SOME PHYSIOLOGICAL FUNCTIONS OF THE GROWTH HORMONE IN HIGHER PLANTS

Thesis by

Folke Skoog

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CONTENTS

The work to be presented deals with some physiological actions of the growth hormone in higher plants. It comprises three different problems in relation to the formation, some physiological functions and the inactivation of hormone in the plant. Although the subject matter of each problem in many respects overlaps on to that of the other two, the material will be presented, closely in the order in which the experiments were done, in three main parts as follows:

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PART I

THE INHIBITION OF LATERAL BUD DEVELOPMENT BY THE GROWTH HORMONE

Chapter I

Bud Inhibition produced by the terminal bud and by applied auxin

Introduction

It is well known that the growth hormone of plants, auxin, promotes the elongation of cells of many plant organs. Furthermore, the work of Went (1928) and Dolk (1930) on Avena has definitely shown that auxin is essential for elongation. From the large number of species that have since been investigated, it may be concluded, although it has not been definitely proved in every species investigated, that the statement "Ohne Wuchsstoff kein Wachstum" applies generally to higher plants. More recent work has shown that auxin, in addition to this primary action of promoting elongation, also is active independently, but perhaps in a more indirect manner, in other growth processes of the plant. Of these "secondary" activities of auxin, the inhibition of lateral buds to be described here was perhaps the first one to be discovered.

When the growing point of a young dicotyledonous plant is removed, the dormant lateral buds lower down the stem begin to develop, but as long as the terminal bud is present the lateral buds remain ~~present~~ dormant. The presence of the terminal bud thus exercises an inhibiting action on the development of buds. To a smaller extent the leaves also inhibit the buds in their axils, as was shown by Dostal (1909) in his experiments on Scrophularia.

This phenomenon of bud inhibition has been studied by many workers. For a brief perspective of the results of this work they may be divided into three main groups according to the kind of interpretation they offered as to the nature of the primary cause of inhibition. But it is clear that such classification is arbitrary, and the views of the individual investigators can be only approximately expressed.

One group claims that the inhibition of buds is due to the lack of food caused by its rapid utilization in the apical part of the main shoot of the plant. Although it is hard to find the origin of this view, it is certainly the oldest one and has ~~has~~ been held since the last century. It has also been advocated in the recent literature as, for example, by Gardner (1925) and Moreland (1934). A second group claims that a higher metabolic activity of the apex which induces an electrical polarity is directly (not through an effect on the transport of substances) responsible for bud inhibition. A third group, finally, claims that there is a special inhibiting substance formed in the apex of the main shoot, which prevents the development of buds further down the stem.

According to Denny, this concept of bud inhibition through the action of a hormone coming from the tip was first brought forward by Errera (1904). It has since been advocated by several investigators, notably by Loeb and by Snow (1925). Snow (1929) found that for example in Pisum the rapidly growing young leaves in the terminal bud are the

most active in inhibiting the growth of the axillaries. In this connection he brought forward evidence, and this among the first of its kind, that the inhibition of bud development might be due to a special substance capable of being transported basi-petally from the growing point throughout the length of the stem of the plant.

Statement of the problem.

Partly in view of the above experiments, Dr. Thimann had reasons to believe that the inhibiting action was due to auxin or at least to a substance closely analogous to the growth hormone. Thus, with his cooperation, experiments were started in the fall, 1932, to determine the presence and distribution of the growth hormone in a suitable dicotyledonous plant, Vicia Faba, and to determine its physiological action in the plant especially in relation to inhibition of bud development. These experiments which definitely prove the inhibition of bud development to be caused by the growth hormone by an action independent of its promotion of growth will now be described.

In order to show that the growth hormone is really the inhibitor of lateral bud development, it must be shown in the plant studied, that:

1. growth hormone is produced by the terminal bud;
2. the lateral buds, as soon as their development begins, also produce it in appreciable amounts;
3. growth hormone is produced in the leaves; and
4. application of growth hormone, after removal of the terminal bud, represses the development of the lateral buds to the same extent as does the terminal bud in intact plants.

Material and Methods

In the earlier experiments young plants of Vicia Faba, grown in the light; in later experiments young plants of Pisum Sativum grown either in the light or in the dark, were used. These plants were both used by Snow, and they are very well adapted for these experiments. For inhibition experiments, the plants were selected to be of approximately equal height, and with as nearly as possible equal numbers of buds and leaves. A typical experimental plant (Pisum) is shown in figure 1.

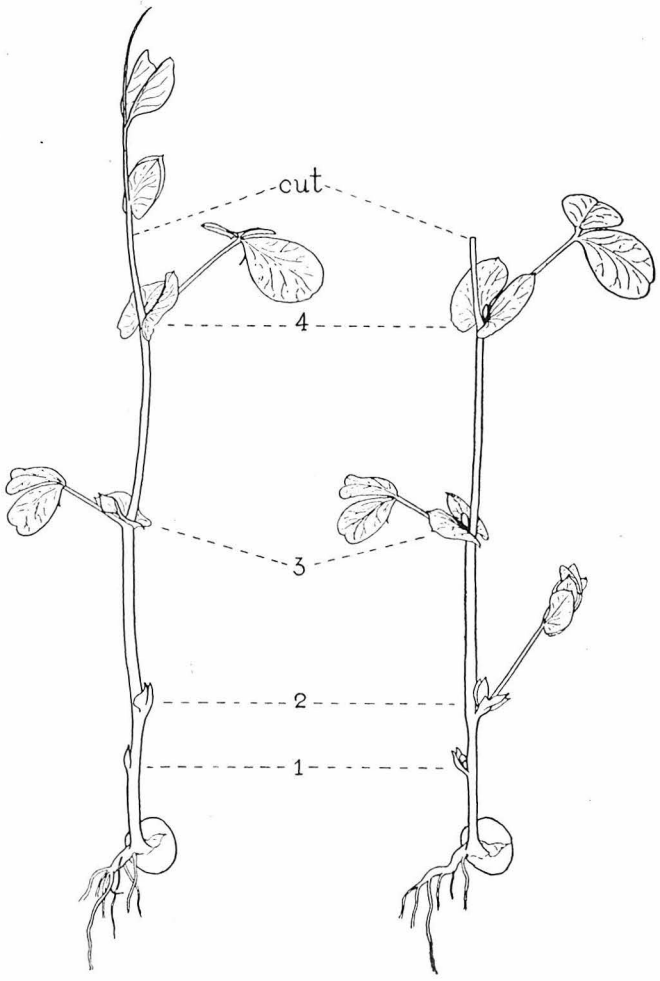


FIGURE 1

In the earlier experiments the growth hormone used was obtained from the growth of Rhizopus suinus. It was prepared from the ether extract of the concentrated and acidified culture medium, by a process involving chilling, fractional extraction at different pH, and vacuum distillation. Its activity was of the order of 12,000 units per milligram, representing a purity of about 5 to 10%, since pure synthetic hetero auxin has activities varying with the time of the year between about 1 to 3×10^5 units per mg. under the standard conditions of testing on Avena described by Went (1928), and using the procedure, size of agar blocks (10.7×10^{-3} ml per block) etc. as given by Dolk and Thimann (1932). In later experiments synthetic hetero auxin prepared by Thimann and Koepfli (1935) and in a few experiments crystalline preparations of auxins A, B, and hetero auxin prepared and kindly submitted by Prof. Kögl were used.

A. Growth hormone in the Plant

1. Production of growth hormone by the terminal bud of Vicia.

In order to determine whether growth hormone was produced by the terminal bud of Vicia Faba, young healthy plants of various ages were decapitated 1 to 2 mm. below the bud, and the bud was then placed upon a block of agar (1.5%) for 4 hours; good contact between the cut surface and the agar block was obtained by moistening the cut surface with either water or gelatin. The bud and agar block were kept at 25°C. and in a covered vessel lined with moist filterpaper to prevent drying out. The agar blocks were then divided into 12 pieces in the standard manner and tested on Avena coleoptiles.

Table I.—Production of Growth Substance by Terminal Bud.

Height of plant	Size of block	Curvature obtained	Plant units diffusing out per hour
(cm.)	(× standard block)	(degrees)	(p.u.)
12·1	12	11·7	35·1
12·4	12	14·0	42·0
13·3	12	11·6	34·8
17·8	6	12·8	19·2
18·8	6	14·0	21·0
20·8	6	10·7	16·1
20·8	6	10·5	15·8
22·3	6	11·1	16·7
37·3	6	0	0

It can be seen from Table I that the amount of growth hormone produced by the terminal bud is considerable, although it varies widely with different plants. In general, the older the plant, the less is the production of growth hormone. Since the production (i.e. at least the amounts obtainable by the technique used) also varies with the season, the experiments were all carried out within a few weeks in the early spring. In the last column of the Table are given the number of plant units produced by the terminal bud per hour, a figure to which reference will be made later. No claim for absolute accuracy is made for these figures; they represent merely the order and the relative amounts of growth hormone diffusing out from the tips of these plants at the particular ages and season.

2. Production of growth hormone by lateral buds.

Since, as will be shown below, developing lateral buds exercise an inhibiting action, while dormant ones do not, determinations of the production of growth hormone by lateral buds when undeveloped and when rapidly growing were made. For this purpose buds of the former type were removed from plants which had been decapitated 10 days previously,

and of the latter type from intact plants. Since the amounts of growth hormone were small, agar blocks of one third the standard size were used, each being subsequently divided into 4 parts for testing. The results, given in Table II, indicate that there is little or no production of growth hormone by the undeveloped laterals (3.5 mm. in length) but when they become longer (21.5 mm.) the production reaches about one half that in the terminal bud.

Table II.—Production of Growth Substance by Lateral Buds.

	Average length of bud in mm.	Time of diffusion in hrs.	Curvature obtained, in degrees, each bud being placed on agar block giving 4 standard *Avena* blocks.							Average number of plant units diffusing out per hour.
			1	2	3	4	5	6	Mean.	
Undeveloped buds	3·5	3·2	0·5	0·5	4·0	0·5	0·3	1·7	1·25	1·6
Developing buds	21·5	3·0	12·0	16·0	8·0	17·5	14·0	19·0	14·4	19·2

The production of growth hormone in the bud of Pisum is completely analogous to that in Vicia, with the exception that the amounts produced per plant are, as would be expected, smaller. For data relative to growth hormone production in Pisum see Table XXV part II.

3. Production of growth hormone by the leaves.

The experiments of Dostál (1909) showed that in a great number of species of plants, when cut sections containing two opposite leaves and their axillary buds were placed in water the leaf inhibited the development of the bud in its axil. In later experiments he further showed that the inhibition by the leaf was only sufficient to disturb the balance in the rate of growth of the two buds, so that of two

oppositely placed buds one in the axil of an amputated leaf would start to develop first, and the subsequent inhibition of the second bud in the opposite axil with an intact leaf was caused principally by the developing bud in the axil with amputated leaf. If the growth hormone is the inhibitor it should be found that it is produced by the leaves in smaller quantities than by the developing lateral buds, providing that the conditions in Vicia are not essentially different from those obtaining in Scrophularia. This was in fact found. The amount of growth hormone produced is roughly in inverse proportion to the age of the leaf. Thus in one set of plants the production of growth hormone measured by diffusion into agar from a number of comparable leaves bound together for each determination was in 4 hours:-

Lowest leaf (oldest)	3.8	plant	units
Second "	3.0	"	"
Third "	15.0	"	"
Fourth " (youngest)	21.6	"	"
Terminal Bud (average)	120	"	"

These results are also in agreement with the observation that the buds in the axils of the lowest leaves are, in Vicia, the only ones which show an appreciable development. The amount of growth hormone produced by a leaf in one hour is thus much less than that produced by a developing lateral bud (cf. Table II), and, if growth hormone is the inhibitor, the inhibition in Dostál's experiments can be ascribed to the amount of this substance produced first by the leaf, and later by the developing bud. Also in agreement with the theory that auxin inhibits buds is the fact that in Vicia and in Pisum, normally the buds in the stipules

are the ones to develop and only very rarely the buds in the lower leaf axil may develop instead and correspondingly in stipules no appreciable amounts of growth hormone could be found. The above evidence relative to the presence and production of auxin in the plant together with the facts that the hormone is completely polarly transported in the plant, especially in the apical portions of the stem, but perhaps to a slightly less extent in the lowest internodes, is thus in complete agreement with the conditions one would expect to find, if the hormone is active in bud inhibition.

B. The effect of applied growth hormone on the development of lateral buds.

1. Entrance of applied hormone into the plant.

From the data in Table I, the amount of growth hormone diffusing from the terminal bud of young plants into agar is of the order of 30 to 40 plant units per hour. Experiments were therefore performed to determine whether growth hormone would diffuse to a corresponding extent from an agar block into the stem. A number of plants were decapitated, and an agar block, containing a known amount of growth hormone, was applied to the fresh cut surface of each stem, the plants being kept at 25°C. in the dark. Contact was ensured by the application of a film of gelatin or of water between the block and the cut surface. After definite times the blocks were removed and the amount of growth hormone remaining in them was determined by application to Avena coleoptiles in the usual way. The results, expressed as the per cent of growth substance entered, summarized in Fig. 2, show that the rate at

Figure 2

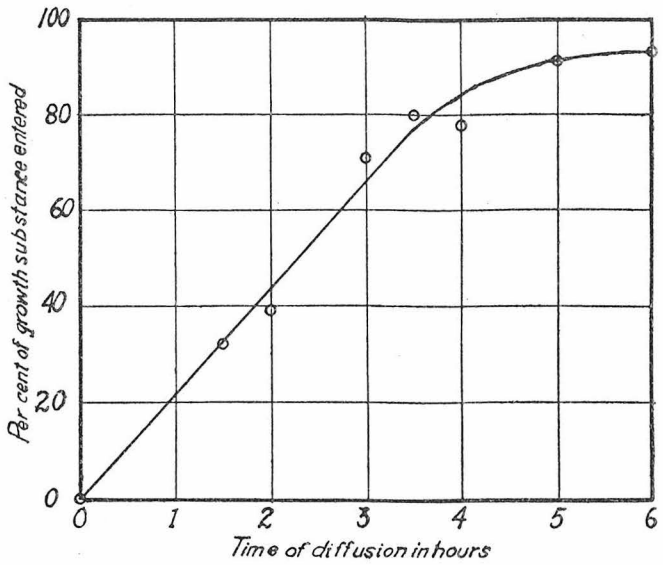


FIG. 1.—Per cent. of initial concentration of growth substance diffusing out of standard agar blocks into stems of *Vicia*.

which the growth substance enters the plant falls off with time. This is to be expected from the fact that the rate of diffusion into the plant is proportional to the concentration of hormone in the agar block at any moment, as previously shown by Thimann and Bonner (1932). After a period of contact of 6 hours, 93% of the growth hormone applied has passed from the block into the stem. The intermediate points of the curve are somewhat irregular, but the diffusion in 6 hours is the mean value obtained in seven experiments, the lowest and highest values of which were 87 and 99% respectively. The application of a fresh agar block containing growth hormone to the plant every 6 hours should thus result in an almost complete uptake of the growth hormone contained in the block, and at the same time should provide a fairly continuous supply of hormone to the plant. Furthermore, this method of application is more quantitative than that employed by Uyldert (1931) and that later used by Laibach and by Müller (1935), and therefore, for this purpose, preferable.

2 The effect of applied hormone on inhibition of lateral buds

Having determined the approximate quantity of growth hormone diffusing out of the terminal bud, we were now in a position to apply this quantity of auxin in place of the terminal bud, and to determine its effect on the development of the lateral buds.

Attempts were first made to carry out these experiments in the dark room at 25°C. and under high, controlled humidity (89%), but during the rather long period required for the experiments the plants became etiolated, were readily infected and were not very satisfactory. Subsequent experiments on Vicia were therefore carried out in the light at 15° to 25°C.

For measurements, the two lowest lateral buds in the stipules, Buds 1 and 2, (see Fig. 1) were selected, firstly because they are free from any inhibiting influence exerted by adjacent leaves, and secondly, when the plant is decapitated, it is these buds which develop first and grow most markedly. The buds higher up the stem, but very seldom higher than those in the axil of the lowest leaf, may also occasionally develop, but do so somewhat later. To facilitate measurements the stipules were removed. The small wounds so produced were covered with a thin layer of paraffin. The plants were grown individually in pots, placed in large trays, in the bottom of which was a layer of water to ensure equal water supply for controls and experimental plants. The plants were selected to be of equal size and vitality (average height 15 cm.), to have the same total number of leaves, and especially to have buds of comparable size. The average length of the

measured buds at the beginning of the experiment was 5 to 7 mm. Plants with buds which had already begun to develop were not used, because, as shown above, such buds are actively producing growth hormone. In a few cases the buds that had developed were removed, and the secondary buds always present at the base of a developing bud were used. Such plants were distributed equally between the controls and the experiments.

The plants were divided into three groups, from two of which the terminal buds were removed. To the smooth cut surface of these were applied agar with and without auxin. The blocks were renewed every 6 hours, this being the time required for nearly complete utilization of hormone as found above. The cut surface was moistened with water to ensure good contact with the block. To prevent drying out the block and the cut surface were completely sealed by a layer of soft paraffin. A mixture of 3 parts of paraffin with a melting point of 50°C. to 1 part of vaseline was brought to the melting point (about 40°C.) and allowed to solidify as a coating around the block and the cut surface, care being taken to prevent the paraffin from coming between the block and the surface of the plant. From the third group of plants the stipules where buds were to be measured were removed, but the terminal buds were left in place.

Measurements were made, each successive day at the same time, of the total lengths of the plants and of the two lowest lateral buds. At the conclusion of each experiment some of the other buds which had gradually developed to a smaller extent were also measured.

After some preliminary trials an experiment (No. 3) was carried

out in which 21 plants were selected. Five were kept undecapitated; the remaining 16 plants were decapitated. On eight of these plants were placed plain agar blocks, on the remaining eight plants were placed agar blocks containing 150 plant units of auxin, i.e. an amount about the same as that diffusing out from the terminal bud in 6 hours. The means of the daily measurements of the lengths of the buds are plotted in Fig. 3.

Figure 3

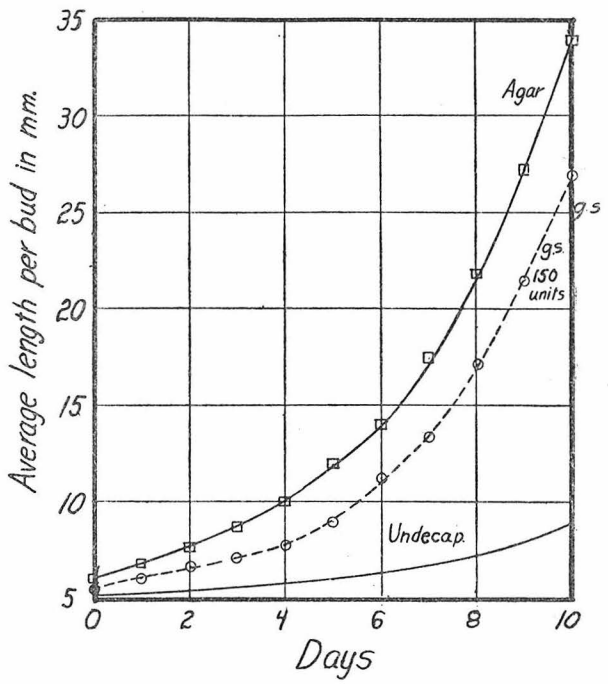


FIG. 3.—Average daily growth of lateral buds in experiment 3.

The mean increase in length of the lateral buds on the plants supplied with growth substance is less than that of the buds on the plants supplied with plain agar, but in each set the growth of the buds was more than that of the buds on the intact plants. There is thus some inhibition, but it is not complete. (See page 16) During the

experiment the buds in the axils of the lowest leaves, and also some secondary buds at the base of those measured also developed to some extent. At the conclusion of the experiment, 10 days after decapitation, there were 20 such buds in the plants to which growth substance had been applied; these had an aggregate length of 95.1 mm., or an average length of 4.8 mm. each; there were 22 similar buds in the plants supplied with plain agar, having an aggregate length of 137.2 mm. or an average length of 6.9 mm. per bud. Thus these buds had also been inhibited by the growth hormone treatment to about the same extent as the more rapidly growing ones. Since these buds were mostly too small to be measured at the beginning of the experiment, their actual increase in length cannot be given; however, if their average initial lengths are taken to be as high as 1 mm., the inhibition by the hormone is 33%. It can thus be seen that an amount of auxin equal to that diffusing from the tip into agar is enough to retard, but not completely to prevent the development of the lateral buds. In order to find whether the application of larger amounts of hormone would lead to a more complete inhibition, a similar experiment (No. 4) was carried out in which about 10 times the amount of hormone, namely, 1670 plant units per block were used. The details of the experiment were exactly as described for Exp. 3. Thirteen plants were selected and placed in corresponding groups 3, 5, and 5 respectively. From the data, given in some detail in Table III, it can be seen that the inhibition is now much more complete, and is in fact even greater than that in the undecapitated controls. The difference between the two groups is small, but especially by taking

into account the closely similar growth rates of the buds in each group in other experiments it appears to be significant.

Table III.—Growth of Lateral Buds in millimetres (experiment 4).

	Plant No.	Bud No.	Length of bud at— Start.	4 days.	8 days.	Total length increase of bud.	Mean length increase per bud.
Undecapitated controls	A	1	7·4	8·5	10·6	3·2	3·4 ± 0·5
		2	3·2	3·2	5·3	2·1	
	B	1	8·5	11·6	12·7	4·2	
	C	1	9·5	10·6	13·7	4·2	
Decapitated, 1670 units g.s. applied	1	1	4·2	4·2	5·3	1·1	1·8 ± 0·6
		2	3·2	3·2	3·2	0	
	3	1	5·3	5·3	5·3	0	
		2	3·2	5·3	6·4	3·2	
	5	1	3·2	4·2	5·3	2·1	
		2	4·2	6·4	9·5	5·3	
	7	1	3·2	3·2	6·4	3·2	
	9	1	3·2	4·2	4·2	1·0	
		2	7·4	8·5	8·5	1·1	
Decapitated, plain agar applied	2	1	7·4	10·6	23·3	15·9	16·0 ± 2·7
		2	9·5	15·9	34·9	25·4	
	4	1	6·4	7·4	18·0	11·6	
	6	1	3·2	5·3	9·5	6·3	
		2	3·2	6·4	10·6	7·4	
	8	1	8·5	11·6	24·3	15·8	
	10	1	5·3	10·6	31·8	26·5	
		2	6·4	9·5	25·4	19·0	

The mean values of the daily bud measurements of experiment 4 are plotted in fig. 4 which also includes measurements made five days after the termination of the experiments on the plants treated with auxin. These showed an immediate increase in rate of elongation of the buds as soon as the application of auxin was stopped indicating that the buds were in no way damaged and were still fully able to develop.

Figure 4

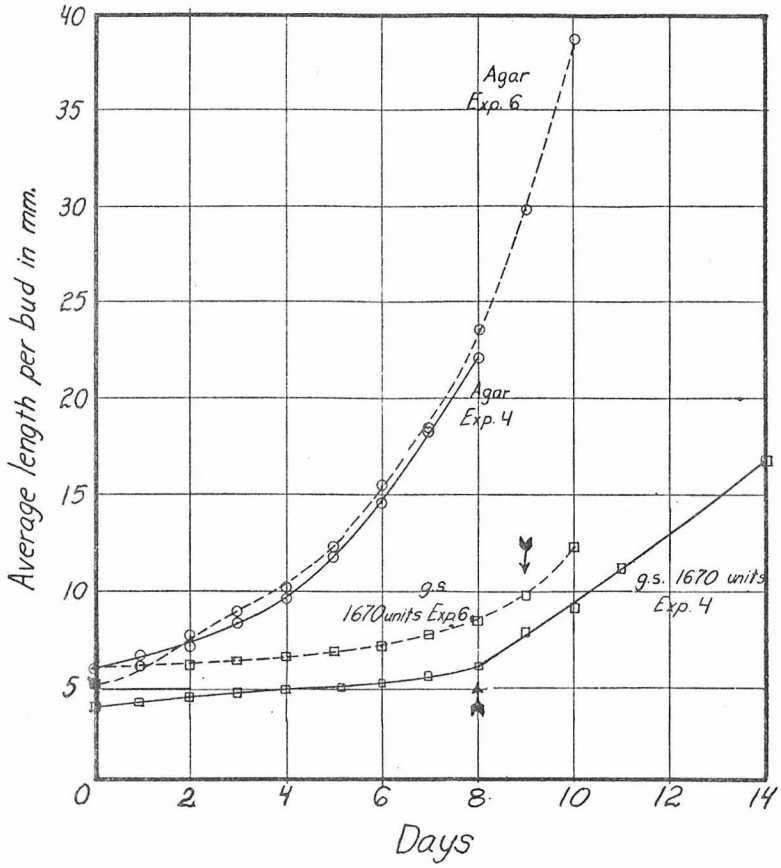


FIG. 3.—Average daily growth of lateral buds in experiments 4 and 6. Application of growth substance was stopped at arrow. For undecapitated controls see fig. 2.

Comparison of the average growth per bud per day in the agar treated plants of figs. 3 and 4 shows that the growth rates are almost identical. This indicates that with plants of the same age and size, results can be duplicated very closely, and small inhibiting effects may be considered significant. It should be noticed that the rate of growth of the buds is rather faster than logarithmic. The so-called "compound interest law" is therefore not entirely true for the development of these buds.

For confirmation, the experiment was repeated. The results of this experiment are given in detail in Table IV, and the mean values

Table IV.—Daily Growth of Lateral Buds in millimetres (experiment 6).

	Plant No.	Bud No.	Lengths of buds at the end of each day. 0	1	2	3	4	5	6	7	8	9	Total length increase of bud.	Mean increase in length per bud.
1670 units g.s. applied	1	1	5·5	6·0	6·0	6·0	6·0	6·0	7·0	7·5	9·5	12·0	6·5	3·7±0·7
		2	2·5	2·5	2·5	2·5	2·5	3·0	4·5	4·5	7·5	8·0	5·5	
	3	1	10·0	10·0	10·0	10·0	10·0	10·0	10·0	10·0	10·0	12·0	2·0	
		2	7·5	7·5	7·5	8·0	8·5	8·5	9·0	9·0	9·0	10·5	3·0	
	5	1	8·0	8·5	8·5	9·0	9·0	9·0	9·0	10·5	12·0	13·0	5·0	
		2	3·0	3·0	3·0	3·0	3·0	3·0	3·0	3·0	3·0	3·0	0	
	7	1	4·0	5·0	5·0	5·0	5·0	5·0	5·0	5·5	6·0	7·0	3·0	
		2	4·0	4·0	4·0	4·0	4·0	4·0	4·0	4·0	4·0	4·5	0·5	
	9	1	5·0	6·0	6·0	6·0	6·0	7·0	8·0	8·5	9·5	10·0	5·0	
		2	2·0	2·0	2·0	2·0	2·5	2·5	3·0	4·0	4·5	5·5	3·5	
	11	1	11·0	11·0	11·5	11·5	11·5	11·5	12·0	14·5	16·0	19·0	8·0	
		2	5·5	5·5	6·0	6·0	6·0	6·5	7·0	9·0	9·0	12·5	7·0	
	13	1	14·0	14·5	14·5	14·5	14·5	15·0	15·5	15·5	17·0	17·0	3·0	
		2	3·5	3·5	3·5	3·5	3·5	3·5	3·5	3·5	3·5	3·5	0	
plain agar applied	2	1	2·0	3·0	4·5	7·0	7·5	8·5	10·0	14·0	17·0	20·0	18·0	24·2±2·7
		2	4·0	5·0	6·0	7·0	7·5	10·0	16·0	20·0	25·5	34·0	30·0	
	4	1	7·0	8·0	9·5	13·0	14·0	16·5	20·0	24·0	32·0	38·5	31·5	
		2	4·0	5·0	6·5	8·5	9·5	12·0	15·0	19·0	24·5	31·5	27·5	
	6	1	5·0	6·5	7·5	8·5	9·5	10·0	10·0	10·0	11·0	11·0	6·0	
		2	9·0	10·5	12·0	14·0	17·0	20·0	23·0	29·5	36·5	45·0	36·0	
	8	1	4·0	4·5	5·5	6·5	6·5	9·0	12·0	14·0	17·0	18·0	14·0	
		2	4·5	5·0	6·0	6·5	8·0	10·0	13·0	16·5	21·5	34·0	29·5	
	10	1	5·0	6·0	6·0	7·0	7·5	8·5	10·0	12·0	16·0	20·0	15·0	
		2	2·0	3·0	4·5	5·0	5·5	6·5	9·0	9·0	10·0	11·0	9·0	
	12	1	6·0	8·0	8·5	9·0	9·0	10·0	12·0	13·0	17·0	23·0	17·0	
		2	9·0	10·5	11·0	12·5	14·0	17·0	22·5	27·0	34·5	43·5	34·5	
	14	1	5·0	7·0	7·5	9·0	9·5	12·5	15·0	19·5	25·0	32·0	27·0	
		2	7·0	8·5	11·0	12·5	15·0	18·0	23·0	30·5	40·0	51·0	44·0	

Bud No. 1 is the lower lateral bud, No. 2 the upper.

are included as dotted lines in fig. 4. They clearly confirm the previous results. The buds of the plants supplied with auxin had a slightly larger average initial size than the others, so that in this case the inhibition is the more striking. It is interesting to note that although the plants of this experiment were more rapidly growing, as shown by their stem elongation measurements, the growth rates of the buds are closely comparable with those of experiment 4, for, from a length of 7.5 mm. the two curves are parallel. At the conclusion of

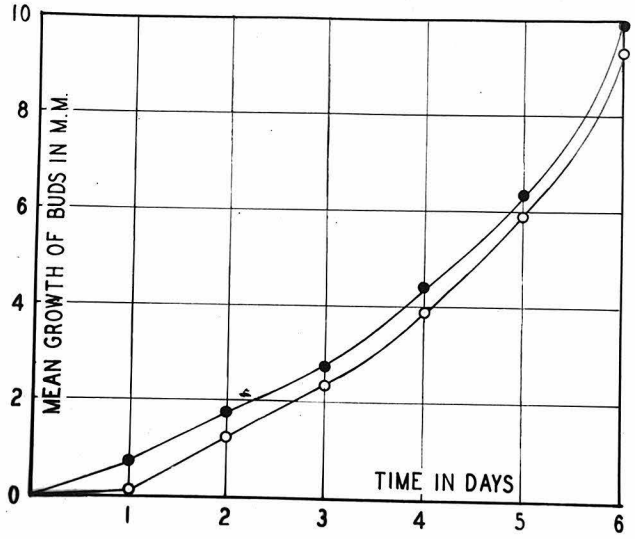
this experiment (8 days), the average increment in length of a lateral bud in the agar treated plants was 24.2 ± 2.7 mm.; in the plants with auxin 3.7 ± 0.7 mm. By treatment with auxin, therefore, the growth of the buds on these plants was made just about equal to that of the buds of the undecapitated controls of fig. 3 and Table III. Further, the buds in the axils of the lowest leaf averaged 6.6 mm. in the agar plants and 0.9 mm. in the auxin plants, while the buds at the side of the upper of the two main lateral buds averaged 5.1 and 2.2 mm. respectively. Both the latter types of buds therefore showed a very marked inhibition by the applied growth hormone.

3. Necessity of continuous supply of hormone

In another experiment, an attempt was made to apply double the concentration of hormone at half the frequency; i.e., 3000 units every 12 hours, to plants kept in the light. However, as shown in fig. 5, the plants thus treated showed a marked inhibition only on the first day. Since it was shown above that the bulk of the applied auxin enters the plant within 6 hours, it is likely that the buds were during the second six hours of every twelve hour period receiving very little auxin. On the basis of the mechanism of the inhibition suggested below, this means that at each six hour interval the buds were given an opportunity to synthesize auxin. Thus the inhibition became less daily, until in four days the corresponding growth rates of buds on both sets of plants were equal. It follows that for effective inhibition the supply of auxin from the apex must be continuous.

Figure 5

Bud inhibition in Vicia
(3000 plant units applied every 12 hours)



4. Inhibition of one lateral bud by another.

It was shown by Dostál, as mentioned above, that a rapidly developing lateral bud may inhibit the growth of a second bud. In Vicia we have found that a lateral bud immediately below and rarely immediately above the rapidly developing bud in a stipule is strongly inhibited. In ten decapitated plants the development of the two lowest lateral buds was measured over a period of five weeks, the means of the initially longer and initially shorter bud of each pair being given in Table V. The bud of each plant which was initially longer develops very rapidly, while the one initially shorter, although in the period immediately after decapitation it grows at a comparable rate, soon shows a marked retardation. However, on the thirty-fifth day, when the longer bud was decapitated or removed, the shorter bud

at once showed a noticiable increase in rate of growth and would eventually become the main shoot. Occasionally the bud in the axil of the lowest leaf developed early, and whenever this occurred neither of the lowest lateral buds grew at the full rate. This experiment provides quantitative confirmation of the fact, observed qualitatively in all our experiments, that one developing bud inhibits another.

Table VI.—Inhibition of One Lateral Bud by another.

Group.	No. of buds.	Upper line : mean length of bud in mm. / Lower line : mean rate of growth in interval per day in mm.							
		0	2	5	7	14	35	37	39
Initially the longer of each pair	10	8·3	9·7	16·2	29·5	107·8	383·1	—	—
		0·7	2·2	6·7	11·2	13·1			
Initially the shorter of each pair	10	4·6	6·0	10·3	17·6	30·2	47·7	49·7	52·7
		0·7	1·4	3·7	1·8	0·83	1·0	1·5	

On the 35th day the longer of each pair was removed.

