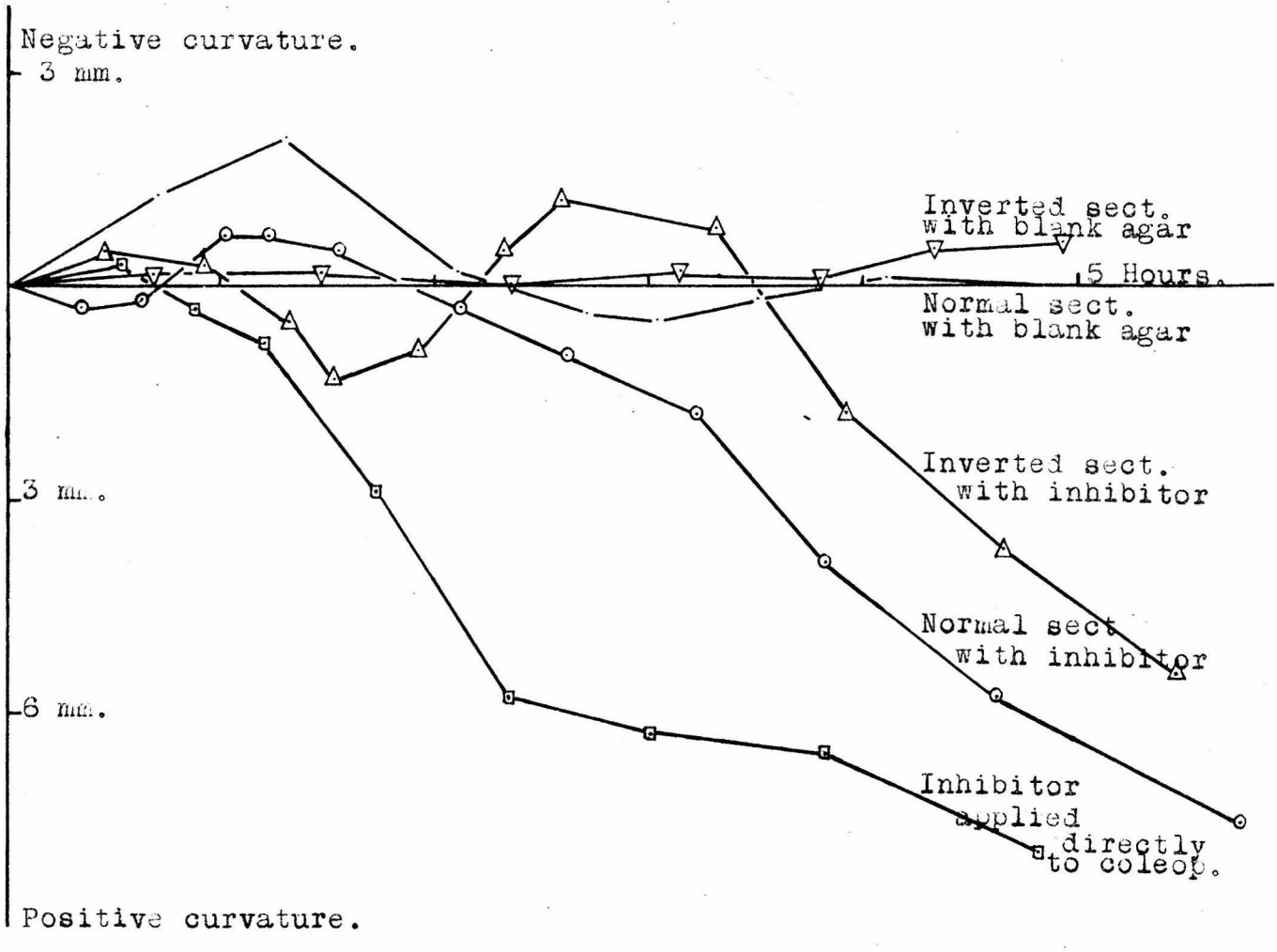


Fig. 25. Reaction rate of twice decapitated Avena coleoptiles to inhibitor transported through normal and inverted 4.2 mm. sections of Radish hypocotyl.

city of transport was found to be 8.1 mm / hour. This should merely be considered an indication of the velocity as only four plants were measured in determining this value.

Summarizing the facts concerning the transport of inhibitor through Avena and radish sections one may say that:

1. Inhibitor is transported non-polarly.
2. It is possible to obtain inhibitor apparently free from auxin by the "Inverse Transport" purification method so that no negative curvatures are found when such purified auxin is tested on the kymograph.
3. The effect of unpurified inhibitor may be duplicated by mixing auxin with purified inhibitor.
4. The more dilute the crude extract of inhibitor, the greater will be any negative curvatures preceding the positive ones.
5. Inhibitor may be hydrolysed at cut surfaces.
6. Inhibitor is transported through inverted and normal Avena coleoptile sections at the rate of about 11 mm. per hour.
7. It is indicated that inhibitor is transported through inverted radish hypocotyl sections at the rate of 4.0 mm. per hour.

Chapter VII. Physiological Role of Inhibitor.

Since it was well established that the inhibitor substance was capable of being hydrolysed into auxin and yet that it could cause growth inhibition in the Avena plant, it was interesting to inquire into its possible physiological role in the radish plant. As pointed out in the introduction, one class of inhibitions are known to be caused by auxin. The questions now raised were:- 1. Could inhibitor act the same way in these inhibitions as the applied auxin?; 2. Could the inhibitor act like an auxin precursor and be hydrolysed to yield auxin in the plant?; and 3. Could the inhibitor have a role as an inhibitor in the physiology of the radish plant?

A. Inhibitor does not inhibit bud growth.

The first problem investigated of the known auxin inhibitions was that of bud growth. This was done in the following way: Eight day old etiolated pea seedlings (strain Alaska) which had been grown in sand, were cut off near the tip just below the third node. On the cut surface of thirty such stems purified inhibitor in lanolin was applied. To a second group of thirty stems inhibitor was applied mixed in the ratio of two parts of inhibitor and one part auxin. To the third group it was applied as one part inhibitor and

two parts auxin. The auxin used above was in a lanolin paste in a concentration of 1 part in a 100. A fourth lot of thirty pea stems were treated with the auxin paste full strength, while a fifth lot were treated with blank lanolin. After six days the outgrowth of the lateral buds was measured. None of the buds developed except those on the stems treated with inhibitor or treated with lanolin alone. These results, given as the growth of the buds with stand. error, on treated stems after six days, were: inhibitor-- 87.7 mm. ± 4.4; control (lanolin only) -- 77.4 ± 6.1. See also figure 26 which shows a toxic effect of the inhibitor as evidenced by the atrophying of the tip centimeter of the stem tissue. From this experiment, which has been repeated with similar results, it is apparent that the inhibitor has no effect on bud inhibition. It might also be said that at the concentrations of auxin used, inhibitor has no effect in counteracting the auxin inhibition.

B. Inhibitor is not active in mesophyll growth.

Since inhibitor was found to disappear during the growth of the leaves it was wondered if it could function as a factor for leaf growth. To settle this point Mr. David Bonner kindly made a leaf growth test according to the method developed by him, (Bonner, Haagen-Smit, & Went.

