GROWTH, AUXIN, AND TROPISMS IN DECAPITATED AVENA COLEOPTILES

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(WITH THREE FIGURES)

With all the work on auxin appearing in the last years it is remarkable that not more investigations are concerned with the actual rôle of the auxins inside the plant. Barring some early opposition, the original statement "Without auxin no growth" (**12**) has stood unchallenged for many years. Probably this was so because all test methods were based on the principle of proportionality between auxin applied and its resultant effect. When BONNER and THIMANN (**2**) established that there was also direct proportionality between auxin used up and growth, the paramount importance of auxin in the growth process seemed firmly established. In later years, however, doubts have been expressed about the necessity of auxin for the growth process in general. AVERY and LARUE (**1**) concluded "that growth hormone is not a necessity for elongation of the coleoptile." The confusion was due to the fact that auxin is present inside the plant in different states. This was pointed out especially by WENT and THIMANN (**15**), and WENT (**13**), who stressed the point that it is essential to distinguish between auxin which can be collected by diffusion (diffusible or free auxin) and the auxin which is obtained by extraction. It is evident that only that part of the auxin which combines in some way inside the cells can be responsible for growth, whereas correlation phenomena such as tropisms can be brought about only by a correlation carrier which moves freely inside the tissues.

Since no attempt has been made to correlate for one experimental object the relations between diffusible auxin, extractable auxin, growth rates, and tropisms the following paper describes experiments in which this was done for the Avena coleoptile. This is an especially favorable object for such studies since its growth rate and tropistic behavior can be radically changed by simple decapitation. The relation between auxin production and growth and tropisms was worked out by DOLK (**3**). After decapitation the growth rate of the Avena coleoptile falls off until two and one-half hours later when the growth rate suddenly rises to a value somewhat below its original rate. Geotropic and phototropic sensitivity show the same general dependence upon decapitation. Whereas tropistic sensitivity disappears immediately after cutting off the tip, it suddenly reappears two and one-half hours later. This behavior can be correlated with the auxin production in a coleoptile. Decapitation removes the production center of auxin, but two and one-half hours after removal of the tip, the cells immediately below the cut surface

regain the ability to produce auxin. This is called regeneration of the physiological tip. Each of these phenomena will be measured quantitatively in the following investigation.

Auxin content of decapitated coleoptiles

The extractable auxin from coleoptile zones 5 to 25 mm. below the original tip was determined by ether extraction. Thirty-six to 48 coleoptiles were cut off and placed in freshly prepared ether and left overnight. According to VAN OVERBEEK (**11**), under those conditions at least 75 per cent. of all free auxin present is extracted from coleoptiles. Extractions were made of five separate lots before, and one, two, three, and four hours after decapitation. The extracts were then taken up in 0.5 ml. of 1.5 per cent. agar and tested on 24 test plants. The results of two experiments are given in table I. They show that the auxin content of coleoptiles gradually decreases to 50 per cent. in the first two hours after decapitation. After 3

TABLE I

AUXIN CONTENT OF AVENA COLEOPTILES, INCLUDING THE REGION 5 TO 25 MM. FROM ORIGINAL TIP ONLY, WHEN EXTRACTED WITH ETHER AT VARIOUS TIMES AFTER DECAPITATION. EXTRACT TAKEN UP IN 0.5 ML. AGAR, AND CUT INTO AGAR BLOCKS OF 12 MM.[3]