5. Is inhibition due to auxin itself?

The above experiments definitely show that the inhibition of lateral buds may be produced artificially by the application of a growth hormone preparation from Rhizopus to the apecis of decapitated plants, and the concept of inhibition by auxin fits the available data from the literature on bud development; nevertheless, it was impossible to determine whether inhibition of lateral buds was due to auxin or to some other substance present in the auxin preparation and also present in conjunction with the growth hormone in the intact

plant. However, within the following two years with the aid of crystalline preparations of auxin from urine (kindly submitted by Professor Kögl) and the chemical synthesis of one of these, hetero auxin, by Thimann and Koepfli (the latter compound found to be identical with the extract from Rhizopus used above) it became possible to show that the inhibiting action is in fact due to auxin itself. These experiments, which with the single exception of experiment 7 were performed on Pisum rather than Vicia, will now be described.

It was found by Snow (1929) that the behavior of Pisum sativum with regard to bud development is very similar to that of Vicia Faba. From an examination we found a similar situation with regard to growth hormone in the two species. Data relative to the production and distribution of growth hormone in Pisum is included in Chapters V and VI. Since peas are much more convenient to grow and to treat in larger numbers, we have used a pure line of peas, the strain "Alaska", exclusively in later experiments.

a. Inhibition by Auxin A

The first preparation from urine, auxin A, (identical with the hormone produced in Avena), obtained by Kögl and Haagen-Smit in 1933, was tested for inhibiting activity on Vicia (experiment 7). This substance unfortunately rapidly loses its growth promoting activity upon standing. Six days before the experiment was started a solution of about 700 units per cc. was prepared. In 16 days its activity had fallen to zero. At the time of the experiment the activity was thus

of the order of 100 to 300 units per cc. The results of this experiment are summarized in fig. 6, which gives the mean length per bud, 16 buds being used in each group. It is seen that the inhibition, although slight, is about the same as that obtained by a similar concentration (150 units per cc.) of the hormone preparation from Rhizopua (fig. 3). However, since the growth of the control buds is identical with those of experiments 3, 4, and 6, the small amount of inhibition is certainly significant. The inhibition is further substantiated by the difference in the growth of the buds in the axils of the lowest leaves, indicated in the block diagram B of the figure.

Figure 6

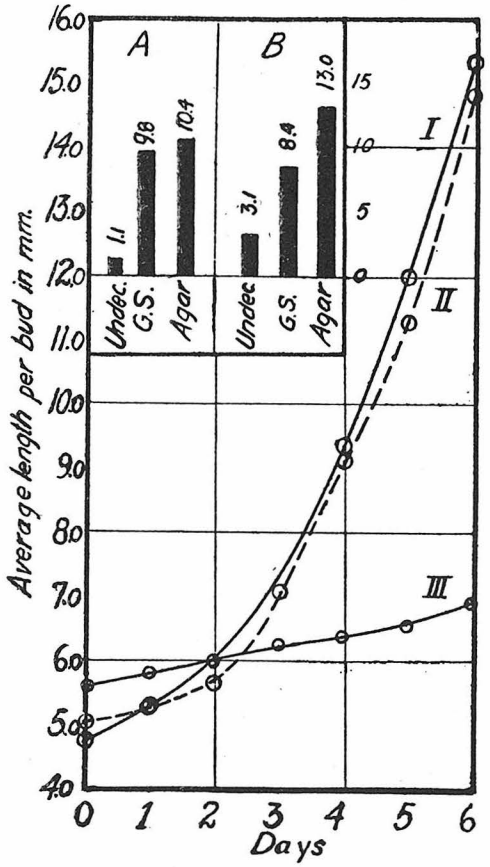


FIG. 6.—Average daily growth of lateral buds in experiment 7. (Growth substance by Kögl and Haagen-Smit.) The inset shows the average increase in length of buds in millimetres at the end of the experiment. A, lateral buds in stipules; B, lateral buds in lowest leaf axil.

b. Inhibition by crystalline hetero auxin. The inhibiting action of crystalline hetero auxin from urine was first tested on very young Pisum plants grown in the green house until the first leaf was completely developed. They were decapitated just below the leaf node, both leaves and terminal buds thus being removed. A small paraffin cup was moulded around an agar block of standard size onto the cut surface. A small opening was made at the top of the cup and the agar block was extracted in small pieces with a needle. Then an aqueous solution of auxin or water was introduced into the cup by means of a glass tube with a drawn out capillary. The cups were refilled with fresh solutions (kept at 0°C) and sealed every eight hours. The experiments were carried out in an atmospheric humidity high enough to prevent rapid evaporation. This technique, although slightly less quantitative than the application of blocks, was found satisfactory for our purposes and was less time consuming. The bud in the upper stipule grew exclusively, and was therefore principally observed.

The results, summarized in table VI show conclusively that complete inhibition is obtained also with the crystalline substance chemically identical to the Rhizopus extract, but from a completely different source.

c. Comparison of activity in inhibition of different preparations. A preliminary trial experiment, using auxin A, (auxin-triolic acid), auxin B (auxenolenic acid), hetero auxin (crystalline indol acetic acid), and Br. F. (about 5 to 10% pure indole-acetic acid) was made. The first three compounds had all been prepared in crystalline form

Table VI

INHIBITION BY CRYSTALLINE HETERO-AUXIN

Pisum: 20 plants, 2 weeks old; no leaves present; applications every eight hours for 6 days; mean stem length at start approx. equal; measurements of Bud No. 2 only.

	LENGTH OF BUD IN MM. DAY 0	DAY 4	DAY 6	LENGTH OF STEM IN CM. DAY 6
Decapitated; water applied	<1.5	5.2 ± 0.8	9.0 ± 2.0	11.2 ± 0.3
Decapitated; 0.05 cc. of Hetero-auxin, 7000 units per cc. applied	<1.5	<1.5	<1.5	11.6 ± 0.3

from urine, the last one was similar to the preparations from Rhizopus, previously used. The following results were obtained: Auxin A which had lost almost all its growth promoting activity when received from Holland, produced no inhibition; auxin B and hetero auxin, at a concentration of about 1000 units per cc. gave about the same inhibition as the Br. F. of the same concentration as measured by growth promoting activity on Avena.

Thus since complete inhibition can also be attained with these crystalline preparations, it only remains to show that for a given concentration in growth promoting units, these substances produce a comparable inhibition. Hence with the small amounts of substances available the activities were re-standardized with Avena, and adjusted to concentrations of 1000, 3000, and 5000 units per cc. The solutions were then tested for bud inhibition activity. The results are given in Table VII. At the start all buds were less than 1.5 mm. long. The experiment had to be discontinued after five days, and for three solutions after four days, owing to shortage of material. Buds other than No. 2 (as designated in drawing fig. 1) also developed to some extent in this experiment, and their measurements are included in

Table VII

COMPARISON OF HORMONE PREPARATIONS AT DIFFERENT CONCENTRATIONS

Plants grown in light; all buds less than 1.7 mm. long at start.

DAY	BUD NO.	DECAP. CONTROLS	AUXIN B 3000	AUXIN B 5000	LENGTH OF NUMBERED BUD IN MILLIMETERS BR.F.(RHIZOPUS) 1000	BR.F.(RHIZOPUS) 3000	BR.F.(RHIZOPUS) 5000	HETERO-AUXIN 1000	HETERO-AUXIN 3000	HETERO-AUXIN 5000	INTACT CONTROLS
4	2	7.8 ±0.6	3.3 ±0.3	2.6 ±0.2	4.4 ±0.6	4.3 ±0.8	3.2 ±0.8	2.6 ±0.1	2.3 ±0.1	2.4 ±0.3	1.7 ±0.1
5	2	14.0 ±0.6	4.8 ±0.8	4.4* ±0.6	7.5 ±1.5	8.1 ±1.8	6.0 ±1.5	3.7 ±0.8	3.8* ±0.3	4.5* ±0.5	1.7 ±0.1
5	1	2.6	1.1	1.2	1.2	1.2	1.2	1.0	1.8	1.0	no development
5	3	6.4 ±1.2	3.7 ±0.4	2.6 ±0.2	4.3 ±0.9	3.6 ±0.3	3.4 ±1.0	2.9 ±0.4	2.8 ±0.3	2.6 ±0.5	no development
5	4	2.6	2.0	1.8	2.4	1.2	1.2	1.9	1.8	1.4	no development
No. of plants		15	10	7	9	7	7	11	5	5	15

* Application of hormone stopped at middle of 4th day.

the table. It is apparent that on the whole in the different groups of plants they developed in the same ratio as the No. 2 buds, except that buds No. 4 are relatively little affected. On account of the small number of plants used the results do not show a smooth variation with concentration. Nevertheless, it is clear that the crystalline compounds are unit for unit of growth hormone, at least as active as the preparation from Rhizopus (Br. F.). The table further shows that hetero auxin produces a more complete inhibition than the other two preparations. This behavior is in accordance with the fact that while the latter preparations rapidly decrease in activity on keeping, the crystalline hetero auxin solutions become inactivated much more slowly, and therefore, retain a higher activity throughout the experiment. Furthermore, also in the plant hetero auxin is much less readily attacked by oxidizing agents than the other compounds. That the loss in activity due to the plant may be considerable has been shown by inactivation by crushed plant tissues (Thimann). That unit for unit of growth hormone the inactivation caused by the plant at its cut

surface is relatively much higher for auxins A, B than for hetero auxin, although in both cases it may be large, has later been clearly shown by Van Overbeek (1936).

On account of the possibility that the plants might have become injured by the application of the high concentrations of growth hormone used, the buds were remeasured nine days after stopping of the experiment. In order to get a measurement of the total amount of bud elongation, the total lengths of buds 1, 2, 3, and 4 were added together. The mean values of the lengths of the separate buds and the mean aggregate lengths in mm. are given in Table VIII. They show that the inhibited buds grow in a normal manner after the application of hormone has ceased. The buds of the plants treated with hetero auxin are still somewhat behind the others; they are, however, rapidly growing, and the difference is to be ascribed to the stability effect discussed above.

Table VIII

Decapitated	Auxin B		Hetero-auxin			Br. F.			Intact
controls	3000	5000	1000	3000	5000	1000	3000	5000	controls
184	174	169	117	155	120	156	194	185	<4

d. Inhibiting action of synthetic hetero auxin; effect of concentration

In the experiments described, a given Avena growth activity of different substances caused comparable inhibition and in general the higher the concentration (activity) the more effective was the inhibit-

ing action of a substance. Now a final proof of the inhibiting action of auxin and its relation to concentration applied was obtained with synthetic hetero auxin, β-indoleacetic acid, (Thimann and Koepfli), dissolved in lanoline in different concentrations.

It was first established in two experiments, carried out in the green house in the same manner as above, that the synthetic compound when applied in relatively high concentrations, renewed daily, was as active in bud inhibition as the extracted preparations previously used. Detailed results need not be given, but for example in one experiment the mean bud lengths ($B_1 + B_2$) of about 12 to 15 plants in each group were in the following groups on the 6th day after decapitation:

Intact control plants	2.7 mm.
Decapitated plants with auxin	2.6 mm.
Decapitated plants without auxin	9.0 mm.

In experiments with decapitated young etiolated Pisum seedlings carried out in the dark room and to which had been added a series of high concentrations of synthetic hetero auxin in lanoline, Dr. Went observed (1) a very high response in bud inhibition to a single application of auxin, and (2) a definite correlation between the concentration of auxin applied and the amount of bud inhibition. With the highest conc. (10 mg./gr.) complete inhibition was obtained, whereas in the light, I had found this concentration to be effective only if fresh applications were made daily. The buds in Dr. Went's experiment were, therefore, measured at the same time as measurements were made of the swellings of

the stem produced by the different concentrations of hormone. The effect on bud inhibition was then checked in a second experiment.

The results of the first experiment are shown in figure 8 and Table XVI. The results of the second experiment are included in figure 8, curve IV. The experiments show conclusively that the amount of hormone necessary for inhibition is relatively high, and that the amount of inhibition although not directly proportional to the concentration applied, nevertheless, over a wide range is a definite function of the concentration. The relation between inhibition and swellings of the stem will be considered (section 2 B, Chapter II)

6. General occurrence of bud inhibition by auxin.

The fact that bud inhibition can be produced by growth hormone has also been independently found by Laibach and Müller (1933) in their experiments on the effect of orchid pollen on the growth of the stem of Vicia. The phenomenon has since been studied in some detail by Müller (1935). She was able to demonstrate inhibition by applied hormone in nine of twelve species used. In the three species failing to respond, there is also normally no inhibiting effect exerted by the terminal bud on the growth of lateral buds. It is entirely possible that auxin cannot act in these plants because through some mechanism present in them the growth hormone will be destroyed. In accordance with this, many attempts by van Overbeek (private communication) to extract growth hormone from Tropaeolum, one of the three, failed to give positive results.

Inhibition has since been observed also in other species, as

for example by Michener in Salix (1935) and especially by Urhova (1935) in Bryophyllum. The latter investigator was able to cause effective inhibition by transporting the inhibiting substance polarly by diffusion from leaves through agar to the buds.

Similarly Katunskij (1935) has confirmed certain phases of our work and also has succeeded in inhibiting the outgrowth of lateral rootlets from the decapitated main root of Vicia by the application of coleoptile tips to the cut surface of the root.

Thus, such work as has been recently done confirms our work as to the effect of auxin on bud inhibition, and the phenomenon of inhibition through auxin may be considered as generally accepted.

CHAPTER II

THE MECHANISM OF THE ACTION OF AUXIN IN THE INHIBITION OF BUD DEVELOPMENT

A. Proposed Mechanisms of Inhibiting Action of Auxin.

It is well known that auxins are determined, and may thus be said to be in one respect defined, by the property of promoting cell elongation. The above experiments have shown that the activity of a growth hormone preparation in causing bud inhibition is a function of its activity as determined by cell elongation. Furthermore, a preparation which has lost its elongation activity, as measured on Avena, has simultaneously lost its activity in causing bud inhibition. Are the two processes then casually related? Does auxin act as a bud inhibitor indirectly through its action in promoting the growth of the decapitated stem, which in turn depletes the supply of nutritive materials in the plant and thus prevents the development of buds, or does the hormone act independently in the two processes? This question has raised a considerable controversy, and three different proposed mechanisms of the action of auxin in bud inhibition must be considered.

1. Mechanism of direct inhibition of buds by auxin.

It may seem at first sight paradoxical that a growth promoting substance should also act as an inhibitor. A possible explanation of this behavior and the evidence on which it is based are briefly as follows:-

Auxin is formed in the terminal bud (especially in the young

leaves) and is polarly transported in relatively high concentrations down through the stem to the buds. As long as the supply of auxin from the apex is maintained the buds are prevented from synthesizing hormone and are thereby prevented from developing.

It is clear from the work of Went and of Dolk (1928 and 1926) that the presence of the tip of the coleoptile (in which growth hormone is formed) prevents the formation of hormone in the sections below it. However, upon removal of the tip, the coleoptile section immediately below it soon acquires the capacity to produce hormone. Now on the basis of various evidence, it was suggested by Dr. Thimann that the prevention of synthesis in the lower regions is directly due to the presence of a supply of auxin coming from above; and further, it was assumed that the same conditions obtain in other plants such as Vicia. A further condition is that the buds are able to grow in response to auxin only when synthesized in their apical cells, and are not able to give a growth response to auxin derived through the stem from the apex. The above proposed mechanism, which was the idea from which our experiments were started, is capable of satisfactorily accounting for all the facts observed in our experiments on inhibition of buds.

2. Mechanism of indirect inhibition of buds through growth of stem.

The above mechanism of direct inhibition by auxin has been denounced by Laibach and by Müller. They consider the inhibition of buds to be only a secondary phenomenon accompanying an increased growth of the stem, which according to them occurs when hormone is applied to

the cut surface of the decapitated plant. This concept will for convenience be referred to as the mechanism of indirect inhibition.

3. Mechanism of inhibition of buds through prevention of cell division.

A third possible mechanism which is in direct opposition to that of Laibach has been brought forward by Katunskij (1935). He considers cell elongation and cell division as two antagonistic processes. Auxin by promoting the former process automatically inhibits the latter. Experimental evidence on which this view is based is briefly the following:-

(a) When to leaf sections of succulents, cultured as by Haberlandt and by Lamprecht, coleoptile tips are applied to the surface, cell division normally occurring in the underlying tissue is prevented. Hence auxin inhibits cell division.

(b) When coleoptile tips are applied to the cut surface of a decapitated main root of Vicia, not only is the normal development of lateral rootlets inhibited, but the root itself grows less. Hence Laibach's mechanism of indirect inhibition cannot be generally correct.

4. Food as a limiting factor in bud development.

The old concept that available food is directly a limiting factor in bud development rather than special inhibiting substances is also the essential idea behind the indirect inhibition mechanism in (2). Thus it need be only briefly separately considered below.

It is evident that the above contributions have left the problem of the mechanism of the action of auxin in bud inhibition in

a rather uncertain state. Since the proposed mechanisms in many respects are contradictory it becomes necessary to choose the one which is in agreement with all the available experimental data and to reject the others. In this chapter, therefore, it will be shown in more detail than would otherwise be necessary that:

1. Auxin acts independently in promoting growth and in inhibiting bud development.
2. The arguments directed against the mechanism of direct inhibition by auxin are inconsistent with the experimental results, and are therefore invalid.
3. The alternative mechanisms of indirect inhibition are inconsistent.
4. The inhibition of buds as caused by auxin and by lack of food can be distinguished.
5. The mechanism of direct inhibition is in agreement with the experimental data, and in terms of it all inhibition effects observed can be explained.

B. Independence in action of auxin in growth promotion and bud inhibition

1. Relation of stem elongation to bud inhibition.

In the experiments on bud inhibition described in Chapter I, it was found that the application of auxin every six hours had no effect on the mean growth of the stems. Not only were both sets of decapitated plants greatly behind the undecapitated controls, but the plants supplied with auxin grew no more than those supplied with plain agar. With the higher concentrations of auxin (1600 units) the exten-

Table IX

Table VIII.—Measurements of Average Stem Elongation in Plants with and without Growth Substance.

Experiment No.	No. plants in each group.	Duration of experiment in days.	Plants treated with g.s.			Plants treated with agar only.			Difference (cm.).
			Initial length (cm.).	Final length (cm.).	Increase (cm.).	Initial length (cm.).	Final length (cm.).	Increase (cm.).	
3	7	10	12·0	15·5	3·5	12·1	15·6	3·5	0
4	5	8	16·5	20·3	3·8	15·9	21·6	5·7	−1·9
5	6	5	14·0	17·8	3·8	15·2	19·1	3·9	−0·1
6	7	9	14·9	18·7	3·8	15·2	21·3	6·1	−2·3
7*b* (in dark)	8	5	14·9	19·6	4·7	14·9	19·4	4·5	+0·2

sion of the auxin treated plants was even less than that of the plants receiving plain agar. The length measurements of the stems in the different experiments are summarized in Table IX. It can be seen that over the total period of an experiment the application of auxin in the light certainly causes no increase in stem elongation. This raised the following points:-

(a) Does auxin have any effect on stem elongation in Vicia?

(b) If so, under what conditions?

(c) Is it possible that growth hormone may be present in the agar treated plants in amounts sufficient to cause stem elongation but not bud inhibition?

In regard to (a) it was observed that, although after 8 to 10 days, the plants supplied with auxin showed no increase in length over the controls, yet during the first two days there was in each series a small increase. There is thus a small response at first, and the effect of auxin on stem elongation was, therefore, further studied.

a. Effect of auxin on isolated stems.

As shown in Table X, A and B, in Vicia and in Pisum the elonga-

Table X

Growth of internodes in mm. (All figures means of 10 plants)

A. Vicia. (Intact)

Day	B1-B2	B2-L1	L1-L2	L2-L3	L3-L4	L4-L5	L5-L6	L6-L7	
0	11.0	19.0	21.0	33.5	26.0	8.2			Lengths
1	0.0	0.0	1.3	1.3	6.3	5.1	3.0		Increase per Interval
2	0.0	0.0	0.3	5.5	9.2	14.3	2.3		
4	0.0	0.0	0.3	4.5	12.1	13.5	11.5		
6	0.0	0.0	0.0	1.8	9.2	18.5	8.0	2.8	
9	0.0	0.0	0.0	0.0	11.0	29.7	22.7	2.5	
9	0.0	0.0	1.9	13.1	47.8	81.1	47.5	5.3	Total Increase

B. Pisum. (Intact)

0	8.3	33.2	63.6	61.0	13.20	?	Initial Lengths
4	8.3	33.3	63.9	66.1	37.8	15.9	Final
4	0.0	0.1	0.3	5.1	24.6	15.9	Increase

(Decapitated)

0	8.5	34.9	68.7	75.0	10.7	Initial Lengths
4	8.5	34.8	68.7	79.4	19.8	Final
4	0.0	-0.1	0.0	4.4	9.1	Increase

tion of the stem is localized to the upper internodes. The lower half of the plant undergoes no or practically no elongation. The upper half of the stem of young, rapidly growing plants was cut off, defolia-

ted and placed in water with and without the addition of auxin. The lengths of the sections were measured at the start and then at intervals up to 48 hours. The results of measurements at 24 hours, after which time practically no further growth was obtained, summarized in Table XI, are rather irregular, but they show that auxin definitely causes elongation in isolated stem sections of Vicia. It is therefore probable that in the experiments in which auxin was applied to whole plants no effect on elongation was obtained, because the controls were, in fact, also receiving supplies of growth hormone sufficient to give a maximum elongation response.

Table XI

Table VIII.—Growth of Isolated 10 cm. sections of *Vicia Faba* Stem, in Water and in Growth Substance Solution.

Experiment No.	No. of sections.	Conditions.	Mean increase in length expressed as per cent. of the original length after 24 hours.	
			In water.	In growth substance, 100 units per c.c.
1	2	Light	−0·9	+4·4
2	2	Light	+9·9	12·7
3	5	Light and dark alternately	1·1	2·8
4	6	Dark	0·5	2·9
5	6	Dark	2·0	1·6
6	5	Light	4·9	9·0
Mean of all experiments			+2·9	+5·6

In experiments on stem sections of young etiolated pea seedlings carried out simultaneously, it was found that the elongation in similar sections placed in given small concentrations of auxin solutions (40 to 100 units per cc.) was considerable when the experiment was performed in the dark room in red light, but was hardly measurable when the experiment was performed in the laboratory in the light.

Now it was shown in Chapter I, section 3 A that the leaves produce from 0.8 to 5.4 plant units per hour. The decapitated plants, treated with plain agar, thus receive from 10 to 15 plant units from their leaves. It is entirely possible that this amount of hormone when produced within the plant itself is sufficient to allow of normal stem elongation, so that the application of further amounts would make no appreciable difference. The effect of auxin on stem elongation in the absence of leaves was therefore determined, both in the dark and in the light, since this would provide an answer to point (c) above.

b. Effect of auxin on defoliated plants.

Selected plants of an average height of 18 cm. were decapitated and all leaves and buds removed. The cut surfaces were covered with paraffin as described above. Agar blocks with and without auxin (800 and 1600 units) were applied, and the stem lengths were measured every 12 hours. The results are plotted as Curves II, III, and IV in Fig. 7. (Curves I and V will be considered below.)

Figure 7

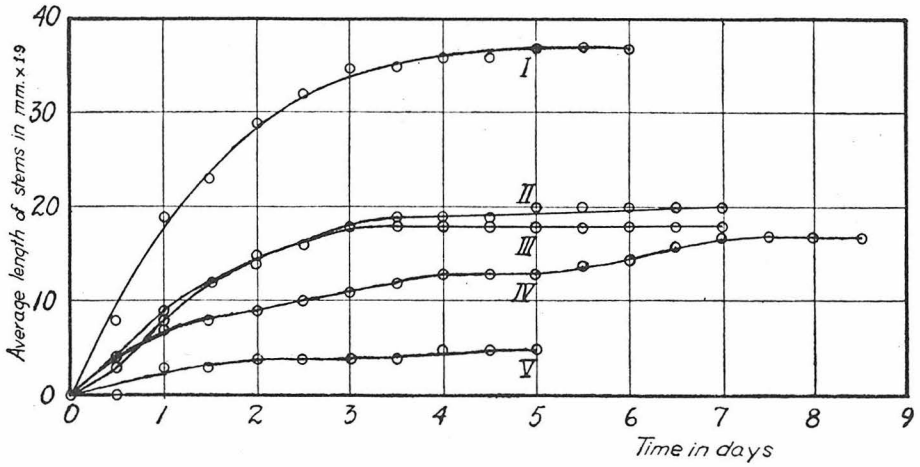


FIG. 7.—Growth of defoliated decapitated plants in the light and in the dark with and without growth substance applied every 12 hours. I, dark (1600 units/c.c.); II, light (800 units/c.c.); III, light (1600 units/c.c.); IV, light (agar control); V, dark (agar control).

Firstly, it is clear that the application of auxin to these defoliated plants increases stem elongation. The plants supplied with auxin (curves II and III) grow considerably more rapidly than the controls (curve IV). Secondly, it is clear that the amount of auxin applied is in excess of that required, since the application of both 1600 and 800 units concentrations in equal volumes give the same rate of elongation. There is indication that the larger amount of auxin causes some inhibition, as in Table IX. Thirdly, while the auxin treated plants reach a constant length after 3 to 4 days, the agar treated controls continue to grow at a very slow rate, and in 7 days closely approach the length of the auxin treated plants, after which time there is no further growth of the stems.

The continued growth of the control plants to the 7th day must be due to the synthesis of small amounts of growth hormone in the stem, which takes place only in the light. This is shown by the growth of a set of plants exactly similar to those of curve IV, except that they were kept in the dark, carried out at the same time (curve V, fig. 7). These plants show practically no growth. Hence either (a) growth hormone is not produced in defoliated, decapitated plants in the dark, or (b) it is produced but does not act in Vicia under these circumstances. But, as is shown by the results expressed by curve I, fig. 7 representing the mean growth of the stems of a similar group of plants carried out at the same time, to which auxin (1600 units) was applied in the dark, it is clear that auxin is active in causing stem elongation in the dark. Hence, it is apparent that the growth hormone is not pro-

duced in the dark.

From a comparison of curves I and V, from plants treated with equal amounts of auxin, it is, furthermore, apparent that the plant gives a higher response to auxin in the dark than in the light. Thus, there are two separate factors in relation to auxin which must be distinguished between, the production and the response.

Table XII

Table IX.—Production of Growth Substance by the Terminal Bud in Dark and in Light.

Series No.	Test carried out after—	Dark.		Light.	
		No. of experiments.	Mean growth substance production in plant units per hour.	No. of experiments.	Mean growth substance production in plant units per hour.
I	4 days	5	0·65	5	17·1
	8 days	6	0·1	3	5·0
II	4 days	6	2·7	4	14·8

c. The relation of light to the production of growth hormone.

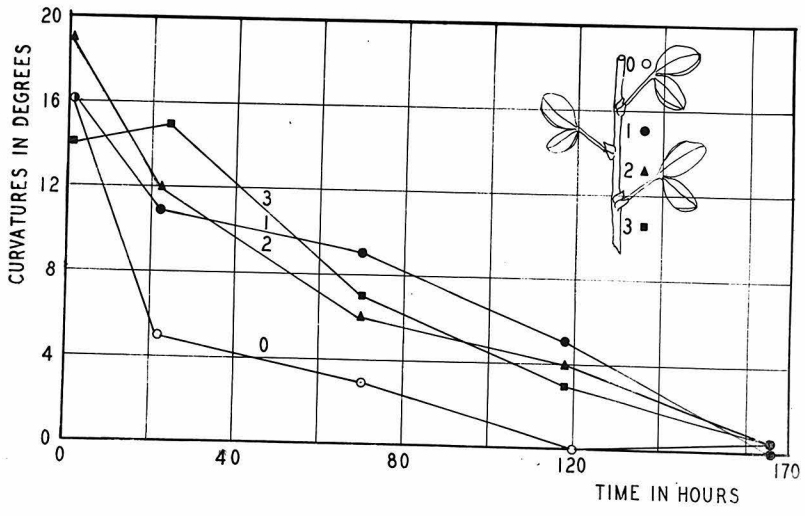
The above deduction that growth hormone is only produced in Vicia in the light was confirmed by the following experiment (Table XII). A number of comparable intact plants were placed in the dark and in the light. After 4 and 8 days the terminal buds were removed and the growth hormone obtained by diffusion into agar was determined as in Chapter I, Table I. It is clear from the results that even after only four days in the dark the production has fallen almost to zero.

In another experiment short sections of stem, 9 mm. long, were

cut from each internode of plants kept in the light and were placed on agar blocks. The amount of growth hormone diffusing out of the basal ends of the sections in one hour was then determined. It was found that when cut immediately after decapitation, 18 to 22 plant units may be obtained from each section after one hour of diffusion, but when cut during successive days after decapitation of the plants, the quantity of hormone obtainable decreases and reaches practically zero in seven days. The results (Figure 8) further show that in the topmost internode above the youngest leaf the growth hormone decreases most rapidly, as would be expected if the transport of the hormone is strictly polar from the tip toward the base. That this is in fact true is shown below. Now when the same experiment was repeated

Figure 8

Auxin from internodes of decapitated Vicia plants



but by placing the plants in the dark immediately after decapitation, the decrease in auxin was much more rapid. Although the values obtained were not very quantitative, they show that the hormone content falls to zero within about three days. Thus while the consumption of hormone by the plant continues in the dark, its production ceases, and consequently the hormone supply is very rapidly depleted in the dark.

d. Difference in response to auxin in the light and in the dark.

Reference to fig. 7 shows that the application of auxin to defoliated plants in the dark causes a much greater increase in stem elongation than in the light. The same fact is also indicated in Table IX, in which the only positive response in stem elongation to applied auxin was obtained in experiment 7 b, carried out in the dark. In another experiment, blocks of 1370 plant units and of plain agar were applied every 12 hours in the dark. The average increase in stem length in the groups of 12 plants with average initial bud lengths of 16.2 and 16.8 cm. respectively were after

24	48	72 and	96 hours
11.4	17.8	23.9	25.0 mm. in auxin treated plants and
5.6	9.8	11.8	11.9 mm. " agar " "

Thus the mean difference in growth was

5.8 8.0 12.1 13.1 mm. respectively at each daily measurement.

Hence the auxin treated plants grew about twice as much as the control plants throughout the experiment until growth stopped in all

plants. From a comparison of the amount of stem elongation obtained with and without auxin in the different experiments, it is thus also clear that the response to a given amount of auxin is much greater in the dark.

e. Bud inhibition in the dark.

It was stated in Chapter I that the early experiments on bud inhibition in Vicia in the dark were unsatisfactory. It was also shown that with young seedlings of Pisum complete inhibition of buds was obtained by a single application of auxin, in the dark, whereas in the light equally high concentrations had to be applied daily in order to produce an effect. Thus in Pisum seedlings it is apparent that also with regard to bud inhibition the plant gives a greater response to auxin applied in the dark.

An experiment was therefore attempted to determine whether a definite inhibition in response to auxin could not be obtained in the dark also in Vicia previously grown in the light as in the experiments above. It was found that the lateral buds of the decapitated controls, although they start to develop, show a steady decrease in growth rate from the first day on, whereas in the light the growth rate increases from day to day. Nevertheless, the rate of growth of these buds is significantly higher than that of buds in plants supplied with auxin. The mean increase of 16 buds in each group is given in Table XIII, which shows that up until the time of complete cessation of growth by both sets of buds, there is a marked inhibition produced by auxin. A

Table XIII

Table V.—Daily Growth of Lateral Buds in the Dark.

	No. of buds.	Average length per bud at the end of each day (mm.).						Average increase in length.
		0	1	2	3	4	5	
Decapitated 1400 units g.s. applied	16	5·37	5·90	5·97	6·09	6·09	6·13	0·78±0·17
Decapitated plain agar applied	16	5·84	6·72	7·10	7·44	7·53	7·71	1·94±0·48

second experiment gave identical results. It must be concluded that this growth of the buds in control plants is due to auxin formed in the buds most likely from a precursor still present in small amounts in the seeds of these plants.

f. Conclusion

The upshot of the experiments in light and in darkness in relation to the effect of stem elongation produced by auxin to its action in bud inhibition is summarized as follows:-

(1) The fact that the application of auxin does not give an increase in stem elongation in plants in the light is due to the fact that sufficient amounts of auxin are being formed in the leaves to allow of maximum growth of the stem.

(2) Auxin can act both in the light and in the dark; but the response of the plant as measured either by elongation of the stem or by bud inhibition is higher in the dark. This increased response might be largely accounted for by a faster rate of destruction of auxin in the plant in the light.

It has thus been clearly demonstrated that although a decapitated plant is able to produce enough growth hormone to allow of maximum growth of the stem, nevertheless, it is not able to maintain an inhibiting action on the development of lateral buds. It follows that the action of auxin is independent in the two processes, i.e., the inhibiting action of auxin on bud development is not through its action on stem elongation. The latter process requires relatively low concentrations of hormone; the former process requires relatively much higher concentrations of hormone.

2. Epicotyl swellings and bud inhibition.

The type of swellings of the epicotyl stump described by Laibach (1933) were also obtained to a smaller extent in experiments on Pisum in the dark. However, they were not obtained in plants grown in the light.

(a) Nature of swellings.

The structure of the swellings has not been investigated in detail, but superficial examinations by several people have shown them to be due to an abnormal enlargement in several directions of pith and parenchyma cells. In a few days numerous cell divisions occur as well, and large masses of new parenchyma cells are formed. Very possibly cambial activity is also stimulated, but this effect is certainly relatively insignificant in determining the size of the swellings. For a given concentration of auxin applied the swellings produced are largest in the apical, most rapidly growing internodes near to the terminal bud; the lower internodes which have practically ceased to

elongate produce at best relatively very small swellings, and as far as observed, do so only when the application is made to the very tip of the epicotyl stump in the dark.