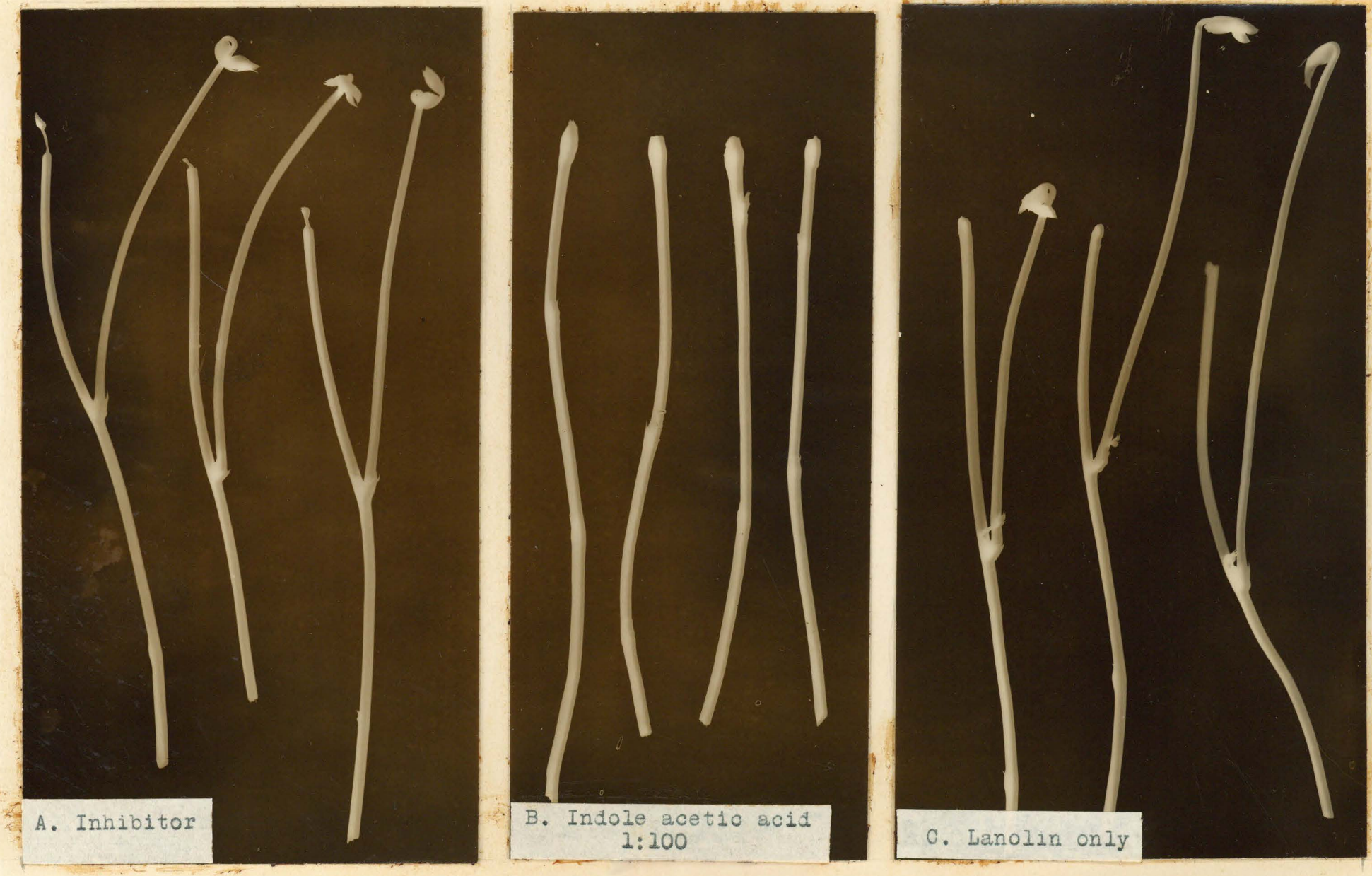


Fig. 26. Etiolated pea stem bud inhibition test ,using: Inhibitor; Indole acetic acid,and;Lanolin only,control. Note lack of bud inhibition in A,also atrophying of its stem tip.

1939). Unpurified inhibitor was used in this test. In this method 5 mm. circular discs of 14 day old first foliage leaves of radish plants are floated on a 1% sucrose solution. Appropriate controls are run. The solutions containing the leaf discs are put in an incubator at 25 degrees C. and allowed to grow for 30 hours at the end of which time they are weighed. The weighing is done in a standardized manner so that any increase in wet weight of the leaves over the sucrose controls is directly indicative of the amount of growth. A dilution series of the unknown substance being tested is generally run. The results of this test for inhibitor showed that the growth of the leaf discs did not increase over the sucrose controls. It is therefore concluded that the inhibitor substance under these conditions is not a factor for mesophyll growth.

C. Inhibitor inhibits root growth.

Since root growth is inhibited by auxin it was desireable to see if applications of inhibitor would act likewise. For this experiment purified inhibitor was used and the technique used by Dr. James Bonner was followed.

Shelled Avena seeds are soaked for two hours in water and are then laid out on moist filter paper for twenty-four hours. At the end of this time the radicle is approximately 2mm. long. From the germinated seeds those

are selected whose roots are of approximate uniform length. They are transferred to Petri dishes containing filter paper and 4 cc. of the solution being tested. Fifteen plants are put in each dish which are then put in an incubator at 25 C. Twenty hours later the increase in their root length is measured.

Inhibitor was tested in the above manner in a 1,. 1 and .01 dilution series. In another similar series of dilutions, inhibitor was hydrolysed into auxin by heating the solution for 10 minutes in a boiling water bath. Duplicate samples at each concentration of both inhibitor and hydrolysed inhibitor were run. Water control solutions were also run. Standard auxin and inhibitor tests were made on the inhibitor and hydrolyzed inhibitor 24 hours after their preparation. During this time they had been kept in an ice box.

The results of the complete test are given in Table II. Here it may be seen that the inhibitor prevented the growth of the roots to the same extent as the auxin from the hydrolysed inhibitor. The Avena test used subsequently to determine the strength of the original solutions showed that the inhibitor solution contained a large amount of auxin although not quite as much as the hydrolysed inhibitor solution. This could only be accounted for by the fact that purified inhibitor is extremely susceptable to hydrolysis. In conclusion of this experiment it can only be

Table II.

Root growth in : inhibitor; hydrolysed inhibitor, and; water. Data is the average length in mm. of 15 roots per concentration tested. All concentrations run in duplicate. Net growth over the original length (2 mm.) is underlined.

	Root length after 20 hours in :		
Conc.	Inhibitor	Hydrolysed Inhibitor	Water
1.00	5.2 5.6 <u>3.6</u> 6.1	6.2 6.5 <u>4.5</u> 6.8	17.7 17.2 <u>15.2</u> 16.8
0.10	10.8 10.7 <u>8.7</u> 10.7	11.6 11.3 <u>9.3</u> 11.1	
0.01	14.7 19.0 <u>12.0</u> 13.3	15.1 15.4 <u>13.4</u> 15.8	

said that under these conditions the inhibitor acts like auxin to cause an inhibition in the growth of roots. There is still a question as to what its effect would be if it were possible to test it in a purified form without having it hydrolyse into auxin.

D. Indole acetic acid does not inhibit root growth by causing an accumulation of inhibitor.

Since the action of auxin on root growth is to cause a growth inhibition it was suggested that it might be possible that the auxin is transformed into inhibitor in the roots. The answer to this point was sought by analyzing indole acetic acid inhibited roots in the following way:

A lot of approximately 165, 26 hour germinated Avena plants were inhibited in their growth by planting them in a 10^{-5} M. indole acetic acid solution. A water grown control was also used. 20 hours after the planting they were divided into roots and coleoptiles and were ether extracted according to the method of Van Overbeek (1938). Duplicate extractions were made in all cases. Just previous to the extraction the material was washed thoroughly on a Buchner funnel; fresh weight determinations were them made. The ether extract was taken up in .3 cc. of hot 1½% agar. This was cast into blocks 1.8 x 2 x 2 mm. and tested on once decapitated Avena plants. After 90 minutes the nega-

tive curvatures were measured. See Table III. After a 150 minutes, as they had not changed appreciably, measurements at this time were not taken. From this data it was seen at once that the inhibited coleoptiles did not show the presence of inhibitor but on the contrary showed only the presence of auxin.

Nearly twice the amount of auxin per gram weight was found in the inhibited plants than in the water controls. The roots of the inhibited plants, however, were only 7.7 mm. long while the controlw were 14.3 mm. long or nearly twice the size. Therefore the amount of auxin per root was approximately the same in both the inhibited and the non-inhibited roots. The coleoptiles were of approximately the same length so in this case, per coleoptile, the amount of auxin in the inhibited plants was nearly twice as much as the controls.

E. Inhibitor is not active in root formation.

Since inhibitor was able to act like auxin in certain instances the question arose as to whether or not it would act like auxin in the formation of root primordia. To answer this question the following rooting experiment was done.

Etiolated 8 day old pea seedlings, which had been grown in sand, had the terminal bud removed and were cut off at the first node (near the base). They were then soaked with

Table III.

Ether extractable auxin present in the roots and coleoptiles of Avena plants inhibited in their growth by treatment with indole acetic acid, 10^{-5} M. Extract taken up in .3 cc. 1½% agar. Auxin given as degrees negative curvature of 12 Avena test plants. All determinations in duplicate.

	Water control.				Indole acetic acid.			
	Roots		Coleoptile		Roots		Coleoptile	
	1.	2.	1.	2.	1.	2.	1.	2.
Fresh wt. in grams	.671	.708	.491	.632	.451	.613	.298	.283
Curvature	11.3	13.6	18.8	18.2	16.5	20.9	14.7	15.7
Curv. per gram wt.	16.6	20.7	38.3	28.8	36.7	34.2	49.5	55.5
Avg. Curv per gr. wt.	18.6		33.5		35.5		52.5	

their bases in water for four hours. At the end of this time they were put, with their bases (basal 1 cm. of the stem), in inhibitor solution. A tenfold dilution series of inhibitor was tested (from 1 to 10^{-7}). Seven stems were used at each concentration. After 24 hours they were transferred to a 2% sucrose solution of the same volume as the inhibitor. (At this time it was noted that the stems which had been in the full strength inhibitor had become flaccid.) Seven days later they were examined for root formation. No roots or root primordia were present in any of the inhibitor concentrations although indole acetic acid controls showed many. The stems were then allowed to stand for a week with their bases in a Vitamin B_1 solution of 1 mg. per liter. At the end of this time there were still no roots present on the inhibitor treated stems whereas the indole acetic acid controls had become well rooted. The water controls showed no roots. All of these treatments were carried out in the Avena testing dark rooms.

