GROWTH SUBSTANCE CURVATURES OF AVENA IN LIGHT AND DARK

By J. VAN OVERBEEK

(From the William G. Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena)

(Accepted for publication, January 13, 1936)

I

INTRODUCTION

Since it had been shown (Van Overbeek, 1932, 1933) that growth substance curvatures of *Raphanus* hypocotyls offered an easy way to study light growth responses, the question arose whether this was true for *Avena* coleoptiles too. By growth substance curvatures are meant such curvatures as appear when growth hormone is applied unilaterally to a coleoptile, hypocotyl, etc. If the growth substance curvatures of *Raphanus* hypocotyls which have occurred in the dark are compared with those that have occurred in the light (exposed from all sides), the former (dark) are always the larger. The ratio of the curvatures in dark and light depends on the amount of light, on the concentration of the hormone applied, etc. Furthermore it has been shown clearly that the difference in growth in light and dark has an important part in the explanation of the phototropic curvature of *Raphanus*.

In *Avena*, on the other hand, the light growth responses seem to have a minor part, if any, in the explanation of the phototropic curvature. Went (1928) was able to show that an unequal distribution of the growth substance, caused by unilateral exposure to light, is sufficient to explain the entire phototropic curvature of the *Avena* coleoptile. This unequal distribution occurs in the tip, which is solid, and, therefore, more fit for lateral transport of the growth substance than the hollow lower parts of the coleoptile. Cholodny (1933) was able to show that it was possible to obtain a phototropic curvature in

Avena under circumstances which did not cause a light growth response.

The growth substance curvatures of *Avena* coleoptiles in light and dark may not be important for the problem of phototropism, but it certainly is worth while to study them in order to increase our knowledge of the physiology of the coleoptiles.

On the basis of the present investigations, moreover, it has become possible to answer practical questions which had never before been answered satisfactorily. (1) Do decapitated coleoptiles such as those used in the growth substance test react in the same way to a given amount of growth substance after they have been photographed; *i.e.*, exposed to a small amount of light? The answer is no; their sensitivity is higher because the formation of new growth substance in the decapitated coleoptiles is inhibited by light. This "regeneration" of the growth substance formation decreases the sensitivity of the coleoptiles to growth hormone. Skoog (1937), however, has developed a method to obtain "regeneration free" plants. These plants can be exposed hourly to a small amount of light without the slightest difference in sensitivity to growth substance.

(2) Another question is answered by the present investigations on the growth substance curvatures in dark and light; *viz.* can hetero-auxin be used without limit as a substitute for auxin-*a* and auxin-*b*? Hetero-auxin is a substance produced by fungi as shown by Kögl and Kostermans (1934) and by Thimann (1935*a*). Auxin-*a* and auxin-*b* are present in higher plants (Kögl, Haagen-Smit, and Erxleben (1934)). In a recent study by Haagen-Smit and Went (1935) and by Thimann (1935*a*) it has been shown that in addition to hetero-auxin a large number of other substances which are not the natural growth substance of *Avena* and pea plants do stimulate the growth apparently in the same way as auxin-*a*, the only difference being that these other substances require a higher concentration to act in the same way that auxin-*a* does. Furthermore it has been shown by Skoog and Thimann (1934) that both hetero-auxin and auxin-*b* are able to inhibit the development of lateral buds. Went (1934) and Thimann and Went (1934) have shown that the root formation of pea cuttings can be increased greatly by application of hetero-auxin as well as of auxin-*a*. Cooper (1935) obtained a stimulation of root formation in lemon and

other woody cuttings after application of hetero-auxin. All these facts seemed to indicate that hetero-auxin can act as a substitute for auxin-*a* in every sense. Below, however, it will be shown that if in *Avena* coleoptiles auxin-*a* is replaced by hetero-auxin, growth is not inhibited after exposure to light. It will be shown, furthermore, that there are considerable differences in the destruction of both hormones.

The present investigations were started at the Ryks Universiteit in Utrecht, Holland, in the early part of 1934. They were continued, after an interval of about a year, at the California Institute of Technology in Pasadena.

II

A. Experiments Carried out in Utrecht

Method.—A detailed description of the experimental set up is given in an earlier paper (Van Overbeek 1933[1]). Briefly the circumstances under which the experiments were carried out were as follows. Temperature 21°C. and humidity about 90 per cent. The experiments in the dark were carried out in a dark room with controlled conditions. The room was lighted with orange-yellow light of wave lengths larger than 546 mμ. The experiments in the light were done in an incubator, which was kept at the same constant temperature and humidity as the dark room. In order to expose the plants uniformly on all sides, they were placed on a disk revolving in a horizontal plane. The light source consisted of two incandescent lamps of 500 watts each, which were placed at 80 cm. distance from the objects. The plants were shielded from the heat of the lamps by a set of glass plates and water containers. Under these conditions one lamp radiates an amount of energy of 200 erg/cm^2. per second upon the plants.

The *Avena* plants used were "Victory" oats from Svalöf. They were grown under standard conditions (see for example Went, 1935*a*).

The auxin used was pure auxin-*a* supplied by Prof. F. Kögl and Dr. A. J. Haagen-Smit.

1. Continuous Exposure Starting at the Second Decapitation.—When the coleoptiles were about 3 cm. long they were prepared in the following way. The tip of the coleoptiles was cut off (first decapitation) and 1½ hours later a fresh cut surface was made (second decapitation) and the primary leaf was pulled loose. Immediately after this an agar block containing auxin-*a* was applied on one side of the cut surface of each coleoptile. After this preparation one set of plants was exposed

[1] P. 550.

on all sides to the light, and another set was kept in the dark room. The curved plants were photographed 110 minutes after the blocks had been put on and the experiment was stopped. Later the curvatures of these plants were measured.[2]

Fig. 1 shows the results of three experiments carried out as described above. In these experiments the concentration of the auxin-*a* is plotted on the abscissa. It is clear that the growth substance curvature of the coleoptiles which were exposed on all sides to light is smaller than those of the non-exposed plants.

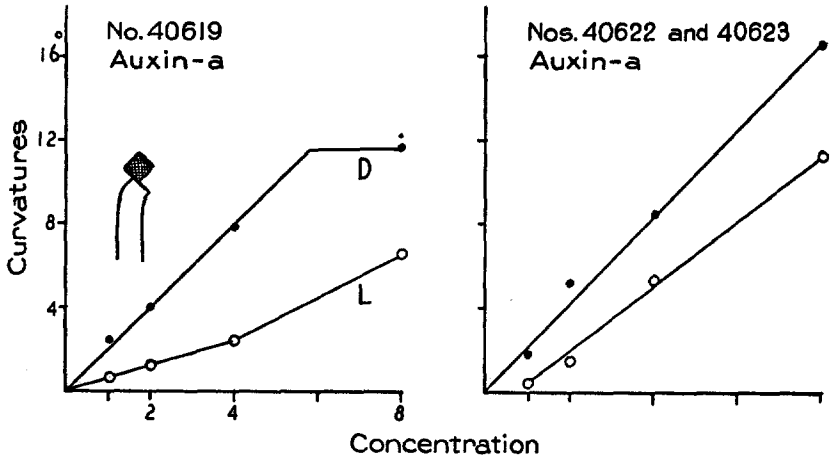


FIG. 1. Auxin-*a* curvatures of twice decapitated *Avena* coleoptiles in dark (black dots) and exposed on all sides to light (circles). Abscissa: relative concentration of the hormone. Each point is the average of the curvatures of twelve plants.[3]

2. Continuous Exposure Starting at the First Decapitation.—In order to see what will happen if the plants are exposed immediately after the first decapitation until the end of the experiment, the following experiment was made. Plants were decapitated and immediately after this decapitation one set of plants was exposed to the light

[2] To secure subjective measurements the photographic records were measured out "blindly" (without knowing to what the record referred).