(b) The relation of swellings and bud inhibition to polarity

Usually in experiments with decapitated plants in the dark swellings were obtained by the application of high concentration of auxin in lanoline paste to the cut surface of the stem, and the swellings occurred immediately below the cut surface. However, if the paste is applied to the periphery of the stem at the cut surface, similar results are obtained. Now if to intact plants auxin paste is applied laterally to the stem at the corresponding place, large swellings are also produced. These are spindle shaped and taper in both directions from the point of application; however, frequently the amount of swelling is greater above the point of application than below it. In addition swellings often occur some distance above the point of application just below the terminal bud. But, if auxin is applied to a freshly decapitated rapidly growing plant laterally 2 to 3 cm. below the cut surface, the swelling frequently occurs principally just below the cut surface in the same way as if the application of auxin had been from above onto the cut surface.

From the experimental results described, it can be concluded that the locus of the stem swellings need not be in accordance with the normal basipetal polar transport of the hormone in the stem. The swelling may occur below, at, or above, the point of application of auxin. Whether in the latter case auxin is transported also in an

apical direction, or whether its action is indirect (not at the place of swelling) has not been determined.

It will now be shown that in the case of bud inhibition, auxin can act only when applied above or at the place of the bud, and not when applied below the bud. Young etiolated Pisum seedlings were decapitated in the upper half of the internode between the lowest leaf and the upper stipule (bud No. 2). To different groups of plants lanoline paste with and without auxin was applied to the stem some distance above Bud No. 2 and some distance below bud No. 1 respectively. The growth of the buds, measured seven days afterwards, is shown in Table XIV. The results show that whereas auxin applied above the buds

Table XIV

Lateral bud development in decapitated Pisum plants supplied with auxin or pure lanoline at different parts of the stem.

All figures are the means of 14 to 22 plants

(Experiment 1)

Place of application	Substance	Mean lengths of buds in cm.				Total
		B_1	B_2	B_{2_2}	$BL_1(B_3)$	
On apex of stem	Control	0.18	5.45	0.36	0.08	6.07
	Auxin	0.12	1.66	0.34	--	2.12
Below B_1	Control	0.17	5.19	0.66	0.17	6.19
	Auxin	0.56	5.13	0.59	0.15	6.43
		(Experiment 2)				
Between B_1 and B_2	Control	0.48	9.73	0.60	--	
	Auxin	0.36	9.74	0.43	--	

has a marked inhibiting effect on their growth, the same concentration of auxin when applied to the stem below the buds is completely ineffective. In another set of plants paste was applied to the stem in the middle of the internode between the two buds. The results, included in the table, confirm the above results. Bud No. 2, above the point of application, grows as much in auxin treated plants as in controls; bud No. 1, below, which although it grows very little also in the control plants, nevertheless, is definitely less developed in auxin treated plants. An independent proof of the fact that auxin is active in bud inhibition only when it is actively transported through the stem from the point of application to the buds was obtained by the following experiments. It was found by Thimann (1936) that two substances closely related in structure to hetero auxin, indene-3-acetic acid and coumaryl-1-acetic acid, are active in promoting cell elongation. Thus they are fully active in pea tests and give considerable activity in promoting elongation of coleoptile sections immersed in solutions. But their rate of transport in the plant is very low, and consequently they produce only small apically situated curvatures in the Avena test. Of the two, however, indene-3-acetic acid is more readily transported. In accordance with this slow rate of transport, as shown in Table XV, these substances inhibit bud development in Pisum qualitatively inversely in proportion to the distance of the stem from the point of application to the buds, and, furthermore, indene-acetic acid, which is relatively more readily actively transported through the plant is also relatively more active in bud inhibition. The activity of hetero auxin

Table XV

Inhibition of bud development by indene-3-acetic acid, applied at two different distances from the bud. (All measurements are in cm.)

Compound (Acid)	Concentration mg/gram of lanoline	Control		Experiment	
		Distance from decapitation point to bud	Length of bud after 8 days	Distance from decapitation point to bud	Length of bud after 8 days
Indole-3-acetic (hetero auxin)	10	3.7 cm	15.3	2.5 - 3.5	0
Indene-3-acetic	ca 1	6.1	11.9	5.5	7.0
		1.2	11.0	0.9	1.5
Coumaryl-1-acetic	10	5.5	15.4	5.7	9.3
		0.6	23.1	0.7	ca 2.-

in bud inhibition, on the other hand, has been found from measurements on large numbers of plants to be independent of the length of the stem to the buds. Nevertheless, under suitable conditions these substances are fully able to inhibit bud development. The behavior of a precursor of hetero auxin (β-indol-ethyl amine), to be described in the last chapter, is also of interest in this connection. This substance which can be changed into auxin and will then produce very large curvatures on Avena is nevertheless completely lacking in bud inhibition activity whether applied to the stem above or below the bud. On the other hand, some experiments indicate that when supplied from below it may stimulate the growth rate of the developing buds. This latter point, however,

has not yet been definitely established.

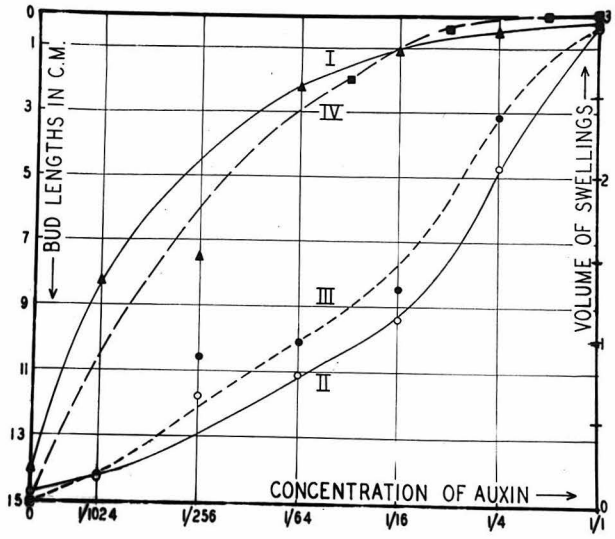
Relative to the mechanism of the action of auxin the above results are in complete agreement with the expectations based on the polar transport of the hormone and the condition of our proposed mechanism that auxin should inhibit bud development only when present in relatively high concentration in the tissue of or immediately in contact with the bud.

c. Absence of correlation between the size of swellings and amount of bud inhibition.

That the swellings of the stem accompanying bud inhibition in the dark grown plants, produced by the application of auxin, are not the cause of its effect in bud inhibition is clear from the following measurements. The mean lengths of the lateral buds of the different sets of plants summarized in Table XVI are plotted against the logarithms of the concentrations of auxin applied, curve I, Fig. 9. At the same time as the bud lengths were determined, measurements were made of the diameters and lengths of the apical swellings of the stems in each set of plants. Approximate values for the mean relative volume of the swellings as calculated in terms of the product of the square of the diameter times the length are plotted in curve II, and to show that the calculated values are accurate enough for the purpose at hand, the volumes of the same sections as determined by Dr. Went by immersing equal numbers of sections of a given length from each set of plants in water are represented by curve III.

Figure 9

Effect of concentration of hetero auxin on bud development and stem swellings in etiolated Pisum. Curves I and IV bud lengths in mm. Curve II calculated, Curve III measured volumes of swellings.



If the two processes, bud inhibition and production of swellings of the stem, were causally related, then (1) the curves I and II, as plotted, should run approximately parallel. On the contrary, it is evident that for a range of concentration of auxin where the rate of decrease of growth of the buds is most rapid, the rate of increase in swelling is relatively slow; and for the range of concentration of auxin where the rate of decrease of growth of the buds is most rapid, the rate of increase in swelling is relatively slow; and for the range of concentration where the inhibition is almost complete, and thus, the rate of decrease in length with concentration is very slow, the rate of increase in swelling is maximal. (2) Within each group of plants, supplied with a given concentration of auxin, in the range

Table XVI

Bud development and stem swellings in etiolated Pisum treated with different concentrations of hetero auxin. Concentration 1/1 represents 10 mg./gram of lanoline. All measurements are in terms of millimeters.

Auxin conc.	Mean measurements of	No. of plants	Bud length	Swelling diameter	Swelling length	$d^2 \times l$ (volume)
1/1	Above average(0)	1	7.0	5.8	18.0	0.609
	Below "	9	0.0	5.54	9.0	0.276
	Mean	10	0.5	5.55	9.6	0.296
1/4	Above average(0)	6	18.4	5.2	8.4	0.227
	Below "	12	0.0	4.90	7.0	0.168
	Mean	18	5.4	5.05	8.0	0.204
1/16	Above average	8	20.1	4.00	8.6	0.138
	Below "	9	1.6	3.96	5.7	0.090
	Mean	17	10.7	3.98	7.06	0.111
1/64	Above average	9	42.3	4.03	5.3	0.086
	Below "	10	4.9	3.49	4.8	0.059
	Mean	19	22.6	3.68	5.1	0.069
1/256	Above average	12	109.7	3.58	5.4	0.069
	Below "	10	33.1	3.41	5.3	0.062
	Mean	22	74.9	3.50	5.36	0.066
1/1024	Above average	13	125.5	2.42	2.8	0.016
	Below "	10	28.5	2.16	2.8	0.013
	Mean	23	83.1	2.30	2.83	0.015
0	Above average	5	164.4	2.0	1.2	0.004
	Below "	5	120.0	1.9	1.0	0.004
	Mean	10	142.2	1.92	1.1	0.004

of concentration where the inhibition is measurable but not complete, there should exist an inverse correlation between the length of buds and the amount of swelling in individual plants. This possibility was tested by comparing separately for each concentration of auxin the average bud lengths and the average diameter and length of swellings in the plants whose individual buds were larger than the mean bud length of the entire group with the corresponding values for plants whose

individual buds were shorter than the mean bud length of the group. (For conc. 1/1 and 1/4, where only a few buds developed and the majority of the buds remained completely dormant, the plants with any bud development were compared with plants without bud development). It is clear from Table XVI that no inverse correlation between amount of swelling and bud development exists. On the contrary, for each concentration, there appears to be a positive correlation. The plants which have larger than average buds have on the average larger than average swellings, and plants which have shorter than average buds have on the average smaller than average swellings.

Conclusion.

The only apparent possible interpretation of the above results is that (1) the action of auxin in producing swellings is independent of its action in producing bud inhibition, and (2) that the capacity (a second factor) for growth varies with the individual plants. Plants with larger capacity for growth (a) produce larger swellings in response to a given amount of applied auxin, and (b) produce larger buds. It is clear that the limiting factor in (a) has to do with sensitivity to auxin, since by the application of higher concentrations of auxin to identical plants larger swellings are obtained. It is likely that the increased size of buds occurring in these plants is also due to a higher growth response to growth hormone formed in the buds.

3. Opposition to and defense of the mechanism of direct inhibition.

The conclusion, drawn from our early experiments on Vicia, that

auxin acts independently of its action on cell elongation in its effect on bud development has been refuted by Laibach. His arguments against our view are included and extended in the work of his student, Müller (1935). After it has first been shown that the differences in technique employed in their experiments and in ours are not such as to have any essential effect on the mechanism of the action of auxin in bud inhibition, these objections will be considered.

a. Difference in technique.

In the experiments of Laibach and of Müller the plants were cut off below the lowest axillaries, thus only cotyledonary buds were left. In our experiments, frequently only the terminal bud was removed and the development of the axillary buds was measured. However, experiments were made to compare the effect of auxin on axillary and cotyledonary bud development. From one group of young Pisum plants the terminal bud was removed, leaving two developed leaves on the stem intact; in a second group of plants the stem was cut below the lowest stipule, leaving only a short stump of the stem. Auxin in lanoline paste and pure lanoline were applied respectively to half the number of plants in each group. A single application of auxin in the dark, or daily application in the light, was found to cause complete inhibition of buds in each group, whereas in plants treated with lanoline, buds grew in each group of plants to an average length of between 20 and 25 cm. in the dark and about 10 cm. in the light within a period of two weeks. No visible difference in the appearance of the effect of the action of

auxin could be detected, and although some of the short epicolyl stumps showed a slight swelling at the point of application in response to auxin, the majority of them showed no measurable increase in size.

(1) Difference in source of auxin. Laibach and Müller used Orchid pollen and also auxin extracted from urine, dissolved in lanoline. Our preparation included the auxins obtainable from urine but were highly purified. As already shown, we found all different preparations of equal growth activity to be of comparable activity in bud inhibition. The fact that we used crystalline preparations can certainly not be a valid argument that our results are inconclusive; the fact rather makes them the more conclusive. Thus, as far as the mechanism of the action of auxin is concerned, the differences in technique employed is in favor of our view being correct and do not give cause for any exceptions either due to differences in the plant material or to possible impurities in the preparations.

b. The further objections raised by Müller to our proposed mechanism of action are as follows:

1. The application of pollen did not cause complete inhibition in spite of the fact that at the end of the experiment the pollen was still active on Avena.

2. Complete inhibition could also not be obtained with auxin in lanoline paste. Hence activity does not depend on the amounts and concentration of hormone used.

3. In order to attain complete inhibition it was necessary to stimulate successively new regions of the epicotyl stump by a successive removal of 3 to 4 mm. apical sections and the fresh application of hormone.
4. The fact that the decapitated stem, i.e., the distance from the point of application of hormone has no essential effect on the inhibiting action of the hormone is in favor of the view that auxin acts indirectly.
5. If the action of auxin inhibition were direct (not through growth) then the buds closest to the point of application should be most strongly inhibited, which is opposed to all observations.
6. The fact that auxin indirectly prevents leaf fall through promoting growth is in favor of the view that auxin acts through its effect on growth of the stem in its inhibition of bud development.

That the above objections are entirely invalid is clear from the experiments described above, but will further be pointed out as follows.
1. The fact that pollen does not give complete inhibition is attributed to the relatively low concentration of auxin (Müller in the same article).
2. Against this argument are several facts. (a) "Wie die Werte der Tabelle zeigen, erhöhte sich die hemmende Wirkung, wenn auch nicht in denselben Masse wie die angewandten Konzentrationen. Leider konnte die Konzentration nur bis zu einem bestimmten Grad gesteigert werden weil dann eine schädigende Wirkung, Faulen des Epikotylstumpfes, durch

andere Bestandteile der Paste eintrat", (p. 507) of the same article and (b) (p. 505) "Mit Pasten der Konzentrationen, die sich wie 1:4 verhielten, behandelte Pflanzen zeigten, dass die Länge ihrer ausgetriebenen Axillarsprosse sich ebenfalls verhielten wie 1:4 (0.24 cm. :: 1.09 cm.) (c) In our experiments complete inhibition was obtainable by the application of auxin either in agar or in lanoline paste both to plants with long stems, leaves and lateral buds; and to plants in which only a small part of the stem was retained. Furthermore, as is shown in Figure 9, for a wide range, the amount of inhibition is a function of the concentration of auxin applied. Amounts of hormone 16 times higher than necessary for practically complete inhibition had no injurious effect on the plants.

3. Experiments on Pisum in the dark in which it was possible to cause complete inhibition by a single application for periods of 1 to 2 weeks definitely invalidates this argument.

4. The logic of this argument is not clear, However, it has been shown that the distance of the bud from the point of application may become important if the inhibiting substance is not readily transportable through the stem.

5. The falsity of this argument is shown by the experiments of Dostál and also from a consideration of bud development in Vicia, showing that the localization of most active bud development is inversely correlated to the distribution of auxin in the plant.

6. This argument is clearly irrelevent.

Conclusion

All arguments proposed to overthrow our interpretation that auxin inhibits buds independently of its effect on the growth of the main stem are invalid.

4. The mechanism of inhibition of lateral buds through inhibition of cell division.

The scanty experimental basis for the theory that auxin inhibits development of adventitious buds through inhibition of cell division has been given in section A, 3. Even if auxin inhibits cell division in leaf cultures it is far from proved that this is a general property of auxin. On the contrary, there is abundant evidence to show that auxin does not inhibit cell division. Thus for example in Vicia a high growth hormone content was found throughout the apical portion of the plant in which cell divisions must certainly occur, and in Tobacco leaves it was found by Avery (1935) that the region of most active cell division corresponds to the region of highest growth hormone content.

There exists furthermore considerable evidence that the application of auxin will induce cell division, although the action of auxin in this process must almost certainly be indirect. Thus, (a) Snow (1935) has found hetero auxin to stimulate cambial activity; (b) In a large number of species auxin has been found to promote callus formation, Laibach (1934), Michener (1935), etc.; (c) and similarly neoformation of roots, Went (1934). In woody cuttings Michener has found bud inhibition, callus formation and root formation simultaneously

induced by the application of hetero auxin to the apical cut surface, and finally in experiments on Pisum in the dark, bud inhibition was accompanied by active cell division in the swelling on the stem.

It should further be pointed out that the action of auxin in inhibition is not the prevention of the formation of buds but the outgrowth of buds already formed. That cell division can be the limiting factor of growth in such a process does not seem likely.

Inhibition of cell division by auxin, therefore, is not a general phenomenon and cannot be the cause of bud inhibition by auxin.

(Whether through the application of auxin it is possible to shift the region of most active cell division within the plant is a different question.)

C. Food and auxin as limiting factors in bud development.

Lack of food of various kinds, particularly carbo-hydrates and nitrogen, has been considered by numerous investigators to be the cause of the inhibition of lateral bud development.

Moreland (1934) from his experiments on Phaseolus draws the following conclusions: Inhibition of lateral bud development exercised by the terminal bud is probably due to its depletion of available food. Nutritive substances, especially nitrogen, is translocated to the apex past the lateral buds, which have relatively much poorer vascular connections with the stem. It is impossible to definitely show that inhibition is not due to a special inhibiting substance coming from the tip, as proposed by Snow. But, if such a substance is active, it is transported through living cells (phloem) and not

through the xylem. It must also be produced in developing leaves but only until they have reached their full size.

In our experiments it was early recognized that food may be a limiting factor in bud development. Thus it was found in Vicia that in the absence of leaves the buds grow more slowly. For example in one experiment the axillary buds grew at an equal rate for the first five days but on the tenth day the mean bud length of defoliated plants was only 21.6 as against 39.8 mm. in controls with leaves, and on the 18th day after decapitation it was only 41.8 as against 151.2 mm in controls. Thus in our experiments ~~the effect of~~ lack of food does not become a limiting factor until after the development of the buds becomes very rapid.

Furthermore, the conditions under which hormone should be able to prevent bud development as deduced by Moreland are precisely those under which growth hormone will be found in the plant. In fact it is possible to interpret nearly all experiments done by Moreland on Phaseolus in terms of the presence or absence of relatively large amounts of hormone as found experimentally in Vicia. It is thus clear that under ordinary growing conditions food is not directly the limiting factor in bud inhibition.

D. Conclusion

In the preceeding sections it has been shown that the action of auxin in bud inhibition is not a stimulation of growth of the stem either through an effect on cell elongation or on cell division and that the bud development in our experiments is not limited by lack of

food. In conclusion it remains to further point out that (1) the experimental evidence is in agreement with the proposed mechanism of direct inhibition through the prevention of auxin synthesis in the buds, and (2) to consider how the inhibition of auxin formation may be accomplished in the plant.

1. Conditions essential for the inhibition of buds by auxin.

From a consideration of the various experiments described above, it becomes clear that auxin can act as an inhibitor only when the following conditions obtain:-

a. The supply of auxin must be of relatively high concentration. (Concentrations just high enough to cause maximal normal growth are not sufficient.)

b. The supply of auxin must be continuous. (The intermittent application of concentrations higher than necessary to cause complete inhibition if the same amounts were supplied more frequently in smaller contrations have no effect.)

c. The auxin must reach the buds, or at least, the tissues of the stem in immediate contact with the basal end of the bud. (Applications below the bud are without effect, and such substances as are not readily transported polarly through the stem must be supplied immediately above the bud in order to have a maximum effect.)

It will be seen that the above conditions are in complete agreement with the concept that auxin inhibits the formation of growth hormone when supplied in relatively high concentrations from above.

2. The prevention of auxin synthesis.

As stated above, the presence of the coleoptile tip prevents the formation of growth hormone in sections below it, which, nevertheless, are potentially able to form it. However, it will be evident from work described in ~~the~~ Part III. that auxin synthesis in the coleoptile is a special case in as much as the material for auxin synthesis is derived from the seed. What is the justification then for the assumption that the same condition obtains generally in green higher plants? One piece of evidence in its favor is the fact found above, that inhibited lateral buds contain practically no or very small amounts of growth hormone, whereas rapidly growing ones produce amounts comparable to production in the terminal bud of the main shoot. A second evidence which confirms our results and thus shows them to be no mere coincidence was obtained by van Overbeek (1933) in Raphanus. He found that in the presence of cotyledons which are apically situated, and produce relatively large amounts of hormone, no hormone was actively formed in the tip of the hypocotyl, but a small amount was present. Now if the cotyledons were removed, the tip of the hypocotyl began within a period of five hours to produce considerable amounts of hormone. Van Overbeek concludes, independently and in complete agreement with our view, that the formation of auxin in the hypocotyl can take place only after the inhibiting effect from the cotyledons has been removed.

The evidence for the prevention of growth hormone synthesis by the presence of an apically located hormone producing center is thus

quite complete. In Avena Thimann and Bonner (1933) have brought forward evidence to show that the inhibition of regeneration is due to auxin, and also Heyn (1932) states that the application of an agar block with auxin has a similar effect on regeneration as has the tip.

In regard to the mechanism whereby the synthesis of auxin in the buds is prevented, no further definite conclusions can be made. It cannot be due to a simple chemical equilibrium since substances of different chemical structure and composition, some presumably identical, others different from the hormone normally produced by the plant, are able to produce the same effect. It has been suggested by Czaja (1935) that auxin in addition to being polarly transported in the plant also is active in maintaining the polarity of the tissues. Of interest in this connection is also the theory extended by Lund (1931) that the difference in redox potential existing between the apex and the lower part of the plant is a factor in the direction of transport of substances in the stem; and such a potential difference, which disappears and is taken over by the lateral buds, has been studied by his student, , in Phaseolus. There is in addition other evidence, not well enough established to be described here, indicating that auxin plays a role in the transport of substances in the plant. Thus, for example, Snow is now of the opinion that the action of auxin in bud inhibition is the control of the transport of some specific bud inhibiting substance. It might equally well be inferred that auxin controls the transport of some substance necessary for the synthesis of growth hormone or merely indirectly affects the conditions under which

synthesis can proceed. In any case this kind of transport is not a question of making food available.

Whatever the details of mechanism of the action of auxin may be, it is clear from the work presented above that auxin controls the development of buds, and the conditions under which auxin is active in inhibition are in complete agreement with our originally proposed concept that auxin prevents the synthesis of growth hormone when supplied to the buds from above in relatively high concentrations.

This work, then, finally proves the theory that the inhibition of buds is due to the action of a hormone. On the other hand, it also demonstrates that alternative hypothesis proposed to account for bud inhibition, such as polarity, transport of nutrient substances, difference in metabolic rates, and potential differences, although insufficient in themselves to account for bud inhibition, nevertheless are not irrelevant, but may be important factors in the mechanism through which the effect on bud development, controlled by auxin, is brought about in the plant. From the experiments on the production of growth hormone and the growth response to auxin of the plant in light and in darkness it has further been shown that in green plants auxin is formed only in the presence of light but the growth response to auxin is higher in the dark.

Finally by experiments with defoliated plants it has been demonstrated that in the stem of Vicia as in the Avena coleoptile auxin is essential for elongation. However experiments not described here also indicate that auxin is not the only hormone controlling the growth of the stem.

PART II

THE EFFECT OF X-IRRADIATION ON AUXIN AND PLANT GROWTH

CHAPTER III

INTRODUCTION

A. STATEMENT OF PROBLEM

During the work on bud inhibition, described in Part I, it was noticed that the effect of auxin on bud development appeared to be exactly opposite to the reported effects of X-irradiation. For example, Lallemand (1930) showed that if the terminal bud of a seedling was irradiated in such a way that the lateral buds were shielded, then the latter would start to grow out.

The action of X-rays on living organisms has been investigated by an increasing number of workers during the last forty years. Many interesting observations have been recorded regarding the effects produced by irradiation on growth and development, but very little has become known about the mechanisms of the action of X-rays on the living cells. As shown in the works of Johnson (1926) and Bersa (1926) which also include extensive references to earlier work, it has long been established that X-irradiation inhibits the growth of higher plants.

Since it is now also well known that the plant growth hormone, auxin, is essential and within limits controls the growth of higher plants, and since X-rays and auxin also both affect bud development, it seemed reasonable to suppose that one effect of X-irradiation on

growth would be in connection with its effect on auxin in the plant. This assumption was found to be correct. Hence, especially, since at this time the experiments on bud inhibition had to be discontinued due to lack of funds, and an opportunity became available to study the effect of irradiation in relation to auxin, this work was continued.*

In the following five chapters will be presented in some detail the results of experiments designed to determine the role of auxin in the immediate effects produced by x-irradiation on growth and development of higher plants. The effect studied is primarily in relation to the control of growth by the growth hormone. Correspondingly, the expression growth used here refers largely to cell-elongation, which is the phase of growth controlled by auxin. The part played by cell division in growth must be treated separately; but effects on growth of factors such as water, nutrient substances, temperature and light have, as far as possible, been eliminated.

B. APPARATUS AND MATERIALS

The high voltage tube of the Kellogg Radiation Laboratories was used as a source of radiation in all experiments. A description of its construction and operation is given by Mudd et al. (1934). It need only be stated here that this tube supplies a uniform beam of hard γ-rays at constant voltage and intensity measured in Röntgen units. All soft radiation is eliminated by successive lead, steel, and aluminum filters. In most experiments the tube was operated at 900

kilo-volts and 3 to 4 milliamperes.

The auxin obtained in highly active purified preparations by Thimann from Rhizopus suinus and from urine have been used. For comparison a synthetic product, Hetero auxin, or β-indolylacetic acid, which is identical to the extract from Rhizopus (1935, 1933) has also been used in some experiments. In addition one sample of auxin A, with activity greater than 300,000 plant units per milligram at time of experiment, has been irradiated in solution.

The plants were grown in the greenhouse or in dark rooms at constant temperature and humidity as specified for the Avena test method (Went, 1928). Seedlings and very young plants were used exclusively.

CHAPTER IV

THE EFFECT OF X-RAYS ON AUXIN IN SOLUTION

A. Inactivation of auxin in agar blocks

Auxin in highly active solutions of known purity, about 500,000 plant units per milligram of dry weight, was diluted with distilled water to a concentration of between 10 and 20 units per milliliter. This is the upper range of the concentrations directly measurable by the Avena method. Details of the technique need not be given here, since the method described by Went (1928) and as applied by Dolk and Thimann (1932) has been used throughout, and will be presented in some detail in Chapter VIII. From the solutions 1.5% agar blocks of the standard size used in the tests were prepared and placed on glass slides in Petri dishes lined with moist filter paper. Series of such dishes were exposed to the X-rays for different lengths of time, and the blocks were subsequently tested on Avena coleoptiles in the usual way. Some blocks treated in the same way, but not exposed to the X-rays, were tested at the same time and were used as controls. The amount of auxin found in the non-irradiated blocks was always taken as 100%. The reduction in activity found in irradiated blocks was then expressed as the percentage of inactivation produced by irradiation. This method eliminates any error resulting from the variations in the plants from day to day. The accuracy of the determination therefore is largely dependent on the uniformity of the curvatures of plants in each test. By the use of a row of 12 plants or more, the

curvatures of the plants seldom have a mean error exceeding 1 degree, i.e., it is usually less than 10%. With a series of experiments it is thus possible to determine with certainty even quite small changes in the activity of the auxin solutions.

TABLE XVII

The per cent of auxin destroyed by x-irradiation in agar blocks

EXPERIMENT NO.	CONTROL CURVATURES	TIME OF EXPOSURE IN MINUTES												
		0.5	1	2	3	5	7	10	15	20	40	45	60	120
I	14.5	30	30	33		34			57			44		
II	22			20			16	25		27			40	
III	18.4		38			36				32				40
IV	12	33	25			22		43			47			
V	17.7				47		41							

Experiments done July to September, 1933. Dosage 50 Röntgen units per minute at 600 kv. and 3 to 4 m.a.

Results from experiments are summarized in Table XVII. From them it can be seen that the auxin in 1.5% agar blocks is definitely inactivated by irradiation. The rate of inactivation is very fast at first, but decreases with time of exposure, so that complete inactivation would require a very long exposure time. It should be noticed, however, that about a third of the activity is destroyed almost immediately, and nearly half by moderate dosages under these conditions. No definite conclusion can be drawn regarding the mechanism of the inactivation. It appears from the table that (1) the solution contains two active auxins of different stability, or (2) the inactivation is indirect, i.e., some substance taking part in the inactivation reaction, at first present in relatively high concentration, is used up and becomes the limiting factor.

B. The mechanism of inactivation

1. Inactivation in water solution. In order to investigate

the role of the above two possible factors in the effect of irradiation it was convenient to use direct exposure of water solutions containing known high concentrations of auxin. A volume of 0.50 milliliters of auxin solution, usually of 1000 units per milliliter concentration was placed in series of small glass tubes with small capillary openings which could be sealed with a flame without heating the solutions. The tubes were exposed to the X-rays for different lengths of time and subsequently diluted with a given volume of water, such as to bring the concentration of auxin of the non-irradiated control solutions within 10-20 units per milliliter, and from these solutions blocks tested on Avena coleoptiles were prepared as above.

TABLE XVIII

The per cent of auxin destroyed by x-irradiation in water solution

EXPERIMENT NO.	CONTROL CURVATURES	TIME OF EXPOSURE IN MINUTES											
		1	5	10	15	20	40	60	80	100	120	180	240
		A. Hetero auxin from Rhizopus											
I	12.6°	14				15	29	54	54	60			
II	18.2°					22	28	35			43	48	
III	16.5°						30						
IV	14.0°	38	43	55					57				
V	11.0°	27	27	23	32				64				
VIa	9.2°												57
b	17.0°												62
		B. Synthetic hetero auxin (β-indolyl acetic acid)											
I	10.1°	24		28		57	68	59	80				
II	6.3°					45			85	92			
III	18.2°		27					41	38				
IV	18.1°			19		33	33	29	39				

All solutions exposed in concentration of about 1000 units per milliliter. Exposure to 50 R. units per minutes at 900 kv. and 3 to 4 m.a.

a. Comparison of different preparations. In Table XVIII A and B are summarized results obtained by exposing highly purified preparations of the hormone from Rhizopus and the identical synthetic product, indolyl acetic acid, respectively. They show that the inactivation by the X-rays is similar to that in the agar blocks. However, with comparable concentration the inactivation becomes much more complete with increasing dosages in the water solutions (see Table XIX) Furthermore, since the inactivation is similar in the extracted and synthetic preparations, it is clear that there is only one active substance present in the extract and that possible impurities have no effect on the inactivation. Thus possibility (1) above is excluded.

b. Effect of concentration. The second possibility was next studied by irradiation of different concentrations of a given auxin solution. The tubes for exposure were prepared from an auxin solution of known activity by successive dilutions so that the lowest concentration would be within 20 to 10 units/ml. After exposure this solution was tested without further dilution and the stronger solutions were

TABLE XIX

The per cent of auxin destroyed by x-irradiation in water solution at different concentrations

A HETERO AUXIN (THIMANN) DOSAGE 900 KV., 3.1 M.A., 50 R./MIN.					B AUXIN A (KÖGL) DOSAGE 900 KV., APROX. 3 M.A., 50 R./MIN.					
Dosage R. units	Per cent loss in activity at concentrations of				Dosage R. units	Per cent loss in activity at concentrations of				
	640°	160°	32°	10.5°		1125°	375°	125°	62°	20.8°
500	24	45	75	90	500	14	33	(96)	43	52
5000	29	67	86	84	3000	22	46	(94)	57	59
0	0 (10.2°)			0 (10.8°)	0	0 (20.7°)				0 (20.8°)

diluted so as to have the same original concentration as the last dilution. The means of the activities of control tubes from the highest and lowest concentrations tested at the same time were taken as 100% activity. The results are illustrated by the experiment given in Table XIX A. They show that the total amount of auxin destroyed by a given dosage is greater in the more concentrated solutions, but the percentage of auxin destroyed increases with dilution of the solution. Thus the view that the inactivation is indirect is supported by these experiments.

2. Oxidative inactivation. It is known that auxin is readily destroyed by peroxides. It has also been shown that when water is irradiated in the presence of air a considerable concentration of hydrogen peroxide is produced (Risse, 1930, Taylor et al, 1933). It therefore seemed likely that the auxin would be destroyed by the hydrogen peroxide formed by irradiation in the solution. That this is in fact the mechanism of inactivation is borne out by several experiments.

a. Effect of oxygen. Series of tubes containing auxin at a given concentration in freshly boiled water solutions were prepared. After bubbling oxygen-free nitrogen gas saturated with water vapor through capillary tubes into the solutions for eight to ten minutes the vessels were sealed in an atmosphere of nitrogen. They were then exposed to the X-rays together with similar sets of tubes containing the same concentration of auxin in the presence of air. All the solutions were diluted to a given volume and tested on Avena at the same time. The percentage inactivation in each case is shown in Table XX.

TABLE XX

The per cent of auxin destroyed by x-irradiation in the presence and absence of oxygen

	EXPERIMENT NO.	TIME OF EXPOSURE IN MINUTES						
		1	5	10	15	20	75	80
Air	I	27	27	22		32		64
	II	36	41		54		56	
Nitrogen	I	0	0	0		0		27
	II	11	11	11	11	11	33	

Aqueous auxin solutions, 1000 units/ml. con., in the presence of air or nitrogen gas in sealed glass vessels exposed to 50 R. units/min. at 900 kv., and 3.5 m.a.