From this experiment it was concluded that with the unpurified inhibitor there was no effect on root formation of Pea stem cuttings.

F. Inhibitor inhibits seed germination.

Another question was raised as to the action of inhibitor on seed germination. It is well known that auxin

can inhibit the germination of seeds. To investigate this point with respect to inhibitor the following experiment was done using purified inhibitor. This test was kindly made by Dr. James Bonner who has standardized the procedure.

In making this determination the percentage of germination of tomato seeds is used as an indication of the effect of the solutions being tested. The percentage of germination is compared to that with water alone. The technique is to soak tomato seeds for 1 hour in water, then to lay them out separately on filter paper in a Petri dish containing 4 cc. of the solution being tested. After 48 hours, during which time they are kept in an incubator at 25 degrees centigrade, the number of germinated seeds are counted and the percentage germination determined.

In this experiment one such test was made of purified inhibitor, one of indole acetic acid 10^{-4} M., and two duplicate water control tests. In each determination there were 100 seeds used. The purified inhibitor was not tested for its amount of inhibitor activity at the conclusion of the experiment but in view of the former experiments where it was found that it was extremely susceptible to hydrolysis, it seems very likely that much of the inhibitor had probably formed auxin. The results of this experiment showed 66% germination for the controls, 46% germination for the indole acetic acid, and 46% germination for

the inhibitor. It is seen that the effect of the inhibitor was exactly the same as that of indole acetic acid. These would be the expected results if the inhibitor had, as suggested, hydrolysed into auxin.

Dr. James Bonner has reported (unpublished) the presence of a substance in canned tomato juice that inhibits the germination of tomato seeds. It was accordingly of interest to determine whether or not this substance might be the same as the inhibitor. To determine this point an ether extraction was made of 300 cc. of canned tomato juice. The extraction was made according to the standard technique for inhibitor extraction. It was taken up in 1½% agar and tested by the kymograph technique. Only auxin curvatures were indicated as a result of this test. The inhibitor of tomato seed germination is, then, certainly not the same as the radish inhibitor.

G. Growth inhibition by inhibitor, in tissues other than the Avena coleoptile.

In another experiment designed to investigate the response of various kinds of plant tissue to inhibitor, the inhibitor was applied to the plant parts unilaterally in lanolin. The concentration of the inhibitor was such that when it was applied unilaterally at the middle of the coleoptile, after 2 hours a 20 degree positive curvature

was present. Unilateral applications of this paste caused growth inhibition responses from such diverse tissues as the petiole of Bryophyllum; tendrils stems, and petioles of etiolated and normal peas; stems of etiolated Vicia fabe; and hypocotyls of radish. In the case of the Bryophyllum the treatment was unquestionably toxic as after 24 hours the treated tissue was flaccid and a few days later the leaf broke off at the stem. In the other cases no toxic effect was noted during the week following the treatment. Observations were discontinued after this time. Even though positive curvatures appeared as a result of the inhibition of growth at the site of application of the inhibitor, the tissues continued to grow beyond that place.

The conclusion indicated from the above results would be that the growth inhibiting effect of inhibitor was not specific for Avena plants alone.

H. Inhibitor does not affect the phototropic or geotropic response of Avena coleoptiles.

Inhibitor was found not markedly to affect the response of Avena coleoptiles to phototropism or geotropism although further experiments on this point are necessary. This was shown in the following way. For the phototropic investigation four sets of 12 plants each were used. The plants were 30mm. tall at the time of the beginning of the experiment. To the first set inhibitor in lanolin was

applied uniformly over the tip of the coleoptile two hours before exposing the coleoptile to light. The second set was a control for the first and was similarly treated but with pure lanolin alone. The third set was treated in the same manner with inhibitor in lanolin but it was applied just before the light exposure, while the fourth lot were controls for the third and were accordingly treated with lanolin alone. All four sets were then exposed at a distance of 3½ meters, for 15 minutes, to the radiation from a 60 W. Mazda lamp. They were then returned to the dark room and the curvature of the plants toward the side which had been exposed was measured 90 minutes later. The curvatures were:

Inhibitor applied immediately	9.7 ± .05
Lanolin control applied immed.	7.6 ± .04
Inhibitor applied 2 hours previous	7.8 ± .04
Lanolin control applied 2 hrs. prevs.	6.4 ± .01
Inhibitor same conc. as above applied unilaterally, curvature after 2½ hours	13.9

To study the geotropic response of Avena coleoptiles after inhibitor treatment a similar group of four sets of six plants each were used. Inhibitor in lanolin was applied to one set two hours before, and to another set just

immediately before exposing the plants to the geotropic stimulus. The regular Avena holders were turned so that the long axis of the coleoptile was perpendicular to the force of gravity. The negative geotropism resulting from this stimulus was measured after 75 minutes. The entire procedure was carried out in the Avena testing dark room with plants that were about 35 mm. tall. The geotropic curvatures measured in the four sets of plants were:

Inhibitor applied immediately	18.7 ± 1.7
Lanolin applied immediately	21.5 ± 1.1
Inhibitor applied 2 hrs. previous	21.3 ± 2.0
Lanolin applied 2 hours previous	24.4 ± 1.6

From this data it is clearly seen that inhibitor did not effect either the geotropic or phototropic response of the plant. Other experiments have likewise indicated this conclusion.

I. Inhibitor does not permanently stop growth.

It was also of interest to inquire into the capacity for growth of the plant after the application of inhibitor. This was done by applying inhibitor in agar blocks to both once and twice decapitated plants. The degrees of positive curvature after 150 minutes were then measured and the agar locks removed. Twenty four hours later the curvature

was again measured. The plants were kept in the dark room throughout the course of the experiment. The results were:

Positive curvature,	once	decapitated	plants,	2½ hrs.,	24.1 ± 1.4
"	"	"	"	"	24 hrs. 14.8 ± 1.3
"	"	twice	"	"	2½ hrs. 27.1 ± 2.2
"	"	"	"	"	24 hrs., 16.6 ± 1.7

Each value is the average of twelve inhibitor test plants.

It is seen that in both instances there was about 10 degrees less positive curvature after the 24 hour period. This indicates that growth has taken place on the side that was inhibited after the removal of the inhibitor even at a greater rate than on the normal side. This may have been the result of geotropic stimulation as well as the effect of an increased supply of food fact~~o~~r which had not been used during the period that the inhibitor was active. This experiment again demonstrates that inhibitor is capable of inhibiting growth and yet not permanently stopping it.

J. Auxin-like action of inhibitor in the pea stem growth test.

Attention was now turned to the action of inhibitor in

in the Pea test for growth promoting substances as given by Went & Thimann (1937). In this test the amount of growth of the third internode of etiolated pea plants is used as a measure of the growth activity of the substance being investigated. This internode is longitudinally split into two equal halves down to within a centimeter of the second node. It is then cut off below the second node and put in the solution being tested. The growth curvature of the split internode is manifested by an inward curling of its tips. Seven split stems, per solution tested, were used. As each split stem had two tips that could curve, this gave 14 stem curvature measurements. The inward curvature is proportional to the amount of growth that has taken place and is measured after 24 hours.

It has been shown by Went (1939) that certain compounds, phenyl butyric acid, cyclohexane acetic acid, are capable of causing a "preparatory" growth reaction in the Pea Test. By preparatory reaction is meant that the ability of the material to grow in response to auxin is increased after a treatment with these substances. Accordingly in testing the purified inhibitor for its activity in the Pea test the combinations of pretreatment and after treatment as given in Table IV. were made. Here it is seen :1. That the inhibitor was used for a preparatory reaction; 2. That it was tested on material which had been given a preparatory reaction; and 3. That its action alone was tested. The

Table IV.

Effect of purified inhibitor in the Pea growth test. The first number is the average degrees of inward curvature of the tips. In parenthesis is the number of tips showing no curvature ,either inwards or outwards,-- indicative of slight growth promoting activity. The underlined number is the number of tips curving outward,-- indicative of no growth activity.

Pretreated 2 hrs. with:	Water	Phenyl butyric acid	Inhibitor
After tested in:			
Indole acetic acid,.5 mg./l.	68 (0) 2	108 (0) 0	63 (0) 1
Indole acetic acid,.2 mg./l.	34 (2) 2	87 (0) 1	37 (0) 1
Inhibitor	11 (4) 6	38 (1) 3	28 (6) 5
Water	0 (0)		

results are shown in the same table. From this data it is seen that the inhibitor is able to act like an auxin. It responds to the pretreatment as does the auxin, is unable to give any pretreatment itself under these conditions, and actually causes a growth promotion. A kymograph analyses of inhibitor solution afterwards showed that the inhibitor had been hydrolysed into auxin so that its auxin-like action was quite understandable.