	Experiment 1				Experiment 2				
	Number of coleoptiles extracted	Mean Avena test curvatures per 12 test plants	Curvature per 100 extracted coleoptiles	Auxin content; percentage of intact coleoptiles	Number of coleoptiles extracted	Avena test curvature	Curvature per 100 extracted coleoptiles	Auxin content; percentage of intact coleoptiles	Auxin content; mean of expts. 1 and 2
		deg.	*deg.*	%		*deg.*	*deg.*	%	
At the moment of decapitation	36	7.3, 7.9	21.0	100	36	6.7	18.6	100	100
1 hour after decapitation	48	6.9, 6.9	14.4	69	48	6.7	14.0	75	72
2 hours after decapitation	48	4.5, 5.0	9.9	47	48	5.0	10.4	56	51
3 hours after decapitation	48	8.1, 8.3	17.1	81	48	8.2	17.1	92	86
4 hours after decapitation	36	4.9, 6.4	15.7	75	36	6.9	19.2	103	89
25 γ/L indoleacetic acid	.....	7.2	.....	.....	.....	.....	.....	.....	.....
50 γ/L indoleacetic acid	.....	13.7	.....	.....	.....	18.7	.....	.....	.....
100 γ/L indoleacetic acid	.....	.....	.....	.....	.....	24.2	.....	.....	.....

hours there is a sudden increase to more than 80 per cent. of the original auxin content. How reproducible these results are is evidenced by the fact that BONNER and THIMANN (2) found that, irrespective of whether coleoptiles are in contact with the seeds or cut off, about 50 per cent. of the extractable auxin has disappeared 2 hours after decapitation. After 3 hours they found that the auxin content had risen again to 83 per cent. of normal. Their experiments were carried out 5 years earlier with the chloroform extraction method, but their Avena plants had been grown under essentially the same conditions of temperature, light, and humidity.

Growth rate after decapitation

Coleoptiles of the same age and size as used for the auxin extractions were measured with the horizontal microscope to determine their growth rate. The results of these measurements are presented in figure 1 and

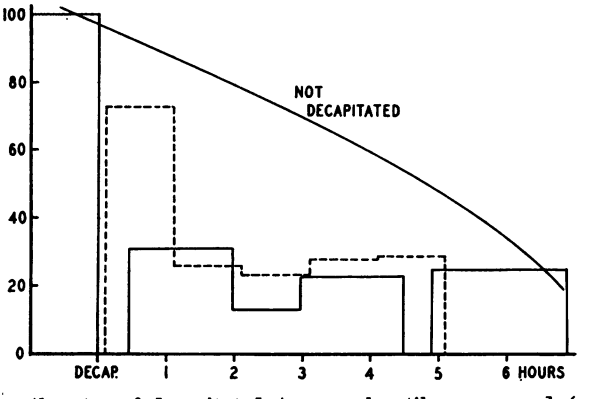

FIG. 1. Growth rates of decapitated Avena coleoptiles, expressed (on ordinate) in per cent. of the rate before decapitation. The upper curve represents growth rate of intact control plants. For actual values see table II. The dotted block diagram gives the results of another experiment.

table II; their behavior can be compared directly with the plants used for the auxin extractions in table I. After decapitation, growth decreases first slowly and then more rapidly until after 3 hours an increase occurs due to regeneration of the physiological tip. Reference to the data of DOLK (3) shows that the plants behaved normally. With the help of his data the block diagram of figure 1 has been smoothed to obtain the growth rate curve which is used for comparison with the auxin content, auxin production, etc. (fig. 3). It will be seen from table II that the growth rate of the non-decapitated control plants fell off considerably after six hours, due to the grand period. Therefore, the growth rate of the decapitated plants was not expressed in percentage of the original rate but as the percentage of the growth rate of the non-decapitated controls. Without this correction the

shape of the curve is essentially the same but remains at a somewhat lower level.

The growth rate of coleoptiles depends not only on their auxin content but also upon their response to the available auxin. To obtain figures which would permit one to estimate the sensitivity of coleoptile cells to auxin after decapitation, the same concentration of auxin (agar blocks containing 50γ

TABLE II

GROWTH RATE IN MM./HOUR OF NORMAL AND DECAPITATED AVENA COLEOPTILES* (SAME GROUP AS IN EXPERIMENT 1, TABLE I)

	GROWTH RATE				
	BEFORE DECAPITATION	AFTER DECAPITATION			
	1 HOUR	0–2 HOURS	2–3 HOURS	3–4½ HOURS	5–7 HOURS
	mm./hr.	*mm./hr.*	*mm./hr.*	*mm./hr.*	*mm./hr.*
Not decapitated, intact plants	1.41	1.17	1.05	0.79	0.46
Decapitated plants	1.46	0.45	0.19	0.34	0.36
AUXIN APPLIED†	PERCENTAGE INCREASE IN GROWTH RATE DUE TO AUXIN APPLICATION‡				
	%	%	%	%	%
2 hours after decapitation	.....	.....	37	100	53
3 hours after decapitation	.....	.....	.....	38	30
5 hours after decapitation	.....	.....	.....	.....	25

* Only the zones 5 mm. below the tip are measured.