From the results it is clear that in the absence, or in the very low concentrations of oxygen attained, there is no immediate inactivation of auxin comparable to that in the presence of air. The fact that prolonged irradiation gives rise to some inactivation is probably to be ascribed to incomplete removal of oxygen. A 1000 unit per milliliter hetero auxin solutions contain 1 to 2 x 10^{-8} mols per milliliter. If the solubility of oxygen is taken as that from air at 20°C, the solution saturated with air contains 2.6 x 10^{-7} mols per milliliter of oxygen. Assuming that two molecules of auxin react with 1 molecule of oxygen to become inactive, then the amount of oxygen required to reduce the activity 33% is only about 3 x 10^{-9} mols per milliliter, or about 1 percent of the amount present in water saturated with air. With the simple method of removing oxygen from the solutions used above it is likely that enough oxygen would be left to have an effect after prolonged irradiation. However, since this point does not invalidate the results, no attempt has been made to use a more refined method.

3. Inactivation in chloroform solution. In the above experiments the rate of inactivation in aqueous solution of high concentration

rapidly decreases with time of exposure, so that complete inactivation was not obtained. If the indicated mechanism of oxidation is correct, however, it should be possible to increase the destruction by the use of a suitable solution giving rise to larger quantities of oxidizing agents on exposure to radiation. It is well known that when chloroform is irradiated it decomposes, giving rise to peroxides and other strong oxidizing agents. Chloroform is particularly suitable for these experiments, because it has been used for keeping highly active auxin preparations for long periods of time in this laboratory. When such chloroform solutions were irradiated and tested in the same manner as the water solutions above they were found to be almost completely inactive after 40 minutes exposure, either in the presence or absence of air. In fig. 10 curve I (continuous line) representing the mean of several experiments, percent inactivation is plotted against time of exposure. The fact that the destruction here is complete strongly supports the view that the inactivation is caused by oxidation. The uniform rate of the reaction also indicates that the hormone solution contains only a single active component.

Since a chloroform solution contains the auxin in only the undissociated form, the possibility exists that the ions might be stable and thus account for the less extensive inactivation in water. However, this possibility is excluded by the fact that in buffered water solutions the inactivation is at least as great in pH ranges well above the pK of the acid as in the range below it.

Figure 10

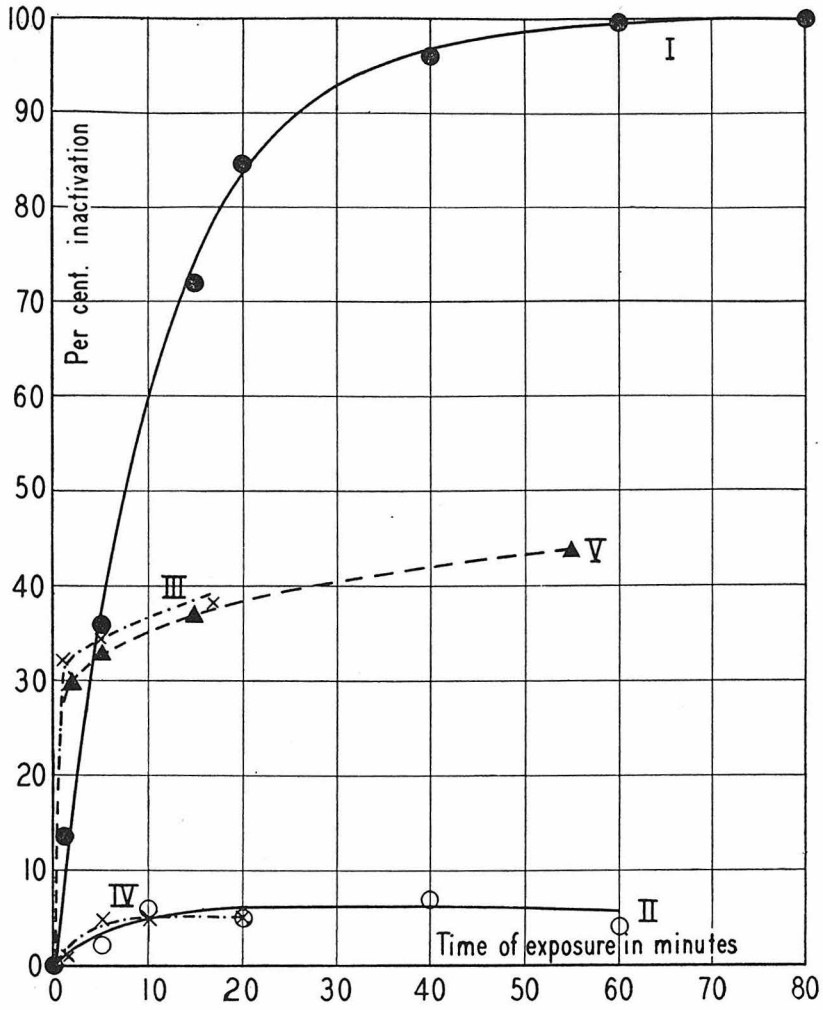


Fig. 1 Effect of irradiation on inactivation of auxin in solution. I. 1000 u./ml. concentration hetero auxin, in chloroform. II. 1000 u./ml. concentration hetero auxin, in chloroform, + trace of sealing wax. III. 1000 u./ml. concentration hetero auxin, in water in presence of air. IV. 1000 u./ml. concentration hetero auxin, in water in presence of nitrogen. V. 10 to 20 u./ml. concentration hetero auxin, in 1.5 per cent agar blocks. Dosage approximately 50 R./min. at 900 kv., 3 to 4 m.a.

Further proof that the inactivation of auxin is only indirect was obtained by the following experiments. When the glass tube containing the chloroform solutions of auxin were sealed with a small amount of sealing wax of which a trace dissolved in the solution, there was no inactivation produced by the X-rays even after very long

exposures. The same is true if a trace of sealing wax was dissolved in the solution in glass sealed vessels either in the presence or absence of air. The mean values of such experiments are expressed by Curve II (continuous line) in fig. 10. It is assumed that some component of the wax present in the solution in minute concentration stabilizes the activity of the auxin through its action as an anti-oxidant, because it is known that the addition of small amounts of certain organic compounds prevents the photochemical production of oxidizing agents in chloroform (Chapmen, 1935). Moreover the addition of finely dispersed sealing wax to a water solution does not prevent oxidation, but, on the contrary, as shown in Table XXI, makes the destruction of the auxin more complete.

Table XXI

The effect of addition of finely dispersed sealing wax on the inactivation of auxin by irradiation in water solution

Time of exposure min.	Substances present		Curvatures in degrees	Loss in activity in %
	Air	Sealing wax		
0	+	-	11.0	----
0	+	+	11.5	0
60	-	-	11.7	0
60	+	-	9.8	16
60	+ +	-	8.8	22
60	+	+	4.2	63
60	+	+ +	1.8	84

Thus inactivations of 63 and 84 per cent were obtained with small and large amounts added compared to about 20 per cent in tubes without

sealing wax, and no inactivation in tubes with sealing wax but not exposed to the X-rays.

4. General occurrence of auxin inactivation. It has been shown that extracted and synthetic hetero auxin are similarly inactivated by X-irradiation. In order to show that this occurs generally and is not dependent on the individual preparations, one experiment with auxin A, kindly submitted by Professor Kogl (1935) is given in table XIX B. Furthermore, in order to show that the inactivation is not limited to high voltage X-irradiation, one experiment was carried out with a small quantity of radium. The auxin vessels were so placed

Figure 11

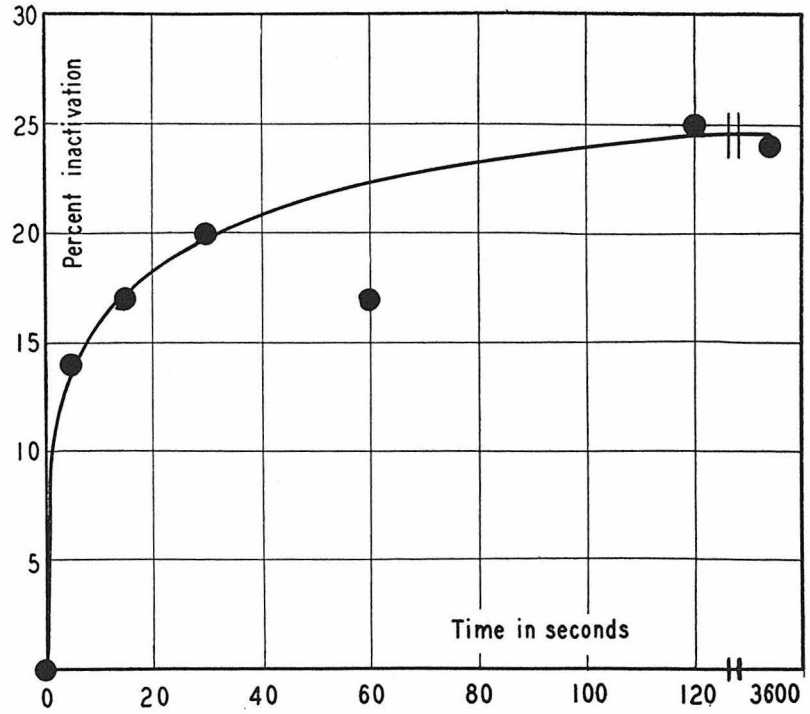


Fig. 2 Effect of radium irradiation on auxin in water solution. Tubes containing 1000 units/ml. auxin solution placed 0.5 cm. from 75 mg. radium bromide tube. Dosage = 0.8 R./sec.

that the dosage in Röntgen units would correspond to that from the X-ray tube. In this experiment the initial, fast rate of inactivation was determined by making some of the exposure times very short. The results, Figure 11, show that the inactivation is comparable to that produced by the X-rays, and that the rate at first is very fast.

C. The photochemical inactivation of auxin in dilute eosin solutions.

In work on irradiation of various substances it has been shown that eosin and similar dyes catalyze photochemical oxidation reactions. Furthermore it has been shown by Boas (1933) that Avena seedlings grown in eosin solutions in the dark have a nearly normal growth rate, but when exposed to light they fail to give a phototropic response. It is known that the phototropism in the Avena coleoptile depends on the distribution of auxin. Therefore, if it could be shown that auxin in the presence of eosin is inactivated in the light, and only in the light, this would be a case of inactivation similar to that by the X-rays but by a different method.

Experiments were done as follows. Two identical sets of tubes containing a given concentration of auxin and various dilute concentrations of eosin were prepared in weak red light. One set was exposed either to sunlight or white light, and in one experiment to X-rays for different lengths of time. The other set was kept in the dark. One tube of each set containing no eosin was kept as control. The solutions were subsequently tested at the same time by the Avena method. Results of the experiments are summarized in Table XXII. It can be seen that a solution containing as little as 1×10^{-5} parts of eosin was completely inactivated

TABLE XXII

Photo-chemical inactivation of auxin in presence of eosin in water solution

EXPERIMENT NO.	DATE (1934)	CONCENTRATION DYE PER CENT × 10^4	TIME OF EXPOSURE IN MINUTES	CURVATURES IN DEGREES	LOSS IN ACTIVITY IN PER CENT
I	1/14	0	S	18.2 ± 1.6	0
		0	X 40	13.1 ± 1.6	28
		25	X 40	0.0 ± 0.1	100
		25	D 10	1.6 ± 1.4	93
II	5/26	0	S 15	12.2 ± 0.8	0
		25	Dark	12.8 ± 1.5	0
		75	Dark	11.7 ± 0.8	4
		25	S 15	0.1 ± 0.1	99
		75	S 15	0.7 ± 0.8	94
III	5/28	0	S 15	17.6 ± 0.9	0
		0	Dark	17.6 ± 1.2	0
		50	Dark	14.6 ± 1.2	17
		50	S 15	0.0 ± 0.7	100
IV	6/4	0	D	14.3 ± 1.4	0
		50	Dark	15.4 ± 1.3	0
		50	E 0.5	15.9 ± 0.9	0
		50	E 5	10.9 ± 1.4	24
		50	D 10	8.0 ± 0.8	44
V[1]	6/7	0	S 18	13.2 ± 1.1	0
	a	50	Dark	11.5 ± 0.6	13
	a	50	S 5	0.9 ± 0.7	93
	a	50	S 15	0.7 ± 0.9	95
	b	50	Dark	13.2 ± 1.1	0
	b	50	S 5	2.9 ± 0.9	78
	b	50	S 15	2.8 ± 1.1	79
VI[1]	6/8	0	S 15	13.5 ± 0.8	0
	a	25	S 15	0.2 ± 0.5	99
	a	10	S 15	1.4 ± 1.3	90
	a	1	S 15	1.1 ± 1.2	92
	a	0.1	S 15	3.6 ± 0.7	73
	b	25	S 15	2.3 ± 0.8	83
	b	10	S 15	1.1 ± 0.9	92
	b	1	S 15	1.4 ± 0.9	90
	b	0.1	S 15	3.3 ± 1.2	76

The source of light used for exposure is designated as follows: S, sunlight; D, diffuse daylight; E, light from 40 watt Mazda lamp at a distance of 20 cm.; X, x-irradiation 50 R. units per minute at 900 kv. and 3.5 m.a.

[1] In experiments V and VI the tubes marked b contained a concentration of 0.0025 and 0.0100 normal $Ti_2(SO_4)_3$, respectively.

in the light, whereas a solution containing no eosin remained fully active. The tubes in the dark (red light), on the other hand, showed no, or practically no, inactivation in the presence of eosin. It is therefore clear that the eosin causes a complete destruction of auxin

in the presence of light or X-irradiation.

After these experiments were completed a paper by Boysen-Jensen (3) appeared which shows that the action of eosin in preventing growth or phototropic response is in fact through its destruction of auxin in the plant.

D. Conclusion

From the above experiments it is clear that auxin is inactivated by X-irradiation. The mechanism of the inactivation is an oxidation. Thus in aqueous solutions the presence of oxygen is necessary, and the extent of the reaction is limited by the strong oxidizing agent made available by the X-ray treatment. In chloroform solutions where appreciable concentrations of oxidizing agents are produced through photochemical decomposition, the inactivation is complete even in relatively strong auxin solutions both in the presence and absence of air, but can be prevented by anti-oxidants. A similar inactivation in water solution is carried out catalytically by eosin in the light.

CHAPTER V

THE EFFECT OF X-RAYS ON AUXIN IN THE PLANT

A. Loss of auxin

Since auxin is inactivated in solution by X-rays, experiments were performed to determine to what extent it will be affected by X-irradiation in the plant. It is well known that auxin was first isolated by placing tips of Avena coleoptiles on agar blocks, thus allowing the auxin to diffuse from the tips into the agar (Went, 1928). This procedure was followed. The amount obtainable from one tip per hour is quite small, but when a set of 12 tips are placed on a standard full size agar block subsequently divided into 12 equal test blocks, the amount obtained is usually enough to give a good quantitative test.

It must be borne in mind that in the plant the loss of auxin resulting from irradiation may be due to any of three different causes. (1) Inactivation as in the water solutions above, (2) inhibition of auxin transport in the plant, and (3) damage to the auxin synthesizing system in the plant. After it has been established that auxin is lost by irradiation of the plant, the nature of the effect in relation to the above three processes will be studied separately.

The first experiments were on Avena coleoptiles. The plants were grown in zinc boxes containing a mixture of leaf mold and sand in the dark room under standard conditions. On the third day after the beginning of germination when the coleoptiles were 2.0 to 2.5 cm. long, the boxes were covered with black paper envelopes and brought to the

X-ray tube room. Half of them were exposed to the X-rays. The others were used as controls. Immediately after treatment the boxes were returned to the dark room. The tips were cut off and placed on standard agar blocks, twelve tips on a block, and allowed to diffuse for a given time, usually two hours. The blocks were then divided into twelve

Table XXIII

The effect of irradiation on the amount of auxin diffusing out from Avena coleoptile tips

EXPERIMENT NO.	DATE (1934)	DOSAGE IN R.U.	DIFFUSION TIME	NUMBER OF PLANTS	CURVATURES	PER CENT LOSS
			minutes			
I	3/7	0	90	24	14.9 ± 1.1	..
		1200		24	12.9 ± 0.8	14
II	3/16	0	180	11	25.8 ± 1.2	..
		1200		24	17.4 ± 0.7	32
III	3/22	0	120	22	15.6 ± 0.9	..
		1200		23	12.9 ± 1.1	18
IV	6/29	0	120	23	4.3 ± 0.6	..
		600		23	2.8 ± 0.4	35
		1200		23	2.0 ± 0.4	53
V	7/2	0	90	21	5.5 ± 0.6	..
		600		22	4.6 ± 0.5	17
		1200		32	2.7 ± 0.3	51
VI	7/19	0	120	24	4.4 ± 0.4	..
		1200		23	3.3 ± 0.5	25
VII	8/7	0	150	23	11.1 ± 0.6	..
		2100		22	9.0 ± 0.6	19
VIII	8/8	0	180	22	10.6 ± 0.5	..
		1800		22	7.3 ± 0.6	32
IX	8/9	0	120	18	6.9 ± 0.8	..
		1200		18	2.7 ± 0.7	61

Plants grown in dark room at 25°C. exposed at 900 kv. and 3.5 to 4 m.a. at 30 R. units/min. Lapse time between exposure and placing on blocks 10 to 30 minutes. Twelve tips placed on a full-size block.

equal parts and tested in the usual way. The effect of X-irradiation on the amount of auxin diffusing out from Avena tips is shown in Table XXIII. The percentage of auxin lost varies considerably in the different experiments. This variation might be due to several causes such as the temperature of the tube room and variation in the material from day to day. It is therefore impossible to express the amount of inactivation as a function of the dosage, although it appears to increase with increasing dosage. However, by taking a mean of all the experiments it can be said that in the Avena coleoptile one third to one half of the auxin is lost by treatment of the plant with a moderate dosage.

Figure 12

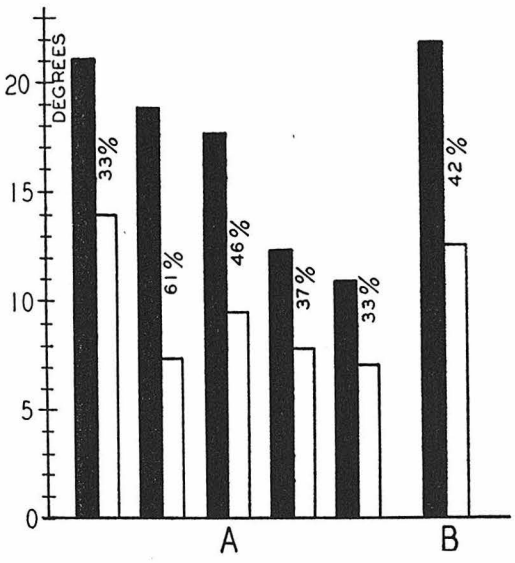


Fig. 3 Effect of irradiation on the amount of auxin diffusing out from A) terminal buds, B) stem sections of Vicia faba. Plants irradiated for 20 minutes at 750 kv., 3 to 4 m.a., and 30 R./min. Time of diffusion 120 minutes immediately after exposure. Black columns represent controls. Inset figures represent mean per cent inactivation by irradiation.

It has been shown in Chapter I, Table I, that the terminal buds of young Vicia faba plants, grown in the light, yield relatively high concentrations of growth hormone by diffusion into agar in the same manner as the Avena tips above. In figure 12 are shown the results of determinations of the amount of hormone obtained from tips (A) and stem sections (B) of control and irradiated plants. Results of experiments with Pisum, which is very similar to Vicia in regard to auxin are shown in Figure 13. The results show that both in Vicia and in Pisum between 30 and 40% of the auxin was lost by irradiation under the conditions of the experiments. It is thus clear that in the plant the loss of auxin is quite large and compares favorably with the order of inactivation in water solutions by the same dosages.

Figure 13

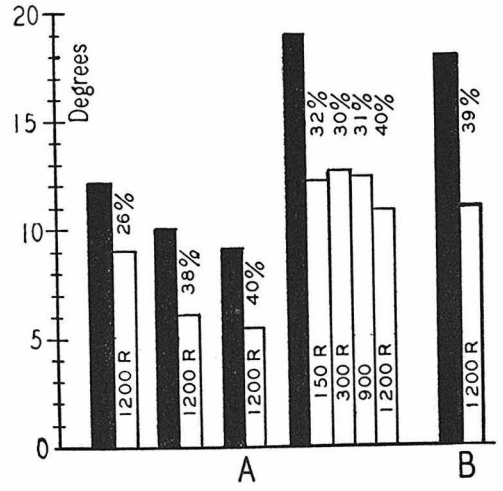


Fig. 4 Effect of irradiation on the amount of auxin diffusing out from A) terminal buds, and B) stem sections of Pisum sativum. Exposure 30 R./min. at 900 to 925 kv. and 3 to 4 m.a. Lapse time between exposure and placing on blocks 10 to 30 minutes. Diffusion time approximately 1 and 2 hours. (Heights in B multiplied by 5.)

B. The effect of X-irradiation on the transport of auxin.

It has been shown that the transport of auxin in the plants used is strictly polar. Auxin formed in the tip of the plant or in the leaves is transported basipetally. If the plant is inverted the transport will remain from the tip towards the base, thus against the force of gravity and not in the opposite direction. The same behavior is shown by isolated short stem sections. As in the experiments of van der Wey (1932), the amount of transport was quantitatively determined by standing stem sections on standard 1.5% agar blocks and placing blocks of agar containing a given concentration of auxin at the upper cut surfaces of the sections. Subsequent determinations with the Avena test of the amount of auxin in the lower blocks after a given time of diffusion give the amounts transported. Such experiments were made using sections from the two topmost internodes of Pisum and from the upper halves of the hypocotyls of Helianthus. These plants were grown in the light and irradiated under the same conditions as those used for the auxin determinations above and for other experiments below. In these plants the stem sections themselves yield appreciable amounts of auxin. The amount actually transported, therefore, was determined by the difference in activity in the lower blocks when auxin was supplied to the upper surface and that of controls to which pure agar was applied. Alternate sections from the same plants were used for transport and controls. Since irradiation affects the auxin content of the stem sections (see figure 13 B) these controls were carried out separately for irradiated and non-irradiated plants. In order to get uniform

results and a large amount of transport in a short diffusion time, 20 sections of Pisum and 12 sections of Helianthus respectively were placed on a single block. In some cases an identical set of experiments containing the sections in inverse position was carried out at the same time. All sections were kept in the same large Petri dish lined with wet filter paper to prevent drying out during the time of diffusion, which was usually two hours. About an hour after the sections had been

TABLE XXIV

The effect of irradiation on the transport of auxin in Pisum and Helianthus

EXPERIMENT NO.	DATE	TIME OF		AUXIN BLOCKS APPLIED				PURE AGAR BLOCKS APPLIED			
		Exposure	Diffusion	Irradiated		Controls		Irradiated		Controls	
				Norm.	Inver.	Norm.	Inver.	Norm.	Inver.	Norm.	Inver.
		minutes	*minutes*								
I	3/23/24	60	120	11.2	0	14.4	0.2	2.5	0.1	2.8	0.0
II	3/9/34	40	150	11.1		12.5		1.0		2.6	
				9.2		11.0		1.6			
				10.2		11.8		1.2		2.6	
III	5/20/34	40	120	4.6		6.1		2.3		4.4	
IV	6/12/34	20	150	8.9	1.0	9.8	1.8	4.9		5.8	
Mean total diffusion				9.2	0.5	10.9	1.0	2.2	0.1	3.6	0.0
Amount of transport				7.0	0.4	7.3	1.0				
Difference in transport				0.3	0.6						
I	2/7/34	40	120	9.0	0.2	9.0	0.9	3.1	0.8	4.6	0.0
II	2/14/34	40	120	7.2	0.2	10.4	0.0	0.5	0.0	0.9	0.0
Mean total diffusion				8.1	0.2	9.7	0.4	1.8	0.4	2.8	0.0
Amount of transport				6.3	—0.2	6.9	0.4				
Difference in transport				0.6	0.6						

A. Pisum

Alternate sections cut from the two topmost internodes of young Pisum plants exposed to 30 R. Units per minute at 900 to 925 kv. and 3.0 to 3.5 m.a. Twenty sections 6 mm. (except in I, 10 mm.) placed on a full-size agar block. Standard 40 to 50 u./cc. auxin blocks used for application. All figures are the means of twelve or more test plants

B. Helianthus

Alternate sections cut from the upper halves of hypocotyls of seedlings exposed to 30 R./units per minute at 925 kv. and 3.3 to 3.5 m.a. Twelve sections placed on full-size agar block. Standard 50 u./ml. full-size blocks used for auxin application. All figures are the means of about twelve test plants

removed and the auxin had become distributed throughout the blocks, they were divided into twelve equal parts and tested on Avena. Results of experiments are given in Table XXIV A for Pisum and B for Helianthus. The amounts of auxin diffusing out from the sections themselves are quite small, but there is a definite loss of hormone due to irradiation, and this corresponds to the difference in total amount of hormone obtained in blocks on which had been placed sections, supplied with auxin, from control and irradiated plants respectively. When correction is made for the hormone derived from the sections themselves, therefore, the amount of auxin transported in control and irradiated plants is so closely the same that it can be said with certainty that the transport of auxin in the plant is not at least immediately affected by moderate dosages of irradiation. (There is some indication from other experiments that a long time after irradiation, the transport may have become decreased.) From the experiments with sections inverted, it is also clear that the transport is strictly polar and that the polarity is not influenced by X-irradiation.

It will be shown from a consideration of the growth response to auxin in irradiated Avena coleoptiles that also in these the transport is not affected.

The loss in growth hormone in the irradiated plants must, therefore, be due either to its destruction, to the inhibition of its formation, or to the combination of both in the plant.

C. The effect of X-irradiation on the formation of auxin.

In order to determine whether the mechanism of formation of auxin

in the plant is affected through the action of the X-rays either of two methods may be used. (1) A direct determination of the amount of auxin present in the plant at different successive intervals after exposure. (2) A comparison of the growth rates of control and irradiated plants. The latter method, however, is only applicable when it is known that the amount of auxin produced by the irradiated region strictly limits the growth rate. This condition is approximated in the growth of the Avena coleoptile in the dark at constant temperature and humidity. However, if intact coleoptiles are irradiated, only a small decrease in growth is to be expected from the experiments of Thimann and Bonner (1933), in which are shown the relative growth rates of decapitated coleoptiles with the application of different small concentrations of auxin. Furthermore, even in the Avena coleoptile at least two factors control growth, one of which is the auxin concentration. In the apical portion the hormone is in excess and does not limit the growth, so that in irradiated plants the auxin destruction is likely to be greater than is indicated by the decrease in growth. Nevertheless, if the measurements are continued for some time after treatment, it is at least possible to determine whether a permanent effect on the formation of auxin has been produced. Measurements were therefore made on a large number of control and irradiated coleoptiles. The plants were grown and in all respects treated in the same manner as those used for auxin determinations above. They were measured with a horizontal microscope just before irradiation, and then at intervals. In figure 14, curves I, one of five essentially identical experiments, is represented by

Figure 14

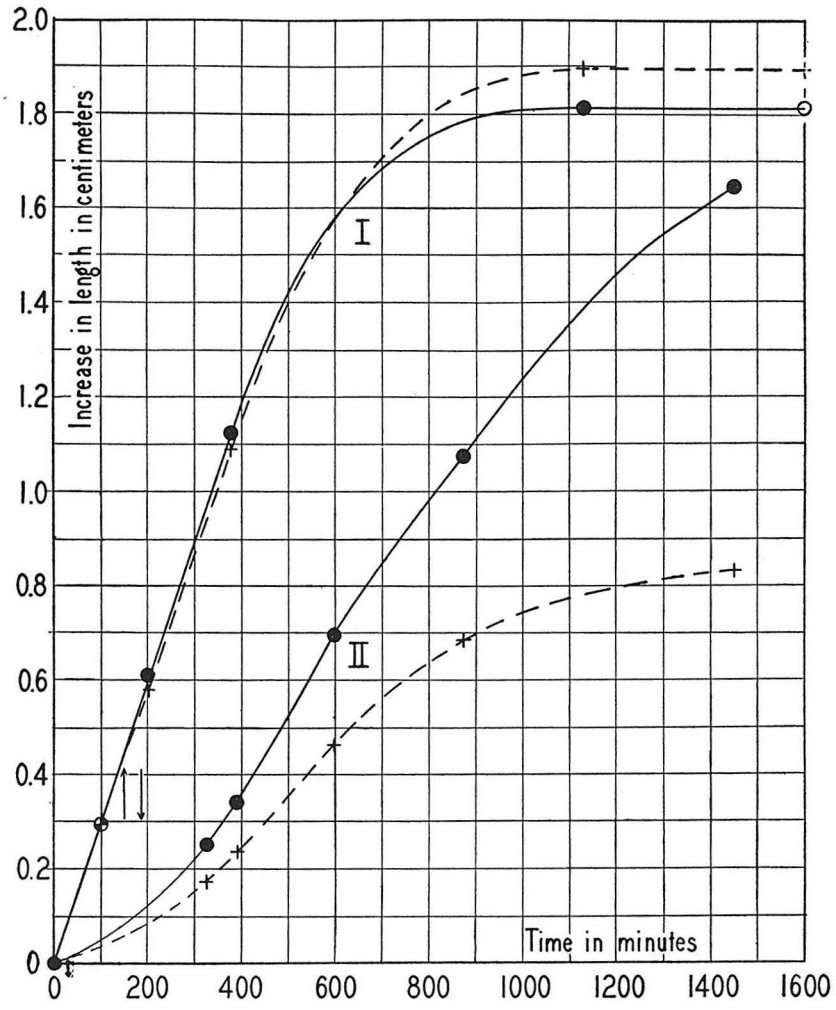


Fig. 1 Effect of irradiation on the growth of Avena coleoptiles, curve I, and primary leaves curve II. Broken line, irradiated. Scale for II reduced so that length = cm. × 10 and time = min. × 5. Each curve represents the mean of fifty plants. Dosage 30 R./min. at 900 kv. and 3 to 4 m.a.

plotting the increase in lengths of the coleoptiles against time. (The growth of the primary leaves is also included, curves II). It is seen that there is a definite but very small immediate decrease in growth due to irradiation.

However, after treatment the plants quickly regain their

normal growth rate and soon overtake the controls, so that some time after irradiation the total lengths of the exposed coleoptiles are the greater. This apparent stimulation of growth is a secondary effect of irradiation. It is mainly due to the fact that the growth of the primary leaf is much retarded by the action of the X-rays, so that it will break through the coleoptile some time later in the greated plants, thus allowing them to continue growth for a longer time. It has been shown cinematographically that as soon as the coleoptile is broken through by the primary leaf its growth immediately decreases very fast and soon ceases. Vice versa, the retardation of the growth of the coleoptile by decapitation results in increased elongation of leaves subsequently developed. Thus there is an inverse balanced relation between the growth of the coleoptile and of the leaves. It might also in part be due to the effect of irradiation on the activity of enzymes in the plant known to be destroyed by irradiation, so that the effective auxin concentration becomes increased. By experiments with maize seedlings van Overbeek (1935) has demonstrated enzyme inactivation of auxin in the plant, and has shown that the rate of this inactivation may become the limiting factor in growth.

The above results indicate that the mechanism of auxin formation in the coleoptile tip is not destroyed by irradiation. Moreover, since the percentage of auxin lost in the coleoptile is of the same magnitude as that destroyed in water solution by a comparable dosage, it is concluded that the loss in auxin is due entirely to its inactivation by irradiation.

In green plants, although auxin is known to control growth, the quantitative relationship between the amount of auxin and growth is less well worked out. But, since in green plants such as Pisum auxin production continues for a long time at about a constant rate, it is here possible to estimate the effect of X-irradiation on the formation of auxin by a series of determinations at different times after treatment. For this purpose 15 - 20 day-old Pisum plants grown in the green house as above, were divided into two identical groups. One was irradiated, the other was used as control. The amount of hormone obtainable from the terminal buds by diffusion into agar blocks was determined on successive days after exposure. Six buds were placed on one full size agar block in each case. Results are given in Tables XXV A, B, and C, representing exposures of 10, 20, and 40 minutes respectively. From these it is evident that in Pisum there is a definite permanent inhibition of auxin formation produced by irradiation. It also appears that the large decrease in auxin obtained immediately after irradiation is due largely to the inactivation of auxin in the terminal bud of Pisum in the same manner as in the tip of the Avena coleoptile. This conclusion is drawn because firstly, the immediate loss in activity is much greater than would be expected simply from an inhibition of formation of the hormone in view of the subsequent, relatively slow rate of decrease in formation, and secondly, the fraction of auxin lost is of the same order of magnitude as that destroyed by comparable dosages in irradiated stem sections (see Figures 12 and 13) in which there is no synthesis of the hormone. Set A was

Table XXV

The effect of irradiation on the amount of auxin diffusing out from Pisum tips at different times after exposure

	DAY	LAPSED TIME	NUMBER OF PLANTS	CURVATURES, DEGREES	AUXIN PER HOUR	INACTIVATION
		hours				*per cent*
A	0	0.5	23	17.5 ± 0.9	8.8	..
			34	13.0 ± 0.6	6.5	26
	1	24	33	9.0 ± 0.7	4.5	..
			35	4.6 ± 0.2	2.3	49
	2	47	36	3.6 ± 0.5	1.8	..
			34	0.6 ± 0.2	0.3	83
	3*	72	30	8.8 ± 0.5	3.5	..
			34	1.3 ± 0.3	0.5	85
	4	96	28	6.7 ± 0.7	3.4	..
			35	0.6 ± 0.2	0.3	91
	5	120	23	4.9 ± 0.5	2.5	..
			24	0.8 ± 0.1	0.3	89
	9*	216	24	10.2 ± 0.7	4.1	..
			23	2.5 ± 0.5	1.0	76
B	0	0.3	34	18.5 ± 0.8	9.3	..
			35	14.5 ± 0.8	7.3	22
	1	24	36	14.0 ± 0.6	7.0	..
			35	9.9 ± 0.6	5.0	29
	2	48	23	9.5 ± 0.8	4.8	..
			21	6.3 ± 0.6	3.2	34
	3	72	35	13.0 ± 0.7	6.5	..
			35	8.6 ± 0.7	4.3	38
	4	96	33	11.0 ± 0.7	5.5	..
			35	4.1 ± 0.3	2.1	63
	13	312	48	4.1 ± 0.5	2.2	..
			48	1.0 ± 0.3	0.5	76
C	0	0.5	22	13.1 ± 0.8	6.6	..
			23	8.2 ± 0.6	4.1	37
	1	24	34	13.6 ± 0.7	6.8	..
			35	3.1 ± 0.5	1.6	77
	2	48	20	13.8 ± 0.9	6.9	..
			36	0.8 ± 0.2	0.4	94
	4	96	34	11.7 ± 0.7	5.9	..
			36	0.8 ± 0.3	0.4	93
	7	168	32	8.5 ± 0.6	4.3	..
			24	0.5 ± 0.3	0.3	94
	8	192	22	9.6 ± 0.8	4.8	..