[3] All figures in the graphs and tables of this publication represent average values of twelve plants, unless the contrary is stated.

(without growth substance being applied). 1½ hours later the plants were decapitated again and auxin-*a* blocks were applied. Photographs were made 110 minutes after this and the experiment finished. The results, shown in Table I, show that the exposed plants have on an average the same curvature as the non-exposed controls. Table I also shows the results of experiments similar to those described in the section entitled Continuous exposure starting at the second decapitation.

To study the different behavior of *Raphanus* as compared to *Avena*, the same experiments which were carried out and described in this section for *Avena* were repeated with *Raphanus*. The preparation of

TABLE I

Auxin-A Curvatures of Twice Decapitated Avena Coleoptiles

Auxin application after second decapitation. Three sets of plants. (1) Exposed continuously from the first decapitation, (2) exposed from the second decapitation, and (3) control in dark. Temperature 21°C. 2 lamps of 500 watts at 80 cm. Photographed 110 minutes after the growth substance was applied.

No.	(1) Exposed from first decapitation until end of experiment	(2) Exposed from second decapitation until end of experiment	(3) Dark
40,416	10.5	6.4	10.5
	—	5.8	11.8
	—	—	13.0
40,614	11.6		9.4
	14.2		13.9
40,625	4.3		3.6
	4.8		4.0
Average.....	**9.7**	**6.1**	**10.2**

the *Raphanus* hypocotyls was the same as has been described in the earlier paper. Table II shows the results. Whether the hypocotyls were exposed 2 hours before and 2 hours during the growth substance action, or the plants were exposed during the action of the hormone only, did not have any influence upon the growth substance curvature. In both cases the average value of the growth substance curvatures in the dark was 12, and 5 in the exposed ones. The same value was found during the experiments described in Fig. 27 in the earlier paper.

3. Plants Exposed between the First and Second Decapitation Only.— After the first decapitation one set of plants was exposed. 1½ hours later the plants were brought back into the dark room and decapitated again, where upon auxin-*a* was applied on one side of the cut surface

TABLE II

Auxin-A Curvatures of Raphanus Hypocotyls

Auxin applied 2 hrs. before the end of the experiments. Exposed 4, 2, and 0 hrs. before the end of the experiment. Temperature 21°C. 2 lamps of 500 watts at 80 cm. Photographed 2 hrs. after application of auxin. Nos. 40,606, 40,611, and 40,613.

4 hrs. exposed (average of 9 x 12 plants)	**4.9** ± 0.26
2 hrs. exposed (average of 7 x 12 plants)	**5.2** ± 0.60
Not exposed (average of 8 x 12 plants)	**12.1** ± 0.44

TABLE III

Auxin-A Curvatures of Avena Coleoptiles

Auxin applied after second decapitation. One set of plants exposed between the first and second decapitation (before growth substance application), other set is dark controls. Temperature 21°C. 2 lamps of 500 watts at 80 cm. Photographed 110 min. after growth substance application. No. 40,423. Average of 6 × 12 plants for each set.

Exposed (between first and second decapitation only)	**8.9** ± 0.33
Not exposed	**6.8** ± 0.31

TABLE IV

Auxin-A Curvatures of Raphanus Hypocotyls

2 hrs. before the growth substance was applied one set of plants had been exposed for 2 hrs., another set was kept in the dark room all the time. Growth substance application 2 hrs. before the end of the experiment. Temperature 21°C. 2 lamps of 500 watts at 80 cm. No. 40,612. Average of 4 × 12 plants for each set.

Exposed (2 hrs. before growth substance application only)	**12.0** ± 0.42
Non-exposed	**12.5** ± 0.63

of the coleoptiles. The controls, which had not been exposed, were treated in the same way. The curvatures were photographed 110 minutes after the blocks had been put on. The results are shown in

Table III. The plants that had been pre-exposed show a larger curvature than the non-exposed controls.

In one further respect *Raphanus* also reacts differently from *Avena* coleoptiles. It does not show an effect of exposure to light before the auxin-*a* had been applied as Table IV shows. *Raphanus* obviously reacts only (with a growth inhibition) if the growth substance acts during the exposure.

B. Experiments Carried out in Pasadena

4. Method.—We have tried to carry out these experiments under conditions resembling those in Utrecht as closely as possible. The experiments in light were carried out in a room with controlled conditions. The lamps were placed in the room itself and were cooled by running water. Two to four lamps of 200 watts each were used at a distance of 125 cm. from the objects. The oats were Victory oats from Svalöf. Two important differences, however, had to be made. (1) Due to the instability of auxin-*a*, which is made only at Prof. Kögl's laboratories in Holland, the active pure substance could not be obtained here. Therefore pure synthetic hetero-auxin was used which was supplied by Drs. K. V. Thimann and J. Koepfli. (2) Due to the hot summers in Pasadena, it was impossible to maintain a constant temperature below 27°C. in the experimental rooms, which are not equipped with a cooling system.

5. Continuous Exposure Starting at the Second Decapitation; No Difference between the Hetero-Auxin Curvatures in Light and Dark.—If the experiments of the section entitled Continuous exposure starting at the second decapitation were repeated, but at 27°C. and with hetero-auxin instead of auxin-*a*, no difference between the curvatures in light and dark could be found. In order to find which one of the two factors was responsible for the non-occurrence of the smaller curvature in the light, the temperature of the rooms was lowered. By bringing ice into the rooms and stirring the air by means of electric fans a fairly constant temperature around 23°C. could be maintained. The experiment was then repeated. The *Avena* coleoptiles were decapitated, and after 1½ hours again decapitated. Agar blocks containing hetero-auxin were put on one side of the cut surface of the coleoptile stumps. One set of the plants treated in this way was exposed on all sides and a control set was kept in the dark room. The experiments were stopped 110 minutes later and the plants photographed. Fig. 2 shows the results. The curvature in the light is

still not smaller than in the dark. Hence the kind of growth hormone used must be an important factor in these experiments.

6. Continuous Exposure Starting at the Second Decapitation; the Auxin-A and B Curvature is Smaller in the Light Than in the Dark.—As no pure auxin-*a* was available a less refined product had to be used. In order to obtain such a product a method was followed similar to that used by Kornmann (1935) who extracted corn starch with water. Coarse corn meal was extracted with distilled water for about 12 hours at 3°C. Plain agar blocks were soaked in the extract for a few hours.

