THE ACTION OF THE PLANT GROWTH HORMONE

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INTRODUCTION

Although the control of cell elongation in plant tissues by a special growth-promoting substance or substances has been well established for some time, the processes by which this substance is able to bring about growth have remained obscure. Since the general properties of the response to growth substance by plant tissues, in particular of the *Avena* coleoptiles which have been most extensively studied, have been recently summarized by Thimann and Bonner (1933), only the principal points of interest for the present discussion need be given. These are briefly as follows:

(*a*) The growth-promoting substance of the *Avena* coleoptile is produced only in the coleoptile tip and passes from there downward (Went, 1928). After removal of the tip new growth substance is formed by the uppermost cells of the stump ("physiological regeneration," Dolk, 1926).

(*b*) The growth of the *Avena* coleoptile is for some time proportional to the amount of growth substance supplied to it (Thimann and Bonner, 1933).

(*c*) The growth substance which enters the plant and causes growth cannot be recovered; *i.e.*, is used up (Went, 1928).

(*d*) Growth substance is an unsaturated acid of empirical formula $C_{18}H_{32}O_5$ (Kögl, Haagen-Smit and Erxleben, 1933) and readily loses its growth-promoting activity by oxidation.

(*e*) The growth substance is a true hormone, *i.e.*, it acts in minute amounts and bears no direct stoichiometrical relationship to the number of molecules of soluble substance transformed during growth into, for example, cell walls. Thus one molecule of growth substance causes an amount of growth of the *Avena* coleoptile at 27°C. which requires

the changing of 3×10^5 molecules of hexose to cellulose in cell walls (Thimann and Bonner, 1933).

The changes in the physical properties of coleoptiles under the influence of growth substance have been studied to some extent. Heyn (1931), and independently, Söding (1931, 1932) have shown that the plasticity, and also to a considerable extent the elasticity, of the coleoptile is increased after action of growth substance, and that this increase is independent of whether growth has occurred or not; *i.e.*, this action of growth substance is preliminary to active elongation. Heyn also found an increase in extensibility in coleoptiles which had been plasmolyzed after growth substance action, so that it is the physical properties of the cell wall, and not of the protoplasm, which are changed. The action of growth substance has now been further studied, and a few of the results will be described in the present paper. This study has been made easier by discovery of the fact that short sections of coleoptiles grow at a rapid rate if immersed in a growth substance solution of suitable concentration.

This method of using coleoptiles is convenient because, under proper conditions, a large amount of growth takes place in a relatively short time, and the "physiological regeneration" mentioned in (*a*) occurs slightly or not at all. It has the added advantage that the effect of known concentrations of growth substance upon the growth of younger and older portions of the same coleoptile may be examined independently.

Methods

Avena plants of the pure line "*Siegeshafer*" were used and were kindly supplied by Dr. Åkermann of Svalov. The plants were grown in sand in the dark at a temperature of 25°C. and a relative humidity of 85–90 per cent, and were used when 4 days old. Tips 3 to 5 mm. long were removed from the plants 2 hours before using. During this period the plants use up a large part of the growth substance already present in them and the production of growth substance by the stump does not commence. At the end of the 2 hours they were cut with a special cutter into sections in general 3.1 mm. long and immersed in the solution to be investigated.

The growth substance solutions, for which the author is indebted to Dr. K. V. Thimann, were prepared from large scale culture of the fungus *Rhizopus suinus* (Bonner, 1932; Thimann and Dolk, 1933). They were purified to an activity of the order of 2×10^{-6} mg. per plant unit.

Some of the growth measurements were made with a 12 power binocular equipped with an eyepiece micrometer. With this arrangement, the growth of free sections which were placed in small open dishes could be conveniently measured. For more accurate measurements, a horizontal microscope having an enlargement of 15 diameters and equipped with a stand mounted upon a micrometer screw was used. The coleoptile sections were placed upon thin glass rods of just the diameter of the interior of the coleoptile. These rods were in turn mounted in paraffin inside a rectangular vessel with glass sides through which the growth of the sections could be easily followed.