† Various times after decapitation an agar block containing 50γ/liter indoleacetic acid was applied to the cut surface and renewed every two hours.

‡ Growth increase due to auxin application is indicated as percentage of growth rate of decapitated plants in the same period.

indoleacetic acid per liter, renewed every 2 hours) was applied to the entire cut surface of coleoptiles at various periods after decapitation. The growth rate of decapitated coleoptiles treated in this way was expressed as the per cent. of the growth rate of coleoptiles which did not receive this additional auxin. From table II it appears that the greatest response is obtained when auxin is applied two hours after decapitation; after 3 and 5 hours, however, the response was reduced. This phenomenon has been investigated before and has been called aging (**4**). In the regular Avena test the sensitivity of auxin greatly increases during the first three hours after decapitation, but this phenomenon is due to the decreasing auxin content of the cells (**7**).

Diffusible auxin

Avena coleoptiles were cut into a number of cylinders and the auxin diffusing out of different levels of the coleoptile was measured. This was done by cutting 24 coleoptiles into 2-mm. sections; these cylinders cut at the same level were placed together with their bases down on the standard 8 × 10.5-mm. agar plate. The latter was cut into 12 blocks and each block was applied to a standard Avena test plant. After the cylinders had stood for 2 hours on one agar plate they were transferred to another. In this way the amount of auxin diffusing out of the coleoptile cylinders in 2-hour periods was measured. This value corresponds with the auxin production. Some of the results are shown in table III. Coleoptile tips of 2 mm. length continued to produce auxin for the first 4 hours, but in the 2 hours immediately following decapitation the coleoptile cylinders, whether cut 2, 4, or 8 mm. below the tip, did not produce any measurable amount of auxin. This is in com-

TABLE III

AUXIN PRODUCTION BY COLEOPTILE CYLINDERS. THE AUXIN DIFFUSING IN TWO HOURS TIME FROM 24 TWO-MM. LONG COLEOPTILE CYLINDERS, IS EXPRESSED IN DEGREES STANDARD AVENA TEST CURVATURE. THE CYLINDERS WERE EITHER CUT OFF IMMEDIATELY OR TWO HOURS AFTER DECAPITATION. FIGURES IN ITALICS INDICATE AUXIN REGENERATION

	Distance of cylinders from original tip	Hourly period after decapitation		
		0–2	2–4	4–6
	mm.	*deg.*	*deg.*	*deg.*
Tips of plants 2 mm. long	0	21.6	19.8	.....
Coleoptile cylinders from same plants	2–4	0.3	*4.6*	.....
Next cylinders	4–6	+ 0.3	*3.0*	.....
Cylinders from same plants	8–10	0.0	0.3	.....
Apical coleoptile cylinders cut off 2 hrs. after 2-mm. decapitation	2–4	.....	*2.8*	6.9
Apical coleoptile cylinders cut off 2 hrs. after 8-mm. decapitation	8–10	.....	0.6	*8.8*

plete accord with earlier work (**12**). In the next 2-hour period these same coleoptile cylinders started to produce considerable amounts of auxin (15 to 25 per cent. of the production by tips). Comparison with other data (**11**) shows that the same holds true for corn coleoptiles. Only when the cylinders were more than 8 mm. removed from the tip did the regeneration of auxin production not occur within the first 4 hours. It was conceivable that auxin was produced by the coleoptile cylinders in the first 2 hours after cutting, but in such small amounts that the standard Avena test did not indicate them. Therefore, the same experiment was repeated using the deseeded Avena test (**9**). Table IV shows the results of a comparison of both tests, carried out on the same day and with the same material. The amounts

of auxin are expressed in indoleacetic acid equivalents, which rules out the individual differences in sensitivity of both tests.