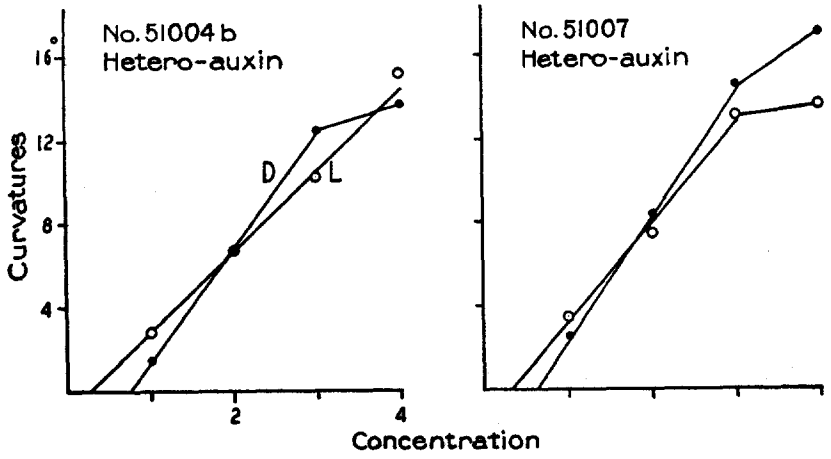


FIG. 2. Hetero-auxin curvatures of twice decapitated *Avena* coleoptiles in dark and exposed on all sides to light.

If these agar blocks were diluted twice, blocks of 1 x 2 x 2 mm. gave a curvature of about 10° in the standard test. From the experiments of Kögl, Erxleben, and Haagen-Smit (1934) it is known that corn oil contains a large quantity of auxin-*a* and *b*. It is very probable therefore that the growth hormone which we extracted from corn meal consists of a mixture of the auxins-*a* and *b*. The growth hormone extracted from corn meal will be called in this paper auxin-*a* and *b* and the curvature caused by this hormone an auxin-*a* and *b* curvature.

If now the experiments described in the last section were repeated again with this auxin-*a* and *b* as growth hormone, results similar to those obtained with pure auxin-*a* (Section 1) were obtained. Fig. 3

shows two of these experiments, the curvature in light is smaller than in the dark. Figs. 4*a* and 4*b* show similar results with deseeded (regeneration free) plants. In the experiment of Fig. 4*a* auxin-*a* and

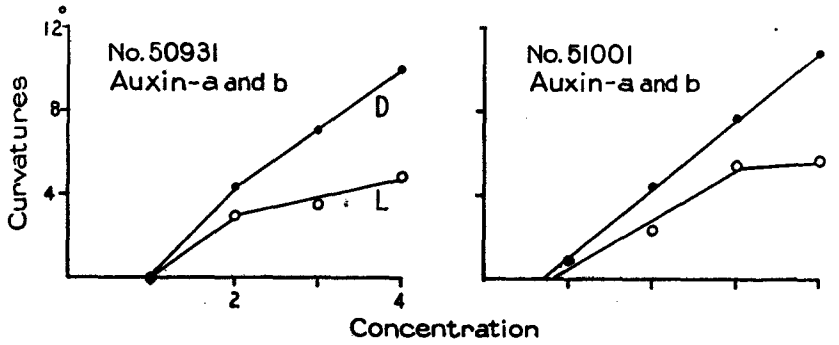


FIG. 3. Auxin-*a* and *b* curvatures of twice decapitated *Avena* coleoptiles in dark and exposed on all sides to light.

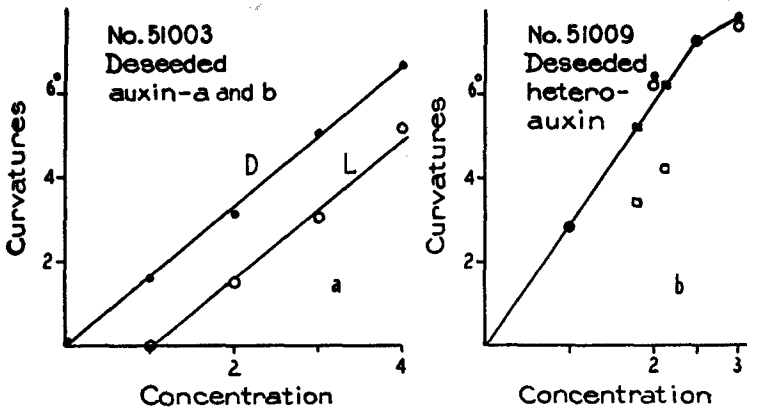


FIG. 4. Growth substance curvatures of twice decapitated and deseeded *Avena* coleoptiles. (*a*) Auxin-*a* and *b* curvatures. (*b*) Hetero-auxin curvatures, but with auxin-*a* controls. The black dots indicate the hetero-auxin curvatures in the dark. The open circles indicate the hetero-auxin curvatures in the light. The black squares indicate the auxin-*a* curvatures in the dark and the open squares indicate the auxin-*a* curvatures in the light.

b was used, while in the one of Fig. 4*b* hetero-auxin was used. In the latter experiment, however, a control with auxin-*a* and *b* was made at the same time. The black squares show the curvatures with corn meal auxin in the dark, the open squares show the same in the light.

7. Continuous Exposure Starting at the First Decapitation; and Exposure between the First and Second Decapitation only; in Both Cases the Hetero-Auxin Curvature is Larger in the Light Than in the Dark.—In Section 2 it has been shown that if *Avena* coleoptiles are exposed continuously from the first decapitation and the auxin-*a* blocks are put on after the second decapitation, the curvatures obtained are the same in the light as in the dark. If the experiment is repeated, however, with hetero-auxin instead of auxin-*a*, the curvature is larger in the light than in the dark as is shown in Table V. In this table the results of experiments similar to those described in Section 3 are also given. The experiments described there were with plants which were exposed on all sides between the first and second

TABLE V

Hetero-Auxin Curvatures of Twice Decapitated Avena Coleoptiles

Three sets of plants, (1) exposed continuously from first decapitation, (2) exposed between first and second decapitation only, (3) controls in dark. Temperature 23°C. 4 lamps of 200 watts at 125 cm. Photographed 1½ hrs. after growth substance application. Nos. 51,125, 51,126. Average of 7 × 12 plants for each set.

1. (Exposed from first and after second decapitation)	**9.6** ± 0.81
2. (Exposed between first and second decapitation only)	**8.9** ± 0.61
3. (Dark)	**6.6** ± 0.30

decapitation (1½ hours) only. After the second decapitation auxin-*a* blocks were put on the cut surface of the coleoptile stumps and 110 minutes later the pre-exposed plants had a larger curvature than the non-exposed controls. Table V shows that this experiment can be repeated with hetero-auxin with the same result.