K. Possibility of inhibitor acting like an auxin precursor

The next question was now as to whether or not the inhibitor acted in the radish cotyledons as an inhibitor or as an auxin precursor hydrolysing to auxin. It has already been shown by Van Overbeek (1933) that auxin diffuses out of the cut surfaces of the petioles of the cotyledons. It was felt that if it were possible to determine whether this auxin were auxin a or b, or indole acetic acid (which is probably the auxin from inhibitor) it would furnish evidence that this diffusing auxin could or could not come from the inhibitor. As the best means of distinguishing between the auxins is by their molecular weights, molecular weight determinations were made: for the auxin diffusing out of the cut surface of the radish cotyledon petiole; for indole-3-acetic acid; and for purified inhibitor. The

molecular weights of inhibitor and of the auxin diffusing from the cotyledons were determined in three separate experiments.

The method used was that described in Chapter IV. In every determination a graph of the concentration-curvature relationship was made using two concentrations of the of the substance being determined, 1 and $\frac{1}{8}$. This was done in order to be sure of the relative concentration of the substance in the agar plates. In the inhibitor test this graph crossed the abscissa at the origin so that the angles measured were a direct indication of the relative amount of inhibitor in the plates.

The results of these determinations are given in Table V . Here it is readily seen that the evidence would indicate that the auxin diffusing out of the cotyledons was indole acetic acid. The results obtained here for the molecular weight of the inhibitor are lower than those given by Mr. Redemann who used the same method. The results in Table V . were obtained after considerable practice with the technique and it is felt that they are valid within the limits of the method. It should be pointed out that this method is not considered accurate to more than 20%; because of this it is useful not so much for establishing the exact molecular weight of a substance as it is for indicating substances of known molecular weight. In this case

Table V.

Molecular weight determinations of:
A. Purified inhibitor.
B. Auxin diffusing from radish cotyledons.
C. Indole acetic acid.
("n" is number test plants measured for each determination. For method see Chapter IV.)

A. Purified inhibitor.

1.)

Relative conc.	If Tot.is 10,000.	"x"		
10.2	3950	.1412	n 36	h .98 mm.
7.1	2750	.0800	time 41 min.	Mol. Wt. 117
4.9	1900	.1440	Temp. 19	
3.6	1400	.1195	Avg. x .1213	
			D .694	

2.)

15.1	3950	.1412	n 36	h .98 mm.
9.6	2560	.0460	time 42 min.	D .822
8.4	2200	.0885	Temp. 20	Mol. Wt 83
5.1	1340	.1235	Avg. x .0998	

3.)

15.3	3890	.1415	n 48	h 1.03 mm.
12.1	3080	.1536	time 42 min.	D .675
8.5	2160	.0951	Temp. 22	Mol. Wt. 142
3.4	860	.1798	Avg. x .1372	

Table V. continued.

B.) Auxin diffusing from radish cotyledons.

Relative conc.	If Tot.is 10,000. "x"					
1.)						
10.8	3940	.1459	n	12	h	.98
8.0	2920	.1137	time	40 min.	D	.631
5.0	1820	.1600	Temp	23	Mol. Wt.	144
3.6	1312	.1274	Avg. x	.1368		
2.)						
10.7	4020	.1534	nn	12	h	.98
6.9	2595	.0540	time	42 min.	D	.741
5.6	2105	.1060	Temp.	23	Mol. Wt.	104
3.4	1280	.1300	Avg. x	.1109		
3.)						
14.4	3532	.1132	n	12	h	.98
12.7	3110	.1649	time	40 min.	D	.678
8.9	2180	.0917	Temp.	22	Mol. Wt.	125
4.8	1178	.1402	Avg. x	.1273		

C.) Indole acetic acid.

1.)						
14.6	3815	.1354	n	12	h	.98
11.2	2929	.1137	time	44 min.	D	.600
6.9	1802	.1709	Temp	19	D,Theoretical	.563
5.6	1462	.1038	Avg. x	.1309	Mol. Wt.	159
					Actual Mol Wt.	175

it is clearly shown that the the auxin diffusing from the cotyledons is neither auxin a nor b. Since the only other known naturally occurring auxin is indole acetic acid the conclusion may be drawn that the auxin coming from radish cotyledons is indole acetic acid. In Chapter IV it was pointed out that the differential destruction test of the auxin obtained from inhibitor by alkaline hydrolysis indicated that it too was indole acetic acid. Thus this substantiates the evidence from the molecular weight determinations and indicates that the naturally occurring auxin in radish cotyledons is indole acetic acid. From this data there is no reason to believe that this indole acetic acid could not come from the inhibitor.

L. Effect of inhibitor on auxin production in Avena coleoptile tips, and Radish cotyledons.

It was next thought that if the inhibitor were being hydrolysed to form the auxin diffusing from the radish cotyledons, as indicated, that it might be possible to increase this diffusion by applying inhibitor externally. To do this purified inhibitor was taken up in lanolin, which was then uniformly spread over a microscope slide. Cotyledons from seven day old radish seedlings were harvested and soaked in water for ten minutes. Then their upper surface was

pressed down into the lanolin paste on the microscope slide. 1½% agar blocks 1 x 2 x 2 mm. were put on the cut surfaces of the petioles and in this way the substance diffusing out during the next two hours was collected. It was then analyzed by the Avena test. Control cotyledons were run at the same time being treated with pure lanolin alone. During the diffusion period, the cotyledons were in Petri dishes lined with moist filter paper. The dishes were left in diffuse room light. The experiment was done in duplicate using twelve cotyledons in each series. The results were:-

Treatment	Curvature
Inhibitor	9.5
	8.4
Control, Lanolin only	18.6
	18.4

From this data it is seen that there was no effect of the inhibitor to increase the yield of auxin but that on the contrary it decreased it.

A similar experiment was tried using the tips from 30 mm. tall Avena coleoptiles. In this experiment the tips were cut off of the coleoptile and kept on moist filter paper for half an hour. They were then placed on 1½% agar plates, 1 x 6 x 8 mm. and each tip treated with a

small drop of inhibitor in lanolin. A control group using lanolin alone was run. Both sets were allowed to stand on the plates for 75 minutes. At the end of this time they were cut into 1 x 2 x 2 mm. blocks and analyzed by the Avena test. The results, as degrees of negative curvature per 12 test plants with standard error, were:- Avena tips treated with inhibitor, 15.7 ± 1.2 Avena tips treated with lanolin only, 12.9 ± .9. As these results are derived from only one experiment they can not be regarded as being established. They indicate however, that application of inhibitor to Avena coleoptile tips does increase the auxin production.

M. Conclusions.

It is concluded then, that in many instances inhibitor exerts physiological inhibitions like auxin because it actually is being hydrolysed into auxin. It is also concluded that circumstantial evidence indicates that it could act like an auxin precursor in the radish cotyledons.

Chapter VIII. Mechanism of the Inhibiting Action.

A. Effect of inhibitor on food factor distribution.

According to Went (1939) there are two possible mechanisms by which a growth inhibition may come about. First, the inhibitor may interfere with the growth process itself directly as seems to be the case for the radish inhibitor, or secondly, it may eliminate one of the subservient factors necessary for the growth process. Since the process of growth is so little understood it is useful to classify these secondary factors under the general heading of "Food Factors". In the above reference Went has shown that certain substances, gamma phenyl butyric acid and cyclo hexane acetic acid, are capable of inducing positive curvatures in less than 2½ hours in the Avena test by causing a redistribution of these food factors. These positive curvatures are only found in the basal regions of the Avena coleoptile, however, and are never found near the tip. Plants so treated show a negative curvature at the tip. This S shaped curvature is just the reverse of the S shaped curvature caused by inhibitor as mentioned in Chapter VI. Here the plants were curved negatively at the base and positively at the tip. This different shaped S curvature is explained by

Went on the following basis.

The unilateral application of these certain substances, phenyl butyric acid cyclo hexane acetic, changes the normal distribution of the food factors so that they tend to accumulate near the tip of the coleoptile, i.e. the place of application. This causes a deficit in the food factors, for the base and results in a growth inhibition and positive curvature at this place. Near the tip the opposite phenomenon occurs and here one finds a surplus of food factors and a negative curvature. This explanation was proven by the following experiment.