TABLE IV

AUXIN DETERMINATIONS, BOTH WITH THE STANDARD AND DESEEDED AVENA TEST, OF THE MATERIAL DIFFUSING OUT OF AVENA COLEOPTILES. AUXIN EXPRESSED AS INDOLEACETIC ACID EQUIVALENTS, WHICH OBVIATES DIFFERENCES IN SENSITIVITY BETWEEN THE TWO TESTS

	Experiment 1		Experiment 2		Mean of both experiments	
	Standard	Deseeded	Standard	Deseeded	Standard	Deseeded
Auxin produced by 36 coleoptile tips	90	72	51	69	70	70
Auxin produced by 36 coleoptile cylinders 2 to 4 mm. from tip, 0 to 2 hrs. after cutting	<11	22	<10	16	<10	19
Auxin produced by same coleoptile cylinders 2 to 4 hours after cutting	62	51	42	40	52	46

It is evident that whenever auxin is produced by coleoptile tips and coleoptile cylinders after regeneration, the standard and deseeded Avena tests give approximately the same results. Only the values for auxin production in coleoptile cylinders during the first 2 hours after decapitation differ. This indicates that for the first period the curvature in the deseeded test is

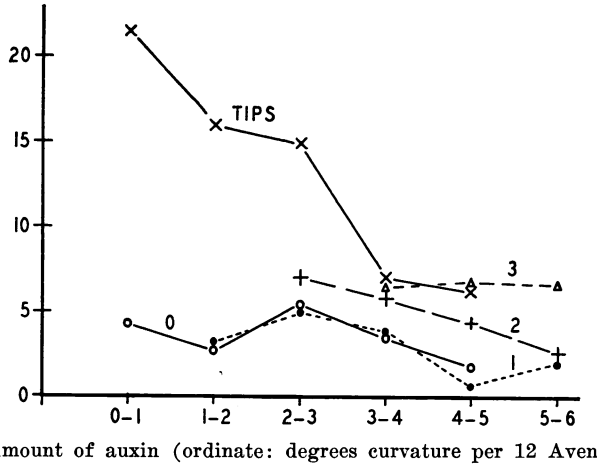

FIG. 2. Amount of auxin (ordinate: degrees curvature per 12 Avena test plants), diffusing out of 36 coleoptile cylinders in successive hourly periods (abscissa). Two millimeters of tip cut off, the next two-mm. sections were cut in curve 0 (circles) at the moment of decapitation, curve 1 (solid dots) one hour, curve 2 (plusses) two hours, and curve 3 (triangles) three hours after decapitation. For comparison the amount diffusing out of tips is plotted (crosses).

almost exclusively due to auxin precursor and not to free moving auxin, for the deseeded test responds not only to auxin but also to auxin precursor.

With this fact in mind we can now consider the data of table V and figure 2. Because of the greater sensitivity of the deseeded test the 24 cylinders were moved once every hour to a fresh agar plate, and thus the auxin production could be followed more closely. Since we have seen that the response of the deseeded plants in the first 2 hours after decapitation is due to auxin precursor we can conclude that the apical coleoptile cylinders give off more precursor, which is in excellent agreement with the experiments of VAN OVERBEEK (**11**) who found that the region nearest the tip contained most precursor. Regeneration of auxin production occurs after 2 to 3 hours

TABLE V

AUXIN PRODUCTION BY COLEOPTILE CYLINDERS. CURVATURES IN DEGREES PER 24 TWO-MM. SECTIONS PER AGAR PLATE PER HOUR DIFFUSION, TESTED ON DESEEDED AVENA PLANTS. THE FIRST FOUR GROUPS WERE TIPS AND SECTIONS ALL CUT FROM THE SAME PLANTS; THE NEXT THREE GROUPS WERE APICAL SECTIONS FROM THREE SEPARATE GROUPS OF 2 HOURS PREVIOUSLY DECAPITATED PLANTS. VALUES ITALICIZED CORRESPOND WITH REGENERATED AUXIN

