STUDIES ON THE GROWTH HORMONE OF PLANTS

VII. The Fate of Growth Substance in the Plant and the Nature of the Growth Process

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Since the early work of Went (1928), it has been known that the growth substance of the *Avena* coleoptile may be obtained in the usual way, *i.e.* by diffusion into agar blocks, only from that part of the plant which produces it, namely the tip. The hormone is not recoverable in this way from those parts of the plant, such as the lower zones, which only make use of it. Recent work (Thimann, 1934) has, however, shown that by extraction with chloroform some growth substance is recoverable from the lower zones of the coleoptile. The results of this extraction method confirmed the earlier view that there is a marked concentration gradient of growth substance from tip to base.

The fact that there is less growth substance in the rapidly growing middle zones than in the hormone-producing tip has been commonly interpreted to mean that growth substance is actually destroyed in the growth process. It is clear, however, that this is not of necessity the case. It could equally well be that this inactivation is carried out in the plant by processes quite apart from those of growth. In an earlier paper (Thimann and Bonner, 1933), it was shown that the growth of the coleoptile is under certain conditions proportional to the amount of growth substance entering the plant. If it could further be established that, at least under some conditions, the growth appearing is proportional not only to the amount entering but to the amount actually inactivated, this would indicate strongly that the hormone is destroyed, or at least transformed, in the growth process. To show that this is actually the case, the extraction method, which

makes it possible to determine the amount of growth substance destroyed, has been used, and experiments of this kind will now be described.

EXPERIMENTAL

The chloroform extraction of coleoptiles was used as described in a previous paper, (Thimann, 1934). The previous procedure was modified by the use of a specially designed mechanical grinder. From the coleoptiles used the tip, 5 to 7 mm. long, was in all cases removed. About 200 plants were used for each extraction.

In the course of the experiments, considerable variation in the amount of growth substance present in the plants was encountered. This variation is apparently in part normal, but is also in part due to the presence of variable small amounts of peroxides in the chloroform. These quantities were too small to be detected by the titanium sulfate test (less than 5×10^{-5} mols per liter), but were nevertheless sufficient to inactivate small amounts of growth substance, occasionally even a significant fraction of the total amount. That there were small amounts of peroxides present was shown by the partial inactivation of known small quantities of growth substance by shaking with the chloroform. Redistillation, washing with water, and addition of titanous sulfate to the chloroform did not completely remove this power of inactivation.

RESULTS

(*A*) *Growth Substance Used and Growth Resulting, after Decapitation.* —The first point to be investigated was the decrease of growth substance in the plant after the removal of the hormone-producing tip. It is known that for approximately 2 hours after removal of this tip the growth rate falls steadily. At the end of this time, the topmost zones of the stump begin to produce the hormone and an increase in the growth rate is observed. Table I shows that correspondingly after 2 hours the amount of growth substance in the plant has fallen to about one-half of its original value. After 3 hours, on the other hand, the amount of growth substance is, due to the "regeneration" described above, approximately the same as in the freshly decapitated plant.

Table I shows that in 2 hours approximately 2.7 units of growth substance disappear. The data of the earlier paper (Thimann and Bonner, 1933) show that over the range in which the growth response is proportional to the growth substance entering the plant, 2.7 units

of growth substance in the plant would give 0.73 mm. of elongation. If growth is proportional to the amount of growth substance which disappears, and if all growth substance entering the plant ultimately disappears in this way, these decapitated coleoptiles should then grow 0.73 mm. Reference to Table II of the earlier paper reveals that in 2 hours approximately 0.79 mm. of growth occurs, a very satisfactory agreement with the expected value.

