STUDIES ON THE GROWTH HORMONE OF PLANTS. II. THE ENTRY OF GROWTH SUBSTANCE INTO THE PLANT

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1. *Introduction.*—In recent years methods have been developed whereby the growth substance or hormone which controls the elongation of plant cells may be prepared. Furthermore, it has been shown that this substance may be quantitatively determined. This was first done by F. W. Went (1928) who obtained the growth substance by allowing tips of Avena coleoptiles to stand upon agar. He found that if the agar was then placed upon one side of a decapitated coleoptile, the increase in growth on that side produced a curvature which was, he found, a measure of the amount of growth substance in the agar. Since the amount of growth substance in the agar was determined by the number of tips which were placed upon it for a given time, he was able to establish that there was a proportionality between the growth substance added and the curvature produced. This proportionality held up to a certain limiting curvature which has been called the "maximum angle."

Recent studies have made it possible to obtain the growth substance in much larger amounts. Nielsen (1930) showed that it was produced by the growth of molds and Bonner (1932) studied the conditions under which it was produced by Nielsen's culture of *Rhizopus suinus*. Studies on large scale production were carried out by Thimann and Dolk (1932), using the same mold.

The partially purified extract from this source has been used in these experiments. The use of a solution of high activity, from which dilutions may be made, enables repeated tests to be carried out with accurately controlled quantities of growth substance. This procedure is much more quantitative than the method of allowing a given number of coleoptile tips to stand on agar. In a preceding communication the chemical properties of the growth substance were studied and a method of partial purification worked out (Dolk and Thimann, 1932). It is of interest that Kögl and Haagen-Smit (1931) have obtained either the same substance or one of a similar physiological action from urine and have been able to prepare a crystalline product of high activity.

In the course of experiments to elucidate the relationship between growth substance and growth in the plant, it was necessary to determine whether all of the growth substance applied to the plant actually entered, or, if not, what proportion remained in the agar. The experiments of Went (1928) seemed to show that the curvature is proportional to the *amount* of growth substance in the agar block. Thus, he found that if its concentration was reduced by some factor, and the size of the block increased by the same factor, the resulting curvatures appeared to be the same. However, van der Wey (1932) found that the curvature was proportional to the *concentration* of the growth substance in the agar and nearly independent of the size of the blocks. In general, we have confirmed the finding that the curvature is proportional to the concentration of growth substance and not to the amount of it present in the block. It will be shown, however, that if it be assumed, as seems justified, that the rate of entry of the substance into the plant is proportional to its concentration in the block at each moment, then the variation of curvature with size of block is a necessary consequence, and that hence an explanation of the divergent results of different investigators is found.

2. *Methods and Materials.*—The concentrated growth substance was prepared from a culture of the mold, *Rhizopus suinus*, grown upon a large scale as described by Thimann and Dolk (1932). The filtered and evaporated medium was extracted with ether, and the product of the first three extractions, freed from ether and separated from insoluble oils, was used in these experiments.

The pure line of Avena, "Siegeshafer," which was used was kindly supplied by Dr. Åkermann of Svalöv. The quantitative assay of the extracted substance depends, as already pointed out, upon the curvature produced by the difference in growth between the two sides of a decapitated coleoptile, when one side is supplied with the growth substance. This technique was originated by Went (1928), who has also described the determination of the resulting curvature by direct measurement of the angle. The method used by Nielsen (1930) and by Boysen-Jensen (1931) to determine the curvature involves three measurements: radius of curvature, length of curved portion and thickness of the coleoptile, and is on this account more troublesome. It was also pointed out in the preceding communication that since radii of curvature greater than 8 cm. have to be neglected, the relationship between growth substance and curvature is no longer linear at high dilution. In a paper recently received, (1932), Boysen-Jensen has objected to this criticism, but it seems to us still to be valid.

The standard agar blocks were made up using the cutting instruments of Dolk (1930); each had a volume of 10.7 cubic millimeters.