	Length of decapitated tip	Hours elapsed between decapitation + cutting of sections	Distance from original tip	Hourly periods after decapitation				
				1	2	3	4	5
	mm.	*hr.*	*mm.*	*deg.*	*deg.*	*deg.*	*deg.*	*deg.*
Tips	...	0	0–2	24.8	.....	17.4	.....	6.0
Next 2 mm.	...	0	2–4	6.3	2.5	*3.9*	2.4	1.4
Next 2 mm.	...	0	4–6	4.9	2.0	*4.6*	.....	1.2
Next but 2 mm.	...	0	8–10	3.5	0.2	0.2	*3.2*	.....
Apical sections	2	2	2–4	.....	.....	*6.8*	6.8	4.6
Apical sections	4	2	4–6	.....	.....	2.6	*7.5*	3.8
Apical sections	8	2	8–10	.....	.....	1.6	1.1	*1.9*

in sections 2 to 6 mm. removed from the tip, whereas sections 8 to 10 mm. away regenerate auxin only after 3 to 4 hours. This compares very well with the data from TSI TUNG LI (**6**) who found at 20° C. (with growth measurements) regeneration after 143, 166, and 182 minutes for 2-, 4-, and 6-mm. decapitated plants, respectively. When the apical sections are removed 2 hours after decapitation, these times are somewhat longer which fact was confirmed in other experiments.

From these same experiments it also follows that the amount of auxin which is regenerated by the apex of decapitated plants is larger the longer the sections are left on the plants. This is particularly clear in figure 2, and has been confirmed in other experiments such as shown in table VI.

TABLE VI

AUXIN PRODUCTION (IN DEGREES PER 12 DESEEDED PLANTS) PER 24 APICAL COLEOPTILE SECTIONS, 2 TO 4 MM. BELOW THE ORIGINAL TIP, WHEN THEY ARE CUT OFF AND PLACED ON AGAR 0, 1, 2, OR 3 HOURS AFTER DECAPITATION. SECTIONS REPLACED ON NEW AGAR PLATES EVERY TWO HOURS

	TIME AFTER DECAPITATION	HOURLY PERIODS AFTER CUTTING APICAL SECTIONS					
		EXPERIMENT 1			EXPERIMENT 2		
		0–2	2–4	4–6	0–2	2–4	4–6
	hr.	*deg.*	*deg.*	*deg.*	*deg.*	*deg.*	*deg.*
Coleoptile tips	...	24.1	10.3	2.3	31.8	29.5	14.5
2-mm. apical section	0	1.4	6.1	0.5	7.7	6.3	3.0
" " "	1	4.0	3.8	.....	10.2	8.2	.....
" " "	2	6.0	10.8	.....	14.2	7.2	.....
" " "	3	8.4	.....	.....	12.7	.....	.....

This means that after decapitation auxin precursor continues to accumulate in these apical sections. The same conclusion was reached by VAN OVERBEEK (**11**) for corn coleoptiles.

Geotropism and phototropism of decapitated coleoptiles

DOLK (**3**) found that within the first hours after decapitation only very slight curvature resulted from geotropic stimulation. Moreover, these curvatures appeared mainly in the basal zones. Six hours after decapitation completely normal geotropic curvatures could be observed. A number of

TABLE VII

GEOTROPIC AND PHOTOTROPIC CURVATURES OF DECAPITATED AVENA COLEOPTILES, MEASURED 70 OR 120 MINUTES AFTER BEGINNING OF EXPOSURE. EACH FIGURE IS THE MEAN OF 10–12 PLANTS. THE FIRST FIGURES SHOWING REGENERATION HAVE BEEN ITALICIZED