8. Growth Substance Curvatures of Once Decapitated Avena Coleoptiles; Explanation.—Van der Wey (1931) in a detailed investigation on the influence of decapitation upon the growth substance curvature, showed that the growth substance curvature obtained with the same amount of growth hormone is larger in plants that had been decapitated two or three times than in plants which had been decapitated only once, especially if the agar blocks containing the hormone are applied right after the first decapitation. If the tip of a coleoptile is

cut off the stump still contains a large amount of hormone which disappears gradually. 1½ hours after the tip has been cut off almost all the hormone in the stump has disappeared, but at the same time the stump is starting to produce growth hormone again. This regeneration, however, can be suppressed by the second decapitation. Therefore if the cells of the stumps are empty as far as growth substance is concerned a larger growth substance curvature occurs than if the cells have a high growth substance content though in both cases the same amount of growth substance is used.

Let us analyze now the results obtained from the following experiment. *Avena* plants are divided into four groups. The coleoptiles

TABLE VI

Hetero-Auxin Curvatures of Once and Twice Decapitated Avena Coleoptiles

Temperature 27°C. 4 lamps of 200 watts at 125 cm. Photographed 110 min. after growth substance application.

No.	Once decapitated		Twice decapitated	
	Exposed	Dark	Exposed	Dark
50,902	6.7	3.0	5.8	6.6
	—	3.8	5.3	6.0
	—	3.0	5.3	—
50,909a	7.7	2.8	7.6	6.0
	5.0	2.2	6.7	7.8
Average.....	**6.5**	**3.0**	**6.1**	**6.6**

of two of them are decapitated, the remaining groups are left intact. After 1½ hours the first two groups are decapitated again and agar blocks containing hetero-auxin are applied on one side of the cut surfaces. One of these groups is placed in the light, the other one remains in the dark room. The two other groups are now decapitated also, but for the first time, and hetero-auxin of the same concentration and in the same way is applied to these groups right after the first decapitation. One of these groups is placed in the light too, whereas the other one stays in the dark room. The plants were photographed 110 minutes after the blocks were put on. Table VI shows the surprising result that the curvatures of twice decapitated plants and the just once decapitated but exposed plants are the same. The

curvatures of the just once decapitated plants in the dark have a smaller curvature than the twice decapitated plants in the dark. As has been shown above, this is due to the fact that during the period between the first and second decapitation the growth hormone left behind in the plant after the tip is cut off, has disappeared. It is reasonable to suppose that the growth hormone left behind in the plant after first decapitation, has disappeared also in the plants that have been exposed to light. This growth hormone is very probably auxin-*a* or (and) its close relatives, because Kögl, Haagen-Smit, and Erxleben have shown that the hormone present in *Avena* coleoptiles has the same molecular weight and other properties as auxin-*a*. Another interesting fact which can be concluded also from Table VI, is that hetero-auxin apparently is much less affected by light than

TABLE VII

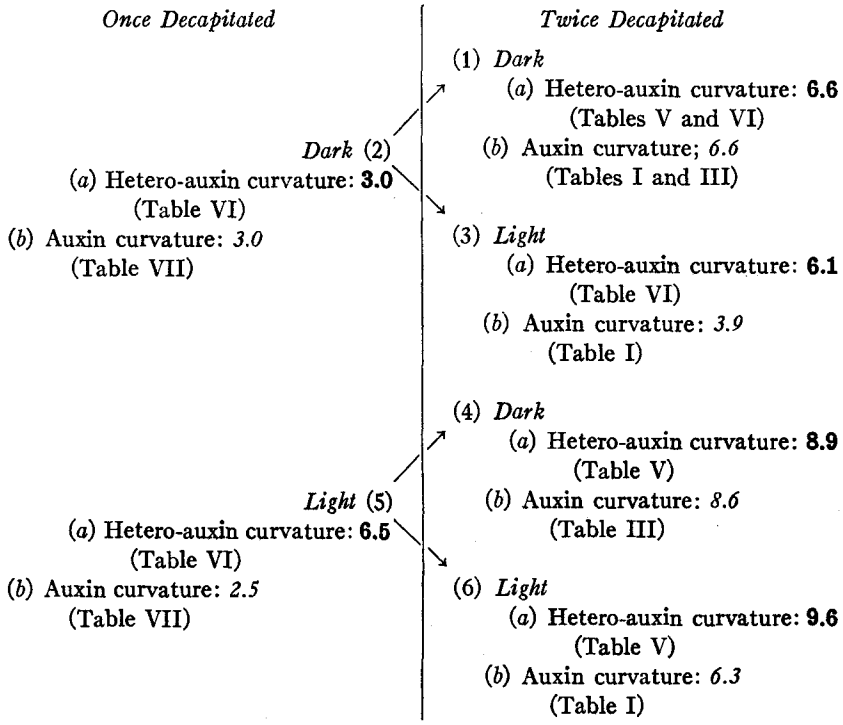
Auxin-A and B Curvatures of Once Decapitated Plants

Temperature 23°C. 4 lamps of 200 watts at 125 cm. Photographed 1½ hrs. after growth substance application. No. 51,127. Average of 3 × 12 plants for each set of plants.

Exposed	**8.6** ± 0.64
Dark	**10.3** ± 0.71

auxin-*a*. If this point of view is right, once decapitated plants to which auxin-*a* and *b* is applied must show a smaller $\frac{\text{Light}}{\text{Dark}}$ ratio than the once decapitated plants to which hetero-auxin has been applied (Table VI). Table VII gives the answer. The curvature of the plants which have been exposed to light and to which auxin-*a* and *b* has been applied is even smaller than that of the non-exposed controls. Apparently also a part of the auxin-*a* and *b* which was added to the plants has disappeared before it could accelerate the growth of the cells of the coleoptile stump, therefore the auxin-*a* and *b* probably is inactivated (destroyed) in the light. Another support for the idea that the auxin-*a* is destroyed in the light, is the fact that it is much more easily destroyed by enzymes of the plant than hetero-auxin, as

will be shown below. The results of the preceding sections are summarized in the following scheme.



On the left-hand side of the scheme is indicated whether the plants between the first and second decapitation were in the light or in the dark. On the right-hand side is indicated whether after the second decapitation the plants were in the light or in the dark. There are two groups of dark plants (1) and (4), each of which has a different previous history. The group (4) has been exposed before the second decapitation, the group (1) has not. The same holds for the "light groups" (3) and (6). In each group the hetero-auxin curvature (*a*) and auxin-*a* or auxin-*a* and *b* curvatures (*b*) are indicated. Each figure in this scheme is directly comparable with any other figure in the scheme. The values for the hetero-auxin curvatures are obtained directly from the tables as indicated in each group, the (1) group serv-

ing as a "standard." The auxin-*a* curvatures, however, are reduced to this standard (1*a*).