A. Started 7/27/34, 10 minutes irradiation at 900 kv. and 4.0 m.a., 30 R./min. Dosage= 300 R.

B. Started 6/13/34, 20 minutes irradiation at 900 kv. and 3.4 m.a., 30 R./min. Dosage = 600 R.

C. Started 5/29/34, 40 minutes irradiation at 925 kv. and 2.9 m.a., 30 R./min. Dosage = 1200 R.

Diffusion time = 120 minutes except * = 150 minutes.

done in July when the daily maximum temperatures were above 95 to 100 degrees F. during several days. Under these conditions the plants have an unsteady, abnormal growth rate, and the amount of auxin obtainable by the Avena tests varies greatly from day to day. Nevertheless, since control and irradiated plants were always tested together, the results of each experiment are consistent among themselves, but the three experiments are not comparable with each other. Thus no conclusion can be drawn regarding the exact relationship between the amount of inactivation and the dosage.

Several attempts to determine the difference in the amount of auxin diffusing out from the tips of irradiated and control Helianthus seedlings have been unsuccessful, because the amounts of auxin obtained have been so small that the difference is of the same order as the experimental error. It will be clear from other experiments, however, that also in this plant auxin is inactivated by irradiation.

Conclusion

From the above results it is concluded: 1. Moderate dosages of X-irradiation produce an immediate inactivation of from 20 to 40% of the auxin obtainable by diffusion from both the tip of the Avena coleoptile and from the terminal buds of Vicia and Pisum. 2. These dosages do not affect the mechanism of auxin formation in the Avena coleoptile. In Pisum, on the other hand, they produce a gradual, almost complete inhibition of the auxin synthesis. This difference between the two will be discussed later in relation to the source of auxin in the plants.

CHAPTER VI

THE RELATION OF AUXIN INACTIVATION TO THE INHIBITION OF GROWTH BY X-IRRADIATION

The purpose of the experiments to be described in the following chapter is to determine how far the inactivation of auxin and the prevention of its formation in the plant are responsible for the inhibition of growth produced by X-irradiation. It is possible that the main action of irradiation on growth is not the destruction of auxin but that the cells are affected in other ways which would result in a decrease in growth. This possibility can be investigated by measurements of the response of irradiated plants as compared with controls to applied auxin.

A. The effect on the sensitivity to applied auxin.

It is, for instance, possible that the X-rays would cause a decrease in the sensitivity of the growth response of the cells to applied auxin. If, on the other hand, the inhibition of growth is due only to loss in auxin, then the growth response to applied auxin should be as great in irradiated as in control plants.

1. Experiments with Avena.

The curvatures of the Avena test gives an accurate measure of small amounts of growth in the coleoptile. The first experiments were therefore to determine whether irradiated coleoptiles give quantitatively the same auxin curvatures as non-irradiated controls. Plants about 3 cm,

long were decapitated and treated similarly to those used above. Immediately after the exposure they were redecapitated, and blocks of a given concentration of agar were applied as in standard tests. Results of such experiments are given in Table XXVI in which are also included experiments on coleoptile sections mounted on thin glass rods with the bases in water, and one experiment on geotropic sensitivity. From the table it can be seen that the irradiated plants have the same capacity for growth as the controls. Indirectly the experiments also show that the transport of auxin in the coleoptile is not affected.

Table XXVI

The effect of irradiation on the sensitivity of growth response to applied auxin in Avena coleoptiles

EXPERIMENT NO.	DATE	LAPSED TIME	DOSAGE		NUMBER OF PLANTS	DIFFERENCE	CURVATURES	
			At	In R.				
		hours						
I	10/33	½–1	750 kv.	0	9	—1.0	5.4	Sections
			30 R./m.	300	9		6.4	
II	10/33	½–1	750 kv.	0	16	0.2	5.4	Sections
			30 R./m.	300	18		5.2	
II	After 5 hours			0	16	—1.3	15.0	Sections
				300	18		16.3	
III	11/33	½–1	900 kv.	0	15	—0.7	13.0	Intact seedlings
			30 R./m.	700	19		13.7	
IV	11/33	½–1	900 kv.	0	44	—0.2	16.1	Intact seedlings
			30 R./m.	900	26		16.3	
V	8/34	0.15	925 kv.	0	16	—1.6	35.5	Geotropic response in intact seedlings
			30 R./m.	2000	26		37.1	

Thus if auxin be supplied to the plant in such a way that the partial inactivation by irradiation is counteracted by the presence of an excess concentration, then there should be no decrease in growth resulting from exposure to the X-rays. In order to show the effect of

applied auxin to irradiated plants it is necessary to work with material that on the one hand exhibits a marked decrease in growth due to irradiation, and that on the other hand has a marked growth response to applied auxin. It has been shown that the decrease in growth of the intact irradiated coleoptile is slight, and that the synthesis of the hormone in the plant continues. In order to show a decrease in growth by irradiation it is necessary as far as possible to stop the formation of new auxin after treatment. Since the earliest work on auxin the view has been held (see also Part III) that the hormone formed in the extreme tip is derived from a precursor transported from the seed to the tip. In accordance with this, the upper part of the coleoptile removed from the lower portion of the plant will exhaust its supply of auxin. If such coleoptile sections exhibiting a decreasing rate of auxin synthesis are X-rayed soon after they have been cut, and while they still contain appreciable amounts of auxin, they should show a marked decrease in growth compared with similar non-irradiated sections.

Sections of given lengths, 2.00 or 1.50 cm. were mounted on small glass rods projecting from the bottom of small vessels coated with a thick layer of paraffin and partially filled with water, so that the bases of the sections were below the liquid surface. Two other sets of two vessels were also prepared at the same time containing sections of the same lengths as the above, but differing from them in that 1 to 2 millimeters of the tip was removed. They were mounted inversely so that the apical end was projecting into the liquid. In

one case the liquid phase consisted of a 20-unit per milliliter auxin in tap water solution, in the other set only of tap water. The vessels were covered with black paper boxes brought to the X-ray tube, and one vessel of each set was exposed as above. In order to keep the auxin supply nearly constant the solutions were changed immediately after treatment and then twice daily. An example of results of measurements of growth at intervals after the exposure is shown in figure 15. It can be seen that especially in the period immediately after irradiation there is a marked difference in growth between control and irradiated plants with tips intact but not supplied with auxin. In the decapitated

Figure 15

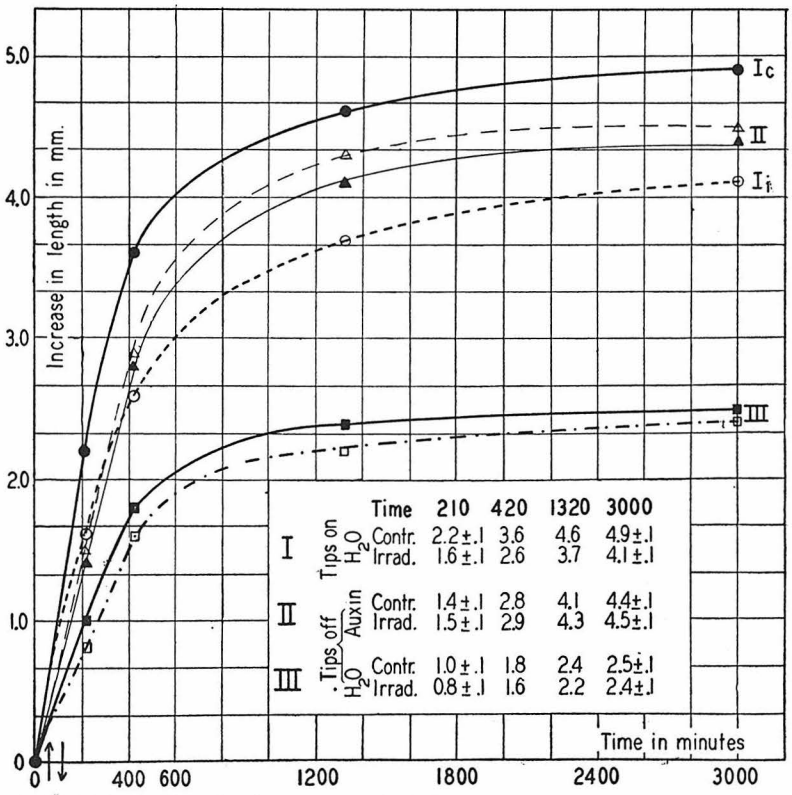


Fig. 6 Effect of irradiation on growth of 1.50 cm. sections of Avena coleoptiles, dosage 30 R./min., 900 kv., 3 to 4 m.a. for 40 minutes. Each curve the mean of about forty plants.

sections to which auxin was added there is on the other hand no decrease in growth after irradiation. In some experiments the sections were not placed in auxin solution until after irradiation. Under these conditions there is at first a decrease in growth in the treated plants, but this loss is recovered soon after the addition of the hormone. However, by such treatment the total growth of the sections is slightly less. In decapitated sections not supplied with auxin the growth is so small that a difference between the control and irradiated plants cannot be determined with certainty.

Conclusion

From these experiments, therefore, it is clear that the destruction of auxin by irradiation will cause a decrease in growth, and that if auxin is supplied in excess to the irradiated plants, they are able to grow as much as non-irradiated controls. Thus the decrease in growth of the Avena coleoptile caused by irradiation is due entirely to the destruction of auxin in the plant.

2. Experiments with Helianthus

Many investigators have used Helianthus in X-ray work. It was found that decapitated Helianthus seedlings give a marked growth response to applied auxin. They are therefore a very suitable material for determining whether the decrease in growth produced by X-irradiation can be counteracted by the addition of auxin to the plant. Seeds were soaked for a few hours, peeled and planted in a mixture of leaf mold and sand in small zinc boxes in the green house. When the hypocotyls were on the average 4 to 6 cm. long, the plants were divided into two

equal groups. One was irradiated, the other used as control. Each group was further subdivided into three sections, a, b, and c. The plants of a were left intact; those of b were decapitated within a millimeter below the point of insertion of the cotyledons, and blocks of auxin were applied to the cut surface; those of c were similarly decapitated and blocks of pure agar were applied. The blocks were of the size of standard test blocks but contained as much as 800 and 1000 units per milliliter concentration of the hormone. To insure contact between the block and the hypocotyl, the cut surface was moistened with a minute drop of water. To prevent drying out, the block was sealed to the plant with a layer of low melting paraffin. New application of the hormone was made twice daily. It is well known that the growth rates of individual seedlings are quite variable, but by the use of a number of plants very consistent measurements may be obtained. Thus a large number of plants were used, and repetition of the experiment four times gave essentially identical results. Determinations with 40 and 20 minutes exposures are given in Table XXVII A and B and C and D respectively. They demonstrate that although irradiation produces a marked decrease in growth of the intact plants, especially immediately after treatment, decapitated seedlings to which auxin has been added immediately after treatment show no effect of irradiation, but have at least as great a growth rate as similarly treated control plants. In decapitated seedlings with only pure agar blocks applied there is very little growth, so that it is hardly possible to measure a difference in growth between control and irradiated plants, although, in one

Table XXVII

The effect of irradiation on the growth of Helianthus hypocotyls with and without application of auxin

A. Plants exposed for 40 minutes at 30 R./min. at 920 kv. and 3.0 m.a. Auxin applied 1000 units/ml. blocks twice daily. Auxin application stopped on fifth day

	SET	NUMBER OF PLANTS	3/31 0 0	1 24.5	2 49	3 72	4 103	5 125	6 150	4/13 13 319	DATE DAY HOUR
I	a_1	39	6.01	0.94	1.61	2.34	2.75	3.18	3.48 ± 0.22	3.89	9.90
	a_2	40	6.79	0.56	1.17	1.65	2.10	2.42	2.72 ± 0.01	3.13	9.92
	b_1	41	6.56	0.42	0.73	1.01	1.15	1.26	1.33 ± 0.03	1.36	7.92
	b_2	39	6.73	0.47	0.78	1.08	1.24	1.34	1.41 ± 0.02	1.43	8.16
	c_1	4	6.08	0.07	0.15	0.22	0.25	0.25	0.27 ± 0.05	0.45	6.53
II	a_1–a_2			0.38	0.44	0.69	0.65	0.76	0.76	0.76	
	b_1–b_2			—0.05	—0.05	—0.07	—0.09	—0.08	—0.08	—0.07	

B. Plants exposed for 40 minutes at 30 R./min., 920 kv. and 3.5 m.a. Auxin applied 800 units/ml. blocks twice daily

	SET	NUMBER OF PLANTS	2/28 0 0	1 21	2 45	3 69	4 94	5 117	6 141	8 189	3/10 10 238	DATE DAY HOUR
I	a_1	36	5.32	0.80	2.04	2.85	3.29	3.52	3.71	3.91 ± 0.17	4.10	9.42
	a_2	30	4.59	0.44	1.18	1.98	2.24	2.49	2.58	2.68 ± 0.17	2.81	7.50
	b_1	40	4.54	0.28	0.96	1.33	1.46	1.57	1.59	1.66 ± 0.09	1.67	6.21
	b_2	44	5.23	0.27	0.95	1.32	1.48	1.58	1.60	1.65 ± 0.06	1.65	6.88
	c_1	6	4.77	0.06	0.11	0.21	0.31	0.31	0.31	0.31 ± 0.13	0.43	5.20
II	a_1–a_2			0.36	0.85	0.87	1.05	1.03	1.13	1.23	1.29	
	b_1–b_2			0.01	0.01	0.01	—0.02	—0.01	—0.01	0.01	0.02	

C. Plants exposed for 20 minutes to 30 R./min. at 750 kv. and 4.8 m.a. Auxin applied 300 units/ml. blocks twice daily. Last application 10/16

	SET	10/14 0 0	0 8	1 19	1 32	2 44	3 68	10/18 4 95	DATE DAY HOUR
I	b_1	5.39	0.24	0.47	0.72	0.91	0.95	0.97	6.36
	b_2	4.92	0.29	0.60	1.01	1.12	1.17	1.17	6.21
	c_1	3.49	0.05	0.07		0.08	0.10	0.11	3.60
	c_2	4.96	0.01	0.02		0.04	0.04	0.05	5.01
II	b_1–b_2		—0.05	—0.13	—0.29	—0.21	—0.22	—0.20	
	c_1–c_2		0.04	0.05	0	0.04	0.06	0.06	

D. Plants 8 days old exposed for 20 minutes to 30 R./min. at 910 kv. and 4.0 m.a.

	SET	1/10 0 ½	1 26	2 50	3 77	4 98	1/16 6 122	DATE DAY HOUR
I	a_1	3.84	2.15	3.78	4.70	5.01	5.28	9.12
	a_2	3.96	1.81	3.14	3.94	4.33	4.54	8.50
II	a_1–a_2		0.34	0.64	0.76	0.68	0.74	

The plants are arranged in the following sets:

a_1 Intact controls — b_1 Decapitated + auxin — c_1 Decapitated, no auxin
a_2 Intact irradiated — b_2 Decapitated irradiated + auxin — c_2 Decapitated irradiated, no auxin

All measurements are in centimeters. Cotyledons are not included in measurements.

I. Mean total growth of hypocotyls in the different sets. The mean initial and final lengths of the hypocotyls are given in the first and last columns.

II. Decrease in mean total growth due to irradiation.

experiment where measurements were made, there appears to be a significant difference. A few plants of group c were included in all experiments merely to show the effect on growth of the application of auxin.

From a comparison of the growth per intervals of the intact plants it is also clear that the effect of the X-rays on the growth of the hypocotyls is only temporary. Three to four days after treatment the growth rate of the irradiated plants is again about the same as that of the controls.

The growth of the epicotyls were also measured. In this portion of the plant recovery is slower. For example, in the plants of Table XXVII B on the nineteenth day after treatment, the lengths of the stems from the cotyledons to the first leaves were 3.70 and 5.97 cm. in irradiated and control plants respectively. The irradiated plants also showed abnormal development of leaves, and in many cases splitting of the stems, etc., as described by Johnson (1926). The flowers and seeds produced by these plants were also abnormal.

3. Experiments on growth of Pisum

In Pisum grown in the light it is harder to study the effect of irradiation on growth in relation to auxin, firstly, because the stem grows in sections (the internodes near the terminal bud grow rapidly, whereas the more basal internodes show hardly any increase in length (see Table X B); secondly, because there is a continuous formation of new tissue at the apex of the plant; and thirdly, because after the removal of the terminal bud the growth of the stem is still independent of the amount of auxin artificially supplied at the apical end. In Vicia faba, which is similar to Pisum, it has been shown (in Chapter II) that in decapitated plants in the light the leaves supply as much auxin as can be utilized in stem elongation. Only by complete defoliation

of the stem is it possible to obtain a growth response to applied auxin. Addition of auxin was therefore not attempted. Instead, measurements were made on the relative decrease in growth produced by different dosages of X-irradiation. From the results, summarized in Table XVIII, it can be seen that within the same group of plants the decrease in growth is a function of the dosage applied. The 10 minutes exposure group is given in parenthesis, because some of these plants were attacked by insects and were thus damaged considerably.

Table XXVIII

The effect of different dosages of irradiation on the growth of young Pisum plants

A. Mean growth per interval of the different sets. Mean initial and final total stem lengths are given in first and last columns

EXPERIMENT NO.	NUMBER OF PLANTS	8/10 0 0	1 22	2 53	4 97	5 126	8 189	8/23 13 319	DATE DAY HOUR
I	50	10.97	2.54	3.32	3.44	2.91	4.27	4.66	32.11
II	18	12.51	2.33	2.40	1.83	1.59	1.91	0.97	23.54
III	33	10.49	2.36	2.72	2.13	1.11	1.76	1.27	21.84
IV	25	11.90	2.26	2.16	1.28	0.40	0.32	0.01	18.27
V	22	12.04	1.99	2.00	1.01	0.30	0.20	—0.07	17.48

B. Mean lengths of the internodes of the plants in the different sets on 8/23 in order from the base to the apex of the plant. The internode between the two scales, about 2 cm., is not included

EXPERIMENT NO.	NUMBER OF PLANTS	I [1]		II [2]						
		b_2–L_1	L_1–L_2	L_2–L_3	L_3–L_4	L_4–L_5	L_5–L_6	L_6–L_7	L_7–L_8	L_8–L_9
I	50	2.37	4.42	6.73	6.65	6.29	4.50	1.43	0.12	0.002
II	18	2.64	4.96	6.43	5.46	3.14	0.65	0.04		
III	33	2.53	4.54	6.75	4.80	2.37	0.66	0.05		
IV	25	2.61	4.94	6.08	3.71	0.48	0.01			
V	22	2.53	4.56	6.43	3.36	0.29				

Seeds planted 7/30, irradiated 8/10 at 30 R. units/min. at 900 kv. and 3.5 m.a. The plants were arranged according to dosages as follows: I, controls; II, III, IV, V, irradiated 10, 20, 40 and 60 minutes, respectively. All measurements of length are in centimeters.

[1] I, development before irradiation.

[2] II, development after irradiation.

Figure 16

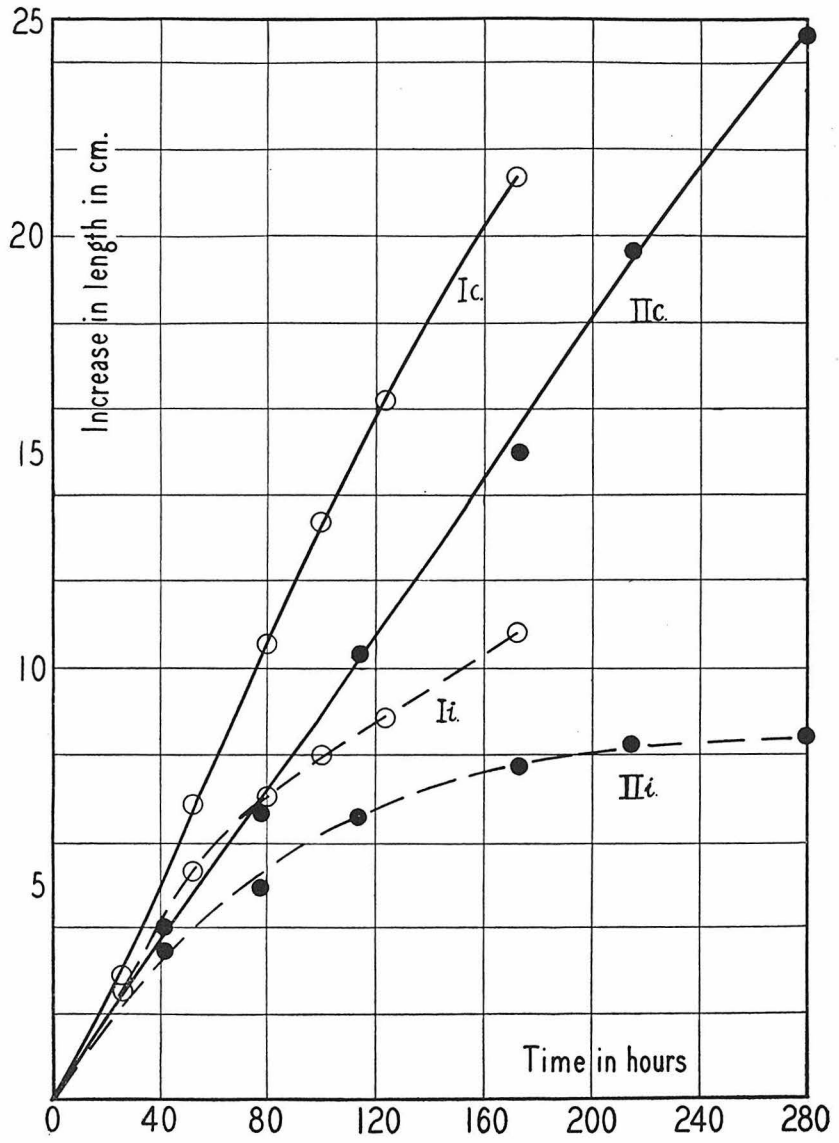


Fig. Effect of irradiation on the growth of stems of Pisum. Curve I. Plants age 8 days exposed 20 minutes at 30 R./min., 910 kv. and 4.0 m.a. on 1/10/34. Curve II. Plants age 11 days exposed 30 minutes at 30 R./min., 920 KV. and 3.5 m.a. on 2/7/34. Curves Ic and IIc controls.

The effect of two different dosages, in the most sensitive range, on the rate of growth is shown by additional experiments presented in figure 16. Each curve is the mean increase in length of fifty or more plants. Curves I show temporary or partial, curves II irreversible inhibition of growth.

Measurements of the number and mean lengths of the internodes of the plants of the different sets are included in part B of Table XVIII. They show that there is more than one single effect produced by irradiation. The internodes which were developed before the time of the treatment (L_1) are of approximately equal length in all groups. The internodes (L_3 to L_5) which were slightly developed at the time of exposure show a decrease in length according to the dosage applied. This effect of irradiation is most probably due to the loss of auxin which occurs, since it has been shown in Chapter II that auxin is necessary for the elongation of internodes. A very considerable decrease in growth, however, seems to be due to the fact that fewer leaves and internodes are formed in treated plants. Although auxin may also be indirectly involved in this process, it is more likely that here a different factor is affected by irradiation. In Pisum the problem of the growth of the stem is further complicated by the fact that as soon as the auxin supply in the plant is reduced, the lateral buds in the stipules begin to develop and thus may interfere with the growth of the main stem. Therefore, although it appears that the removal of auxin plays an important part in the inhibition, it is impossible to conclude from these experiments whether or not it is the main cause of inhibition

of growth.

4. Conclusion

From the results of the application of auxin to irradiated Avena and Helianthus seedlings it can be concluded that in these plants 1) the growth response to auxin is not affected by irradiation, and 2) the loss of auxin is mainly responsible for the immediate inhibition of growth produced by irradiation. Determinations of the growth of irradiated Pisum plants show that here the inactivation and lack of formation of auxin probably accounts for a part of the inhibition, but the effect of irradiation on other factors is, as well, of importance in the inhibition of growth. In the immediate inhibition of growth by irradiation, the effect can be referred to definite reactions on a specific substance rather than to the morphological structure of the tissues. And since the distribution of auxin and auxin formation in the plant are correlated with the regions of highest sensitivity to irradiation, the effect of irradiation on auxin is also capable, at least in part, to account for the difference in sensitivity so frequently reported.

CHAPTER VII

THE RELATION OF AUXIN TO THE DEVELOPMENT OF LATERAL BUDS BY IRRADIATION

Many workers have reported that the outgrowth of lateral buds is promoted by the treatment of the plant with radium or X-rays. The problem has not been investigated in detail as to the mechanism of the action of X-rays. However, since the time of the experiments of Molisch (1912), who could also obtain similar effects with application of hot water, etc. in woody cuttings, this type of effect has been referred to generally as a stimulation of growth caused by irradiation. As mentioned, above, the effect of irradiation of bud development led to the view that auxin might be affected by irradiation. That auxin is in fact affected by X-irradiation has been shown above. It remains to show that the original assumption of the indirect effect of irradiation on bud development through its effect on auxin is correct.

1. Bud development produced by irradiation of terminal bud and stem.

That the development of lateral buds in irradiated plants is not due to a stimulation of growth by the X-rays, but is an indirect effect due to the destruction of the growth hormone is indicated by the next experiment.

From a large group of young Vicia Faba plants, about 20 cm. in height, and with three leaves fully developed, twelve identical plants showing no bud development were selected and divided into four equal groups a, b, c, and d. From the plants of a the terminal buds were removed. The plants of b and c were irradiated. In b the terminal buds and the upper portions of the stems, in c, only the middle portions of the stems were exposed. The terminal buds in c were shielded with

several inches of lead, but it is possible that they also received a very small amount of scattered radiation. The plants of d were left intact, and together with those of a were used as controls. The lengths of the buds were measured at intervals. The final measurements on the fourteenth day after irradiation, given in Table XXIX, show that in a where the hormone supply from the terminal buds was immediately stopped, the development of the lateral buds is the greatest. In b, in which the supply of hormone was practically stopped within two days,

Table XXIX

The effect of irradiation on bud development in Vicia faba

GROUP	PLANT	LENGTH OF BUDS IN CENTIMETERS					TOTAL
		1	2	3	4	5	
a	1	15.25	2.15	1.65	0.85	....	19.85
	2	8.75	2.00	....	....	....	10.75
	3	10.00	2.00	....	....	....	12.00
b	1	3.15	0.75	0.45	....	2.85	7.90
	2	7.00	1.75	1.15	0.65	....	10.55
	3	5.50	1.65	0.50	....	....	7.65
c	1	3.80	0.55	....	....	....	3.35
	2	1.55	0.85	0.55	....	....	2.95
	3	0.95	0.80	....	....	....	3.60
d	1	0.65	1.00	....	....	....	1.65
	2	0.95	0.55	....	....	....	1.50
	3	1.40	0.80	....	....	....	2.20
a	Mean	11.33	2.05	0.55	0.28	....	14.22
b	Mean	5.22	1.38	0.70	0.22	0.95	8.47
c	Mean	2.72	0.73	0.18	....	....	3.63
d	Mean	1.00	0.79	....	....	....	1.79

The plants are grouped: a) Decapitated, not irradiated. b) Intact, terminal bud irradiated. c) Intact, short sections of stem only irradiated. d) Intact controls.

The x-rayed plants exposed for 2 hours, receiving about 2000 R. units at 750 kv. and 4 m.a. The buds are numbered from the base up as 1, 2, 3 and 4. Buds coming from cotyledons are marked as 5. At the time of treatment buds 1 and 2 in the stipules were not protruding, but had a mean length of 0.5 cm. Other buds had not reached a measurable size in any plant. Measurements made 14 days after treatment.

the development is also very great, but, as should be expected, less than that in a. In c, where the hormone supply was only partially disturbed, the development of the buds is much less and only slightly more than that of the intact controls in d.

Although the number of plants in each group was small, and the results might be merely a curious coincidence, it should be pointed out that also the bud development in the leaf axils is in complete accordance with expectations from an effect of irradiation on auxin. Thus, in group a, where the supply of hormone from the upper leaves is not affected, only the two lowest leaf axillary buds of one plant developed; in b where the hormone supply from the leaves is also affected all the plants show some development of leaf axillaries; in c where the hormone supply from the upper portion of the plant has not been affected, only a single leaf and the bud below the point of exposure grew; and in d, where the hormone supply was not affected, no leaf axillary buds grew.

2. Bud development produced by different dosages of irradiation

It has been shown (figure 13) that when the apex of a young Pisum plant is exposed to X-rays, the production of growth hormone by the terminal bud is inhibited, and the growth of the main stem is reduced. Parallel with this the buds in the stipules begin to grow rapidly. Measurements of the mean total lengths of the lateral buds of the plants, used in Table XXVIII, receiving different dosages of radiation are given in Table XXX. They show that for small dosages the lengths of the lateral buds are roughly an inverse function of the

Table XXX

The effect of different dosages of irradiation on the growth of lateral buds of Pisum

GROUP	NUMBER OF PLANTS	EXPOSURE IN MINUTES	BUD NO. 2	BUD NO. 1	AXILLARY BUDS	TOTAL BUDS
I	50	0	<0.2	....	....	<0.2
II	18	10	0.51	0.23	....	0.74
III	33	20	3.43	0.72	0.03	4.18
IV	25	40	6.28	1.54	0.04	7.86
V	22	60	6.07	1.65	0.06	7.78

Mean lengths in centimeters of the lateral buds on the thirteenth day after treatment, 8/23, in the different groups of table 11 irradiated 0, 10, 20, 40 and 60 minutes, respectively, at 30 R./min.

dosage. However, after an optimum dosage has been reached, additional irradiation does not cause a further increase in the growth of the buds although it does cause a further decrease in the growth of the main stem. This behavior is in accordance with the experiments in Chapter II, which showed that in decapitated plants the growth of the lateral buds is independent of the growth of the main stem, and depends only on the extent of removal of the growth hormone from the plant.

3. Inhibition of buds in irradiated plants by applied auxin.

The results of the preceding sections show that the bud development in irradiated plants must depend on the destruction of auxin and the mechanism of its formation. An independent proof, although not absolutely essential, for the correctness of the above statement, should most likely be obtained and was in fact found by counteracting the effect of irradiation on bud development by the application of auxin to irradiated plants.

Young Pisum plants, grown first in the dark and then in the light, were divided into two groups. The terminal buds and the upper

portion of the stems of plants in one group were irradiated; the other group was used as control. Half the number of plants of the former group, and two thirds the number of the latter group were decapitated, and in each (except intact controls) of the four different groups thus obtained auxin was applied to the uppermost portions of stems and to the rest pure lanoline was applied within about thirty minutes after the exposure to X-rays. The results of the experiment, Table XXXI, show that it is possible to inhibit the development of buds in irradiated both intact and decapitated plants in the same way as in normal decapitated plants by the administration of auxin in high concentrations.

Table XXXI

Inhibition of lateral buds by applied auxin in irradiated plants

Plants		Mean increase in length of buds 8 days after treatment (in cm.)	
		without auxin	with auxin
Decapitated	Controls	11.9	0.9
	Irradiated	12.2	1.0
Intact	Controls	0.0	---
	Irradiated	3.0	0.2

Pisum, 18 days old dark grown and light adapted seedlings, irradiated 20 min. at 900 K.V. and 3 m.a. Total dosage about 600 R. units. Crude synthetic hetero auxin applied in lanoline. Conc. 10 mg./g. Each figure mean of about 10 plants.

In another experiment in the green house, partial but not complete inhibition of bud development was obtained in irradiated intact plants supplied with hormone. In a similar experiment carried out in the dark room with young etiolated plants hardly any effect was obtained, as also intact irradiated plants without auxin added showed hardly any bud development. It might be that in these plants hormone is derived from the seed and that the X-rays in this case as in the Avena coleoptile have no influence on the synthesis of hormone. This possibility, however, has not been investigated. Nevertheless, it is clear that the application of auxin tends to completely counteract the effect of irradiation on bud development.

4. Conclusions

It is clear from the material presented in this chapter that the development of lateral buds in irradiated plants is not due to a stimulation of growth by the X-rays, but is directly dependent on the removal of the relatively high concentrations of auxin normally derived from the terminal buds and apical parts of the plants. It is thus only indirectly due to the action of the X-rays.