(*B*) *Growth Substance Used and Growth Resulting, after Application of Additional Growth Substance.*—If growth substance be applied, in agar blocks, to a decapitated coleoptile, an immediate increase in growth rate occurs. In addition the coleoptile continues to grow faster than normal for some time after removal of the block. In the

TABLE I

Amount of Growth Substance Present in Coleoptiles at Different Times after Decapitation

Experiment	Time after decapitation	Growth substance units per plant	Time after decapitation	Growth substance units per plant
			hrs.	
1	0	5.0	2	3.1
2	0	6.4	2	2.9
3	0	7.7	3	6.4
(Mean of two)				

present experiments, agar blocks containing 47 units of growth substance were allowed to remain upon coleoptiles during 2 hours. During this time approximately 37 per cent (extrapolation from data for 110 minutes in Table I of Thimann and Bonner, 1933), or 17.4 units of this 47 units, enter the plant. Table II shows that, however, only a small proportion of this may be recovered at the end of 2 hours. The average of several experiments (see also Table III) gives 6.6 units as the amount recoverable. According to the earlier paper, this 6.6 units should cause a further 1.78 mm. of elongation before the coleoptile returns to the normal growth rate. Interpolating in Table III of the same paper, (1933), (for 47 unit blocks), one finds that approximately 1.72 mm. occur, again a very satisfactory agreement with the expected value.

We have now two cases in which the growth appearing is proportional, not only to the growth substance entering the plant, but also to the growth substance used up. In these two cases it appears, then, that the growth is proportional to the amount of growth substance which has undergone transformation into an inactive form.

In the earlier paper it was shown that with agar blocks containing as much as 47 units, the growth per unit of growth substance is less than

TABLE II

Amount of Growth Substance (in Units per Plant) Recoverable from Coleoptiles Supplied with 47 Unit Agar Blocks during 2 Hours

Experiment	Plants with 47 unit blocks	Plants without 47 unit blocks
1	9.4	3.7
2	3.5	1.7
3	5.5	2.4

TABLE III

Average Number of Growth Substance Units Recovered from Freshly Decapitated Plants and from Plants Supplied during 2 Hours with 47 Unit Agar Blocks

Units per plant	
Fresh plants	47 unit plants
5.0	9.4
6.4	3.5
4.2	5.5
3.2	8.7
3.4	5.0
3.3	7.6
Mean.....4.3 ± 0.5	6.6 ± 0.9

with smaller amounts in the block; *i.e.*, some factor other than growth substance is limiting growth. Considering the first 2 hours of the present experiment, 17.4 units will enter the plant from the block. In addition there were originally present 4.3 units, (Table III). During the 2 hours, then, 21.7 less 6.6, or 15.1, units of growth substance have disappeared. Although if the response to growth substance were linear this would result in 4.06 mm. of growth, Table III of the earlier

paper (Thimann and Bonner, 1933) shows that only 2.55 mm. appear. There is, then, in the presence of high growth substance concentrations, more growth substance destroyed than is used in growth. This was confirmed by the application for 2 hours of blocks containing 310 units. At the end of the 2 hours, the plants were found to contain only 9.6 units per plant. The amount of growth in plants with 310 unit blocks is only about 25 per cent more than in plants with 47 unit blocks, while the amount of growth substance inactivation is about 7 times as much. Thus, in this case, the bulk of the growth substance entering the plant is destroyed without resulting in growth. This excessive destruction of growth substance in the presence of high concentrations is more than sufficient to account for the deviation from

TABLE IV

Extraction of Growth Substance from Cut Off Coleoptiles

Treatment	Plants with bases in water		Plants with bases dry	
	Growth substance in units per plant		Growth substance in units per plant	
	Before treatment	After treatment	Before treatment	After treatment
Decapitate, leave 2 hours	3.2	1.6	4.2	1.9
Apply 47 unit block for 2 hours		8.7		7.0
		5.0		6.0
Mean.....		6.8		6.5

linearity of the growth-growth substance curve given in the earlier paper.