Avena coleoptiles were treated at the tip with lanolin pastes of the substances causing the food factor redistribution. After two hours, during which time controls with blank lanolin had also been treated, the plants were decapitated and cut into 4.2 mm. zones which were kept in the same sequence as they occurred in the plant. These consecutive zones were then put into a 1½% sucrose solution for 24 hours and at the end of that time their growth in length measured with an ocular micrometer. In the untreated plants the greatest amount of growth was found to take place in the second and third zones, with still considerable growth in the fourth zone. With the treated plants, however, the first two zones showed nearly all of the growth, while the subsequent ones grew only slightly. This indicates that the treatment had caused an accumu-

ulation of the food factors in the upper part of the coleoptile and a deficit in the lower part and thus could have brought about a positive curvature in the lower part and a negative curvature in the upper part.

It was of interest to see if any food factor redistribution occurred with radish inhibitor. The same experiment was performed as described above using two concentrations of inhibitor. Fifteen plants were used at each concentration. The data are given in fig 27. The experiment has been repeated three times with similar results. From this graph it is clearly seen that the inhibitor substance does not act by causing a redistribution of the food factor. These results would have been expected from the difference in the type of S curves caused by the inhibitor and the aforementioned substances. It indicates again that the inhibiting action of the inhibitor is on some phase of the primary growth process itself rather than on some of the secondary factors for growth.

A second possible hypothesis for the action of the radish growth inhibitor might be based on the assumption that the inhibitor itself could act as precursor of auxin. (See also Chapt. IX) Under ordinary conditions this precursor could be changed into auxin but when applied to the plant tissues at an abnormally high concentration, as in this case, it would be unable to be converted, would accumulate in the cells and inhibit the normal growth

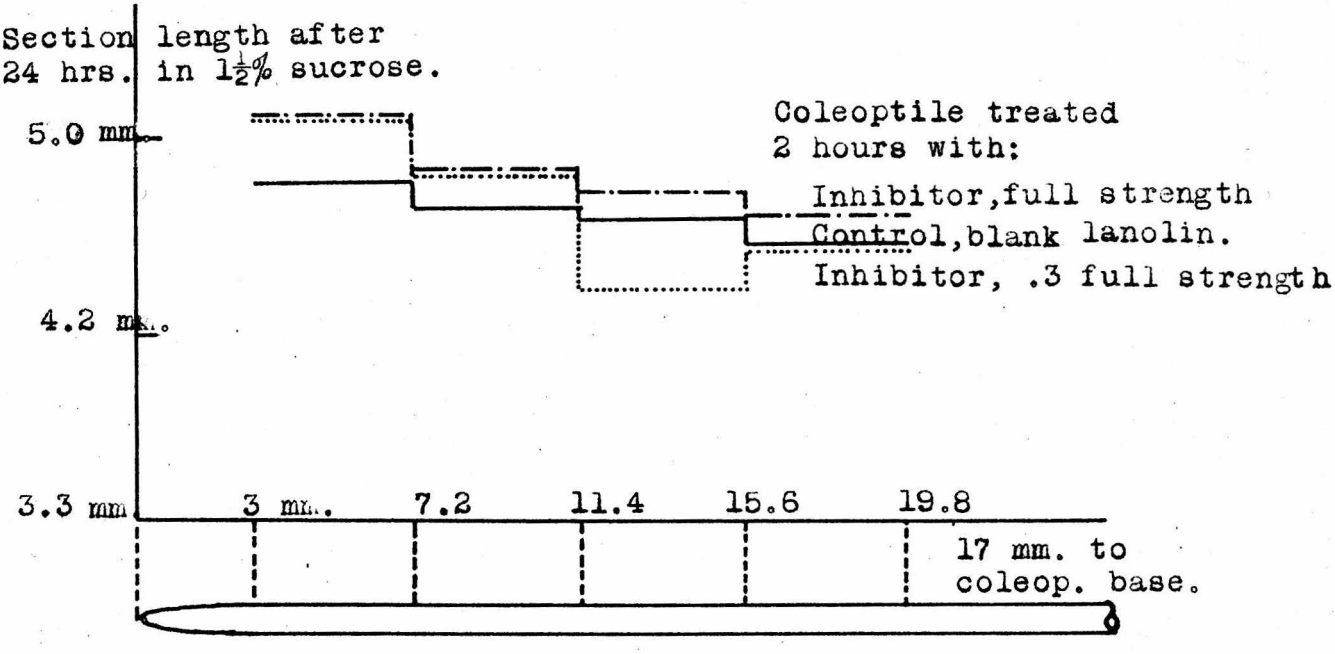


Fig. 27. Food factor distribution in Avena coleoptiles as indicated by increase in length of 4.2 mm. sections. Dash-dot line full strength inhibitor, dotted line .3 of full strength of inhibitor, solid line blank lanolin control. For full details see text.

process. The conversion into auxin might be visualized as taking place through enzyme action. Inasmuch as the indications of the chemical structure of the inhibitor molecule indicates that it is an ester, with auxin constituting one part of the ester linkage, this proposal bore further consideration.

B. Action of lipase preparations on purified inhibitor.

It was felt that if the inhibitor might be converted by enzyme action into auxin, possibly such an enzyme would be a lipase. To investigate this possibility a lipase preparation was made from castor bean endosperm according to the method given by Longenecker & Haley, (1935). The same technique was used for the preparation of a presumed lipase active fraction from radish seeds. The essentials of the technique were to grind the ungerminated seeds as finely as possible and then to extract them with low boiling petrol ether. (Boiling point 20 - 40°C). The ether was then removed from the extracted material, which was dried and ground to a fine powder, This preparation from the radish seed will be referred to as the"radish seed" preparation while the castor bean seed preparation will be spoken of as the "lipase" preparation.(Activated at pH 4.8)

To test the radish seed preparation for its activity

in converting the inhibitor into auxin the following experiment was performed.

To three test tubes each containing the same concentration of purified inhibitor, (sufficient to cause 15 degrees positive curvature), the following substances were added: To the first test tube was added 2 cc. of water and 100 mg. of radish seed preparation; to the second was added 2 cc. of water but no radish seed preparation; to the third was added 2 cc. of water which contained 100 mg. of the radish seed preparation that had been heated for 10 minutes in a boiling water bath. To a fourth test tube was added 2 cc. of water and 100 mg. of radish seed preparation but no inhibitor. All four test tubes were put in an incubator at 37 C. for 18 hours. At the end of this time plain 1½% agar plates (1 x 6 x 8 mm.) were soaked in the solutions and after three hours they were cut into smaller blocks 1 x 2 x 2 mm. and the growth activity determined by the kymograph analysis method. The results are seen in figure 28.

Here it is clearly seen that the presence of the radish seed preparation prevented the inhibitor from changing into auxin instead of causing it to change as it was expected to do. This experiment has been repeated three times with the same results.

A similar test was made using the lipase preparation. This preparation, as with the radish preparation, showed no effect of increasing the hydrolysis of the in-