In Table I for example for the twice decapitated plants which were not exposed, a value 10.2 is found. This value is reduced to 6.6 in order to make the auxin-*a* curvatures directly comparable to the hetero-auxin curvatures. The value 6.1 of Table I must be multiplied with the factor $\frac{6.6}{10.2}$ in order to be comparable with the standard values of group (1) and, therefore, 3.9 is obtained for (3*b*). The same is done for the 9.7 value of Table I which gives 6.3 in group (6) under (*b*). In Table III we find a dark value of 6.8 which has to be reduced to 6.6. Therefore the value in group (4*b*) will be $\frac{6.6}{6.8} \cdot 8.9 = 8.6$. At last the value 10.3 (Table VII) was reduced to 3.0 of group (2*a*) and, therefore, the value 8.6 of Table VII is reduced to $\frac{3}{10.3} \cdot 8.6 = 2.5$ of group (5*b*).

In the beginning of this section the conclusion was drawn that auxin-*a* and closely related substances (which are stable to light by themselves) are destroyed within the plant on exposure to light. The hetero-auxin is not destroyed, or more probably is much less destroyed than the auxin-*a*. These conclusions were drawn from experiments on once decapitated plants.

The experimental data summarized in the scheme may now be considered in regard to these conclusions. If the groups (1) and (3) are compared, we find the hetero-auxin curvature in the light slightly lower than that in the dark. This may indicate that indeed a small amount of hetero-auxin is destroyed in the exposed plant. The auxin-*a* curvature of group (3), however, is much smaller than that of group (1). This is caused according to the conclusions mentioned above by the destruction of auxin-*a* in the exposed plants.

If group (4) is compared with group (1) a higher curvature in group (4) is obvious. Both the hetero-auxin and auxin-*a* curvatures are higher in the plants which had been exposed previously. Because, during the growth substance action the plants were in the dark, no auxin-*a* was destroyed and therefore the value (4*a*) is practically the same as the value of (4*b*). The results show another aspect, however, if the plants are exposed to light during the action of the growth substance. This is manifest if the groups (1) and (4) are compared with

group (6). The values of (4*a*) and (6*a*) are practically the same (mean error rather high, Table V). The auxin-*a* curvature of group (6), however, is considerably lower than in group (4). This is easy to understand if it is assumed that the auxin-*a* is destroyed in plants which are exposed to light. Why the response to growth hormone is higher in the groups (4) and (6) than in the group (1) can also be explained in terms of destruction of growth substance by light. The plants of groups (4) and (6) have been pre-exposed and hence have a smaller growth hormone content than group (1) which has been kept in the dark all the time. Consequently the plants of groups (4) and (6) are more sensitive to growth substance than the ones of group (1) as is explained at the beginning of this section.

The following general conclusions can be drawn from these considerations. (1) The response of the plant to growth hormone (both types of auxins) can be shown by the hetero-auxin curvature. (2) Superimposed upon this response is the destruction of auxin-*a* in exposed plants.

If the hetero-auxin curvature in the dark is *Hd*, the one in the light *Hl*, and the auxin-*a* curvature in the dark *Ad*, then according to these general conclusions the auxin-*a* curvature of exposed plants can be expressed $\frac{Hl}{Hd} \cdot (Ad - Dl)$. In which *Dl* is the destruction of the auxin-*a* in the exposed plant.

9. The Destruction of Hetero-Auxin and Auxin-A and B.—In an earlier paper dealing with dwarfs of corn (Van Overbeek (1935)) a method was developed to determine the destruction of growth substance by sections of plants in agar blocks. Sections of coleoptiles, mesocotyls, etc. were placed with their basal cut surface on agar blocks containing growth hormone. If after a certain time the sections were removed and the blocks analyzed, some of the hormone had disappeared from the blocks. Since then Kornmann (1935) has published similar results. It is certain that enzymes set free from the cells at the cut surface have at least a part in the destruction of the hormone, because rinsing of the cut surface with water reduces the destruction. The following evidence indicates that this inactivation of the growth hormone is due to an oxidative process. Sections (about 5 mm. long) were cut from the apical part

and from the basal part of a mesocotyl of corn. These sections were placed with their basal cut surface on wet filter paper for 1½ hours in order to set free the growth hormone they might contain. These sections were then placed on blocks containing hetero-auxin for 1 hour. After the sections were removed, the blocks were analyzed and it could be shown that more hormone was destroyed in the blocks with which the basal sections had been in contact than in the blocks on which apical sections had been put (Table VIII). If with similar sections a peroxidase test was made, the peroxidase activity was proved to be higher in the basal sections than in the apical sections. This peroxidase test was carried out as follows. Agar blocks were soaked in a benzidine solution to which a small amount of H_2O_2 had been added. If peroxidase is present in the cut surface of the sections the benzidine in the block will be oxidized when the sections are

TABLE VIII

Inactivation of Hetero-Auxin by Apical and Basal Mesocotyl Sections

No. of experiment	41,201	41,130	41,204
Concentration of hormone started with	9.0	18.0	16.0
Left over in blocks with,			
Mesocotyl tips	7.2	11.3	15.0
Mesocotyl bases	1.5	3.8	6.0

brought into contact with the blocks. Since the oxidized benzidine is colored, the darker the color in the block is, the higher the peroxidase activity on the cut surface of the section was. Fig. 5 shows a photograph of such a peroxidase test. The picture is a negative and therefore the whiter the spots (places where the sections made contact with the agar block) are, the higher the peroxidase activity was.

If the destruction of auxin-*a* and hetero-auxin are compared by the method mentioned in the beginning of this section, a higher percentage of the auxin-*a* and *b* than of the hetero-auxin is destroyed. Table IX shows the results of these experiments for *Avena* coleoptiles. In Experiment 51,029*a* 5 day old plants were used. The coleoptile was cut off and decapitated and placed with its basal cut surface in water for 1½ hours. Then from the apical part of the coleoptiles two sections 5 mm. long were cut, which were placed with their basal

cut surface on blocks containing hormone. After 2 hours the sections were removed and the hormone content of the blocks was determined. The result of this experiment was that the sections placed in the dark on blocks containing auxin-*a* and *b* had lost 16 per cent of the original amount of hormone, whereas in the experiment in which the sections were placed in the light 42 per cent of the original amount had disappeared from the blocks. If the sections were placed on blocks containing hetero-auxin, no growth hormone disappeared from the blocks, but 23 per cent was added to the amount originally present in the block. This growth hormone obviously came from the sections. If the sections contain growth hormone,

FIG. 5. The result of a peroxidase test, showing the higher peroxidase activity in the mesocotyl base (below) as compared to the mesocotyl tip.

this hormone is auxin-*a* or *b*. If this hormone is destroyed in the exposed sections, the result will be that less hormone is given off by the exposed sections than by the sections in the dark. The amount of hormone left over in the block on the basal cut surface is determined by the amount destroyed in the block, and by the amount given off bp the sections into the block. The amount of hormone destroyed in the blocks is the same in the light as in the dark. This is shown by Experiment 51,030, which is the same as the other experiment of Table IX, but the coleoptiles were put overnight with their basal cut surface in water after they had been cut off. By this procedure sections free from hormone are obtained. From Experiment 51,029*a*

therefore it can be concluded that the hormone in the sections themselves is destroyed if the sections are exposed to light.