(*C*) *Growth Substance Used without any Accompanying Growth.*—From the preceding section it is necessary to conclude that in the presence of small growth substance concentrations and of external conditions favorable to growth there is a quantitative relation between growth substance converted to an inactive form; *i.e.*, used up, and growth resulting. In the presence of excess growth substance, however, the latter disappears without the appearance of a corresponding amount of growth. This fact suggests that if factors other than growth substance were made to limit growth the hormone would nevertheless disappear. Experiments were therefore carried out in which

available water was made the limiting factor. In Table IV is summarized the disappearance of growth substance in coleoptiles cut from their roots and placed (1) with their bases in water, and (2) with their bases dry, in a saturated atmosphere. The decrease of growth substance after decapitation takes place to about the same extent as in the normal plant in both cases. In addition, if 47 unit blocks be applied to these plants for 2 hours, then the amount of growth substance which disappears is about the same as in normal plants, despite the fact that, as will be shown in Table V, the plants with inadequate water supply grow only about one-fourth as much as those with their bases in water. The logical conclusion is that under conditions unfavorable to growth the conversion of growth substance to an inactive form nevertheless takes place.

(*D*) *Growth Substance Used in Relation to Subsequent Growth.*—It is of interest to discover whether this growth substance which disappears without any accompanying growth is available for subsequent growth if the conditions are again made favorable. For this purpose the growth, in 2 hours, of plants to which 47 unit agar blocks were applied, with and without adequate water supply, was measured. At the end of this time water was supplied to all the plants and the agar blocks removed from one-half of the plants of each group. The growth rates were then measured during the next 4 hours. We know that in the "wet" plants there was in the first 2 hours a certain amount of growth substance converted to inactive form and presumably used, at least in part, for growth. In the "dry" plants, on the other hand, the same amount of growth substance has been destroyed in the first 2 hours but a much smaller portion of it used in growth since less growth has occurred, (Table V). If the uselessly destroyed growth substance is still available for growth, these formerly dry plants should, upon the addition of water, grow faster than the previously wet plants. Table V shows that on the contrary they grow more slowly. This is not because they are for other reasons incapable of fast growth, since those to which growth substance is continuously supplied grow much more swiftly. It is necessary to conclude that growth substance, in order to be used in growth, must be inactivated at the time at which the actual growth takes place.

(*E*) *Nature of the Inactivation Reaction.*—Growth substance is

known to be readily oxidized *in vitro* both by ordinary oxidants (*e.g.* hydrogen peroxide) and by plant oxidase preparations, (Thimann, 1934). It is also known that at least one of the growth processes

TABLE V

Effect of Temporary Water Lack upon the Growth of Coleoptiles (Agar Blocks Containing 47 Units)

Bases wet		Bases wet		No. of plants
Growth in 1st 2 hrs.		Growth in		
		2nd 2 hrs.	3rd 2 hours	
per cent		*per cent*	*per cent*	
6.6	Blocks left on	8.4	5.5	9
	Blocks taken off	4.0	0.3	9
Bases dry		**Bases wet**		**No. of plants**
Growth in 1st 2 hrs.		**Growth in**		
		2nd 2 hrs.	**3rd 2 hrs.**	
per cent		*per cent*	*per cent*	
1.6	Blocks left on	5.8	4.1	7
	Blocks taken off	2.0	0.8	9

TABLE VI

Disappearance of Growth Substance in the Presence of 10^{-2} N HCN

Experiment	Growth substance (units per plant) in plants:		
	Freshly decapitated	In HCN 2 hrs.	In water 2 hrs.
1	2.1	0.4	0.3
2	3.0	3.0	2.2
3	4.8	0.8	0.8
4	3.7	1.6	1.8
Means.....	3.4	1.4	1.3

of the coleoptile can be inhibited by HCN in the same manner as can the respiration (Bonner, 1933). It was therefore of interest to determine whether the inactivation of growth substance in the plant is inhibited by HCN. Table VI shows that the destruction of growth

substance is not significantly affected by concentrations of HCN sufficient to inhibit growth almost completely. The destruction of growth substance is not, therefore, the cyanide-inhibited reaction of the growth process.

DISCUSSION

The reactions taking place in the cell elongation of the *Avena* coleoptile may be included under the following heads:

1. The Passage of Growth Substance into the Growing Portions of the Plant.—This process has been studied particularly by Van der Wey (1932, 1934). It has been shown not to be a simple diffusion of the hormone. Unpublished experiments have shown that in the absence of oxygen the transport of growth substance does not take place. It is clear that the transport of the hormone from the tip or agar block is a prerequisite for the reaction of growth substance in the growing regions of the plant.