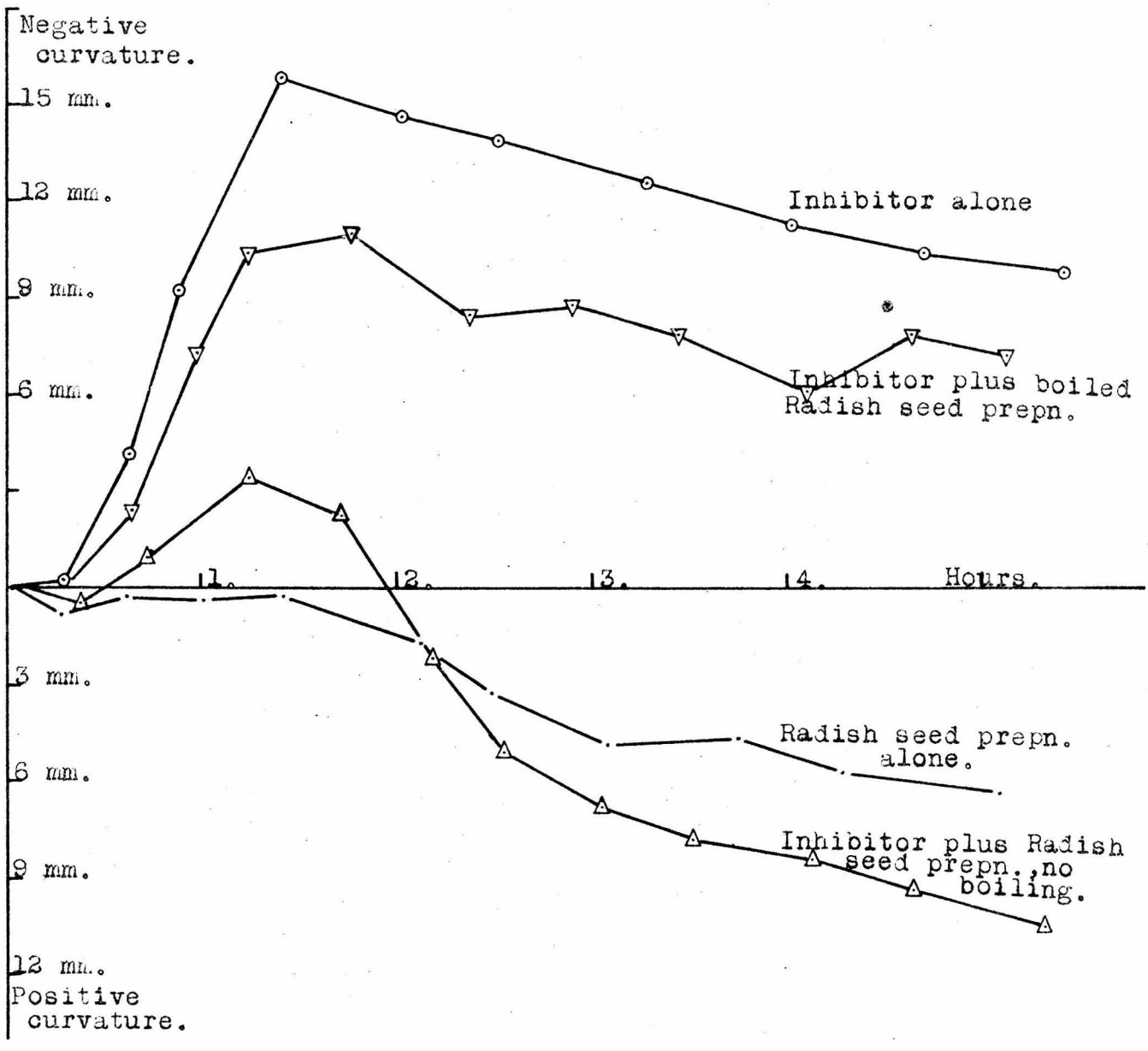


Fig. 28. Curvature rate of twice decapitated Avena coleoptiles to: 1.) Purified inhibitor alone; 2.) Purified inhibitor plus boiled Radish seed preparation; 3.) Radish seed preparation alone, and; 4.) Purified inhibitor plus Radish seed preparation, no boiling. All four solutions kept at 37°C. for 18 hours prior to testing. Each point is the average of 12 plants.

hibitor. In fact as in the radish seed preparation, just the contrary was found to be the case. In the second and third test tubes where the lipase preparation had been added, there was strong inhibitor activity, (although in the third test tube where the lipase preparation had been heated before being added, there was less activity than in the second test tube where it was added with no heating.) The first test tube, however, showed no inhibitor activity. The control on pure lipase preparation alone, likewise caused no curvatures. The conclusion is that the presence of the lipase preparation prevented the hydrolysis of the inhibitor.

C. Prevention of inhibitor hydrolysis by impurities in the extract.

It has also been found that when there are many impurities, chlorophyll, etc. in the crude extract, the inhibitor molecule is not readily hydrolysed, whereas when these have been removed it hydrolyses very easily. This was shown by the following experiment:

The crude inhibitor extract was prepared in the usual manner from radish cotyledons and on evaporating off the ether, a syrupy green mass of chlorophyll and fatty substances was obtained along with the inhibitor. This extract was divided into two portions. One portion was kept in the

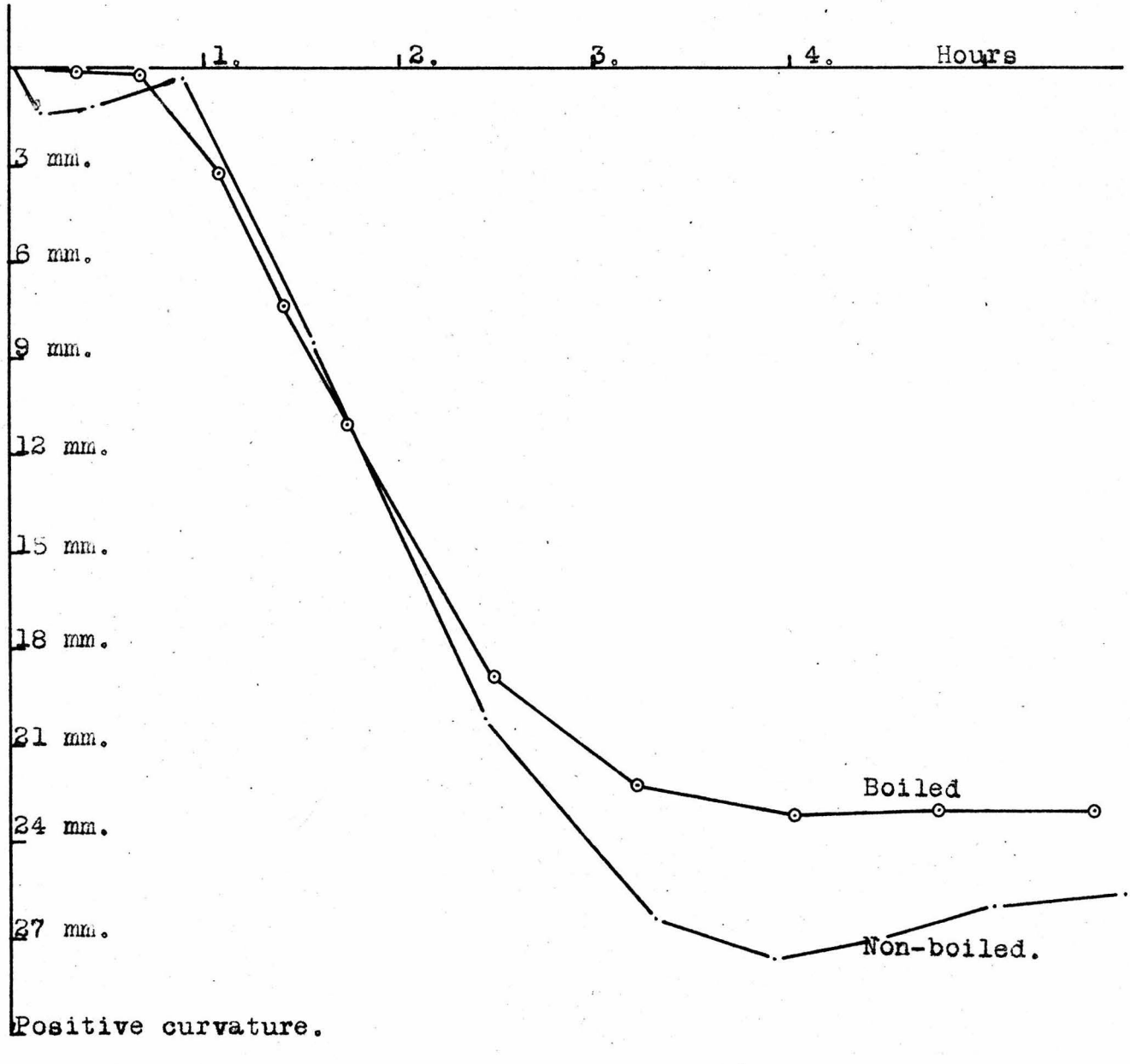
ice box overnight while the other portion was heated in a boiling water bath for 10 minutes and then kept at 37 degrees C. until the next day. Both solutions were then tested on the kymograph machine. The results, figure 29. showed that there had been no hydrolysis of the inhibitor in either case. The same type of experiment using purified inhibitor, with most of the extracted impurities removed, shows however, complete hydrolysis. See figure 28: - 1.

It is suggested that both in this experiment and in the experiments under section B, the inhibitor was prevented from hydrolysing by being adsorbed on the substances added to the inhibitor solution. In this last experiment these substances would be the extracted impurities.

D. Conclusions

From these last two sets of experiments the indications are that there is no lipase-like enzyme in the radish seed which hydrolyses the inhibitor and thus forms auxin. Moreover these experiments give neither an insight into the mechanism of growth inhibition nor an explanation of the action of large amounts of inhibitor in influencing any specific phase of the growth process. In view of the fact that it was found that inhibitor does not cause a redistri-

Fig. 29. Reaction rate of once decapitated Avena coleoptiles to crude inhibitor extract boiled 10 minutes, and with no boiling Each point is average of 12 coleoptiles.



bution of the food factors one must believe that it acts by affecting the growth process directly.

Chapter IX. Discussion and Conclusions.

A. Discussion.

From the previous work it is seen that the indications are that the inhibitor, as it occurs in the radish plant, acts in no manner as an inhibiting agent. It apparently only has the property of inhibiting growth when it has been extracted from the plant and concentrated to many times its natural concentration. In speculating as to what function, if any, that the inhibitor substance might have, one would thus rule out that of inhibition. Since, however, it was found that it is readily hydrolysed into auxin this may be an indication that the inhibitor can function as an auxin precursor. It must be borne in mind that the auxin formed from inhibitor is probably indole acetic acid and not auxin a nor b.