3. *The Proportion of Growth Substance Entering the Plant under Defined Conditions.*—To determine what proportion of the applied growth substance actually enters the plant, agar blocks containing growth substance in suitable dilution were prepared and placed upon decapitated coleoptiles in the usual way. At the expiration of the test the curvatures were photographed, the blocks removed and placed upon another set of de-

capitated coleoptiles. After 110 minutes the curvatures were again photographed. The preliminary results, given in table 1, show that

TABLE 1

CURVATURES FROM REPEATED APPLICATIONS OF BLOCKS

FIRST APPLICATION		SECOND APPLICATION	
CURVATURE	NO. OF PLANTS	CURVATURE	NO. OF PLANTS
13.2°	12	12.2°	12
12.2°	12	8.5°	12
6.8°	6	7.2°	6

the curvatures given by blocks which have been, for a second time, 110 minutes upon coleoptiles, are not greatly smaller than those given the first time. Since these results are the reverse of those which would be expected according to Went, the experiments were repeated using growth substance obtained from the tips of Avena coleoptiles. This was done to preclude the possibility that the behavior of this preparation might be different from that of the growth substance obtained from Rhizopus. Tips were therefore cut off, placed upon agar, the agar left 30 minutes to allow for the attainment of uniform distribution, divided into twelve and tested as before. Table 2 shows that the results, although somewhat irregular, were essentially the same, i.e., while a curvature was produced at the second application, it was distinctly less than that produced at the first.

TABLE 2

EXPERIMENTS WITH THE GROWTH SUBSTANCE FROM AVENA TIPS

	TIME ON AGAR	FIRST APPLICATION		SECOND APPLICATION	
NO. OF TIPS	MINUTES	CURVATURE	NO. OF PLANTS	CURVATURE	NO. OF PLANTS
8	85	3.4°	10	3.5°	10
16	90	6.3°	10	6.0°	9
14	120	9.8°	12	7.5°	11
30	122	11.7°	12	8.5°	10

The next step was to measure as accurately as possible the decrease in curvature at the second application. For this purpose a standard growth substance solution was made up and assayed by the curvature method. The curvatures produced at the first and second applications were then measured upon a large number of plants. Table 3 shows that the fractions of the growth substance which leave the block at different concentrations are reasonably constant, (13–21%), particularly when it is considered that the quantity involved is a rather small difference between two determinations which are both subject to some variation.

In the case of the experiment at 19.3 units concentration, the initial curvature was calculated from assay at lower concentration, and the second curvatures were very close to maximum angles and are therefore somewhat low. This experiment is therefore not included in the mean.

4. *Theoretical Considerations.*—Let it be first assumed that:

The rate of gain of growth substance by the plant is proportional at any moment to its concentration in the agar block at that moment..........(1)

TABLE 3

DECREASE OF CURVATURE FROM REPEATED APPLICATION OF BLOCKS

CURVATURE FIRST APPLICATION	SECOND APPLICATION	PERCENTAGE DECREASE	NO. OF PLANTS
9.0°	7.6°	16%	20
9.0°	7.7°	14%	20
9.5°	8.3°	13%	22
9.2°	7.7°	16%	19
15.2°	12.5°	18%	20
14.1°	12.0°	15%	14
13.9°	12.1°	13%	14
* 19.3°	15.7°	19%	23
* 19.3°	15.2°	21%	25
		Mean of first seven determinations = 15%	

* Assay at lower concentration.

This assumption is not in conflict with the views of any investigator in this field, and it will be shown that the results to which it leads, both in regard to repeated applications of the same block, and in regard to the variation in curvature with size of block, are in accord with those obtained by experiment.

Let the concentration of the substance in the block at any moment be x/v, where x is the amount in grams, and v the volume of the block. Then from (1),

$$dx/dt = - k\,x/v. \qquad (2)$$

On integration this becomes

$$\ln x_1/x_2 = k(t_2 - t_1)/v.$$

In experiments carried out, as were ours, over a constant period of time, (110 minutes) we can put $k(t_2 - t_1)/2.303$ as a new constant K, i.e.,

$$\log x_1/x_2 = K/v. \qquad (3)$$

Now the excess growth produced by the growth substance must be proportional to the amount of substance entering the plant, or, in the case of unilateral growth, the curvature θ is proportional to Δx, the amount of growth substance which enters the plant in the 110 minutes allowed, i.e.,

$$\theta = c(x_1 - x_2) \qquad (4)$$

where x_1 is the amount initially in the block, x_2 the amount remaining in the block at the end of the test and c the proportionality constant.