EXPOSURE OF COLEOPTILES AFTER DECAPITATION	CURVATURES IN DEGREES				
	GEOTROPIC CURVATURES 15 MINUTES HORIZONTAL EXPOSURE			PHOTOTROPIC CURVATURES 5 MM. DECAPITATED 10 MINUTES EXPOSURE	
	2 MM. DECAP.	5 MM. DECAP.	8 MM. DECAP.	60 METER-CANDLES	2 METER-CANDLES
	deg.	*deg.*	*deg.*	*deg.*	*deg.*
Not decapitated	10.2	9.1	9.1	7.4	28.9
2 minutes	6.8	.....	.....	.....	.....
10 "	5.1	3.0	.....	1.8 (base)	−0.4
30 "	1.6	3.0	.....	2.3 (base)	0.9
60 "	1.8	1.7	.....	.....	.....
80 "	.....	.....	.....	1.6	*3.6* (top)
90 "	*4.6*	1.2	1.5	.....	.....
120 "	7.3	*3.5*	0.4	*2.0* (top)	4.3 (top)
150 "	7.6	4.1	*3.8*	.....	.....
180 "	.....	.....	.....	3.3 (top)	3.0 (top)

experiments were carried out to determine the extent of geotropic curvature in coleoptiles of which 5 mm. had been removed. Only a few results are reproduced in table VII. It was found that immediately after decapitation a fair geotropic sensitivity still existed which rapidly disappeared within the next half hour. All these geotropic curvatures after decapitation appear low down in the base, but after 120 to 150 minutes ordinary geotropic sensitivity reappears resulting in curvatures near the apical cut surface. The shorter the decapitated tip was, the earlier the geotropic sensitivity reappeared.

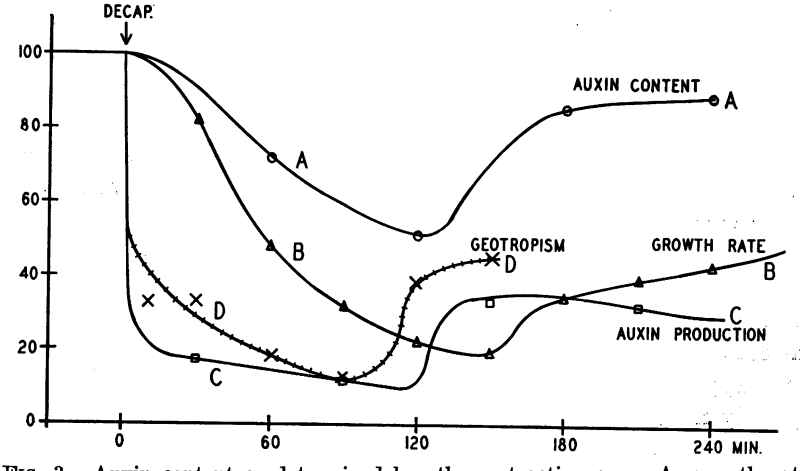

FIG. 3. Auxin content as determined by ether extraction curve A, growth rate B; curve C represents auxin production of coleoptile rings 5 mm. below tip as determined by diffusion into agar, and D geotropic response of Avena coleoptiles, in the 4 hours following decapitation of a 5-mm. long tip. All values expressed as percentage of values before decapitation (ordinate). For further explanation see text.

Almost the same behavior was observed for phototropic curvatures provided that only limited amounts of light were used and when a differentiation between the basal curvatures and the curvatures first appearing near the apex was made. Under such conditions it is easy to determine when the phototropic curvatures increase again as a result of regeneration of auxin by the apical cut surface. Because of the complexity of the phototropic response, however, no further data will be given since the details of the experiments would only confuse the main issue. It suffices to state that immediately after decapitation the phototropic falls off more rapidly than the geotropic sensitivity. After 2 hours, however, a small part of the phototropic sensitivity is restored.

Discussion

As was already known from the literature, regeneration of auxin production, growth, and tropisms are closely related in decapitated Avena coleop-

tiles. In figure 3, all data from this paper on 5-mm. decapitated plants have been summarized, in all cases taking the intact plant as reference (100). The growth rate curve (B) is the smoothed curve derived from figure 1, corrected for the decreasing growth rate of the intact plants. The auxin content, curve A, is obtained from table I; the curve drawn between the points, which give the actual auxin content at 1, 2, 3, and 4 hours after decapitation, is purely arbitrary.