A discussion of the present status of the work on an auxin precursor is given in Phytohormones (1937). Here it is pointed out that evidence indicates there must be an auxin precursor which is able to be stored in seeds, , and move through the stem acropetally. A suggestion is made that this precursor might be an ester of auxin a inasmuch as such esters are in themselves inactive and yet could conceivably be hydrolysed

in the plant to form auxin. The presence of such esters in plants is also indicated. When, however, esters of auxin a are actually tested for growth activity they elicit no response. Esters of indole acetic acid on the other hand are able to undergo hydrolysis and do cause growth promotion.

Inhibitor has been seen to parallel the requirements set forth above for an auxin precursor. It is stored in seeds, and is able to move through stem tissue acropetally. It is also an ester w ich is readily hydrolysed in vitro into auxin. It seems to have a fairly wide distribution. It is, however, not a precursor of auxin a. At this point it may be well to point out that while the present evidence indicates that auxin a is the growth hormone found in higher plants the amount of evidence is rather meager for such a wide generalization. Furthermore the experiments presented in this paper show that the auxin diffusing from the radish cotyledons may be indole acetic acid. Against the viewpoint that inhibitor could act as precursor of indole acetic acid in the radish plant is the fact that when esters of indole acetic acid are tested in the Avena test they have never been recorded as causing any positive curvatures. These experiments were not done, however, with a view toward observing positive curvatures. In fact it has been found by Skoog (1936) that, while not an ester

of indole acetic acid, indole ethyl amine at a concentration of 1 mg./cc. is capable of causing positive curvatures of 5.4 degrees in 1.9 hours, and of 6.5 degrees in 6.1 hours. In 18 hours the curvature was still 6.0 degrees. Lower concentrations (1 mg. / 83 cc.) first caused positive curvatures of 2.9 degrees in 1.9 hours, which became negative curvatures of 5.2 degrees after 18 hours. This reappearance of a negative curvature was not found with inhibitor when the inhibited plants were observed 24 hours after their treatment. (Chapt. VI). This may have been due to the fact that the inhibitor concentration was so high that the plant was unable to form sufficient auxin to overcome the inhibition. This same effect may also be seen in the above example of the positive curvature with the high concentration of indole ethyl amine. Further growth curvature experiments should be performed with more dilute inhibitor concentrations than those used in Chapter VI. and observed over a longer period of time to see if the negative curvatures found by Skoog for indole ethyl amine might also appear with inhibitor.

It is safe to say that from the evidence presented in this work that inhibitor could act as an auxin precursor but until further evidence is obtained, even this point will have to remain an open question.

B. Conclusions.

From the experiments described in this work the conclusions regarding the inhibitor are:

1. That there is an ether extractable substance in the cotyledons and leaves of radish plants that is capable of causing positive Avena curvatures and growth inhibitions. Chapter I.

2. That it is possible to analyze for the amounts of this substance on a quantitave basis using the standard inhibitor test. Chapter II.

3. That the occurrence of this inhibiting substance is not restricted to the radish plant but that it is found in other plants as well. Chapter III.

4. That with the growth of radish leaves their inhibitor content decreases and is finally apparently absent being replaced by auxin. Chapter III.

5. That it has no acidic or basic groups and can be hydrolysed to yield auxin. Chapter IV.

6. That it is probably an ester of indole acetic acid with some unknown fraction. Chapter IV.

7. That the positive Avena curvatures may be completely explained on the basis of the amount of growth inhibition caused by the inhibitor. Chapter V.

8. That in the unpurified condition it has no effect on the growth of sections of Avena coleoptile and radish

hypocotyl. Chapter V.

9. That it has no polarity of movement in the radish or Avena plant. Chapter VI.

10. That it is possible to remove contaminating auxin from the crude extract by use of the "Inverse Transport" purification method. Chapter VI.

11. That it may be hydrolysed at cut surfaces to form auxin. Chapter VI.

12. That it is transported through Avena coleoptiles at approximately 11 mm. per hour, or the same rate as auxin. Chapter VI.

13. That it does not cause growth inhibition as a result of food factor redistribution. Chapter VIII.

14. That it is possible to prevent its hydrolysis into auxin in water solutions by the addition of impurities on which it apparently may be adsorbed. Chapter VIII.

15. That in the purified condition it is readily hydrolysed in water. Chapter VIII.

16. That in many tests for physiological activity it behaves like auxin, but it does not act like auxin and cause bud inhibition. Chapter VII.

17. That its molecular weight is possibly between 100 and 175. Chapter VII.

18. That it appears that the hydrolysis of the inhibitor in the radish cotyledons could account for the presence of auxin diffusing out of them. Chapter VII.

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Part 2. Extensibility of Cell Wall Material in Indole-3-acetic Acid.

Introduction

Since 1931 when Heyn (1931) pointed out that auxin increased the extensibility of the cell wall (including both plastic and elastic extensibility), the question of the mechanism of its action has been the center of much discussion. Two viewpoints on this subject are possible: either that the auxin acts directly on the cell wall or that it acts indirectly through the cytoplasm. The evidence for the latter viewpoint is well summarized by Went and Thimann (1937). On the other hand, Robbins and Jackson (1937) performed experiments which suggest that the action of growth hormone is on the cell wall directly. In these experiments it was found that when .2 per cent indole acetic acid in lanolin was applied to living or dead cell wall materials it caused an increase in their extensibility. It was also found that the same treatment decreased the extensibility of both fresh and dried root cell wall materials. However, they point out that the effects obtained are not necessarily specific for the growth hormone used (indole-3-acetic acid) as no controls of pH were used; and furthermore, since the water content was not known to be the same in the lanolins used, the results attributed to growth hormone may have partly been due to such differences. In view of these circumstances and because of the importance of theoretical conclusions regarding growth mechanisms which might be based on such data, the experiments were repeated

with as nearly identical material as possible. Several new experiments were also devised.

Experiments AND Results.

I. Twelve pieces of No. 8 cotton thread approximately 2100 mm. long were suspended in a dark room with a 120-gram weight attached to each. Three pieces were untreated, three were rubbed with lanolin only, three with .2 per cent indole-3-acetic acid in lanolin, and three with .2 per cent acetic acid in lanolin.

Four pieces of hemp, approximately 1000 mm. long and each consisting of six single fibrils, were subjected to the same treatment. To eliminate the possibility of differences in water content, lanolin from the same container was used to make the mixtures mentioned above. These mixtures were then used in all the subsequent experiments.

The increase in length of the material after three weeks is shown in table 1.

TABLE 1. *Lanolin treatments on cotton and hemp.*

Treatment	Cotton: % total length increase in 21 days			Cotton: Ave. %	Hemp: % total length increase in 23 days
No treatment	.41,	.38,	.14	.31	0.00
Lanolin only	.54,	.46,	.51	.50	0.00
Lanolin — .2% indole acetic acid	.60,	.46,	.41	.49	0.19
Lanolin — .2% acetic acid ..	.66,	.60,	.55	.60	0.18

The indications are that in various mixtures applied to cotton thread, lanolin alone is the cause of the increased extensibility, whereas in hemp the increased extensibility is due to the organic acid in the lanolin.

II. Strips of different materials were rubbed with lanolin, lanolin-.2 per cent (by weight) indole acetic acid, lanolin-.2 per cent acetic acid, and lanolin-.2 per cent water. They were 7.5-15 cm. long, depending on the material. Within any one group they were the same length. Each strip in turn was fastened in a horizontal position on a wooden block with 2.5 cm. of its base abutting on a piece of wood. The remainder of the strip extended horizontally and free in the air. A small lead rider was attached to the end of each piece, and the angle through which each strip bent from the plane of the wooden block was measured by a protractor. The pieces were then laid on a table, in diffuse light, at a temperature of 23 C. After a period of time the angle of bending of each strip was again measured. Except in the cases of the cardboard strips, the fresh Chenopodium album roots, and the Ambrosia psilostachya stems and roots, all the material had been previously dried in an herbarium press. The results are presented in table 2.

An inspection of these data shows that there are no constant, specific effects of any of the treatments for the material used.