Evidence in favor of the assumption that the inactivation of the growth hormone in plants that are exposed to light is also an oxidative process is as follows. Skoog (1935) showed that if a small amount of eosin was added to a growth hormone solution the hormone was inactivated if this mixture was exposed to the light. He showed that this was due to oxidation. Boas (1933) showed that if a seedling is infiltrated with eosin it is unable to show phototropic curvatures. Boysen-Jensen (1934) showed that if roots are infiltrated with erythrosin (yellow eosin) they fail to respond geotropically. He was able to show that eosin treated roots give off a smaller amount of growth hormone than normal ones.

TABLE IX

Destruction of Auxin-A and B and Hetero-Auxin by Sections of Avena Coleoptiles in Light and Dark

Temperature 23°C. Time 2 hrs. Twenty sections per block of 8 x 6 x 1 mm.

No.	Hormone	Concentration started with	Left over		Disappeared	
			Light	Dark	Light	Dark
					per cent	*per cent*
51,029*a*	Auxin-*a*	9.0	5.5	7.7	**42**	**16**
		7.7	4.3	6.4		
	Hetero-auxin	6.0	—	8.0	—	*—23*
		6.4	—	7.1		
51,030	Auxin-*a*	10.2	5.8	4.5	**43**	**48**
		10.3	6.0	6.3		
	Hetero-auxin	12.1	8.0	8.8	*27*	*18*
		11.3	9.2	10.3		

If in Table IX the figures for the destruction of hetero-auxin are compared with the ones for auxin-*a*, we find in the dark a destruction of 18 per cent for hetero-auxin to 48 per cent for auxin-*a* and *b*. In the light these figures are 27 per cent for hetero-auxin to 43 per cent for auxin-*a* and *b*.

Table X shows the same for *Raphanus* sections. If the sections are exposed and the blocks contain auxin-*a* and *b* the amount of hormone left over in the blocks is even more than in the dark. An ex-

planation of this fact is lacking as yet. One thing, however, is shown clearly in these experiments, *viz.* that the percentage of hormone which disappeared from the blocks is in every case larger for auxin-*a* and *b* than for hetero-auxin.

The fact that hetero-auxin is more difficult to destroy than hormones of the type of auxin-*a* is not surprising if the results recently obtained by Kögl and Kostermans (1935) are considered.[4] They measured the activity of hetero-auxin as compared to the auxins-*a* and *b* and found that the activity of hetero-auxin is about half that of

TABLE X

Destruction of Auxin-A and B and Hetero-Auxin by Sections of Raphanus Hypocotyls

Temperature 23°C. Time 4 hrs. Twenty sections per block of 8 x 6 x 1 mm.

No.	Hormone	Concentration started with	Left over		Disappeared	
			Light	Dark	Light	Dark
					per cent	*per cent*
51,025	Auxin-*a*	8.0	6.1	4.2	**36**	**50**
		—	5.0	4.0		
	Hetero-auxin	10.5	6.3	6.5	*34*	*30*
		—	7.5	8.1		
51,026	Auxin-*a*	8.2	6.6	5.3	**15**	**40**
		7.3	—	4.3		
	Hetero-auxin	6.6	—	7.4	—	*−10*
		6.7	—	7.1		
51,028	Auxin-*a*	8.9	4.2	3.6	**46**	**64**
		9.3	5.5	3.0		
	Hetero-auxin	8.7	5.3	5.6	*23*	*20*
		7.4	7.1	7.4		

auxin-*a*. When they took the molecular weight into account, the difference was still larger. The "molecular activity" of hetero-auxin is 3.75 times smaller than that of auxin-*a*. This means that in order to get the same curvature with hetero-auxin as with auxin-*a* 3.75 times more molecules of the former substance are required. Assuming, for instance, that in the process of destruction 1 molecule of

[4] After this article was in press, it came to my attention that Thimann and Went (1934) had shown that auxin-*b* is more easily inactivated by hydrogen peroxide than hetero-auxin (p. 459).

hetero-auxin is as easily oxidized as 1 molecule of auxin-*a*, it is clear that under the same conditions the activity of the auxin-*a* will decrease 3.75 times as fast as that of the hetero-auxin.

10. The Light Growth Responses of the Avena Coleoptile.—From studies by Van Dillewyn (1927), Went (1925), and a recent study by Haig (1935) we know that two types of light growth responses can occur in *Avena* coleoptiles. One of them is called the tip response because it occurs only when the tip is exposed to light. Even a very small amount of light is able to produce a tip response. Went proved that amounts of light able to produce a tip response also decrease the amount of growth substance given off by the tip. He assumes very logically that this decreased amount of hormone given off is the direct cause of the tip response. Since in the present investigations the tips were cut off, tip responses should be excluded. It will be shown, however, in the next section that the formation of growth substance in the decapitated coleoptile (regeneration) is inhibited by small amounts of light in the same way as the production of growth substance in the tip. In a coleoptile stump growing on regenerated growth substance it must be possible therefore to produce a tip response even though the tip of the plant has been cut off. In the experiments described in the previous sections the regeneration has been suppressed by decapitations.

Besides the tip response a base response can occur in the coleoptile. To produce a base response the base (which means here any part of the coleoptile but the extreme tip) must be exposed to relatively high amounts of light. The base response under conditions of continuous exposure consists of two parts. (1) Almost immediately after the plants are exposed the growth rate decreases and about three-quarters of an hour after the exposure was started the growth rate has reached a minimum. (2) Then the growth rate increases again, but stays lower than before the exposure started. Koningsberger (1923) considers this increase in growth rate following on the growth inhibition only indirectly linked to the growth inhibition. According to him the latter is superimposed on the former one.

It is not difficult to draw a comparison between this base response and the results obtained with growth substance curvatures in dark

and light.[5] The first part of the base response, the inhibition, can be compared with the experiments (1*b*) and (3*b*) of the scheme. The inhibition is caused by the destruction of auxin-*a* under influence of the light. The second part of the base response, the increase, can be paralleled with (6*b*) and (3*b*) of the scheme. The increase in growth rate (and increase in curvature) as it is measured in the intact plant (or plant with auxin-*a* blocks) is the result of 2 antagonistic processes. (1) The response to growth hormone itself is larger after a long exposure (as can be shown with hetero-auxin curvatures (6*a*) and (3*a*). (2) Superimposed on this is the destruction of auxin-*a* under influence of the light. This confirms Koningsberger's (1923) point of view, which does not consider the light growth response as a whole, but discriminates strongly between the inhibition and the "anti-reaction."

Besides the tip response and the base response a third type of response in *Avena* has been described (Tollenaar, 1923; Van Dillewyn, 1927). This third type of light growth response is the so called "dark growth response" which occurs if the exposure of a plant which has been exposed to light for a long time, is stopped. The response is an increase in growth rate. A parallel between this response and the experiments (4*b*) and (1*b*) of the scheme can be drawn. This response is due to the increased response to growth hormone. It must be borne in mind, however, that this dark growth response has nothing to do with the bringing back of the plants into the dark because if the plants are continuously exposed they show the response too as can be shown by using hetero-auxin instead of auxin-*a* (6*a*). By bringing back the plants into the dark the destruction of the auxin-*a* stops, whereas the higher response to growth hormone lasts, which results in an increased growth rate in the intact plant and an increased curvature if it is an auxin-*a* curvature.