It should be mentioned here that considerable variability was found, in the reaction of sections to growth substance, both among plants of different experiments, and also to some extent among individual plants of the same experiment. A part of this variability is due, as will be shown, to slight differences in the ages of coleoptiles, but a considerable portion is probably due to causes not yet understood which also bring about variations in the standard *Avena* tests for growth substance activity from day to day. For this reason only the means of the results from a number of plants and several experiments can be regarded as significant.

EXPERIMENTAL

The growth of sections of coleoptiles, prepared in the manner already described, and immersed in pure water was first investigated. Table I gives the results of two series of measurements, and also a comparable series of measurements of sections with their bases in water, but their tips in air. It may be seen that the growth rate of the sections completely immersed in water falls steadily until after 7–8 hours it reaches a very low value. There is no sudden rise after 2 hours corresponding to a production of growth substance by the "physiological tip" as is the case with the sections whose tips were in air. The growth which took place in the sections immersed in water is to be attributed principally to growth substance in them when they were removed from the plants. Table II shows that the elongation of sections in growth substance solution is marked, being as great as 29 per cent in 4 hours and 55 per cent in 24 hours.

It was conceivable that with these sections immersed in solutions, the rate of diffusion of growth substance to the cut surface which it enters, or the rate of diffusion of oxygen to the tissue, might prevent

a maximum growth response. Therefore measurements of the growth rates of sections both with and without stirring of the solution by air were made. These results are given in Table III, and show that stirring of the solution is not necessary.

The effect of the original position in the coleoptile of a given section upon its response to growth substance was then investigated. That such an effect exists is shown by Table IV, which gives the per cent

TABLE I

Growth of Coleoptile Sections in Water

Experiment	Plants	Growth in per cent of original length per hr.						
		1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.	7 hrs.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1 (tops in water)	6	1.8	1.5	1.2	0.7	0.6	0.4	0.4
2 (tops in water)	16	0.8	0.4	0.1	0.3	0.0	—	—
2 (tops in air)	6	2.5	2.5	4.0	4.3	4.7	3.3	—

TABLE II

Growth of Coleoptile Sections in Growth Substance Solution and in Water

(Each value is a mean from twelve–fifteen sections)

Solution	Growth in 2 hrs.	Growth in 4 hrs.	Growth in 24 hrs.
	per cent	*per cent*	*per cent*
Water	3	4	9
Water	3	5	7
Water	3	5	12
Growth substance	15	24	55
Growth substance	13	26	45
Growth substance	14	29	48

growth per hour of sections from the tops and bottoms of a series of previously decapitated coleoptiles. Table IV is taken from one of seven experiments, all of which gave the same result. Similar measurements upon coleoptiles divided into more sections showed that the two 3.1 mm. sections nearest the apex of a plant decapitated 3–5 mm. from the tip have almost equal reactivity. The lower zones showed, as in Table IV, a lower ability to grow in the presence of growth substance. It has been known from measurements upon the growth rates of

entire coleoptiles (marked into zones with ink or paper marks) that the lower zones do grow more slowly than those nearer the top. This has been attributed, however, to a lack of growth substance in the lower zones which must receive it through a long portion of coleoptile actively using the growth substance. That this is not the only

TABLE III

Growth Rates of Coleoptile Sections in Growth Substance Solution with and without Stirring by Air

Experiment	No. of sections	Growth per 2 hrs.					Total growth after 24 hrs.
		2 hrs.	4 hrs.	6 hrs.	8 hrs.	10 hrs.	
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1 (no air)	7	8	6	3	1	0.2	16
2 (no air)	6	9	4	3	2	0.3	19
3 (no air)	7	7	3	2	—	—	16
4 (air)	5	7	5	2	—	—	22
5 (air)	7	6	4	1	1	2	14
6 (air)	7	8	5	3	—	—	21
Mean of 1, 2, and 3	—	8.0	4.3	2.7	—	—	17.0
Mean of 4, 5, and 6	—	7.0	4.7	2.0	—	—	19.0

Growth substance concentration = 10 units per cc.