The auxin production curve, (C), is taken from figure 2. It should be drawn as a block diagram, but it has been smoothed for convenience. It shows that after 2 hours auxin is being produced, and this production continues at a constant or even increased rate. The curve for the geotropic sensitivity (D) is taken from the 5-mm. decapitated plants of table VII. The time of return of geotropic sensitivity lies somewhere near 120 minutes.

In comparing the 4 curves of figure 3, one fact stands out: curves A and B are very similar, as are curves C and D. From this figure it is evident that growth rate B and extractable auxin content A of coleoptiles parallel each other, with an increasing lag of the growth rate. This lag would be expected, since the growth response of coleoptile cells to auxin gradually decreases after decapitation (table II).

Curves C and D, which resemble A and B in their sudden increase around 120 minutes, are very different in other respects. Instead of slowly falling off in the first two hours, this drop is very sudden, and is even much more pronounced than in figure 3; for the actual *auxin* production in decapitated plants should be measured with the standard Avena test, and not with the deseeded test. Since only data for 2-hour auxin production are available with the standard Avena test (table III) no curve could be drawn with them, and the data obtained with the deseeded test have been substituted; these, as discussed before, are too high for the period before regeneration. The drop after three hours is not real, but occurs only in sections cut at the moment of decapitation. For in table VI it is shown that many hours after decapitation auxin production by the regenerated tip increases instead of decreases as in cut sections.

Curve D is also too high for the first 100 minutes, since the geotropic curvatures still appearing after decapitation occur only in the most basal zones. On the other hand, the curvatures of intact and regenerated decapitated plants are mainly in the more apical zones.

A number of conclusions can be drawn from this figure 3:

a) The sudden suspension of auxin production after decapitation is only gradually reflected in a decreased auxin content of the whole coleoptile, and soon after auxin production is resumed the auxin content of the tissues increases again. BONNER and THIMANN (**2**) have shown that the decrease in auxin content in the first two hours after decapitation corresponds quanti-

tatively with the amount of growth which has taken place in the coleoptile. Considering this quantitative correlation, and the similarity between extractable auxin content and growth rate, it cannot be doubted that the growth rate of the Avena coleoptile is regulated by the amount of *extractable* auxin in the tissues.

b) The growth rate of the decapitated coleoptile is *not* correlated with the amount of diffusible auxin; in other words, with the *rate* of auxin *production.*

c) The sudden drop in geotropic (and phototropic) response, to less than one half the initial value within 10 minutes after decapitation, cannot be accounted for by the drop in auxin content (probably 1 to 2 per cent. within the first 10 minutes).

It closely parallels the amount of diffusible auxin, however, and comes up as soon as auxin production is resumed in the regenerated physiological tip.

Consideration of all these facts leads to the main conclusion: Diffusible or free moving auxin and extractable auxin are quantitatively different in the plant; and as far as their effects go, also qualitatively. The diffusible auxin in the Avena coleoptile is correlated with tropisms. Since it has been concluded before that these are due to changes in the path of transport of auxins (CHOLODNY-WENT'S theory) this might have been expected. Besides, since geotropism, and phototropism in part, are correlation phenomena, it is evident that they could be brought about only by a correlation-*carrier,* or moving agent; in other words, by free moving auxin.

The extractable auxin, comprising both free moving and bound auxin, is quite evidently *not* correlated with tropisms. But the growth rate closely parallels the extractable auxin; we must thus conclude that growth is due to the bound auxin. Therefore, the two methods of obtaining auxin, by diffusion and by extraction, are not interchangeable, and are not even comparable. This fact is brought out very clearly by VAN OVERBEEK (**11**) on completely different grounds.