2. The Chemical Transformation of Growth Substance.—It has been shown above that under conditions which are favorable to growth, growth substance inactivation is strictly proportional to growth. It is therefore most reasonable to assume that the chemical transformation involved is an essential reaction in the growth process. Under conditions unfavorable for growth the inactivation of growth substance nevertheless continues. This is most easily interpreted to mean that the inactivation is the first member of a chain of growth reactions, and that hence it may take place even if the subsequent reactions do not. Concerning the nature of the change we have no information other than that it is not inhibited by cyanide. It has been shown above that, in order to be of use in growth, this chemical transformation must take place at the same time as the actual elongation; *i.e.*, the products of the reaction cannot be stored for any appreciable time. This reaction is, then, closely linked to those succeeding it.

3. The Cyanide-Inhibited Reaction.—It has been shown (Bonner, 1933), that the growth of the *Avena* coleoptile is inhibited by HCN in approximately the same way as is its respiration. Since, however, most of the energy of respiration is liberated as heat, the respiration as a whole can hardly be said to be a part of the growth process. On the contrary it may be said with fair certainty, on this as well as on

other grounds, that the respiration-like reaction of the growth process forms but a small portion of the total respiration. It has been shown above that the inactivation of growth substance is not the reaction which is inhibited by HCN, but that these two distinct reactions are necessary to the growth process.

4. The Physical Process.—This portion of the growth process is probably the actual mechanical stretching of the cell walls. Some experiments on temperature coefficients, which will not be given in detail here, indicate that the Q_{10} of coleoptile elongation as a whole is markedly below 2, at least for some time after removal of a coleoptile from a higher temperature to a lower. During the first 2 hours after decreasing the temperature, the average Q_{10} between, for example, 25° and 10° was found to be 1.7 while the Q_{10} of the respiratory reaction, as measured by the Q_{10} of the respiration as a whole, was under the same conditions 2.6. This indicates that the respiratory process with its high Q_{10} is linked to a process having a very low Q_{10}, perhaps close to 1; *i.e.*, a physical process. It is of interest that Heyn and Van Overbeek (1931) have shown that the Q_{10} of plastic stretching of the cell walls of the coleoptile is also below 2.

5. The Uptake of Water and the Resulting Visible Increase in Cell Size.—It is of course clear that water uptake is necessary for an increase in cell size. This is well illustrated in Table V, where plants having an inadequate water supply elongate but little.

If reaction 4 is the mechanical stretching of the cell wall, it is clear that 4 and 5 must go on at the same time. In Section *D* above it was shown that reaction 2 must go on at the same time as 4 and 5, and it has been previously and independently shown that reaction 3 must take place at the same time as reactions 4 and 5. The last four components of the growth process are, therefore, closely linked.

Heyn (1931) and Heyn and Van Overbeek (1931) have shown that the principal change in the properties of the coleoptile which accompanies the action of growth substance is an increase in the plasticity of the cell wall. Since reactions 4 and 5 are concerned with the actual elongation, it is clear that this increase in wall plasticity must take place as a *result* of reactions 2 and 3.

In view of the fact that, up to the present, only information of a rather negative nature has been obtained concerning the more inti-

mate qualities of the above components of the growth process in the *Avena* coleoptile, a further discussion would be unprofitable. It is of interest, however, to summarize in a diagram the reactions which are already known, particularly for comparison with the schemes recently proposed by Söding (1934) and by Strugger (1934). It is clear from this diagram that the growth process may be stopped at any one of a number of places. When it is so stopped, the reactions preceding the one which is inhibited may continue to take place, but no growth occurs.

The Growth Process

	Preliminary	Preparation of the cell wall		Visible growth	
The reactions:	1. Growth substance transport and uptake by the cell.	2. Growth substance transformation.	3. Reaction inhibited by HCN	4. Physical or mechanical process (wall stretching).	5. Water uptake (or other mechanical force).
Growth is inhibited by:	1. Lack of growth substance. Lack of oxygen.		3. Presence of HCN. Lack of oxygen.		5. Lack of water.

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