Furthermore, the results obtained are in complete accord with the mechanism of action of auxin in inhibition proposed in Chapter II. In view of these facts it is now possible also to account for the often observed but hitherto not understood difference in the effect of a single and successive dosage on bud development. A single dosage will give rise to the development of one or a few buds, which grow

out strongly and inhibit further development of others. However, if a second dosage is administered a day or two after the first one, then a large number of buds develop, none of which grow very strongly. Evidently the first dosage only affects the hormone supply from the main shoot; a second dosage, on the other hand, affects also the synthesis of hormone in the buds which have started to develop most. There is then, due to the second treatment, a longer interval without inhibition in which all buds may start to develop until they become large enough to seriously interfere with the development of each other. The same type of effect has been quantitatively studied by Dickson (1932) with regard to the formation of adventitious growing points, "buds", from gemmi in Marchantia, the development of which he also established to be inhibited by a polarly transported substance derived from apical growing points. From the above point of view his results are also readily understood.

CHAPTER VIII

CONCLUSION

A. DISCUSSION

It is difficult to compare the results of the experiments described here with the literature on the effects of irradiation on plants. Most work generally deals with the effects produced on the structure of the cell, cell division, and especially the effects produced on the nucleus. These experiments on the other hand deal primarily with the effects produced on cell elongation.

Because of the indirect way in which auxin is destroyed by the action of the X-rays, and because of the difficulties in carrying out experiments under exactly comparable conditions, no attempt has been made to determine any physical or chemical constants involved in the inactivation reactions. This could be done only under more rigidly specified conditions, hardly obtainable without the installation of X-ray equipment at the place where the plants are grown and studied. However, it is clear that so many factors control the extent to which auxin in the plant will be affected by a given dosage of X-radiation, that little could be gained for present purposes except by very extensive such investigations.

In view of the many variable and different observations that have been recorded from irradiation of plants, and furthermore, in view of the nature of the effect of irradiation on auxin itself, it would be futile to suppose that all the effects produced by irradiation in plants were the results of the destruction of auxin. Still, it is

also evident that the growth hormone directly or indirectly influences many processes in the growth and development of the plant, and it is worth considering how far the destruction of auxin will be in agreement with some of the frequently observed facts. It is known that very young cells are extremely sensitive to irradiation, whereas older cells are relatively resistant.

Similarly auxin is produced exclusively in the young cells and is distributed throughout the plant by a polar transport, so that relatively only small amounts are present in older cells. In general the stages in which the most rapid growth is taking place are the most sensitive to irradiation. This has been ascribed to a greater rate of cell division 'activity'. It may as well be associated with greater auxin growth 'activity'. This is especially true since in view of the recent work of Went and Thimann (1934) on the action of auxin on neoformation of roots in cuttings it is evident that this hormone exerts a definite influence on the early stages in the development of cells in addition to its promotion of cell elongation. Furthermore, it has been generally established that dry seeds are very resistant to irradiation, but after being soaked in water they become extremely sensitive. Komuro (1924) and others have shown that the sensitivity increases with increase in water content of the seeds. Parallel with this it has been shown by Cholodny (1935) that in dry seeds auxin is not present. But as the seeds are soaked in water large quantities of the hormone are set free in the endosperm. Even if the auxin should be present as such in the dry seed, it is likely to be less sensitive

to irradiation than in the presence of water. According to work on the effect of X-rays on the development of Helianthus, it has been shown that many of the normal functions of the cells are changed by irradiation. Moreover, some of the effects on development that were produced by X-ray treatment such as fasciation and dichotomous branching of the stems, etc., could be obtained by three other methods: 1) By mechanical injury to the growing point; 2) by insect attack of the growing point, and 3) by certain environmental conditions conducive to very rapid growth. Since the growth hormone is essentially supplied from the growing point, all three of these methods as well as X-irradiation will have a severe effect on the hormone supply in the plant. It is reasonable, therefore, to suppose that the main effect of these agents is the destruction of the normal supply of hormone in the plant, and that this defect in turn leads to the subsequent abnormal development. The closely analogous case of laferal bud development so frequently observed, has already been treated above in terms of the known inhibition of buds by auxin and is definitely to be ascribed to the destruction of the auxin in the plant. As for malformation of leaves it can only be pointed out that auxin is present in leaves and, although its function is not clear, exerts some influence on their growth.

The much disputed question of stimulation of growth by irradiation might also be considered in terms of auxin inactivation. Although the claims for a stimulative effect by weak dosages of X-rays are numerous, the only well-established cases are those in which a rearrange-

ment of chromosome material has been produced by irradiation. In such material the effect is on the genes and is thus hereditary in nature. It is clear that this kind of stimulation of growth is an accidental, indirect rather than a normal specific effect of irradiation. Other reported cases of stimulation of growth have been found inconclusive when studied statistically, as is shown for instance in the work of Johnson (1931). Nevertheless, the experiments of Bersa have shown that in some species a local stimulation of growth at the expense of some other part of the same plant can be produced by X-irradiation. The increase in size of intact irradiated coleoptiles over controls as shown above may come under this type of stimulation. However, as was also pointed out by Bersa, this shifting of the growth from one region to another in the same plant cannot be called a real stimulation.

Cholodny (1934) and others have investigated the functions of auxin in the growth of roots. It has been established that also in these photo- and geotropisms are controlled by auxin, but the mechanism of the action is opposite to that in the stem of the same plant, i.e., the application of auxin will cause an inhibition of the growth of the roots. Especially in view of this dual action of the auxin, it is conceivable that small amounts of irradiation administered at the proper time may have an effect on auxin inactivation and auxin formation that could be utilized advantageously for growth by the plant. In this connection it is of interest to note that in experiments by Kisser and Possing it has been found that the administration of hydrogen peroxide

to plants in very low concentrations cause a stimulation of growth but in higher concentrations cause an inhibition of growth.

In the experiments described, admittedly not especially adapted to the purpose, no real stimulation of growth could be observed. The question of stimulation of growth by irradiation in relation to auxin must, therefore, be left open until a more complete knowledge of the mechanism of the action of the auxin in the individual cells of the plant has been obtained. However, as has also been pointed out by others, the function of auxin in the plant is not merely promotion of cell elongation, but also a control and maintenance of balance in the development of its various organs.

It is, lastly, of interest to compare the described auxin destruction mechanism to other proposed mechanisms of the effect of irradiation on the living cell. This mechanism does not fit in with the old concept of direct mechanical destruction of a small living body, at first assumed to be the nucleus, later, on the basis of statistical studies of lethal dosages, etc., assumed to be a still smaller body such as a centrosome, within the cell.

It compares more favorably with the more recently proposed theories postulating that the mechanism is a linked chemical reaction, the first step of which is photochemical, and which results in the formation of toxic substances. Such a view is proposed from the experiments of Schechtmann and Klupfel (1932) on dividing eggs of Rana esculenta, and from the experiments of Fischer (1931) on tissue cultures in vitro.

It is in some respects similar to the view of Hammett (1932) that the effect of irradiation on the growth of animal tissues is the oxidation of sulfhydryl compounds within the cell that normally stimulate cell proliferation.

The mechanism of auxin inactivation by X-irradiation is, finally, closely in agreement with the results of experiments on the protozoon Colpidium campylum by Taylor, Thomas and Brown (1933). They demonstrated that the lethal effect of X-irradiation on this organism is due to the production in the medium of the presence of air of small quantities of hydrogen peroxide which in turn cause the death of the organism.

Chesley (1935) has already considered indirect evidence for and against the proposed role of auxin in x-ray inhibition of growth. His conclusions as to the action of auxin may be relevent but do not necessarily follow from his experiments. On the assumption that light promotes the formation of auxin, based on evidence from other plants, he studied the effect of light as a protecting agent against X-irradiation in wheat seedlings. He found in agreement with predictions that exposure to light before X-irradiation protects against small dosages and is without effect against large dosages. However, he measured growth in terms of respiration. It is now clear that auxin does not affect respiration. The effect of auxin can, therefore, be measured only in terms of linear growth unless previously shown to be correlated with other processes. Furthermore, his experiments are meaningless with regard to the action of auxin, because the effect of X-rays and

light on auxin growth cannot be interpreted merely in relation to the formation of auxin. It is well known from the work of van Overbeek (1933) and has been shown above (Chapter II) that light markedly decreases growth response of the plants to auxin. Critical evidence of the kind intended by Chesley can be obtained only from direct determinations of the amounts of auxin formed and the amounts of growth obtainable with corresponding amounts of applied auxin in the light and in the dark and under such conditions that auxin is the limiting factor in growth.

B. Summary

Since the experiments have already been considered in relation to the role of auxin in X-ray inhibition of growth it is only necessary in conclusion to give a short review of the main results.

By the use of a high voltage X-ray tube operated at about 900 kilo volts and 3 to 4 milliamperes the effect of hard γ-rays on the plant growth hormone in solution and in the plant has been studied.

Auxin is inactivated in solution indirectly through the oxidation by peroxides and other possible strong oxidizing agents formed by irradiation in the solvents. In aqueous solution, therefore, immediate inactivation takes place only in the light. A comparable inactivation can be carried out also in white light by the use of small concentrations of eosin as a catalyst. Extracted and synthetic hetero auxin, as well as auxin A seem to be qualitatively similarly inactivated by irradiation in water solution.

In the plant auxin is affected by irradiation in two ways.

Through the partial destruction of the hormone present and through the inhibition of its formation, the mechanism of transport of hormone in the plant is not immediately affected by irradiation. In organs such as the Avena coleoptile, where the precursor of hormone is derived from the seed, and in the Helianthus hypocotyl the effect of destruction of the hormone is apparently the only important one involved in the inhibition of growth by irradiation. In the Avena coleoptile the formation of hormone is not affected. And the application of auxin to decapitated Avena coleoptiles and to Helianthus hypocotyls shows that the capacity for growth is as great in irradiated as in control plants.

In green plants, on the other hand, such as young Pisum and Vicia and presumably also in the epicotyls of Helianthus and the leaves of Avena, where the storage materials from the seed have been depleted, and the growth hormone is synthesized in the terminal buds and in the actively growing regions only in the light, the mechanism of inactivation of hormone, although present, is of relatively less importance. Here the major effect of irradiation in relation to auxin growth is the more or less permanent inhibition of hormone synthesis. Measurements of growth of irradiated Pisum plants show that the decrease in growth is a function of the dosage. The mechanism of the effect, however, is complex involving the destruction and the inhibition of formation of growth hormone with a corresponding inhibition of the elongation of the internodes as well as markedly reduced formation of new tissues in the apex of the plant.

The development of lateral buds caused by irradiation is correlated directly with the removal of growth hormone from the plant and is not the result of any stimulation of growth by the x-rays.

It is concluded from the experiments that the effect of irradiation on auxin and on the process of its formation is a major factor in the immediate inhibition of growth generally obtained by x-ray treatment of plants.

In accordance with this view the possibility of explaining some frequently observed effects of irradiation in terms of the above processes is discussed in relation to 1) sensitivity and resistance to irradiation, 2) the parallelism between the processes influenced by auxin and by irradiation, and 3) a possible stimulation of growth by irradiation.

Finally the relation of the demonstrated auxin destruction mechanism of inhibition of growth to other proposed mechanisms of the effect of X-irradiation on the growth and development of living organisms is briefly considered.

PART III

EXPERIMENTS ON THE FORMATION OF AUXIN

CHAPTER IX

Introduction

The chemical nature and the structure of the growth hormones auxin A and B have been made clear by the work of Kögl et al. These substances were obtained in crystalline form from urine and from corn. A third hormone, hetero auxin, β-indolacetic acid, was also obtained in crystalline form from urine. This substance was found to be identical with the hormone obtained from extracts of culture media of Rhizopus suinus by Thimann (1935). Since the three active substances were found to have different stabilities when treated with acid and alkali etc. they could be readily distinguished, and a comparison of the behavior of growth hormones obtained from various natural sources with these crystalline preparations showed that auxin A and B are produced in Avena and generally in higher plants, whereas hetero auxin is produced by lower organisms. The structure of the hormones in higher plants indicates a relationship to sterols but has given no clue as to the specific source or mechanism of formation of the growth hormone in higher plants.

The work to be described was started on the suggestion of Dr. Went in 1934 as an attempt to determine the origin of auxin in the Avena coleoptile. At this time it was known from the work of van Overbeek

on Raphanus and from the work described above, Parts I and II, that there are in the plant two sources of auxin and connected with each of these sources is a different mechanism of auxin synthesis. In green plants auxin is produced only in the presence of light, presumably directly from special products obtained in photosynthesis. In young seedlings where the storage materials from the seed has not been exhausted auxin can be produced, usually especially in the apex of the seedling, also in the dark. The latter process of auxin formation, which applies to the Avena coleoptile, may for convenience be represented as a transformation of inactive precursor into active auxin, in the same sense as the break-down of inactive tryptophane into active hetero auxin carried out by Rhizopus as later described by Thimann, and also by the plant as will be shown below. It should be pointed out, however, that the types of reactions may be completely different in the two cases.

In accordance with this view are several facts. It has been shown by Went (1928) that the active production of hormone is localized within the uppermost 0.3 mm. of the tip of the coleoptile in intact plants, and to the uppermost zone of decapitated plants. Furthermore, he found that regeneration of auxin in a decapitated plant could be prevented by placing the cut surface in contact with a large volume of water or aqueous solutions. Similarly Gorter (1927) already had attributed the positive curvatures obtained in coleoptiles by the application of agar blocks containing various chemicals to the cut surface as due to an inhibiting effect on regeneration of hormone rather than to

inhibition of growth. Went, therefore, on the basis of this evidence and that from polar uptake of dyes originated the concept that auxin is synthesized in the tip of the coleoptile and is derived from a precursor, different from auxin in its properties, transported from the seed.

Within the last two years, however, attention has been focused on the fact that large amounts of hormone can be obtained from seeds. Thus according to Cholodny (1935), auxin is not present in the dry seed, but immediately upon soaking it is formed from a carbohydrate in the endosperm. Laibach (1935) on the other hand, who objects to this work, claims that auxin is present also in the dry seed and (in corn) not in the endosperm but principally in the aluerone layer. Pohl (1935), who extracted growth hormone in small amounts electrolytically from seeds in water, maintains that auxin is transported as such first up the coleoptile to the tip and then downward. Similarly Avery (1936) proposes that auxin is carried as such in the trachea in solution to the tip of the coleoptile where it becomes dispersed and is transported polarly downward. In short, the evidence or views most recently brought forward point out that there is no actual synthesis of hormone in the tip, but merely transport of auxin in both apical and basipetal directions in the coleoptile. However, no experimental evidence has been presented showing apical transport of auxin.

In the present work, on the other hand, both the source and the transport of the hormone were investigated. A new quantitative

(deseeded) Avena test method was developed whereby very small amounts of hormone can be determined and the substances obtainable from the basal and apical cut surfaces of a coleoptile can be distinguished. Since this method may be of general use in physiological auxin work it will first be separately described in some detail. The results which, although incomplete with respect to the processes involved in the formation of auxin, definitely show that auxin is synthesized in the tip of the coleoptile from a precursor derived from the seed will then be described. In the last chapter the activation of precursors of hetero auxin by the plant will be considered.

CHAPTER X

A TEST METHOD FOR SMALL CONCENTRATIONS OF AUXIN AND AUXIN PRECURSORS

By the use of the standard Avena test method, developed by Went in 1927, the chemistry and many phases of the physiological action of auxin have been studied. In physiological work, however, the amounts of hormone involved are frequently so small that quantitative or even qualitative work has often been very difficult or impossible. A supplementary proceedure with deseeded Avena seedlings will be presented, whereby smaller concentrations of the hormone not detectable by the standard method can be quantitatively determined.

1. The standard method.

Since the deseeded method involves only changes in technique, it need not be described in detail, but rather in relation to the standard method, which will therefore first be reviewed. Oats of the pure line Sieges Hafer of Svalöv are dehusked, soaked in water for two hours, and laid out on wet filter paper in a moist dish in the dark room kept at 25° C., 90 per cent humidity, and free from any phototropically active light or toxic gases. The following day the seeds are planted in individual glass holders (Fig. 17) and placed with the roots in water, 12 plants in a tray. Two days later when the coleoptiles are about 3 to 4 cm. long, they are ready to be used for tests. The plants are decapitated, about 0.5 cm. of the tips being removed, and the primary leaves are pulled loose from the base. The plants are then allowed to stand for 40 minutes, so that the upper portions of the coleoptiles will

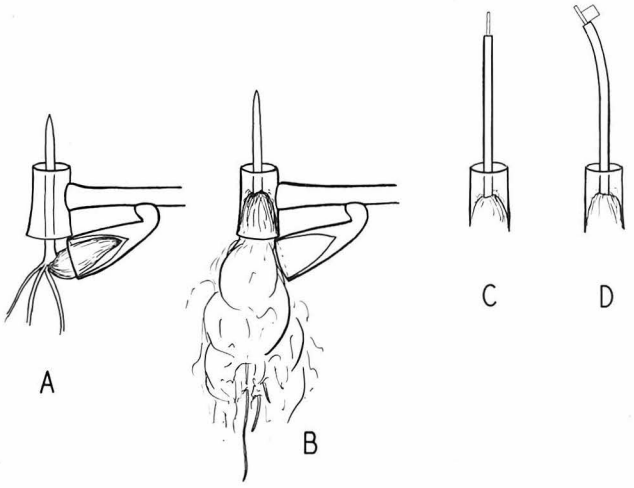


Fig. 17. Diagram of steps in deseeded test showing plant A. ready to be deseeded; B. deseeded and replaced in holder; C. after second decapitation; D. after application of block and ready for photographing.

become largely free from hormone, and so that any individual plants that might show curvatures due to handling can be detected and removed. At this time small agar blocks of standrad size containing the hormone solution to be tested are applied to the cut surface on one side of the coleoptiles. After 110 minutes of application the plants are photographed as shadow pictures on bromide paper. The curvatures produced by the unilateral application of the hormone can then be measured with a goniometer from the pictures. For a single test the mean value of the curvatures of one row of 12 test plants is used. A complete description of the technique is given by Went (1928) and also in some detail in Bot. Rev. (1935a).

It has been established by van der Weij (1932) and by Thimann and Bonner (1932) that under the conditions of the standard test the curvature is proportional to the concentration of hormone in the blocks of a given size in the range of concentrations from 1 to 15 or 25 degrees depending on the daily variation in the maximum angle. The amount of hormone diffusing from the block into the plant in the given 110 minutes of application, however, varies with the size and hormone concentration of the agar block in two respects; through the difference in contact surface and through the decrease in concentration gradient during the time of application. The point is illustrated in the original experiments by Went in which small agar blocks were used and from which 90 per cent of the hormone disappeared during the test, and those by Thimann and Bonner in which standard (8 times larger) blocks were used and from which only about 15 per cent disappeared in

the same time. Thus in the former experiments the curvatures were indeed roughly a linear function of both the concentration and the amount of hormone applied, and therefore also with these blocks smaller amounts of hormone could be determined. For other reasons, however, such as ease of manipulation and especially less susceptibility to change in volume by drying out, larger blocks are superior and have been adopted for standard tests. In this work in accordance with the specifications and units defined by Dolk and Thimann (1932) 1.5 per cent agar blocks of the dimensions $\frac{8}{3} \times \frac{10.7}{4} \times 1.5$ mm. $= 10.7 \times 10^{-3}$ ml have been used. The amount of hormone in one such block that will give a curvature of 1 degree under the above conditions corresponds to 0.4 A. E. (Avena Einheiten) and is therefore about 1×10^{-8} milligrams.

It should be noticed that under the above conditions only a fraction of the hormone applied has been utilized by the plant in the test, which must be completed within 2.5 hours after decapitation. After this time synthesis of auxin is resumed in the new physiological tip of the coleoptile, and since regeneration takes place especially on the side not in contact with the agar block the rate of bending of the coleoptile no longer remains proportional to the concentration of auxin applied. Hence changes in the procedure have been introduced. Most notable are those described by van der Weij (1931), who has developed special tools for decapitation and has introduced the use of a second decapitation one hour after the first one. This technique has also been used in the experiments below. It has been shown by Dolk (1926) that successive decapitations at 2 hour intervals prevent

the regeneration of auxin in the plant. In accordance with this effect the double decapitation delays regeneration of auxin, and makes it possible to work with a larger number of plants. But the actual time of application of the blocks cannot be markedly increased by this method.

2. The deseeded method.

It will be shown below that the auxin synthesized in the tip of the coleoptile is derived from a precursor transported from the seed. By deseeding the source of auxin is removed, thus regeneration is prevented, and as a result more sensitive test plants are obtained. Hence, the changes in the standard procedure are as follows: On the second day after germination, when the coleoptile is about 1.5 cm. long, the plant is taken from the holder, and the entire seed with the exception of the lower half of the scutellum is removed. A small piece of cotton is wound around the lower portion of the seedling which is then reinserted in the holder with a pair of bent forceps (eye forceps). The cotton serves both to hold the plant and to insure a good water supply to the coleoptile. Twelve to eighteen hours later, as in the standard procedure, the plants are ready to be used for tests. Photographs may be taken singly or repeatedly at intervals at any time up to 5 or 7 hours after application of the blocks.

3. Comparison of the deseeded and the standard test.

A comparison of results obtained by the two methods is shown in figure 18. For the sake of clearness the higher concentrations

are given in Fig. A and the values for the lower concentrations are plotted separately on a larger scale in B. These curves, obtained in one of six experiments with practically identical results, represent curvatures in degrees plotted against time in hours of unilateral application of hormone of different concentrations to deseeded and to normal test plants. In this experiment the deseeding was done 15 hours before the time of the first decapitation. Photographs were taken after 110 minutes of application and then at successive intervals.

a. Regeneration and temperature effects.

By comparing each continuous line (deseeded plants) with its corresponding broken line (normal plants), it becomes clear that for moderate concentrations of hormone, above 3 degrees, the curvatures are for a given concentration the same in both tests for the first two hours. However, after this time regeneration sets in. As a result in normal plants the rate of bending is decreased, so that the curvatures recede, remain constant, or continue to increase at a slower rate, according to the concentration of hormone applied. In deseeded plants, where regeneration is practically completely lacking, the curvatures continue to increase linearly with time for several hours, or if the concentration of hormone is smaller, until the supply of auxin from the blocks has been largely depleted. As a matter of fact, in normal plants a small amount of regeneration takes place earlier than 2.5 hours after the second decapitation. Thus in the standard test, when blocks of low concentrations are applied, practically no curvatures will appear; when blocks of very low concentrations or pure

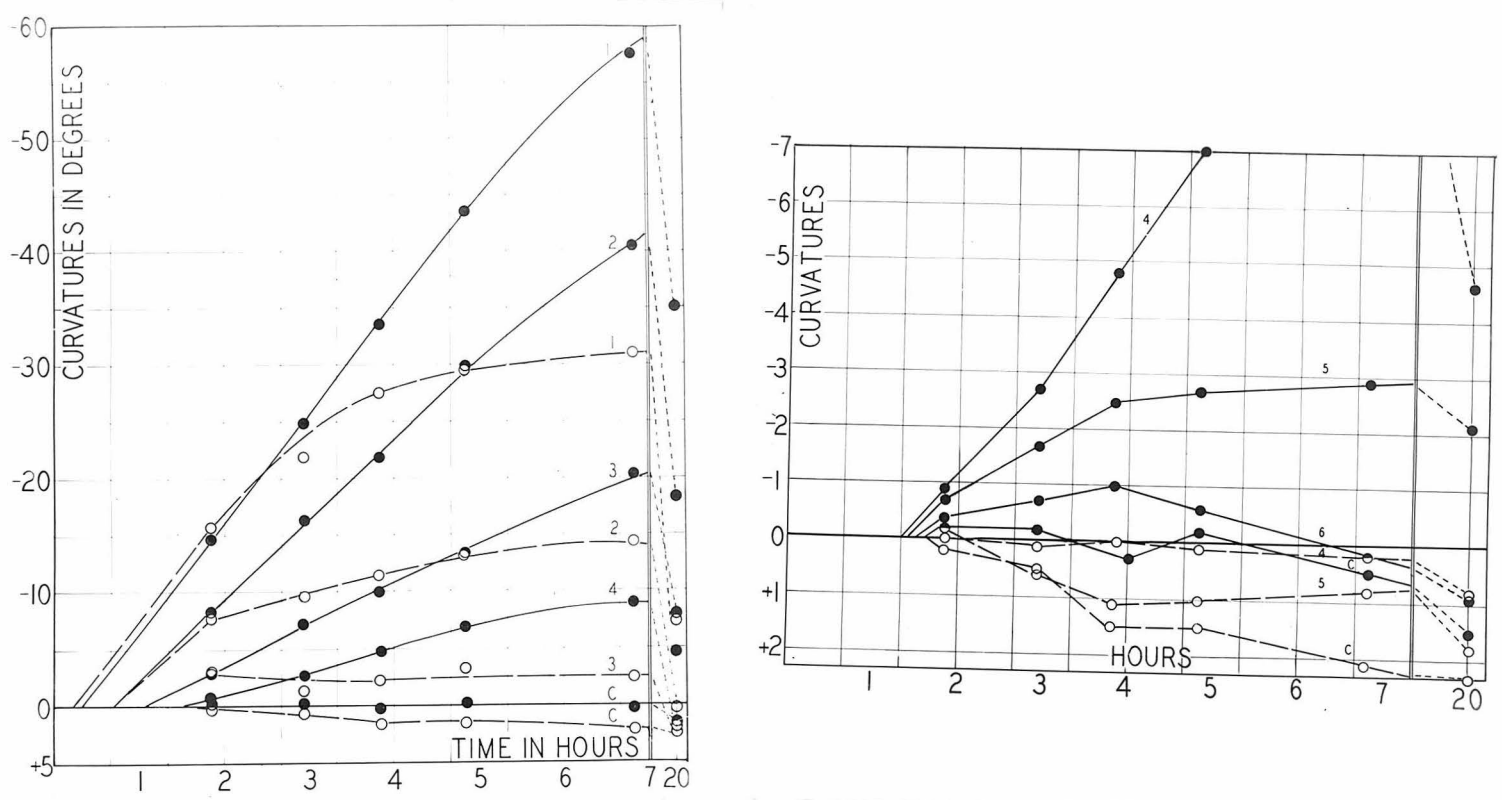


Fig. 18. A and B. Comparison of deseeded (solid lines) and standard (broken lines) tests for different concentrations of hormone.

Curves 1 2 3 4 5 6 and C correspond to concentrations of 1 1/2 1/6 1/18 1/56 1/108 " $H_2\emptyset$ respectively.

agar blocks are applied positive curvatures, i.e., in the direction towards the blocks will occur. The cause of these positive curvatures will be clear from a consideration of the precursor of auxin, which diffuses out into the agar blocks and is not immediately converted into auxin. Also in deseeded plants small positive curvatures may be obtained by the unilateral application of pure agar blocks, but the effect is much less than in non-deseeded plants. Thus for determinations of small concentrations of hormone deseeded plants are relatively even more sensitive than for higher concentrations. This additional sensitivity, appearing already within the first two hours of application, is clearly brought out in figures 19 and 20 in which the curvatures obtained in two experiments as determined by deseeded and standard tests are plotted against concentration of hormone applied. The curves obtained by the use of normal planfs intersect the abscissa some distance away from the origin, whereas the curves obtained with deseeded plants come very close to the origin. The distance from the origin to the point of intersection of the curve with the abscissa for normal plants increases very rapidly with increase in temperature as does regeneration. But the question whether regeneration is the entire cause of the decreased sensitivity or whether there is in addition a small destruction of auxin by the plant (see Van Overbeek, 1936) must be left open, because the curvafures involved are so small that the two effects cannot be clearly differentiated in these experiments. At higher temperatures (27°C) autotropism, perhaps due to regeneration, becomes noticable also in deseeded plants, so that after about four

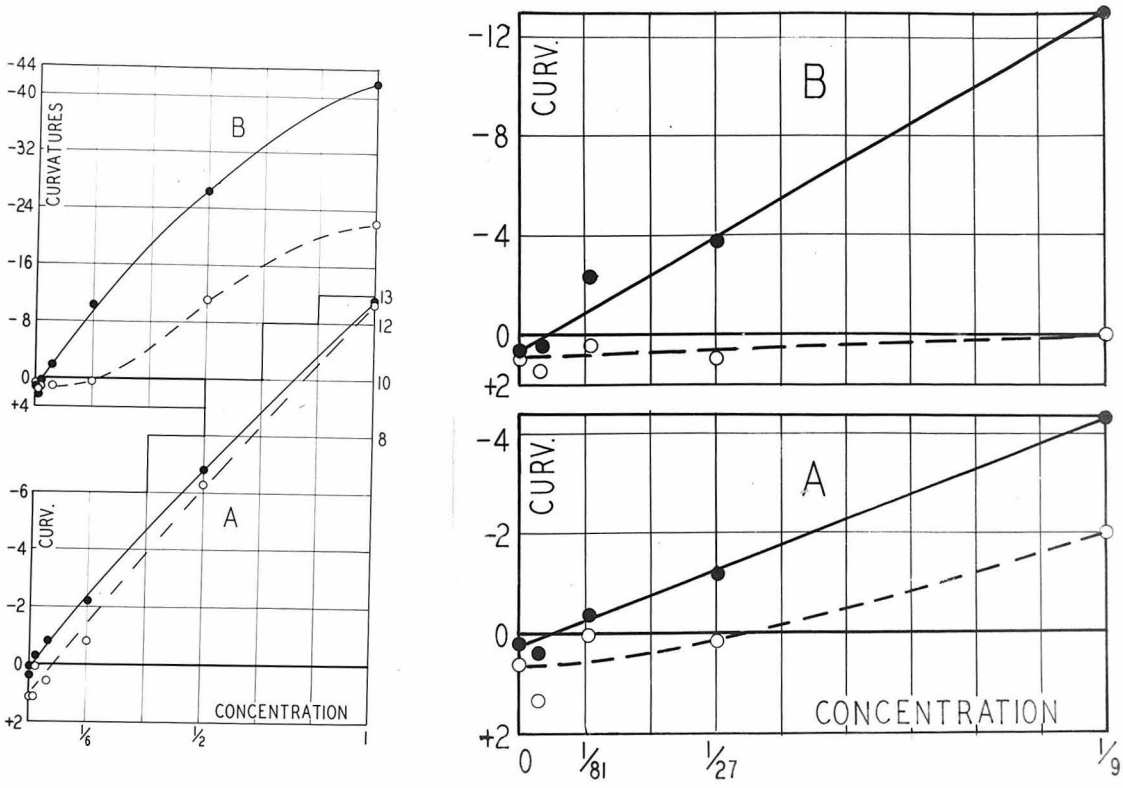


Fig. 19. Comparison of curvatures obtained by deseeded (solid line) and standard (broken line) tests plotted against concentration of hormone applied.

A, photograph taken 110 minutes; B, 390 minutes after application.

Fig. 20. Experiment similar to Fig. 19 showing lower concentrations on a larger scale.

A, photograph taken 110 minutes; B, 390 minutes after application.

hours the linear relationship between curvature and time of application becomes less pronounced.

b. Less physiological aging.

Also contributing to the higher sensitivity of deseeded plants especially long times after decapitation, is the decrease in physiological aging. Du Buy (1933) and Went (1935b) have shown that decapitated coleoptiles prevented from synthesizing auxin and not supplied with auxin for some hours, gradually become less sensitive to subsequently applied auxin. This effect, "physiological aging" is due to the increase in thickness and loss of plasticity of the cell walls. By deseeding, the materials for secondary cell wall formation are largely removed, and the walls of the coleoptiles remain thin and plastic even though the actual amount of auxin in them is less than in normal plants.

c. Limits for concentrations and amounts of hormone.

In general it can be said that with the deseeded method about ten times as small concentrations can be determined as can be detected with the standard test. This fact is readily understood, if we consider that in the standard test only 15 per cent of the auxin passes from the agar block into the plant, whereas in the deseeded test if photographs are taken after about 5 hours, nearly all the auxin in the block is utilized. For example, if two blocks containing very low concentrations are placed one on top of the other on a deseeded test plant about twice as large curvature is obtained as by a single block.

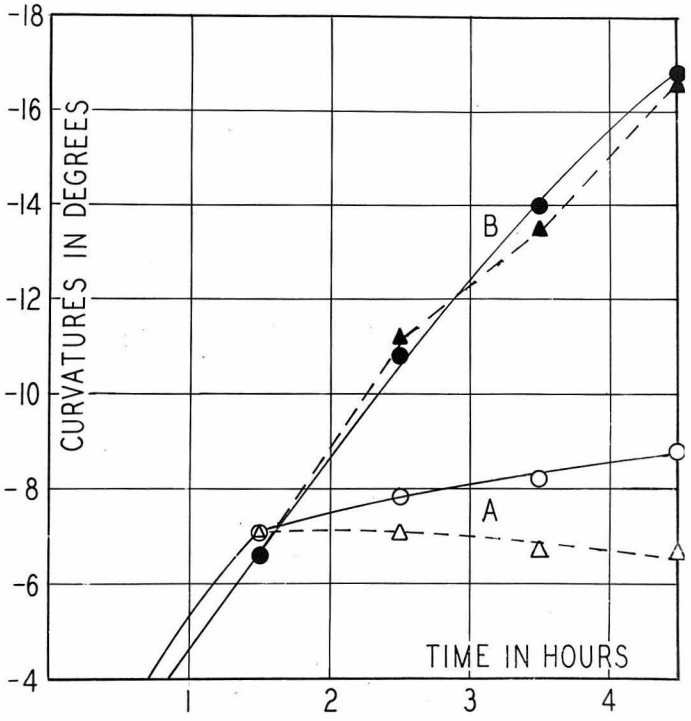


Fig. 21. Effect of light on curvature in normal (curves A, open points) and deseeded (curves B, solid points) plants. Values represented by circles (solid links) are from repeatedly exposed plants. Values represented by triangles (broken line) are from different sets of plants of the same group and not previously exposed.