TABLE 2. *Lanolin treatments on stem and root materials.*

Material	Number of strips	Hours of treatment	Average difference in degrees between original & final curvature (& stand. error) in: Lanolin	Lan-indole acetic	Lan-acetic acid	Lan-water
STEM:						
Ambrosia	5	12	.2 ± 1.2	—.2 ± .7	—.4 ± .9	.2 ± .4
Potato	4	30	0 ± .6	0 ± .4	0 ± 0	.25 ± 1.3
Cardboard	5	14	.6 ± .12	.8 ± .09	.2 ± .05	1.0 ± .5
ROOT:						
Carrot	5	34	7.6 ± 3.3	3.6 ± 3.0	3.4 ± 2.0	2.2 ± 1.0
Willow	4	30	1.7 ± 1.1	.25 ± .1	.25 ± .1	—1.0 ± .7
Chenopodium	1	17	3.0	1.0	4.0	0
Corn Branch	10	20	—.6 ± .8	—.1 ± .1	—.4 ± .6	.3 ± .5
Corn Brace	4	18	2.0 ± 3.3	2.8 ± .8	2.8 ± .9	2.5 ± .7
Ambrosia	4	13	4.5 ± 3.5	2.0 ± 3.4	4.5 ± 4.2	.3 ± 1.2
Onion	8	29	5.5 ± 3.0	5.1 ± 2.2	6.5 ± 2.2	6.6 ± 3.2
Pistia stratiotes	4	32	24.0 ± 7.7	24.0 ± 10.5	17.0 ± 9.0	27.0 ± 1.1
Ludwigia sp.	5	32	2.0 ± .7	3.2 ± 1.4	3.8 ± 1.6	7.0 ± 2.8
Pea	2	20	7.5	13.0	12.5	14.0

III. In view of the great variability found in the experiments carried out according to the methods of Robbins and Jackson (1937), it was decided to adopt other methods by which more consistent results might be obtained. It was desirable to have (1) a means of controlling the water content of the material used and (2) a more accurate method of measuring the extensibility. Accordingly the material was subjected to experimental conditions in water solutions, thus eliminating any differences in water content of the original material. The extensibility was measured accurately to .1 mm. by means of a horizontal microscope.

The apparatus consisted of a vertically mounted glass

tube, 60 cm. long and 1.6 cm. in diameter. It was possible to secure one end of the material under investigation at the bottom by means of a wire hook. The other end was fastened to a fine copper wire which ran up and over a pulley (having but little friction) and down to a pan for weights. Supporting the weight pan was a vertically movable stage mounted on a screw in order to lower the weight pan gradually and thus avoid a sudden application of the stretching force which otherwise might break the material. At the top of the glass tube a millimeter scale was mounted parallel to the copper wire. Any change in length of the material was read with the microscope by observing movement along the scale of an indicator which was perpendicularly attached to the wire. The bottom of the tube was equipped with a drain to facilitate changing solutions. The ramie, cotton, and artificial silk threads were secured with small loops to the wire hooks at the top and bottom. The root material was cemented to wire loops by means of hard De Khotinsky cement. These loops were then easily placed on the wire hooks in the tube.

Two-thread strands of ramie, 35 cm. long, and single strands of No. 8 cotton thread the same length were stretched by a 100-gram weight. The solutions used were water, 0.001 N HCl and .2 per cent indole acetic acid (pH 3.3). For ramie the results expressed as percentage total length increase after 1,000 minutes were: water, .75; .001 N HCl, .62; .2 per cent indole acetic acid, .60. The results for cotton are given for the first 10-minute period since only .5 per cent of the percentage total increase in length occurred during

the next 99 hours. They were: water, 2.78 per cent; 0.001 N HCl, 2.06 per cent; .2 per cent indole acetic acid, 1.98 per cent; .2 per cent acetic acid, 2.08 per cent. Because of the slight extensibility of these materials, more statistically significant data were not sought. From these values there is no indication of any specific increase of the extensibility due to pH or the presence of indole acetic acid.

IV. Since ramie in particular is known to have nearly perfect orientation of the cellulose micelles parallel to the longitudinal direction of the fiber axis, it was thought desirable to investigate some material with less complete micellar orientation. For this purpose artificial silk (regenerated cellulose) was used. The strands consisted of two threads, each of which was composed of fifty fibrils. They were stretched according to the manner described above. The results are presented in table 3.

TABLE 3. *Extensibility of artificial silk in various solutions.*

Solution	Average percentage total length increase in 10 minutes with stand. error
Water	13.5 ± .2
0.001 N HCl, pH 3.0	13.7 ± .2
.2% Acetic acid, pH 3.3	16.1 ± .5
.2% Indole acetic acid, pH 3.3	16.2 ± .4
.1% Trans Cinnamic acid	15.4 ± .1
.2% Oxalic acid	15.6 ± .4
.2% Propionic acid	14.5 ± .3

The figures show that indole acetic acid does increase the extensibility of artificial silk, but the effect is not specific, as shown by the fact that other organic acids

act similarly.

This phenomenon can not be explained on the basis that the organic acids esterify the free hydroxyl groups on the 2,3,6 carbon atoms of the glucose rings of the cellulose of artificial silk. This is shown by the following experiment: Strands of artificial silk were weakly acetylated by treating with acetic anhydride. Their extensibility was determined by stretching them with a 100-gram weight for 10 minutes. It was found to be 15.6 per cent, or within the same range as that for .2 per cent acetic acid. Furthermore, allowing the artificial silk to soak in water, 0.001 N HCl, .2 per cent acetic acid, or .2 per cent indole acetic acid for 21 hours and then stretching for 10 minutes gave values similar to those obtained above. They were as follows (data as percentage total length increase): water, 13.4; 0.001 N HCl, 13.9; .2 per cent indole acetic acid, 16.2; .2 per cent acetic acid, 16.2.

It may be concluded that certain non-growth-promoting organic acids increase the extensibility of artificial silk over that in water or in .001 N HCl. The solutions are effective only during the time the stretching force is acting. This is shown by the fact that pretreatment with the solutions in question is without effect.

V. Roots, 30 cm. long, from an onion bulb growing in water and from the water plants Pistia stratiotes and Ludwigia sp. were thoroughly dried in an herbarium press. The roots were less than 2 mm. in diameter. They were stretched (with

equal forces) in water, in .2 per cent indole acetic acid, and in .2 per cent acetic acid. The results, in the order noted for the solutions, were (as percentage total length increase in 10 minutes): onion, 2.52, 3.10, 3.93; Pistia stratiotes, 4.20, 4.68, 4.64; Ludwigia sp., 8.70, 9.80, 12.20. It may be seen that there is no indication that indole acetic acid has caused a decreased extensibility of root walls.

Fresh water cultured onion roots were stretched in the manner described in section III. A weight of 20.8 grams was used. The roots were selected for uniformity of diameter and were about 13 cm. long. Since it was found that the slope of the extensibility-time curve was constant after the first 77 minutes, the readings presented were taken at that time. The results (expressed as percentage total length increase with standard error) were: water, 3.97 .09; .2 per cent acetic acid, 6.60 .68; .2 per cent indole acetic acid, 8.50 1.50. From these data it is clear that the root material in .2 per cent indole acetic acid and in the .2 per cent acetic acid had an increased extensibility over that in water. As the values obtained for the acetic acid and the indole acetic acid do not show a significant statistical difference, the effect observed is probably a general one for organic acids at pH 3.3.

DISCUSSION.

It was found difficult to draw definite conclusions regarding cell-wall extensibility based on experiments in-

volving dried stem and root materials with lanolin applied to them. The results of such procedures showed variability and inconsistency. This was probably due to individual differences in the materials. With an improved technique, in one case (that of artificial silk) statistically significant differences were obtained. If this be accepted as an example of stem cell wall material, then .2 per cent indole acetic acid does cause a small increase in the extensibility, but likewise so do other organic acids known not to be growth-promoting substances in plants.

No indication of a decreased extensibility of root wall material as suggested by Robbins and Jackson (1937) was found. On the contrary, it was observed that root material had an increased extensibility in .2 per cent indole acetic acid as well as in .2 per cent acetic acid. An "acid effect" has been noted by Bonner (1934) who found that Avena coleoptiles at a pH of 4.1 have a much greater plasticity than those at a pH of 7.1.

While the results given in this paper are concerned with concentrations (.2 per cent or 1.14×10^{-2} M.) of hetero-auxin much higher than exist physiologically, it is interesting, nevertheless, to compare them with the effects of the lower ones. Auxin at a physiological concentration has been shown by Heyn (1931, 1934) to increase the plastic extensibility of Avena coleoptiles and Lupinus hypocotyls. Söding (1933) has found a similar action of physiological concentrations of auxin on flower stalks. Amlong (1937) has observed

that a concentration of hetero-auxin between 10^{-8} to 10^{-6} M increased the extensibility of roots of Vicia Faba. Concentrations of 10^{-6} to 10^{-3} M caused a decrease in their extensibility, while a concentration of 10^{-2} M was found, in agreement with the work presented here, to result in an increased extensibility of non-living roots.

SUMMARY

The experiments of Robbins and Jackson (1937) concerning the extensibility of stem and root walls were repeated in as nearly an identical manner as possible and found to give no conclusive evidence as to the effect of growth hormone upon them.

Artificial silk was found to have an increased extensibility in certain organic acids known not to be growth substances, as well as in .2 per cent indole acetic acid.

Onion roots were found to have an increased extensibility in a .2 per cent indole acetic acid and in .2 per cent acetic acid.

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