11. The Regeneration and Its Influence upon the Growth Substance in Light and Dark Curvature.—This section is more or less independent of the previous ones in this paper. If the tip of a coleoptile is cut off the ability of the coleoptile to produce growth hormone is not damaged beyond repair, because about $1\frac{1}{2}$ hours after the tip

[5] In the meantime it has been proved that they are identical (Van Overbeek (1936)).

has been removed the cells in the apical part of the stump start to produce the hormone. This is called in botanical literature the "regeneration of the physiological tip" or briefly the regeneration. Van der Wey (1931) showed that if the regeneration was suppressed by repeated decapitations the growth substance curvature was larger than if regeneration occurred. Regeneration therefore inhibits the growth substance curvature. Tsi-Tung Li (1930) investigated the effect of temperature upon the regeneration. According to his figures

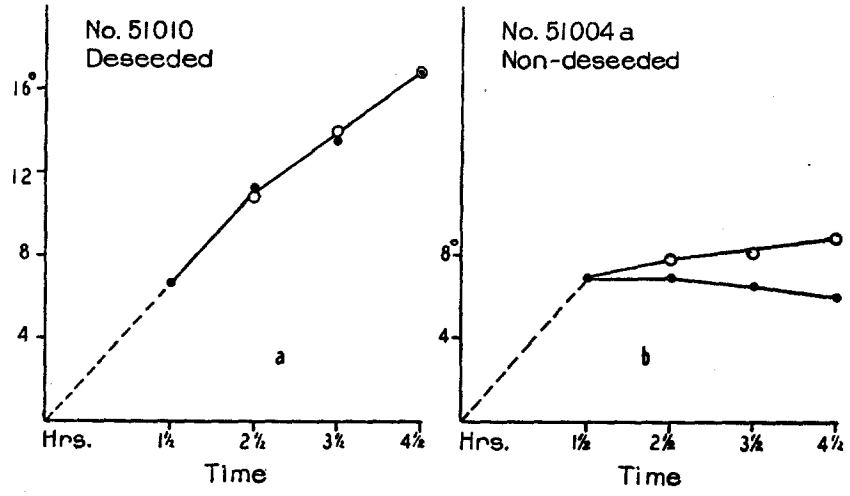


FIG. 6. Growth substance curvatures with (*b*) and without (*a*) the interference of regeneration in dark and exposed hourly to 25 m.c.s. Abscissa, hours after application of growth hormone. Black dots, dark. Open circles, exposed to light. Mean values of twenty-four plants for each point.

the regeneration starts 210 minutes after the tip has been removed at 15°C. At 25°C., however, this time is only 100 minutes. The newest contribution to the knowledge of regeneration has been made by Skoog (1937). At a certain stage of development of the seedlings he removes the seeds. Such plants do not regenerate as he proves. In using these plants it is possible to study the growth substance curvatures without the interference of regeneration. In Fig. 6 the difference in growth substance curvature with and without the interference of the regeneration can be seen. The abscissa represents the time in hours after the blocks containing auxin-*a* had been applied

to the twice decapitated plants. Fig. 6*b* shows that after 1½ hours the curvature in the dark (black dots) in non-deseeded plants does not increase any more, but decreases. Fig. 6*a*, however, shows that in deseeded plants the curvature increases with time.

What effect does light have upon the regeneration? Tsi-Tung Li (1930) tried to answer this question by measuring the growth rate of decapitated coleoptiles with a horizontal microscope. One set of his plants had been exposed to 44 m.c. for 30 minutes. After about 140 minutes at 20°C. and after 100 minutes at 25°C. the growth rate increased, without showing a noticeable difference between the exposed and non-exposed coleoptiles. Tsi-Tung Li concludes that "light exerts no effect on the moment for the appearance of the physiological tip." This conclusion, however, is not justified because the growth rate of the decapitated plants depends upon two factors at least. (1) The amount of growth substance regenerated. (2) The response of the plant to growth hormone. According to Sections 3, 7, and 10 of this paper, the plants with which Tsi-Tung Li worked were pre-exposed, and, therefore, very probably had a higher response to growth hormone than the non-exposed controls. As will be shown below, in three different ways it can be proved that light inhibits the regeneration.

1. As Gorter (1927) has pointed out, the positive curvature (which is towards the block) appearing when plain agar blocks are put on one side of decapitated coleoptiles, is due to the formation of growth substance in the decapitated plants. If such plants are exposed to amounts of light smaller than are required for base responses, the positive curvature is smaller in the exposed plants than in the controls. Table XI shows the results. Plain agar blocks were put on the cut surface of twice decapitated plants. 1½ hours later the plants already showed a small positive curvature. The plants were photographed with a small amount of light (25 m.c.s.). This amount is sufficient to produce a tip response and insufficient for a base response (Van Dillewyn (1927)). 1 hour after the photograph had been taken, and hence 2½ hours after the plants had been decapitated and the agar blocks put on, these plants show a curvature of only 0.5° whereas the controls which had been kept in the dark all the time show a curvature of about 2°. This proves that regeneration is inhibited by light

even in such small amounts as reduce the amount of growth substance given off by the tip of the coleoptile (Went) and as produce a tip response only (Van Dillewyn).

2. If a set of plants to which a small amount of growth substance has been applied is exposed hourly to an amount of light of about 25 m.c.s., the growth substance curvature of these plants appears to be larger than those of non-exposed controls. The experiment is as follows: eight rows of twelve plants were decapitated twice and growth substance blocks of a concentration of 7° were applied. The plants were then divided into four groups of twenty-four plants each. One group was exposed (and photographed at the same time) $1\frac{1}{2}$ hours after the blocks had been applied. After an hour this group was photo-

TABLE XI

Positive Curvatures of Twice Decapitated Avena Coleoptiles to Which Plain Agar Was Applied on One Side. One Set of Plants Has Been Exposed to 25 M.C.S., $1\frac{1}{2}$ Hrs. after the Agar Was Applied

Curvature $1\frac{1}{2}$ hrs. after the agar was applied	1.1
	0.5
Same plants 1 hr. later	0.4
	0.5
Curvature of non-exposed plants, $2\frac{1}{2}$ hrs. after agar was applied	1.8
	2.3

graphed again and another group which had not been exposed before was also photographed. Again an hour later the first group was photographed and so was a third group, which therefore had been in the dark for $3\frac{1}{2}$ hours. The next hour the first group was exposed again as well as the last group which had not yet been exposed. In Fig. 6*b* the open dots represent the first group, whereas the black dots represent the non-exposed controls. It is obvious that the curvatures of the plants which had been exposed are larger than those of the non-exposed ones. The explanation must be that the inhibition of the growth substance curvatures is less in the case where the plants are exposed, which point of view is justified by the next experiment (Fig. 6*a*). The same experiment as described above was repeated with plants which had been prepared according to Skoog's method.