In the standard test on the other hand van der Weij found the curvature to be independent of the size of the block. However, it is clear that in the deseeded, as in the standard test, there is a distinct limit to the concentration of auxin that can be detected. If the limit for the standard method is taken as 1×10^{-8} mg. per block, then the limit for the deseeded method is about 1×10^{-9} mg. per block.

d. Effect of light.

Since white light has a marked effect on the growth of the Avena coleoptile, its influence on curvatures when successive photographs are taken must also be considered. These experiments, which have a bearing on the light growth reaction, have been done by Van Overbeek, and the data given in figure 21 have been kindly contributed by him. In the standard test the plants are not exposed until the end of the experiment. In the deseeded test, however, it is often desirable to make a series of estimations of the curvatures of a given set of plants. Van Overbeek (1936) has shown that the amounts of light necessary for taking photographs partially inhibit regeneration in the plants of the standard test. In accordance with this as shown by curves A, figure 21, the increase in curvature with time obtained from a series of photographs at consecutive intervals of a given set of normal plants is greater than that obtained from plants of the same group, but of which a different set of plants, not previously exposed to light, is used at each corresponding interval. Curves B of the figure represent the identical experiment with similar plants deseeded 20 hours before the auxin was applied. From the close agreement between the curvatures of

successively exposed and not exposed plants it is clear that the amount of white light necessary for photographing has no effect on the rate of bending of deseeded plants.

4. Application of the deseeded test for auxin determinations.

As illustration of the deseeded test two examples will be given which include some data previously not easily obtainable.

a. Determination of auxin in primary leaves of Avena.

Primary leaves of four day old plants grown in the dark room were pulled out of the coleoptiles and placed with their bases on agar blocks, 12 leaves per 12 blocks, for 2.5 or 4.0 hours. The blocks were then tested on deseeded plants. Control agar blocks on which no leaves

Table XXXII

Auxin from primary leaves

Exp. No.	Time in hours of		Mean curvatures from		
	diffusion into block	application in deseeded test	blocks with diffusate in test deseeded	standard	plain agar in deseeded test
1	2.5	8.0	- 3.9	0.0	+0.1
2	4.0	6.0	- 4.5	---	+0.4
3*	3.5	5.0	- 2.3 - 2.1 - 2.6 - 2.8	 +0.7 *0.7	 +0.7

are

* In exp. 3, 18 leaves per 12 blocks were used but values divided by 1.5 to give degrees in terms of 12 leaves per 12 blocks.

had been placed were tested at the same time. For comparison one standard test was also made. The results of three experiments with leaves grown under different conditions, and which are therefore not comparable with each other, are summarized in Table XXXII. With the deseeded test the presence of auxin is clearly demonstrated, whereas with the standard test none can be found, and, in fact, has been thought to be absent. Furthermore, the close agreement between the values obtained in different tests of the same experiment shows that the method gives quantitative results.

2. Determination of auxin in coleoptile sections.

It has been shown by Thimann (1934) by the use of chloroform extractions from a large number of plants that in coleoptile sections auxin is present in small amounts. This finding has been confirmed by placing 0.3 cm. long coleoptile cylinders on agar blocks, which were subsequently tested with deseeded plants. As shown in Table XXXIII, the amounts of auxin obtained are large enough to give quantitative measurements. Comparable experiments with the standard test give at best only a perceptible curvature.

For a computation of the actual amounts of hormone obtained in the above experiments it is best to compare the curvatures directly with those obtained in similar tests of successive dilutions of an auxin solution of known, relatively low concentration. It was estimated that the auxin obtained from the primary leaf is of the order of 0.05 A.E. per

Table XXXIII

Auxin from coleoptile sections

Three mm. long sections placed 20 sections per 12 blocks

Exp./ No.	Time of diffusion into block	Mean curvatures from diffusate 1st photo	blocks 2nd photo	plain agar 2nd photo	Time after application of blocks to 2nd photo
1a	4.0 hrs.	-2.3	-5.9		
b		-2.7	-6.2	+1.2	5 hrs.
2	3.2 "	-1.2	-4.8	+1.4	8 "
3	3.5 "	-0.4	-3.4	*1.8	7 "
4a	4.0 "	-1.6	-5.7		
b		-1.3	-3.5	+1.6	10 "
5a	4.0 "	-1.0	-4.1		
		-0.7	-2.7	+2.2	5 "
6a	4.0 "	-0.5	-3.0		
b		-0.5	-3.4	+2.6	9 "

leaf per hour, and that from sections about 0.03 A.E. per section per hour. These amounts are only 5 or less per cent of the amount obtainable from the coleoptile tip per hour.

The above experiments, although not carried out in detail, indicate the possibilities of the application of the deseeded test to work concerned with the presence and relative distribution of the growth hormone in plant tissues. They also bring out the fact that the high sensitivity of the deseeded test holds for auxins in general and is not limited to synthetic hetero auxin, which was used exclusively above in the development of the method.

CHAPTER XI

Formation of Auxin in the Avena Coleoptile

A. Physiological factors affected by deseeding

If auxin obtainable from the tip of the coleoptile is derived either directly or from a precursor transported from the seed, then by removal of the seed the supply of auxin in the tip of the coleoptile should be affected. The effects of the removal of the seed on the subsequent development of the coleoptile are of course manifold. They will be discussed here mainly in relation to the growth response and the formation of auxin.

1. Effect of deseeding on linear growth.

The effect of deseeding on linear growth has been studied by Went (1935b). He finds that the growth rate in deseeded plants is decreased about 40 per cent, and that this decrease is due to the lack of two factors, auxin and food. Furthermore, the application of high concentrations of auxin to intact deseeded plants causes about the same percentage increase in growth as in normal plants, but in deseeded plants the growth is more limited to the upper part of the coleoptile. In accordance with this behavior is the fact that in deseeded, decapitated coleoptiles much sharper apical curvatures are obtained by the unilateral application of the hormone, i.e., the basal parts do not grow and thus remain straight even after long times of application of the hormone.

The effect of deseeding on regenerative linear growth is shown in figure 22, in which is represented one of five similar experiments. Measurements of growth were made with a horizontal microscope on plants which had been marked with india ink into three zones. Each curve is the mean of 3 or 4 plants. The ordinates represent the sum of the total increase in length of two zones, which include almost the entire coleoptile in the decapitated plants and the corresponding two zones in the intact plants. Measurements were started one hour after decapitation. Curves I A and B represent intact and decapitated control plants respectively. Curves II and III A and B represent corresponding sets of plants which had been deseeded one and ten hours respectively before the time of decapitation. From these curves the following facts are clear: By deseeding, growth is decreased both in intact and in decapitated plants. The amount of decrease is a function of the time after deseeding. In plants that were deseeded only one hour before decapitation the regenerative growth in decapitated plants is only slightly reduced. In plants that were deseeded ten hours before decapitation regenerative growth is markedly inhibited. However how far the decrease in regenerative growth depends on the lack of auxin alone or in addition on the lack of other factors, food, cannot be safely determined from the above curves.

2. Effect of deseeding on geotropic and auxin curvatures.

It will be shown that curvature growth, i.e., the difference in the relative growth of the two sides of the coleoptile, in deseeded and non-deseeded plants under the conditions described for the deseeded

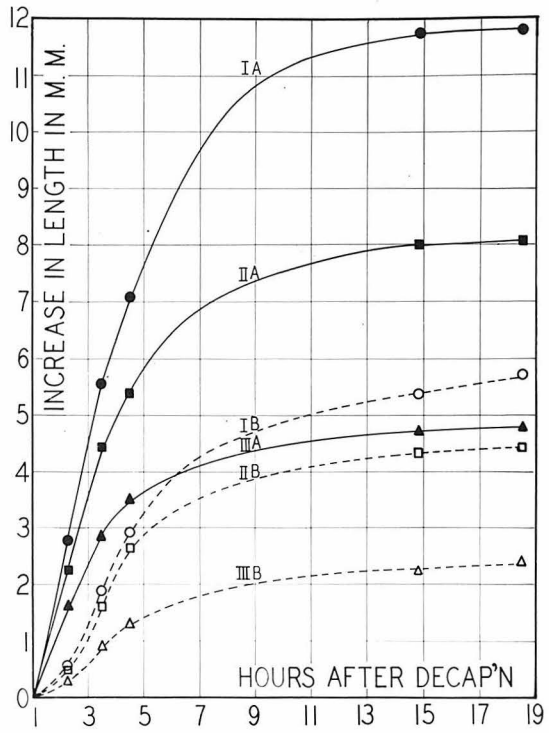


Fig. 22. Linear growth of normal and deseeded intact (solid line, A) and decapitated (broken line, B) plants.
Curves I normal; curves II and III deseeded 1 and 10 hours before decapitation respectively. Measurements started 1 hour after decapitation.

test is independent of food and is a function only of the amount of auxin present in the coleoptile. The following three types of experiments demonstrate this conclusion.

a. Decrease in auxin synthesis after deseeding.

Beginning on the second day after germination, plants were deseeded, 36 at a time, at successive intervals. Then at a given time 3 mm. long coleoptile tips were cut off and placed on standard agar blocks, 15 tips per 12 blocks, for two hours. The amount of hormone diffusing out from the different sets of tips was determined by the standard Avena method. All the blocks were tested at the same time with 24 test plants for each set of deseeded tips and 48 plants for the controls. The amount of hormone produced by deseeded plants expressed as per cent of that produced by normal plants is plotted against time after deseeding in curve II, figure 23, which represents the mean values of several experiments. The curve shows that there is a continuous decrease in the rate of auxin synthesis after deseeding, and at least for a considerable period this decrease is closely a linear function of time.

b. Sensitivity to auxin.

The relative sensitivity to auxin of deseeded plants at different times after deseeding, i.e., the capacity to produce curvatures in response to auxin applied unilaterally in blocks of moderate concentrations (5 to 20 degrees) for 110 minutes as in the standard test, and compared with the curvatures produced by the application of the same concentrations of hormone to normal plants of the same group, is

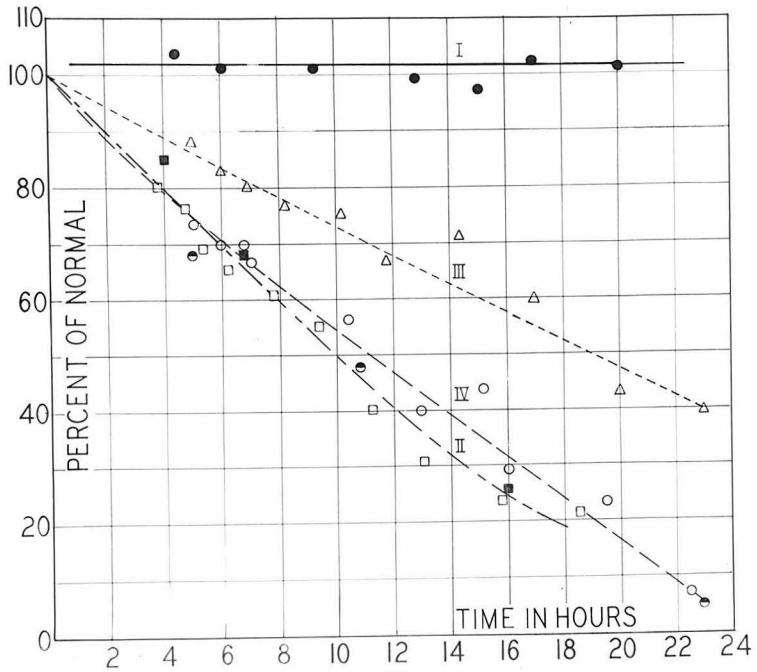


Fig. 23. Effect with time of deseeding on I Sensitivity to auxin; II Synthesis of auxin in tips; III Geotropic response in intact; IV Indecapitated coleoptiles. All values are expressed as per cent of normal controls.

shown in curve I, figure 23. In this curve, which represents the mean values of many experiments with over 600 plants, the curvatures of deseeded plants expressed as per cent of the curvatures obtained in corresponding control plants are plotted against time after deseeding. It is clear that the sensitivity of deseeded plants is at least as great as that of normal plants. Only when the interval between deseeding and the test is made very long (not included in the graph) and also, which is probably more important, when the plants are deseeded in a very early stage of development, is there evidence of a distinct decrease in sensitivity. It appears from the curve that the sensitivity of deseeded plants may be slightly higher than of normal plants. If this increase be real, it is probably due to the fact that in normal plants regenerating has begun less than 2.5 hours after the second decapitation (see also Figures 19 and 20). However, it should be pointed out for moderate concentrations of hormone that this difference is so small that unless a very large number of tests are made, it is well within the experimental error.

c. Decrease in geotropic curvatures in deseeded plants.

It has been shown by Dolk (1926) that the geotropic curvature in normal intact and decapitated coleoptiles is controlled by the amount and relative distribution of auxin in the organ, and is proportional to the concentration of hormone applied. Furthermore, the amount of hormone obtainable by diffusion from the upper and lower sides of geotropically stimulated coleoptiles is proportional respectively to the growth of the two sides. Since it was shown in b that the sensitivity

to auxin is not decreased by deseeding, it can be said with fair certainty that the relations between auxin and geotropic curvature, established by Dolk for normal plants, hold also in deseeded plants. Thus, in conjunction with the experiments of sections a and b, a large number of determinations were made of the relative geotropic response in deseeded plants. At definite times after deseeding, deseeded and normal plants of the same group were placed horizontally. The geotropic curvatures produced in a given time, 1 hour for intact plants and 4 hours immediately after decapitation for decapitated plants, were measured from photographs taken at the end of the specified times. The curvatures of deseeded plants expressed as per cent of those of normal plants and plotted against time after deseeding are shown in curves III and IV, figure 23, representing intact and decapitated plants respectively. The curves demonstrate that in both intact and decapitated coleoptiles of deseeded plants there is a decrease in geotropic response proportional to the decrease in auxin synthesis. Thus, if the plants are decapitated about 15 hours after deseeding, subsequent regeneration of auxin is nearly completely prevented.

From a consideration of the data in figure 23 as a whole, it is possible to draw one further conclusion: The decrease in geotropic curvature in deseeded plants is independent of the sensitivity of the coleoptiles and thus depends only on the decrease in auxin synthesis. Now it also appears that the relative decrease in auxin synthesis in regenerating decapitated coleoptiles is very nearly the same as that in decapitated tips and proportional to that in intact tips. Hence

it follows that the mechanism of auxin synthesis in the tip of the intact coleoptile and the mechanism of regeneration of auxin in the new physiological tip of the decapitated coleoptile are identical.

3. Conclusion

It can be concluded from the above experiments that the removal of the seed influences the growth of the coleoptile in several ways, but its effect on the subsequent formation of auxin can be independently followed either by means of geotropic curvatures or direct determinations of auxin diffusing out into agar from the tips. It is clear that the removal of the seed gradually prevents the formation of hormone in the tip of intact plants and prevents the regeneration of auxin in decapitated plants. The source of auxin is thus in the seed. The results furthermore indicate that auxin present in the tips of the coleoptile must have been formed there and is not derived as such from the seed. If auxin were transported from the seed to the tip in the earlier stages of growth, as postulated by Avery, then there should not be a gradual decrease in auxin formation with time after deseeding, but, on the other hand, a sharp break at some place in the curves. If auxin were continuously supplied from the seed, then there should be no concentration gradient from tip to base as established by Thimann (1934). Hence indirect evidence supports the concept of the formation of auxin at the tip of the coleoptile from a precursor transported from the seed.

B. Demonstration of a precursor of auxin in the Avena coleoptile.

It has been shown above that there is a limit to the concentra-

tions of hormone that can be detected by the standard test. If the concentrations of hormone applied are so small that they will not cause distinct curvatures to appear within the first 5 hours after application, curvatures will not appear at any time later. Furthermore, the transport of auxin in the coleoptile is strictly polar in the direction from the tip toward the base. Even with the deseeded test no detectable amount of hormone has been found to be actively transported in the opposite direction, (10 to 50 degrees) when moderate concentrations of hormone were applied in agar blocks to the basal surfaces of the sections. If extremely high concentrations (about 2000 units or more) are applied, however, auxin is obtained in the apically placed agar block. Whether this auxin has been actively transported through the section or its presence is due to leakage along the surfaces of the cells has not been determined. Nevertheless, it is clear that for a range of concentration far higher than that obtaining in the plant, there is no apical transport of hormone through sections.

Now when to decapitated coleoptiles with the primary leaves removed, agar blocks are applied over the entire cut surface of the stumps, for two or more hours, and these blocks are then tested on deseeded plants, as expected in accordance with the above facts, no visible curvatures were obtained within the first five hours after application. However, within ten to twenty hours after application, distinct negative curvatures can be observed (Table XXXIV). In the determinations made so far, the magnitude of the curvatures have varied greatly from one experiment to the next, but frequently the mean curva-

Table XXXIV

Precursor of auxin from coleoptiles

Exp. No.	Time in hours of diffusion into blocks	Time in hours of application in test	Mean curvatures from apically applied blocks	Mean curvatures from plain agar blocks
1	5.0	17	-2.6	
		20	-6.0	
		20	-4.-	
		20	-7.-	+0.4
2	3 to 4	24	-6.0	
			-7.2	
			-4.6	+0.3
3	1.5 or 3.5	16	-6.5	
			-4.9	
			-4.0	
			-5.9	+0.2
4	?	?	-1.6	
			-2.3	+0.8
5	2 hrs.	18 hrs.	-3.1	-
			-5.3	

tures have been between 4 and 8 degrees. This variability in the results is to be expected, since the optimal experimental conditions must be governed by several factors whose nature is as yet unknown. Thus, for example, in some experiments the curvatures start to appear later than in others, and within a single test the maximum curvatures of the individual plants occur at different periods after application, although in practically every plant the maximum curvature is reached

a long time after application. These results show that a growth substance, different from auxin is obtainable at the apical cut surface of the coleoptile. It should also be pointed out in this connection that the amount of substance obtainable in contra distinction to auxin derived from the basal cut surface, in the results so far, is about the same regardless of how far from the tip the coleoptile has been cut off.

In some experiments the progress of the curvatures was followed by the use of the "kimograph" developed by Dr. Went. By this procedure not only can the rate of bending with time be determined but it is also possible to detect much smaller curvatures than by the usual method. In one such experiment represented in figure 24 the curvatures seem to appear at an earlier time than was usually observed in similar ordinary deseeded tests. However, whereas the curvatures produced by small concentrations of auxin begin to recede also in deseeded tests after 4 to 5 hours, (see figure 18), it is clear that on the average these curvatures continue to increase at a slightly increasing rate for over 15 hours after application. Thus, with respect to time of appearance of maximum curvatures and duration of increase in size, the material derived from the apical surface of the coleoptile or mesocotyl is certainly different from auxin itself.

Perhaps a more striking way of demonstrating the presence of a precursor of auxin in the plant, and which brings out the difference between it and auxin, is by the following arrangement. Sets of 20 coleoptile sections of given lengths usually 3.0 mm., are placed with

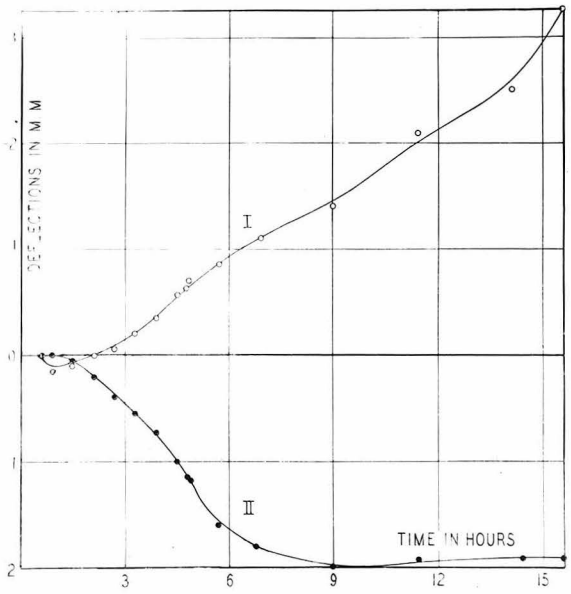


Fig. 24. Precursor from Avena (Curve I) (11 plants) blocks previously on apical surface of decapitated plants; Curve II (7 plants) control agar blocks.

the bases either down or up, but all of a given set in the same direction, between two full size agar blocks. After a given time, varying between 2 and 4 hours the agar blocks are removed, cut into twelve standard test blocks each and tested on deseeded plants. The amount of substance obtainable from the basal and apical ends respectively can be seen from columns 11,12 and 14,15 respectively, Tables XLI and XLII. The mean values of some 15 separate determinations are shown in figure 25. In this figure the curves I and II represent curvatures plotted against time of application in the test obtained from blocks previously in contact with the basal and apical ends of the sections respectively; curve III shows the corresponding curvatures obtained by the application of the pure agar blocks. A survey of the data in the tables and a comparison of the curves in the figure show essentially the same things as was obtained above.

From the data as a whole, then, the conclusion is drawn that the growth promoting substance from the "apical" blocks is a precursor of auxin capable of being transported in the apical direction in the plant and capable of undergoing a chemical change into auxin, whereas auxin itself can be transported in the plant only in the basipetal direction.

2. The relation between positive curvatures and the precursor.

It was shown above that by unilateral application of plain agar blocks to decapitated plants small positive curvatures are produced. As mentioned, Gorter pointed out that these curvatures are not due to

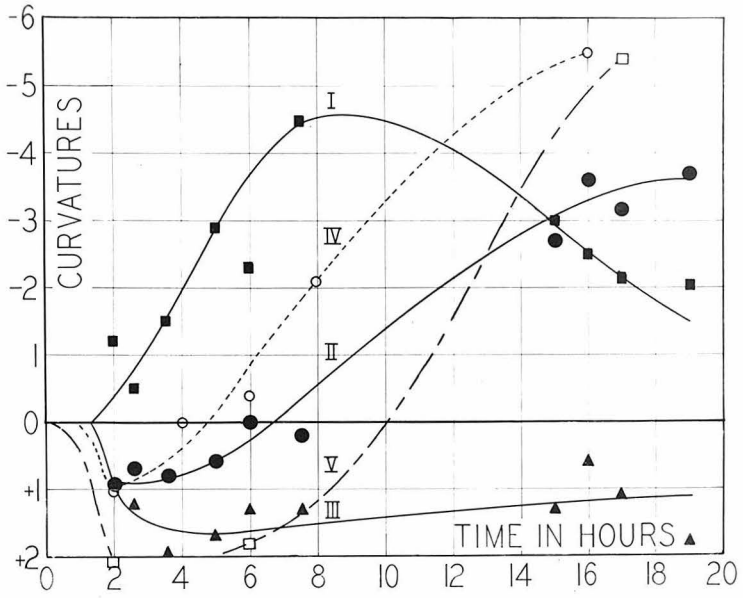


Fig. 25. Curvatures obtained in deseeded plants by the application of blocks containing auxin from coleoptile sections (curve I); precursor of auxin from coleoptile sections (curve II); pure agar (curve III); tryptophane (curve IV), and indolethylamine (curve V).

growth inhibiting substances, but are correlated with the regeneration of auxin in the new physiological tip. Why and by what mechanism regeneration is affected has not been made clear. From a determination of the amount of regeneration in coleoptiles with and without agar blocks, and from a consideration of the precursor of auxin these questions will be answered. About 150 plants were decapitated. To half this number plain agar blocks were applied to the entire cut surface of the coleoptiles immediately after decapitation; the other half was used for controls. Between two and three hours later 1.5 to 2.0 mm. long apical sections were cut off and placed on agar blocks, 24 sections per 12 blocks, for two hours. The amount of auxin produced by the sections was determined by testing these blocks on deseeded plants. The results of three experiments are included in Table XXXV. They show that in the apices of plants on which agar blocks had been placed the production of auxin was significantly less than in the controls without agar blocks. Furthermore, tests of some agar blocks which had been placed on the apical surfaces showed no trace of auxin, but on the other hand indicated the presence of the precursor. The mechanism of the formation of positive, differential regeneration curvatures is, therefore, as follows: On the side of the coleoptile in contact with the agar block a considerable fraction of the precursor of auxin diffuses out into the agar block and will not be immediately converted into auxin. On the opposite side of the coleoptile precursor accumulated and is converted into auxin. The relatively larger auxin production on this side makes possible a corresponding increase in growth. Hence a positive curvature is produced.

Table XXXV

Regeneration in decapitated coleoptiles with and without agar blocks applied to the cut surface

Exp. No.	Time in hours of application of blocks	Mean curvatures with mean error from sections previously		Difference in degrees
		with blocks	without blocks	
	3.0	-4.1 ± 1.2	-9.4 ± 1.2	-5.3 ± 1.7
I	2.5	-2.8 ± 0.8	-9.8 ± 1.2	-7.0 ± 1.4
	2.-	-4.9 ± 0.6	-7.1 ± 1.2	-2.2 ± 1.3
	Curvatures with plain agar blocks +1.4 ± 0.3			
	1.8 to 2.3	-1.1	-2.0	
II		+0.4	-0.7	
		-0.3	-2.9	
		+0.1	-4.6	
		Mean -0.2	-2.6	-2.4
	2.5 (apx)	-7.0	-10.0	
		-1.0	-7.6	
		-0.6	-6.8	
III		-2.2	-5.3	
		-3.3	-6.9	
		-0.5	-3.8	
		Mean -2.4	-6.7	-4.3

3. Attempts to obtain precursor from the seed.

The experiments described above indicate that it might be possible to isolate the precursor of auxin from seeds and different attempts have been made to do so. However, the results are hardly conclusive and will be described only briefly below.

a. Alcohol extractions.

If, as stated by Cholodny, auxin in the seed is present only after soaking in water, it might be possible to extract a precursor of auxin from ground seeds by means of other solvents. Soxhlet extractions with absolute alcohol or chloroform were performed for periods of 3 or 4 days, but each day with a new portion of about 175 cc. of solvent. Five grams finely ground corn meal was placed in a porous thimble and the apparatus was so arranged that the thimble was drained about every 7 to 8 minutes for periods of 5 to 10 hours daily. The extract was then taken over into water solution by means of evaporation of the solvent at reduced pressure and heating to about 80 degrees. Small fresh amounts of water were added to prevent complete drying out of the residue. These water extracts were then tested on Avena in different dilutions. Results of such experiments are shown in Table XXXVI. They show that at least after the extract has been transferred to water, auxin must be present. The fact that especially in the first extract nearly the same curvatures are obtained with 10 times diluted extract as with the highest concentration might indicate that auxin is gradually being formed; on the other hand it might be due to a phase equilibrium of auxin between water and small amounts of oil

Table XXXVI

Activity of extracts from corn meal

Experiment	Extract No.	Volume in cc.	Curvatures obtained from dilutions of 1/1	1/2	1/10	oil 1/10
I	1	20	-10.4	-10.4	-8.4	-6.7
	2	15	-13.3	-6.6	+0.2	
	4	15	-15.3	-16.5	-0.9	
II	1	35	---	-14.3	-6.6	
	2	15	---	-4.3	+0.2	
	3	5	-4.7	---	---	
III	1	25	-7.3	-5.4	-3.0	
			-2.7	---	-2.5	Des. test (2 hrs)
	3	5	+0.8	---	---	
			-0.5	-1.8	---	Des. test
	1	25	+6.4	---	+0.2	
			-7.2	---	-4.2	Des. test (5 hrs)
	3	5	+0.1	---	---	
			-3.1	-5.5	---	Des. test

which could not be entirely removed. When water solutions are made directly the curvatures are proportional to the concentration. Normal plants seem to give higher response than deseeded plants immediately after application. However, this might not be generally true. The

corn meal used in experiment III, which on the third day of extraction gave practically no further hormone by alcohol extraction nevertheless later and after complete drying, when placed in water, 1 gm in 6.0 cc. for three hours in the ice box, the decanted water gave curvatures of 14.5 degrees. However none of these results is conclusive evidence for the presence of a precursor.

b. Transport of substance from Avena seeds or corn meal through coleoptile sections.

It was next attempted to isolate the precursor by allowing it to be transported apically through coleoptile sections. Sections were placed on the surface of agar containing large amounts of ground Avena seed or corn meal. On the apical surfaces were placed plain agar blocks, and these were tested on deseeded plants after given times of transport either in the dark room at 24°C or in the ice box. Although in some experiments the blocks produced delayed precursor curvatures greater than corresponding control blocks from similar sections placed on pure agar, in other experiments no increased curvatures were obtained. Thus from the mean of 7 different such experiments on the whole no significant increase in transport was obtained.

c. Application of seeds to deseeded plants.

Various attempts to increase the production of auxin in deseeded plants by placing soaked seeds at various times after the beginning of germination in contact with the base or more apical regions of the coleoptile have likewise proved unsuccessful. However, these

negative results do not disprove the presence of a precursor of auxin in the seed. It is known that auxin itself is very easily destroyed especially at cut surfaces of the plant. It should further be noticed that all experiments have to be done with the substance in presence of water and air, and it is likely that the precursor under these conditions is even more unstable than auxin itself, and might either be transformed into auxin or become inactivated. Furthermore, by the artificial application of the precursor to the plant as in its determination above it is possible that only a small fraction of the available substance can be detected. That the normal formation of auxin in the tip of the coleoptile can be easily interfered with, presumably through the interruption of the supply of precursor from the seed, can be seen from the following experiment shown in table XXXVII. It was

Table XXXVII

The effect of different extent of removal of the seed

Each figure is the mean of two comparable tests
Plants deseeded 18 hours before the first decapitation

Treatment		Curvatures after time of application in min.				
scutellum	endosperm	135	195	255	315	1045
present	absent	-12.4	-21.4	-29.2	-39.7	-23 apx.
absent	"	----	-18.3	-26.6	-31.7	-27,5
"	present	----	-13.8	-18.8	-24.9	- 9.6
present	punctured	----	-16.0	-20.1	-22.6	+ 0.4
"	present	-12.3	-14.2	-14.7	-17.1	+ 0.8

shown above that the rate of increase in curvature with time is correlated with the amount of decrease in regeneration of auxin. Thus, when a given concentration of auxin is applied to a series of differently treated test plants, as shown in table XXXVII, the larger the curvature the less regeneration of auxin has occurred. It can be seen that merely a small puncture of the seed coat produces nearly as large decrease in auxin formation in the tip as does the removal of the scutellum. But either operation is considerably less effective than the complete removal of the endosperm.

4. Conclusion.

From the material presented in this chapter it can be concluded that the growth hormone present in the tip of the coleoptile is synthesized there from a material transported from the seed. The effects of deseeding on the production of auxin, the gradient of auxin in the coleoptile, as well as direct transport experiments demonstrate that auxin (in its active form) is not transported apically through the coleoptile. Whether auxin is synthesized directly from a precursor or whether it is formed from a more general metabolite from the seed is not clear.

CHAPTER XII

Precursors of Hetero auxin

Since it was impossible to get large amounts of precursor from the plant by the experimental technique used above, attempts were made to find a possible chemical source of hormone. With respect to auxin A these attempts have been unsuccessful. However, two substances, tryptophane and β-indolethyl amine have been found to be converted into hetero auxin by the action of the plant. These substances which behave very much like the precursor described above have, therefore, been studied in some detail.

1. Activation of tryptophane

It has been shown by Thimann that chemically pure tryptophane will not produce curvatures when tested by the standard Avena method. But when applied to sections in solution or to decapitated plants from below, it will promote elongation. Furthermore, from experiments on the synthesis of hetero auxin by Rhizopus suinus, Thimann has shown that tryptophane is a precursor of hetero auxin. The mechanism of the transformation is an oxidative deamination and decarboxylation.

What happens when very dilute tryptophane solution in agar blocks are applied to deseeded test plants? It is evident from curve IV figure 25 that tryptophane behaves in exactly the same manner as the precursor from the plant (curve II). By choosing the proper concentration of tryptophane, which however varies from day to day, the same type of

curve can also be obtained with normal test plants. In this case the amounts of active hormone formed from tryptophane are large enough to more than balance the effect of regeneration and auto-tropism, and may in fact under very suitable conditions cause the plant to be in a state of active bending for more than 30 hours. An approximate idea of the curvatures produced by tryptophane can be obtained from the figures in columns 8, 9 and 13, 14 table XLI. It has been shown by Kögl and Kostermans (1935) that indol pyruvic acid has auxin activity. Thus the possibility exists that this acid rather than hetero auxin (indolacetic acid) is formed from tryptophane. However, these authors point out that the apparent activity on Avena of indolpyruvic acid is likely due to its break down into hetero auxin. They calculate that one per cent break down will account for the measured activity. On the other hand from the experiments of Went on the different homologous acids of this type, the acetic radicle does not appear to be the only active one, and it is possible that more than one active substance is derived from tryptophane.

2. Activation of Indolethylamine

For the above reasons and especially for an investigation of the possible apical transport of the precursor it was desirable to have a precursor not possessing a carboxyl group, but instead in accordance with the theory of Went, a basic group. For this purpose it was thought indolethyl amine might be used. This substance was therefore kindly synthesized by Dr. Koepfli, and it has been found to be very suitable for precursor experiments. It is completely inactive in growth promoting activity on Avena, as determined by the standard test, but in contact

with the cut surface of the plant it can become activated and will then produce curvatures (see curve V, figure 25). Advantageous properties of this compound are shortly as follows: It does not contain a carboxyl group which is supposed to be a prerequisite for activity; it is a weak base (gives blue coloration of red litmus); its only known active possible degradation product is hetero auxin (lower degradation products are, according to the work of Kögl, inactive); its molecular weight is nearly the same as that of hetero auxin.

a. Curvatures in normal plants

It was stated above that the minimum active concentration of tryptophane varied greatly from one experiment to the next. The same behavior is exhibited by the idolethyl amine. Numerous experiments were made to establish a minimum active concentration by different types of tests, and the results obtained will be given below in some detail.