TABLE IV

Growth Rates of Top and Bottom Sections of Coleoptiles in Growth Substance Solution

	Growth per hr.						
	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.	7 hrs.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Top sections	4.5	4.2	4.3	3.7	2.7	2.4	2.4
Bottom sections	1	1	1.1	1.4	2.1	0.9	0.9

Growth substance concentration = 10 units per cc.

factor is shown here directly, since it is clear that the cells at the base of the coleoptile show a much smaller growth response to growth substance than those nearer the top. In the present work, unless otherwise stated, only the two 3.1 mm. sections nearest the tip were used.

The effect of concentration of the growth substance solution upon

growth of the sections was determined, and is shown in Table V. The units are the standard growth substance units of this laboratory (Dolk and Thimann, 1932; Thimann and Bonner, 1933). From Table V it is evident that there is an optimum growth substance concentration in the region of 10 units per cc. Coleoptiles immersed in concentrations as great as 80 units per cc. show a shrinkage after 4 hours and at the end of 24 hours have frequently lost their turgidity due, apparently, to a toxic effect of the high concentration of growth substance. A decrease in growth in very low growth substance concentrations was also found. A simple consideration will show that only in the case of the 0.01 unit solution can this be due

TABLE V

Effect of Growth Substance Concentration upon Growth of Coleoptile Sections
(Each value mean of 50–150 sections)

Growth substance concentration standard units	Growth in		
	2 hrs.	4 hrs.	24 hrs.
	per cent	*per cent*	*per cent*
80	3.3	2.1	0.4
40	4.3	8.0	7.2
20	7.4	10.8	15.4
10	11.7	19.9	31.0
1	8.4	15.7	27.0
0.1	6.5	12.1	17.5
0.01	4.5	7.0	15.5
0	3.3	5.6	11.9

to an insufficient quantity of growth substance, and that even in the 0.1 unit solution the decrease must be due directly to the low concentration. From the data of Thimann and Bonner (1933) 0.00535 cc. of a 29 unit per cc. solution can give under their conditions a maximum of 7.85 mm. of coleoptile elongation. 1 cc. of a 0.1 unit per cc. solution could then give a maximum of 5.05 mm. total coleoptile elongation. Since in general in the present case twelve sections were placed in 4 cc. of solution, these sections should be able to elongate a maximum total of 22 mm., or 59 per cent per section, which is much larger than the 18 per cent observed. In the case of the 0.01 unit solution, however, each section should be able to elongate only

5.9 per cent more than controls in pure water, and Table V shows that the increase is only 4 per cent.

Upon the theory that the action of growth substance is a simple physical change of cell wall, for example by decreasing directly in some way the viscosity of the substance in which cellulose micelles are imbedded (Heyn, 1931), one would hardly expect the action to be stopped by the presence of narcotics or cyanide. If, however, growth substance depends for its action upon processes of a metabolic nature, narcotics or cyanide should inhibit this action. It was easily demonstrated that both KCN and phenylurethane stop growth. Table VI gives a summary of two experiments with various concentrations of KCN and of phenylurethane and shows how marked is the stopping of growth. Further experiments showed that the concentration of KCN which brought about cessation of growth is 1–2 $\times$ 10^{-3} N, and that 0.05–0.1 per cent of phenylurethane brought about the same result. The concentrations of KCN are of the same order as those necessary to affect the respiration of other plant tissues (Schwabe, 1932).

TABLE VI

Inhibition of Growth of Coleoptile Sections by KCN and Phenylurethane

Solution	Growth	Solution	Growth
	per cent		*per cent*
Growth substance alone	23	Growth substance alone	20
Growth substance + 2 $\times$ 10^{-4} N KCN	5	Growth substance + 0.001 per cent phenylurethane	23
Growth substance + 10^{-3} N KCN	2	Growth substance + 0.01 per cent phenylurethane	14
Growth substance + 2 $\times$ 10^{-3} N KCN	−4	Growth substance + 0.1 per cent phenylurethane	2
Growth substance + 2 $\times$ 10^{-2} N KCN	−3	H_2O + 0.1 per cent phenylurethane	3
H_2O + 2 $\times$ 10^{-3} N KCN	−4		

That the growth resulting from growth substance does not occur in the presence of substances which stop metabolism suggests that the action of growth substance is itself intimately associated with the metabolism of the cell.