In such regeneration free plants exposure to 25 m.c.s. does not have any effect upon the growth substance curvature.

3. A direct proof that light inhibits the regeneration can be given as follows: coleoptiles were decapitated and were exposed either to a large amount of light (first two experiments of Table XII) or to a small amount of light (last two experiments of Table XII). After about 4 hours the tips of the coleoptile stumps were cut off, and twenty of them were placed on plain agar blocks of 8 × 6 × 1 mm. for $2\frac{1}{2}$ hours. The extraction of the hormone took place in the dark in order to eliminate possible transport and destruction differences between the exposed tips and the non-exposed controls. Later the blocks were tested on deseeded plants, because the amounts of hormone

TABLE XII

Amount of Regenerated Growth Substance Given Off by Top Sections of Decapitated Avena Coleoptiles; in Dark and Light. Analysis on Deseeded Plants, Photographed 5 Hrs. after the Blocks Were Put on. Temperature 23°C.

No.	Amount of light	Amount given off	
		Light	Dark
50,920	500 m.c. during 2 hrs.	2.5	5.4
		2.8	6.2
51,012	25 m.c.s. each hour (4×)	3.5	5.6
51,016	Same	3.2	5.4

given off by the sections are in general too small to be analyzed by the standard method. In every case the amount of hormone given off by the exposed plants was less than the amount given off by the tips of non-exposed coleoptile stumps.

If Figs. 2 and 3 of this paper are compared with Figs. 1 and 4 a striking difference is obvious. The curve of the latter figures starts at the origin (zero point) and is similar to the concentration curves published by Went (1928) and Van der Wey (1931). The curves of Figs. 2 and 3, however, cross the abscissa. This means that the curvatures measured are not proportional to the concentration of the hormone in the block. Scores of other tests made at temperatures between 27 and 23°C. showed the same. Since the

deseeded plants, which do not show regeneration, show a normal curve an explanation of the abnormal curve may be the early start of the regeneration at higher temperatures. In favor of this point of view is the fact that exposed plants more than the non-exposed ones (Fig. 2) show a curve which approximates the regular curve (starting at zero). Against it, however, can be said that Went's experiments are carried out at 25°C. He used much smaller agar blocks though than the ones in these experiments, which may have an effect upon regeneration and growth substance curvature.

III

SUMMARY

An attempt has been made to analyze the base response, one of the light growth responses of *Avena* coleoptiles, by means of growth substance curvatures. The decrease in growth rate (first part of the base response) after exposure to light does not show if hetero-auxin is substituted for auxin-*a* (Sections 5, 6, and 10). This decreased growth after exposure very likely is due to an oxidative inactivation of auxin-*a* (Sections 8 and 9). Hetero-auxin can be inactivated too but in a much lesser degree than auxin-*a* (Section 9). The increase in growth rate following on the decreased growth (second part of the base response) is due to an increase in response of the plant to growth hormone which is independent of the type of hormone (Sections 1, 2, 7, 8, and 10). Under conditions of continuous exposure to light, however, the inactivation of the auxin-*a* under influence of the light is superimposed on this increased response to growth hormone. This inactivation can be eliminated from the light growth response by replacing the auxin-*a* by hetero-auxin. More detailed information on this subject can be found in Section 10. A review of the experiments and their results can be obtained from the scheme in Section 8.

In Section 11 it is shown that light inhibits the formation of growth hormone in the decapitated coleoptile (regeneration). Very small amounts of light (25 m.c.s.) inhibit the regeneration markedly.

LITERATURE CITED

Boas, F., 1933, *Ber. bot. Ges.*, **51**, 274.
Boysen-Jensen, P., 1934, *Planta*, **22**, 404.
Cholodny, N., 1933, *Planta*, **20**, 543.
Cooper, W. C., 1935, *Plant Physiol.*, **10**, 789.
Gorter, C. J., 1927, *Proc. Kon. Akad. Wetensch. Amsterdam*, **30**, 728.
Haagen-Smit, A. J., and Went, F. W., 1935, *Proc. Kon. Akad. Wetensch. Amsterdam*, **38**, 852.
Haig, C., 1935, *Biol. Bull.*, **69**, 305.
Kögl, F., Erxleben, H., and Haagen-Smit, A. J., 1934, *Z. physiol. Chem.*, **225**, 215.
Kögl, F., Haagen-Smit, A. J., and Erxleben, H., 1934, *Z. physiol. Chem.*, **228**, 104.
Kögl, F., and Kostermans, D. G. F. R., 1934, *Z. physiol. Chem.*, **228**, 113.
Kögl, F., and Kostermans, D. G. F. R., 1935, *Z. physiol. Chem.*, **235**, 201.
Koningsberger, V. J., 1923, *Rec. trav. bot. néerl.*, **20**, 257.
Kornmann, P., 1935, *Ber. bot. Ges.*, **53**, 523.
Skoog, F., 1935, *J. Cell. and Comp. Physiol.*, **7**, 227.
Skoog, F., 1937, *J. Gen. Physiol.*, **20**, in press.
Skoog, F., and Thimann, K. V., 1934, *Proc. Nat. Acad. Sc.*, **20**, 480.
Thimann, K. V., 1935*a*, *J. Biol. Chem.*, **109**, 279.
Thimann, K. V., 1935*b*, *Proc. Kon. Akad. Wetensch. Amsterdam*, **38**, 896.
Thimann, K. V., and Went, F. W., 1934, *Proc. Kon. Akad. Wetensch. Amsterdam*, **37**, 456.
Tollenaar, D., 1923, *Proc. Kon. Akad. Wetensch. Amsterdam*, **26**, 378.
Tsi-Tung Li, 1930, *Proc. Kon. Akad. Wetensch. Amsterdam*, **33**, 1201.
Van Dillewyn, C., 1927, *Rec. trav. bot. néerl.*, **24**, 307.
Van der Wey, H. G., 1931, *Proc. Kon. Akad. Wetensch. Amsterdam*, **34**, 875.
Van Overbeek, J., 1932, *Proc. Kon. Akad. Wetensch. Amsterdam*, **35**, 1325.
Van Overbeek, J., 1933, *Rec. trav. bot. néerl.*, **30**, 1325.
Van Overbeek, J., 1935, *Proc. Nat. Acad. Sc.*, **21**, 292.
Van Overbeek, J., 1936, *Proc. Nat. Acad. Sc.*, **22**, 421.
Went, F. W., 1925, *Versl. Kon. Akad. Wetensch. Amsterdam*, **29**, 185.
Went, F. W., 1928, *Rec. trav. bot. néerl.*, **25**, 1.
Went, F. W., 1934, *Proc. Kon. Akad. Wetensch. Amsterdam*, **37**, 445.
Went, F. W., 1935*a*, *Bot. Rev.*, **1**, 162.
Went, F. W., 1935*b*, *Proc. Kon. Akad. Wetensch. Amsterdam*, **37**, 445.