Much confusion will be prevented by adapting the method of collecting and measuring auxin to the specific problem to be analyzed. If a correlation phenomenon is under investigation, it should be related to the diffusible auxin; growth rates should be compared with extractable auxin. This explains also why AVERY and LARUE (**1**) concluded that "Growth hormone secreted by the coleoptile tip has no helpful relationship to growth of coleoptiles in culture" for it is not the auxin formation but the auxin and precursor *content* which determines the growth rate. But it is equally clear that their other conclusion "that growth hormone is not a necessity for elongation of the coleoptile" does not follow from their experiments.

It is perhaps cogent to point out that the data presented in this paper very strongly oppose the suggestion made by SÖDING (**8**) and JOST (**5**) that auxin could be formed as a result of growth. Experimental evidence is overwhelmingly in favor of the conclusion that increased auxin content and growth follows increased auxin formation.

Since we must distinguish between two successive reactions in which auxin takes part, which have very different characteristics (**14**), and since we know that auxin is present in at least two different conditions inside the plant, it is interesting to attempt a correlation of these facts. In table VIII such an attempt is made. Thus far no facts are known which do not fit into this scheme. Some further remarks concerning each of the points of table VIII follow:

TABLE VIII

PROPERTIES OF AUXIN INSIDE THE AVENA COLEOPTILE

FREE MOVING AUXIN	BOUND AUXIN
1. Can be collected by diffusion out of the living organ into water (agar); its movement inside the plant is polar.	Extractable from the killed cells by organic solvents (alcohol, ether, chloroform, etc.)
2. It is derived from an auxin precursor.	Derived from the free moving auxin. Once it is bound it apparently is not converted into free moving auxin again.
3. Responsible for correlation phenomena such as tropisms, bud inhibition.	Responsible for visible cell elongation and root formation.
4. Not activated by light.	Partially inactivated by light.
5. Affects the first stages of growth and root formation, which are all due to the so-called preparatory reaction. This function can also be performed by hemi-auxins.	Causes the growth and root forming reactions proper.
6. Action not dependent on pH.	Reactive only at lower pH.
7. Double bond in molecule not essential for activity.	Only active with double bond in molecule.
8. Affects transport of other growth factors (calines).	Reacts with other growth factors (calines).
9. No stoichiometric relationship between chemically different free auxins and their effects has been pointed out as yet; probably does not combine with other compounds; otherwise it would not be free moving.	Stoichiometric relationship between bound auxin and growth suggests chemical combination with some other cell constituent.
10. Present in protoplasmic interphase?	Present inside protoplasm?

1. Is a description or definition.
2. This is the conclusion already reached (**15**).
3. Incorporates the conclusions from this paper, with additions (**14**).
4. Gives the conclusion reached by STEWART and WENT (**10**).
5. Gives the suggestion mentioned earlier in this paragraph. It is to some extent based on (3) and the discussion by WENT (**14**).
6. Follows from (5) and WENT (**14**).
7. In a paper by KOEPFLI and WENT to appear shortly it will be shown that the double bond in the auxin molecule is not essential for preparatory activity; some hemi-auxins do not have this double bond.

8, 9, and 10 are quite hypothetical; further discussion can be found in (**14**).

Summary

Avena coleoptiles were decapitated, and at suitable intervals, one-half to one hour, a number of their properties were measured. It was found that after decapitation the auxin content of the tissues, as determined by ether extraction, slowly fell off to 50 per cent. after 2 hours and then increased again. A similar curve was found for the growth rate, only the decrease was greater, especially after 2 hours. It was concluded that the growth rate is due to the amount of extractable auxin present in the tissues.

In the case of diffusible auxin from the various coleoptile zones, it was found that immediately after decapitation only small and decreasing amounts of precursor could be collected from any part of the coleoptile, and no auxin. But after 2 hours auxin production again became evident in the apical section. An almost identical behavior was found for geotropism and phototropism, a very abrupt falling off of geotropic sensitivity, which remained at a low level until approximately 2 hours after decapitation when it increased to about half normal. It was concluded that the geotropic curvature is due to an effect of gravity on the diffusible auxin.

This paper is also a complete confirmation of DOLK'S thesis (**3**) that regeneration of the auxin production 2 to 3 hours after decapitation accounts for the reappearance of geo- and phototropic sensitivity and increased growth rate.

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