Experiments were then carried out to determine whether a connection between growth and cell oxidation exists. The action of growth substance in solutions under an atmosphere of pure nitrogen was first investigated. Commercial N_2 was passed over reduced copper in an electric furnace at 600°C. The gas was then cooled by passage through wash bottles, and bubbled through the solution containing the coleoptile sections. A preliminary experiment showed that growth substance is not affected in its activity by prolonged passage of N_2 through it. The sections were freed of O_2 by treatment with N_2 for 2 hours before introduction of the growth substance solution. After this preliminary 2 hours, sufficient growth substance was introduced to make the solution 10 units per cc., and the bubbling was

TABLE VII

Inhibition of Growth of Coleoptile Sections by Nitrogen

Experiment	No. of sections	Growth after 4 hrs. in N_2 + growth substance	Growth after 20 hrs. in air + growth substance	Growth after 20 hrs. in air + H_2O
		per cent	*per cent*	*per cent*
1*	20	4.5	17	—
2	26	2.9	21	—
3	25	1.7	—	3.8
4	33	1.4	28	8.0
5	46	4.5	13	4.5

* In this experiment the sections were not first freed of oxygen.

continued for 4 hours more. The sections were then measured, a portion of them placed in growth substance solution in air, and the remainder in pure H_2O, in air. Table VII shows the results of five experiments. The coleoptiles were not harmed by the prolonged lack of O_2 as is shown by the fact that they grew normally upon being supplied with both growth substance and oxygen. However, a mean growth of only 3 per cent took place in N_2, although from Table V, 20 per cent growth would have taken place in air. That even this 3 per cent growth takes place is probably to be attributed to O_2 remaining in the sections. Therefore, normal growth fails to take place in N_2. Since the sections do not elongate, when placed in pure H_2O, to any greater extent than when immersed in H_2O without the pre-

liminary growth substance-N_2 treatment, it follows that either the action of growth substance has not taken place or else the growth substance has not been taken up by the sections.

The effect of the presence of KCN and of lack of oxygen in stopping growth suggested that a more intimate study of the effect of growth substance upon respiration be made. This was done with the aid of the standard Warburg manometers and using the technique described by Warburg (1926). The rate of respiration of the sections was found to be rather low and therefore it was necessary to use vessels having as small a gas space as possible in order to obtain measurable decreases in pressure due to O_2 uptake. These vessels did not contain alkali wells for absorption of CO_2, but depended for a decrease in pressure due to respiration, upon the greatly different solubilities of CO_2 and O_2 in the liquid present. The absolute amount of O_2 taken up in respiration may be calculated from the observed pressure change in the manner given by Gaffron (1929). This calculation requires a knowledge of the respiratory quotient. Since a preliminary determination showed that this quantity is close to unity for both sections in growth substance and sections in pure buffer, all of the subsequent calculations were based upon the assumption that the respiratory quotient was actually 1. This procedure is justifiable since the measurements are principally for purposes of comparison.

From 89 to 150 sections (including the basal portions of the stump) were placed in each vessel in M/50 phosphate buffer (pH = 4.8). The vessels were then attached to the manometers and placed upon the shaking rack, with the vessels immersed in a thermostat at 25°. These operations were carried out in red light in order not to cause any phototropic reactions in the sections. Some hours were needed for the respiration to reach a constant value after immersion in the vessel. This undoubtedly was due to an initial high rate of respiration of the wounded tissue at the cut surfaces. After a constant rate had been attained, sufficient growth substance to make up the desired concentration was introduced into one of a pair of vessels. The control vessel was either untreated, or, alternatively, a volume of water equal to the volume of growth substance solution used in the first vessel was introduced. Fig. 1 shows the course followed by the rate