In table XXXVIII is shown an example of the type of curvatures obtained in standard test plants. It can be seen that in the early part of the test large positive curvatures are obtained especially with the higher concentrations of amine. These curvatures evidently result from an inhibitive effect on regeneration of hormone in the plant. They may perhaps in part be explained by a pH effect, but not entirely so, because the application of agar blocks containing phosphate buffers in the range from pH 3 to pH 8.5 produced no marked effect of this kind, although occasional plants supplied with the most alkaline buffer showed higher positive curvatures than plants supplied with plain agar blocks. And on the other hand, the acid buffers did not cause any distinct negative

Table XXXVIII

Activity of indolethyl amine ~~as~~ in test with normal plants

Plants decapitated at 2:45 and 3:20 Blocks put on 4:00

Concentration in mg/cc		Mean curvatures in degrees after different time in hours of test		
		1.9	6.1	18.-
Amine	1/1	+5.4	+6.5	+6.0
"	1/3	+2.3	+2.9	-1.5
"	1/9	+1.4	+2.1	-3.6
"	1/27	+1.7	+1.8	-6.2
"	1/81	+2.9	+1.8	-5.2
"	1/243	+1.7	+1.8	-5.4
"	1/729	+2.7	+1.4	-4.3
"	1/2187	+1.8	+2.3	-5.3
Agar		+1.4	+1.7	+1.0
Auxin	1/9	-0.5	0 ?	?
"	1/81	-2 to -3	-1.8	?
"	1/243	-12.6	-13.2	-34.6
"	1/1458	-21.8	-31.4	-19.9
"	1/8750	-3.4	-6.8	0.-
"	1/80000	+1.9	+1.7	0.-

curvatures, when applied in the same way. Furthermore, it is not a toxic effect, since the cells are subsequently able to elongate and show no visible injuries as is observed from the application of toxic substances or high salt concentrations. In the later stages of the test normal curvatures are produced, so that plants which at first exhibited large positive curvatures now assume the shape of question marks. However, there is no marked linear correlation between the concentration of amine applied and the amount of curvature produced.

b. Curvatures in deseeded test

In table XXXIX is shown the type of results obtainable by the use of deseeded plants. By this method, as should be expected, the large positive curvatures are usually entirely avoided. Nevertheless, marked negative curvatures do not occur immediately after application, as do auxin curvatures, but start to appear slowly and then rapidly increase in size. Furthermore, the range of active concentrations is much higher than for auxin, although the limiting concentration is from 10 to 100 times higher. There is an optimum range of concentration in which the highest curvatures are obtained. This is also true for auxin itself, but the cause of the effect is not the same with respect to the two substances. The fact that higher concentrations of auxin give small curvatures is due to lateral transport and when this occurs distinct curvatures appear in the basal regions of the plants. Amine on the other hand gives no such curvatures, but will after a very long time produce apical curvatures.

c. Curvatures in sections.

Although it is possible to get large curvatures of the precursor

Table XXXIX

Activity of indolethyl amine in deseeded and normal test

Plants deseeded 17 hours before 1st decapitation

Concentration in mg./cc.		Mean curvatures in degrees after time in hours of test							
		Deseeded plants				Normal plants			
		1.6	3.5	5.5	16.-	1.6	3.5	5.5	16.-
Amine	1/10	-2.2	-0.3	+1.-	-1.7				
"	1/40	+0.1	-0.3	-0.4	-7.5				
"	1/160	-0.4	-6.7	-12.3	-8.8	+0.5	+0.7	-1.7	-6.0
"	1/640	-2.4	-11.1	-20.9	-14.3	+0.2	+2.0	+1.6	-3.5
"	1/1280	+0.1	-0.7	-0.4	-4.6	-0.4	+0.1	-0.1	-4.3
"	1/2560	+0.3	+1.1	-0.2	+0.2				
"	1/5120	-0.2	+0.7	+0.2	-0.1	+0.7	+3.2	+2.6	+0.4
"	1/10240	-0.3	-0.0	+0.7	+0.2				
Agar		+1.3	+0.4	+0.8	+0.6	+0.9	+1.9	+0.5	+1.0
Auxin	3/500	-2.8	-17.6	-31.8	-43.5				
"	3/5000	-6.8	-20.1	-31.0	-11.9				
"	2/5000	-4.3	-14.5	-20.5	-18.4				

type by the above methods, the results obtainable by a given concentration vary tremendously from one experiment to the next. By the use of decapitated coleptiles placed in paraffin holders as described by Dolk (1930) it was finally possible to obtain fairly uniform and reproducible results. Decapitated coleoptiles were cut at the base and mounted in suitable holes in a paraffin block, with a shallow layer of water at the base of the sections. The plants were then redecapitated, and the test blocks were applied as in the ordinary test. The plants were kept under a bell jar saturated with water vapor, and photographs were taken of the plants at intervals. Results of two such experiments are shown in Table XL. It can be seen that with this technique, which is a compromise of the former two, on the one hand, the large positive curvatures obtained in a are largely avoided, and on the other hand, an effect noticed throughout this work that plants which had been deseeded at an early stage of development, although giving large curvatures, have a smaller capacity to activate the amine, was also at least partly removed. Furthermore, in these experiments the agar blocks remain in better condition, so that the curvatures of the individual plants, although not as good as in auxin tests, were quite uniform. By this method it was therefore possible to maintain large curvatures for periods of six or more days by a single application of high concentrations of amine, whereas the application of lower concentrations results in curvatures which disappear after a shorter time, and even very large curvatures produced by auxin disappear almost completely within one to two days.

Table XL

Activity of indolethyl amine in section test

Exp. III. Plants decapitated 1st time 4:00 p.m. Feb. 20, then cut at base and placed in holders, redecapitated 5:00 and blocks applied immediately afterwards.

Exp. IV. Plants decapitated 1st time 3:00 p.m. Feb. 22, then cut at base and placed in holders, redecapitated 4:00 p.m. and blocks applied.

Concentration in mg./cc.	Mean curvatures in degrees after time in hours in test							
	Exp. III					Exp. IV		
	3.0	15.3	27.2	45.-	65.-	3.7	14.0	25.5
Amine 1/6.67	-1.3	-13.0	-26.3	-35.0	-32.0	+2.6	-16.5	-61.5
						-0.9	-17.7	-54.0
						+0.9	-17.1	-57.8
" 1/20	+0.9	-15.0	-28.4	-20.6		+0.8	- 8.8	-27.1
" 1/60	+0.4	-11.5	-14.7	- 8.6		+0.6	-14.8	-25.9
						-1.4	-13.3	-36.1
						+1.0	-14.1	-31.0
" 1/180	+1.0	- 4.5	- 1.6	- 0.9		+2.6	- 4.0	- 4.5
	+1.0	- 5.7	- 1.8	- 1.7				
	+1.0	- 4.9	- 1.7	- 1.2				
" 1/540	+0.6	- 3.5	- 1.7	- 1.2		+4.6	- 5.3	- 2.2
	+1.0	- 2.8	- 1.6	- 2.0		+3.0	- 3.1	- 8.8
	+0.8	- 3.2	- 1.7	- 1.6		+3.8	- 4.2	- 5.5
" 1/1629	+1.3	- 1.8	- 0.4	+ 0.2		+1.8	- 1.1	- 1.4
	+0.6	- 0.0	- 0.7	- 0.2				
	+ 1.1	- 1.2	- 0.5	+ 0.1				
" 1/4860	+2.8	- 0.6	+ 0.4	- 0.1		+2.5	+ 0.2	- 1.2
	+0.4	- 1.6	- 2.2	- 1.8				
	+2.0	- 0.9	- 0.5	- 0.7				
" 1.14580	+1.4	- 0.2	- 0.7	+ 0.6				
Agar	+0.6	+ 0.3	+ 0.6	+ 0.6		+1.5	+ 0.4	+ 0.4
Auxin 1/?	-20.8	-14.9	- 8.0	- 7.4	-6.-	-17.6	- 6.2	- 5.8
" 1/2?						- 3.1	+ 0.5	+ 0.3

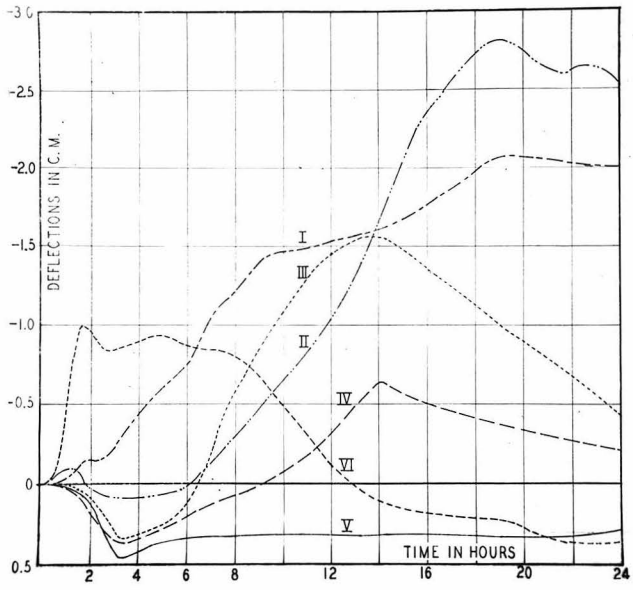


Fig. 26. Curvatures obtained in sections tests with "Kimograph" Curves I, II, III, IV and V produced by indolethyl amine in concentrations of 3/100, 3/500, 3/2500, 3/ 12500 and 0 respectively. Curve VI represents auxin curvatures obtained in a similar experiment. Each curve is the mean of 4 to 9 plants.

3. Transport experiments.

From an interpretation of the experiments described above it will be clear that a basic substance, without a carboxyl group, is capable of being activated to produce curvatures in Avena. It is of interest to determine whether, in accordance with the polarity theory of Went, such a substance can be apically transported within the plant. For this purpose transport experiments of the usual type were performed. Coleoptile sections of given lengths were placed with their basal surfaces on agar blocks containing different concentrations of indol-ethyl amine or tryptophane, and plain agar blocks were placed on the apical surfaces of the sections. After given times of transport the blocks were tested, usually on deseeded plants, together with suitable controls.

Since the results of experiments so far completed are not conclusive enough to be shortly represented, a number of individual experiments are given in tables XLI and XLII for tryptophane and amine respectively. It can be seen that in practically every experiment distinct curvatures are obtained from the apical blocks. However, the apical blocks from sections with tryptophane supplied at the basal surfaces give on the whole not significantly higher curvatures than apical blocks from sections supplied with plain agar. (Compare columns 20 and 22 of table XLI). When allowance is made fof the precursor derived from the sections themselves, therefore, it is impossible to say whether a small amount of transport has occurred. From similar experiments of transport in the basipetal direction (column# 21) it appears that some transport has occurred. But this might be transport of material

TABLE XLI. Transport and activation of tryptophane.

Exp. No.	Conc. of trypto-phane mg/cc.	Length sections in mm.	Time in hrs. diffusion	Time in hrs. test	Plain agar Des. test	Plain agar Standard test	Plain trypto-phane Des. test	Plain trypto-phane Stand. test	Plain auxin	Hormone from sections auxin	Hormone from sections pre-cursor	Transport of tryptophane Normal (basal block)	Transport of tryptophane Inverse (apical block)
1	0.25	4.0	5.0	3.0	+0.7	+1.8	+0.3	+0.8	---	-0.5	-0.2	-1.5	+0.2 +0.2
				5.0	+1.0	+1.6	+0.2	-0.8	---	-1.2	±0.0	-2.3	-0.8 -1.5
2	0.5	4.0	4.0	2.0	+0.3	+0.3	-0.5	+0.5	---	-0.5	---	-3.8	-2.4 --- +2.3*
				15.-	+0.4	+1.4	-2.0	-2.5	---	-0.0	---	-3.4	-3.6 --- +0.9*
3	0.3	4.0	3.5	2.0	-0.1	+1.3	---	+2.2	---	-0.4	-0.1	-1.6	-0.4 --- +0.6*
				15.-	---	+1.8	---	---	---	---	---	-5.9	--- --- ---
4	0.5	3.0	3.5	2.0	+1.2	---	-2.7	---	---	-2.7 -0.2	+0.4	---	-0.7 -0.9 +0.1
				5.0	+2.9	---	-9.9	---	---	-6.2 -1.1	+0.3	---	-3.5 -1.6 -0.2
				19.-	+3.3	---	-18.5	---	---	-2.4 -2.1	-2.4	---	-3.9 -2.6 -5.3
5	0.5	3.0	4.0	2.2	-0.1	---	-0.6	+1.3	-10.3	-2.3	+0.6	-1.6	+0.7 --- ---
				7.3	-0.0	---	-9.7	-1.6	-39.5	-5.9	+2.0	-6.5	-1.1 --- ---
				18.-	+0.7	---	-16.9	-8.0	---	-3.7	-1.6	-1.3	-2.7 --- ---
6	0.5	3.0	3.2 to 3.5	2.0	+2.0	---	-0.9	---	-4.7	-1.2	+0.9	---	+0.1 -0.2 ---
				7.7	+2.1	+1.5	-17.3	-0.4	-40.2	-4.8	-3.4	---	-2.8 +0.1 ---
				22.-	+4.2	---	-14.0	---	-19.0	-0.2	-6.6	---	-4.4 -4.2 ---
7	0.5	3.0	3.5	1.9	+1.6	+1.5	-0.4	-0.4	---	-0.4	+1.5	-3.3	+2.0 +0.7 ---
				7.-	+1.8	+1.1	-8.4	-2.8	---	-3.2	±0.0	-6.8	+0.4 -4.7 -----
				17.5	+0.9	+0.4	-12.0	-5.9	---	+0.4	-0.2	-1.3	+0.6 -1.4 -----
8	0.5	3.0	3.5	2.1	+1.6	---	-1.3	---	---				
				5.1	+0.9	---	-6.9	---	---				
9	0.5	3.0	3.5	2.3	+0.7	+0.4	+1.0	-0.5					
				5.-	+1.0	---	-2.0	---	---				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14

Exp. No.	Transp. of auxin inverse	Applied tryptophane blocks (apical) blocks	Applied tryptophane blocks (basal) blocks	Applied auxin (basal blocks)	Hormone from sections auxin	Hormone from sections pre-cursor	Transport of tryptophane (basal)	Transport of tryptophane apical	Transport of tryptophane auxin (apical)	Tryptophane converted by sections apical blocks	Tryptophane converted by sections basal blocks
1	---	-1.6 -4.8	----- -6.4*	-----	-1.2	-0.9	-1.0	+0.4 +0.3	---	-1.7	-4.6
	---	-2.8 -6.8	----- -8.1*	-----	-2.2	-0.7	-0.9	-1.1 -1.8	---	-3.0	-5.8
2	---	-1.6 -2.3	----- +0.7*	-----	-0.8	---	-3.3	--- ---	---	-1.1	-1.3
	---	-8.0 -9.1	----- -2.3*	-----	-0.4	---	-3.4	--- ---	---	-6.0	-7.1
3	---	-0.3 -1.6	----- -0.2*	-----	-0.3	-0.0	-1.2	-0.3 ---	---	-0.4	-0.7
	---	--- -9.8	--- -1.9*	-----	---	---	---	--- ---	---	---	-7.8
4	---	--- -6.9	-5.1 -3.7	-----	-3.9	-0.8 -1.4m	---	-1.1 -1.4	+0.3m ---	---	-0.9 -0.8m
	---	--- -16.1	-17.7-16.5	-----	-9.1	-2.6 -4.0m	---	-3.8 -1.9	+0.9m ---	---	-0.8 -5.5m
	---	--- -14.1	-19.4-21.1	-----	-5.7	-5.7 -5.4m	---	-1.5 -0.2	-3.4m ---	---	+3.1 -0.2m
5	+0.1	-2.0 -6.4	--- ---	-12.0	-2.2	+0.7	+0.7	-0.1 ---	--- -0.1	-2.0	-3.5
	+0.3	-11.5 -21.8	--- ---	-41.1	-5.9	+2.0	-0.6	-3.1 ---	--- -1.7	-3.8	-6.2
	+0.2	-18.6 -17.5	--- ---	-----	-4.4	-2.3	+2.4	-1.3 ---	--- +1.8	-0.1	+3.1
6	+1.9	--- -3.4	-6.1 ---	-6.4	-3.2	-1.1	---	-0.8 -1.1	+1.0	---	-1.3 -4.0
	-1.0	--- -35.6	-23.6 ---	-45.0	-6.9	-5.5	---	-0.8 +3.5	+2.4	---	-13.5 -1.5
	-2.1	--- -21.8	-6.3 ---	-11.1	-4.4	-10.8	---	+2.2 +2.4	+4.5	---	-7.6 +3.9
7	+0.7	-3.0 -5.5	-5.1 ---	-7.6	-2.0	-0.1	-2.9	+0.3 -0.8	-0.8	-4.1	-4.7 -4.3
	-2.4	-16.4 -23.4	-22.1 ---	-17.0	-5.0	-1.8	-3.6	+0.3 -4.7	+2.4	-8.0	-11.8 -10.5
	-1.4	-13.6 -18.3	-9.0 ---	-7.0	-1.3	-1.1	-0.9	+0.8 -1.2	+1.2	-1.4	-6.7 +2.6
	15	16	17	18	19 (11-6)	20 (12-6)	21 (13-11)	22 (14-12)	23 (15-13)	24 (16- 8+12)	25 (17 - [8+1])

All tests are with deseeded plants except those of columns 7 and 9, and 14* and 17*
20 sections used on each full size agar block
m mesocotyl sections

TABLE XLII

Transport and activation of β-Indolethyl amine

Exp. No.	Conc. of amine in mg/cc	Lengths of sections	Time in hrs. Diffn.	Time in hrs. test	Plain agar Des. test	Plain agar Stand. test	Plain amine Des. test	Plain amine Stand. test	Hormone from sections auxin	Hormone from sections precursor	Transport of amine (apical)	Transport of amine (total)	Amine blocks applied to base of sections.	Hormone from sections auxin	Hormone from sections precursor	Amine transported (- auxin)	Amine converted by sections at basal surface
1	0.50	3.0	4.0	2.0	+1.4	+0.9	+0.4	+2.9	-1.0 -0.7	+0.6 0.0	-3.4	-3.7 -3.1*	-0.9 -2.9 -5.7	-2.4; -2.1;	-0.8; -1.4 -1.1	-3.7; -4.0; -3.1	-1.3 -5.4
				5.0	+2.2	+2.7	+0.8	+4.7	-4.1 -1.7	-0.6 -0.3	-5.6	-5.0 -2.7*	-4.0 -6.8 -5.4	-6.3; -3.9	-2.8; -2.5 -2.7	-5.1; -4.5; -2.2	-2.1 -4.5
				14.5	+0.9	+2.5	+1.5	+8.1	+5.1 -2.5	-3.7 -3.4	-3.9	-3.9 -1.6*	-8.3 -2.6 -5.4	-6.0; -3.4	-4.6; -4.1 -4.4	-0.4; -0.4; +1.0	-1.9 -0.8
2	0.05	3.0	4.0	2.5	+1.5	+2.6	-1.9	-1.1	-0.5 -0.5	+1.2 +2.7	-1.0	+0.9 [-0.0]	-7.8 -9.9 [-8.9]	-2.0; -2.0	-0.3; +1.2; +0.5	-3.0; -1.1 [-2.1]	-6.5
				4.7	+1.6	+2.3	-3.2	+0.4	-2.8 -1.4	-0.5 +2.7	-0.6	+1.5 [+0.5]	-14.7 -16.7 [-15.7	-4.4; -3.0	-2.1; +1.1; +0.5	-2.5; -0.6 [-1.6]	-10.4
				15.5	+0.5	+1.0	-3.8	-0.1	-3.0 -3.4	-1.6 -3.2	-4.9	+1.5 [+0.5]	-18.8 -20.3 [-19.6]	-3.5; -3.9	-2.1; -3.7; -0.8	-4.6; -4.1; [-4.4]	-12.0
	0.50	3.0	4.0	2.5			+2.0	+3.8	-0.5	+2.0	-1.9	-3.5 [-2.7]	- 2.4 -2.3 [-2.4]			-3.9; -4.5; [-4.2]	-3.9
				5.0			+2.2	+3.0	-2.1	+1.1	-1.8	-5.2 [-3.5]	-7.7 -4.8 [-6.3]			-3.9; -6.3; [-5.6]	-6.4
				15.5			+3.1	+3.1	-3.2	-2.4	-4.4	-5.4 [-4.9]	-12.6 -9.4 [-11.0]			-4.1; -5.1; [-4.6]	-10.9
3	0.005	4.0	2.0	2.1	-0.4	---	-0.8	---	-1.3	+1.3	+0.1	+0.4 [+0.3]	-1.7 +0.7**	-0.9	+1.7;	-1.2; -0.9; [-1.1]	+0.4
				6.6	+0.4	---	-1.0	---	-0.6	-0.0	-1.8	-0.0 [-0.9]	-4.5 +1.7**	-1.0;	-0.4;	-1.8; -0.0; [-0.9]	-2.9
				17.5	+0.9	---	-3.7	---	-2.1	-2.5	-4.8	-6.8 [-5.8]	-8.2 +1.5**	-3.0;	-3.4;	-2.3; -4.3; [-3.3]	-2.4
	0.05	4.0	2.0	2.1	---	---	+0.4	---	---	---	+0.7	--- ---	-3.0 ---			-0.6	-2.1
				6.6	---	---	-0.5	---	---	---	+2.5	--- ---	-5.2 ---			-2.5	-4.1
				17.5	---	---	-1.0	---	---	---	-6.2	--- ---	-15.4 ---			-3.7	-12.3
	0.50	4.0	2.0	2.1	---	---	+0.5	---	---	---	+0.7	-0.8 [+0.0]	-3.4 -3.3 [- 3.4]			-0.4; -2.1; [-1.3]	-2.6
				6.6	---	---	+4.6	---	---	---	-0.5	-0.3 [-0.4]	-12.6 -10.6 [-11.6]			-0.5; -0.3; [-0.4]	-16.6
				17.5	---	---	+1.3	---	---	---	-2.7	-2.4 [-2.6]	-9.3 -9.5 [-9.4]			-0.2; +0.1; [-0.1]	-10.1
4	0.50	4.0	2.0	14.-	+0.9	---	---	---	-2.5	-3.1	-7.7 (top)	-3.5 (mid) -3.7* (mes)	-2.0 -1.7* (t-m) (mes)	-3.4	-4.0	-4.6 0.1; 0.6	
5	0.005	3.2	3.0	2.5	0	---	pos	---	neg	0 0				neg	0- 0.-	0.-	
				5.8	0	---	0	---	neg	neg neg	0	0 ---	neg neg	neg	neg neg	?	
				18.-	+1.0	---	-6.0	---	-1.7	-4.1 -3.0	neg	neg	neg neg	-2.7	-5.1 -0.4	+0.1 -1.1	+4.4
											-4.0	-4.1 ---	-2.0 -4.7				
	0.025	3.2	3.0	2.5		---	pos	---								pos neg	---
				5.8		---	0	---			pos	neg ---	neg neg			? ?	---
				18.-		---	-11.2	---			0	neg ---	neg neg			+1.9 -0.5	-2.4
											-2.2	-3.8	-14.5 -4.7				
6	0.05 (apx)	3.0	4.0	3.5	+2.1	---	-0.4	---	-1.6 -1.3	+0.8 +0.8				-3.7 -3.4	-1.3 -1.3	-2.5 -0.2	-14.7
				17.0	+1.6	---	+5.9	---	-5.7 -3.5	-4.3 -2.4	+0.9	-1.5 ---	-16.8 -12.8	-6.7 -5.1	-5.9 -4.0	-3.1 -1.5	-21.0
											-2.6	-5.0 ---	-19.7 -13.6				
1	2	3	4	5	6	7	8		10	11	12		13	14 (10 - 6)	15 (11 - 6)	16 (12 - 8)	17 (13 - [8 + 10])

Exp. 5 1st set of figures for transport at 2°C, second set at 24°C
* mesocotyl sections; ** Standard test plants.
20 sections used on each full size agar block.

which has already been converted into auxin.

In the experiments with amine the amount of actual transport appears slightly higher. But also here the results are not conclusive. In the first place, the differences are small, and secondly, it is necessary to use relatively much higher concentrations than for auxin transport. Leakage of substance by diffusion along the surfaces of the sections are therefore more likely to occur. In control experiments with auxin it was found that no auxin was transported apically under the conditions of the experiments as long as moderate concentrations of hormone were used. But with higher concentrations (about 10,000 and 20,000 units/cc) large auxin curvatures were obtained in the apical blocks.

The possible effect of diffusion of substance is reduced by the fact that relatively higher concentrations of amine than of auxin are necessary to produce curvatures. It is impossible to determine and correct for the possible diffusion of substance until it becomes possible to establish more definitely the minimum active concentration under the conditions of these experiments. The fact that sections of mesocotyl or half coleoptile half mesocotyl also give similar transport indicates that possible transport of substance by diffusion along the surface of the sections might be negligible.

Various attempts to determine apical transport of either amine or of auxin by inducing curvature with unilaterally applied blocks have not been successful. In such experiments with auxin applied unilaterally to the basal surface of the upper half of the coleoptile no trace of

curvature was obtained, whereas similar application of blocks to the apical ends of the lower halves of the same coleoptiles or to the upper halves of identical coleoptiles resulted in curvatures of over 20 degrees. These results then definitely show that auxin itself is not apically polarly transported within the plant. From the negative results with the amine, on the other hand, no definite conclusion can be drawn.

From the above transport experiments, however, one definite result appears. By a comparison of the curvatures produced by the amine and tryptophane blocks which had been placed in contact with sections to the curvatures produced by the corresponding control blocks not previously placed in contact with sections, it is clear that substance in blocks has become activated by the sections, and thus these blocks produce relatively larger curvatures immediately after application. That this increase in curvature is not due merely to the auxin derived from the sections is clear from two facts. 1) Apical and basal surfaces have been found to activate the blocks to about the same extent, and 2) the auxin derived from the sections is not enough to account for the increased curvatures. These results confirm the above evidence that β-indol-ethyl amine and tryptophane are activated by the plant.

4. Activation produced by the plant

In order to show that the activation of the substances is through the action of the plant some additional experiments were carried out.

a. Effect of micro-organisms.

It is well known that micro-organisms are able to break down

indol derivatives, and several investigators since Salkowski (1885) have been able to isolate hetero auxin as an intermediary product. That micro organisms can play a part in the activation process under certain conditions in these experiments was indicated for example in one experiment where the grass stalks stuck into the coleoptiles had not been sterilized in alcohol before they were used, and a considerable growth of fungus appeared. In this experiment the time of appearance and size of curvatures were very irregular and abnormal. However, that the effect of micro organisms in the activation is in general only of secondary importance is indicated by the following experiments.

Blocks of amine in different concentration were placed in contact with apical and basal sections of etiolated Pisum stems for different lengths of time up to four hours. These blocks were then tested. These blocks showed no activation but produced curvatures only a long time after application in the same way as control blocks, when allowance was made for the auxin derived from the basal ends of the sections. If the activation were due to micro organisms, then Pisum sections should be as effective as Avena sections, and the apical blocks from Pisum should also produce curvatures immediately after application. Thimann has recently studied the possible effect of micro organisms in relation to the activation of tryptophane in sections in solution and finds it to have no primary importance (private communication). It is thus reasonable to conclude that the activation is brought about mainly through the action of the plant.

5. Test of activity of amine in solution.

That indol-ethyl amine can produce curvatures only after it has become activated by the plant, and that the delayed curvatures are not due to a slow rate of transport of the substance can be deduced from the transport experiments above and will now also be shown in a different way.

Avena coleoptiles were decapitated, slit longitudinally for about half their lengths, and placed in solutions of various concentrations of amine as in pea tests. The curvatures produced were qualitatively estimated at different times after immersion. The results of one such experiment are given in table XLIII. The results of a regular pea test are given in table XLIV. It is clear that also this "pea test" method in which the capacity of transport of the substance is not an important factor, only the very high concentrations produce visible curvatures within the early part of the test, but after longer times also the more dilute solutions appear to be effective. This behavior indicates that the delayed curvatures obtained in Avena tests is due to gradual activation and is not due to a possible slow rate of transport of the amine. If the substance were active in itself, furthermore, all curvatures should appear, as in standard pea tests, at least within the first eight hours after application. And lastly, since all substances analogous to hetero auxin which produce curvatures to different extent in Avena, nevertheless, mol for mol give the same amount of curvature appearing within a relatively short time (as found by Dr. Went) the different behavior of amine in this respect strongly indicates that it

Table XLIII

ctivity of indolethyl amine on Avena in solution

Conc. mg/cc.	Curvatures after time in hours			
	10	24	48	72
1/125	+++	+++++	+++++	+++++
1/250	++	++++	+++++	+++++
1/500	-	+++	++++	++++
1/1000	-	-	++	+++
1/2000	-	-	+?	++
1/4000	-	-	-	+
1/8000	-	-	-	-
1/16000	-	-	-	-
water	-	-	-	-

Table XLIV

Activity of indol-ethyl amine in pea test
(All figures are rough observations)

Conc. mg/cc	Curvatures after time in hours					
	4	9	12	22	35	103
1/10	+++	400	400	400	400	400
1/40	+	++++	300	300	300	300
1/160	-	-	-	+++	+++	+++
1/640	-	-	-	-	+	++
1/2560	-	-	-	-	+?	+?
1/10300	-	-	-	-	-	-
1/41000	-	-	-	-	-	-
Water	-	-	-	-	-	-
Auxin	++	+++	200	200	200	200

is itself inactive.

An explanation for the apparently contradictory results on activation of amine in pea tests but not by pea sections placed on agar blocks cannot yet be given.

Conclusion and discussion

The results obtained with tryptophane and indol-ethyl amine show that these substances do not possess the properties of auxins but can be activated when in contact with the plant into true auxins. Thus the delayed curvatures produced by these substances in Avena tests are not due merely to a slow rate of transport of these substances in the plant, but they are typical auxin curvatures gradually produced after a chemical transformation. The chemical nature of these substances as well as other evidence indicates that the activation process involves an oxidative deamination and in the case of tryptophane presumably also a decarboxylation in the side chain of the molecule. Most likely the activation takes place extracellularly, since if it occurred exclusively intracellularly no active material would be recoverable from a block applied apically to sections. Nevertheless, the activation must be caused mainly by agents from the plant.

Although these substances are evidently not identical with the precursor from Avena, they lend strong support for the evidence given in Chapters X and XI for its existence and behaviors. How far the relationship existing between these synthetic precursors and hetero auxin can be extended to explain the relationship between the precursor and auxin in the plant is still a speculative matter.

The evidence presented shows that auxin is not likely to be transported as such apically through the coleoptile either in its relatively early or later stages of growth. On the other hand indirect evidence obtained from deseeded plants as well as more direct evidence from the behavior of the material obtainable by diffusion into agar at the apical cut surface of the coleoptile or mesocotyl strongly point to the conclusion that a precursor of auxin is transported through the plant.

The question whether the precursor is transported polarly to the apex of the plant, or whether it is translocated in some different manner, cannot yet be answered. The necessity of chemical change in tryptophane and especially indolethyl amine to become active on Avena is in accordance with the view that a terminal carboxyl group is a prerequisite for auxin activity. However, Glover (1936) has reported skatol to be active in Avena tests, but the data presented do not indicate what kind of curvatures were obtained.

Zimmerman and Wilcoxon (1936) report that a-naphtalene-aceto nitrile is active in causing epinastic movements of tomato leaves in the same way as naphtalene-acetic acid, but the effect is produced a day later. They suggest that the nitrile is inactive but undergoes hydrolysis into a-naphtalene acetic acid.

It has been pointed out by Thimann (1935b) and by Haagen-Smith and Went (1935) that a clear distinction must be made between true auxins and such substances as may promote growth but are not capable of being polarly transported through the plant. It appears that the substances

studied here do not belong to either one of these two groups, so that in addition it is necessary to consider a third group of substances, inactive in themselves, but capable of becoming changed into true auxins by the action of the plant.

SUMMARY OF THE MAIN RESULTS

The results of the experiments are included in the conclusions at the end of different chapters and in the last chapter of each Part. It is therefore only necessary to summarize the main results.

Part I.

1. The inhibition of lateral bud development in *Vicia* and in *Pisum* is controlled by the growth hormone produced by the tterminal bud.
2. The inhibiting effect produced by the terminal bud can be completely substituted by a continuous application of auxin in relatively high concentrations to the stem of decapitated plants.
3. The inhibiting action of auxin is due to its prevention of synthesis of hormone in the buds and is independent of the action of the hormone in promoting growth of the stem.
4. In *Vicia* older than seedlings auxin is produced only in the presence of light, but the growth response of the plant to auxin is higher in the dark.
5. Auxin is an essential factor for the growth of the stem.

Part II.

1. Auxin is destroyed by x-irradiation both in solution and in the plant.
2. Inactivation of auxin in solution is indirectly through the oxidation by strong oxidizing agents formed by irradiation.

3. In the Avena coleoptile X-rays cause only a temporary decrease in auxin.
4. In green plants (Vicia and Pisum) grown in the light the mechanism of formation of auxin is additionally destroyed so that a gradual permanent decrease in auxin is produced by irradiation.
5. The destruction of auxin and the mechanism of its formation in the plant is a major factor in the immediate inhibition of growth of plants caused by irradiation.
6. The development of lateral buds in irradiated plants is due to the removal of auxin and is not due to a direct stimulation of growth by the X-rays.

Part III.

1. A quantitative "deseeded" Avena test method for small amounts of auxin and precursors of auxin has been described.
2. The presence in the Avena coleoptile of a precursor of auxin transported from the seed has been demonstrated.
3. Tryptophane and β-indol-ethyl amine, which lack auxin activity, have been shown to be changed chemically by the action of the plant into hetero auxin.
4. The physiological behavior of these substances in distinction to auxin has been studied.

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