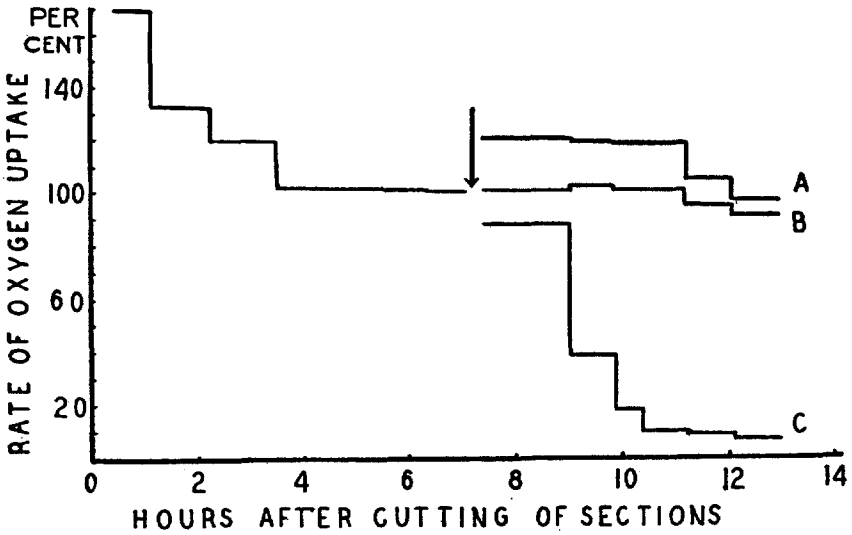

FIG. 1. Effect of growth substance upon the rate of oxygen uptake by coleoptile sections. At the arrow there were introduced: *A*, growth substance, 110 units per cc.; *B*, growth substance, 110 units per cc. inactivated; *C*, growth substance, 1100 units per cc.

TABLE VIII

Effect of Growth Substance upon Respiration of Coleoptile Sections
(Mean values of two to seven determinations)

Growth substance concentration	Increase in O_2 uptake over initial respiration rate, mm.³ per section per min.				Age of coleoptile
	During first 2 hrs.		During second 2 hrs.		
		Increase		Increase	
units per cc.		*per cent*		*per cent*	*days*
1100	−0.0022	−24	−0.0083	−90	4
110	+0.0018	19	+0.0014	15	4
11	+0.0025	27	+0.0024	26	4
1.1	+0.0013	14	+0.0017	18	4
0.11	±0.0000	0	+0.0003	3	4
110	+0.0021	31	+0.0012	21	5–6

of oxygen uptake in three typical experiments. It is clear that the addition of growth substance to a concentration of 11 units per cc., *i.e.* a concentration which causes growth (see Table V), causes a marked increase in oxygen uptake. A similar increase was found in

every experiment in which a suitable concentration of growth substance was used.

The effect of growth substance concentration upon the stimulation of respiration was next investigated. Table VIII gives the summa-

TABLE IX

Effect of KCN and Phenylurethane on Respiration of Coleoptile Sections

Experiment	Solution	Respiration in mm.³ O_2 per min. per section
	Successive additions to buffer solution	
1	(Initial rate)	0.0079
	KCN, 5×10^{-4} N	0.0074
	KCN, 1×10^{-3} N	0.0003
	Growth substance, 110 units per cc.	0.0001
2	(Initial rate)	0.0061
	Growth substance, 110 units per cc.	0.0094
	KCN, 5×10^{-4} N	0.0099
	KCN, 1×10^{-3} N	0.0001
3	(Initial rate)	0.0069
	KCN, 8×10^{-4} N	0.0005
	Growth substance, 110 units per cc.	0.0003
4	(Initial rate)	0.0090
	KCN, 1×10^{-3} N	0.0005
	Growth substance, 110 units per cc.	0.0004
5	(Initial rate)	0.0069
	Urethane, 0.005 per cent	0.0073
	Urethane, 0.025 per cent	0.0088
	Growth substance, 110 units per cc.	0.0107
	Urethane, 0.05 per cent	0.0030
6	(Initial rate)	0.0094
	Urethane, 0.05 per cent	0.0012
	Growth substance, 110 units per cc.	0.0007

rized results for a wide range of concentrations. The maximum stimulation is caused under these conditions, in the neighborhood of 11 units per cc. Stimulation is also caused by concentrations ten times as great, although such concentrations are shown by Table V

to inhibit growth. It should be remembered, however, that the number of sections per cubic centimeter of growth substance solution was in the respiratory vessels five times that in the vessels in which growth was measured. A still higher concentration of growth substance may be seen to be immediately toxic to the respiration. The 1.1 unit per cc. solution also stimulates respiration markedly; the 0.11 unit solution slightly, if at all.

Before further discussion of Table VIII, a few other results will be described. Fig. 1 includes the effects which were found to result from the addition of growth substance inactivated by oxidation with H_2O_2. It was then determined whether the respiration, and the addition "growth substance respiration" in particular, could be stopped by the concentrations of KCN and phenylurethane which stopped growth. That the respiration is so stopped is shown by Table IX. From this table it is also apparent that the additional growth substance respiration does not markedly differ in its sensitivity to the two inhibitors from the normal respiration, and this may be taken as evidence that the two are actually identical.

DISCUSSION

A simple calculation shows that the increase in respiration cannot be due to actual oxidation of the growth substance itself. Under the conditions of the experiment all of the growth substance could be completely removed by the observed increase in oxidation in 2–3 minutes. Therefore the effect of growth substance is due rather to some kind of stimulation.

In Table VIII it was shown that coleoptiles which were used for respiration measurements at the age of 5–6 days instead of the usual 4 days give an increase in respiration upon the addition of growth substance. Measurements upon these sections from "old" coleoptiles showed that they do not, however, elongate in the presence of growth substance. Therefore the increase in respiration upon the addition of growth substance is not due to the process of actual elongation. However, from Tables VI and VII it is clear that in order for elongation to occur, or probably even for growth substance action preliminary to elongation, the presence of aerobic metabolism is necessary.

There remain two possibilities, namely: (*a*) the stimulation of

respiration by growth substance has nothing to do with its action in growth; and is a secondary phenomenon attending its presence or the presence of closely allied impurities in the cell, and (*b*) the stimulation of respiration by growth substance "prepares" the cell for elongation, which may then occur if other conditions are suitable. Schwabe (1932) has shown that various amino acids in minute quantities stimulate the respiration of *Elodea*, *Fontinalis*, and *Potamogeton*. These substances do not, as far as investigated, bring about growth in *Avena* coleoptiles. In favor of the second alternative, the following parallels between the increase in respiration upon the addition of growth substance and the growth of these coleoptile sections may be pointed out: (*a*) Low concentrations of growth substance cause growth; they also stimulate respiration. (*b*) High concentrations of growth substance inhibit growth; they also inhibit respiration. (*c*) The optimal concentrations for the two processes are similar. (*d*) Both growth and (by definition) aerobic respiration cease in the absence of oxygen, even in the presence of growth substance. (*e*) Both growth and respiration (as well as the excess "growth substance respiration") are stopped by the presence of KCN (10^{-3}N), or phenylurethane (0.05 per cent).

These parallels between the increase of respiration by growth substance and the effect of growth substance in promoting growth make it seem possible that the former is a necessary condition of the latter. That the increase of respiration can take place without accompanying growth, as it does in the old coleoptiles, does not exclude this possibility, since the old coleoptiles are stiffer and less plastic (Du Buy, 1932). It might be argued, for example, that although growth substance exerts in these old coleoptiles its general action, yet these sections are already too stiff, due to excessive thickening of the cell walls, to permit of extension by the turgor pressure.

A connection between growth substance activity and respiration is supported from another angle by the work of Van Ameijden (1917), who showed that phototropic and geotropic response do not take place in nitrogen.

The relation between the respiratory activity of growth substance and the increase of the plasticity of the cell walls observed by Heyn and Söding remains obscure, however. There are several conceiv-

able mechanisms by which this result might be brought about and a detailed investigation of this question is now under way.

SUMMARY

1. Sections of *Avena* coleoptiles are found to show a considerable elongation when suspended in solutions of growth substance.

2. This elongation does not take place in the absence of O_2 and is inhibited by KCN and phenylurethane.

3. The rate of respiration of sections of coleoptiles is increased by the addition of growth substance in concentrations which cause growth. High concentrations of growth substance inhibit growth and also respiration.

4. The increase in respiration is inhibited by KCN and phenylurethane in the concentrations which inhibit normal respiration. These concentrations are the same as those which inhibit growth.

5. From 2, 3, and 4, it seems possible that the increase in respiration caused by growth substance may be an essential part of its action in growth.

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