Now consider the case of repeated application of a block of volume v. Equations of the type (3) and (4) may be written for each application:

$$\log (x_1/x_2) = K/v = \log (x_2/x_3)$$

and

$$c(x_1 - x_2) = \theta_1; \quad c(x_2 - x_3) = \theta_2. \tag{4a}$$

Hence

$$x_2 = \frac{x_1}{\text{antilog } K/v}; \quad x_3 = \frac{x_1}{(\text{antilog } K/v)^2}.$$

Substituting for x_2 in equation (4a)

$$\theta_1 = cx_1\left(1 - \frac{1}{\text{antilog } K/v}\right)_\lambda. \tag{5}$$

Similarly, substituting for x_3,

$$\theta_2 = \frac{cx_1}{\text{antilog } K/v}\left(1 - \frac{1}{\text{antilog } K/v}\right). \tag{6}$$

Hence

$$\theta_1/\theta_2 = \text{antilog } (K/v). \tag{7}$$

From the mean value given in table 3, the percentage decrease in curvature is 15%, i.e., the ratio between the angles at the first and second applications is 100/85, or

$$\theta_1/\theta_2 = 100/85 = \text{antilog } (K/v).$$

From this

$$K/v = \log 1.177 = 0.0709. \tag{8}$$

The value of K/v is independent of the concentration of growth substance and size of block, and is a characteristic constant for the reaction here considered.*

The quantity x_1, the amount of growth substance originally present in a block giving a curvature θ, is of some interest. For the block giving a curvature at the first application of 9°, its value is $60°/c$, where c is the relation between grams and degrees. This means that with an infinite number of tests the sum of the curvatures obtained would be 60°.

5. *Influence of Size of Block.*—If now the volume of the block be reduced from the size first assumed, v_0, to $1/n$ of this, then the right-hand side of equation (2) becomes multiplied by n, and hence the rate at which the amount in the block changes is correspondingly increased. Thus, the smaller the block, the larger the percentage of its growth substance which will pass out during the test. The ratio θ_1/θ_2 will therefore be increased for smaller blocks, i.e.,

$$\theta_1/\theta_2 = \text{antilog } (Kn/v_0). \tag{9}$$

It might be objected that a decrease in the volume of the block is accompanied by a decrease in the area of contact between block and coleoptile; however, the accompanying sketch, to scale, figure 1, indicates that even with blocks one-eighth of our standard size, the area of contact is completely unchanged. It is not necessary, therefore, to consider changes in the area of contact in these experiments.

However, it has been found that if the block be placed flat on a coleoptile from which the primary leaf has been pulled out, the rate of entry of the growth substance is more than doubled. In this case the contact area is correspondingly more than twice that shown in the left-hand diagram.

From considerations similar to those above, it is possible to calculate the curvature which would be expected from a block of any given size, provided that the curvature resulting from the use of a block of another size, containing the same concentration of the growth substance, is known.

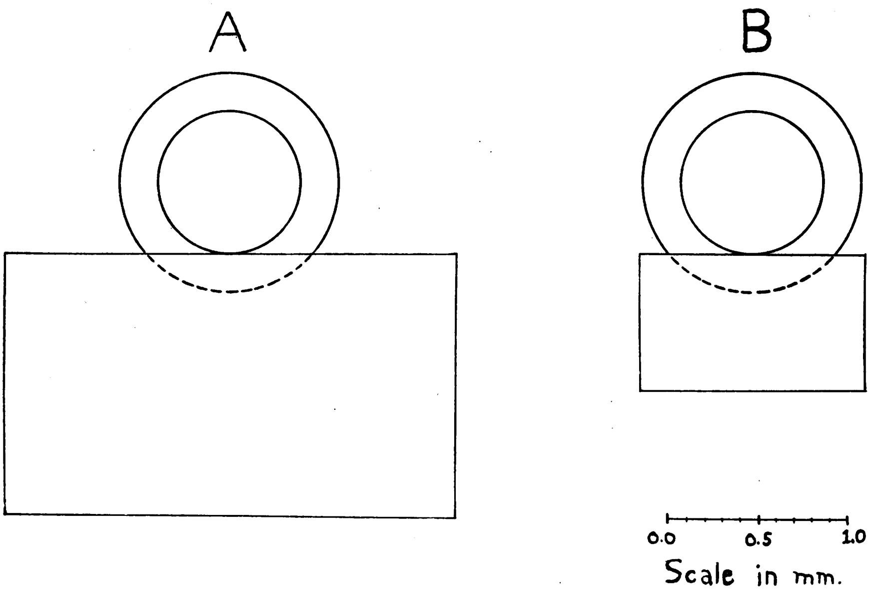

FIGURE 1

Agar blocks resting unilaterally upon decapitated coleoptiles, and in contact with the primary leaf. A. Standard size block. B. One-eighth block. The area of contact is the same in each case.

When a block of our standard size, v_0, is used, the resulting curvature may be designated θ_0. From equation (5)

$$\theta_0 = cx_0\left(1 - \frac{1}{\text{antilog } K/v_0}\right)$$

or, putting in the value of K/v_0 obtained above,

$$\theta_0 = 0.150cx_0. \tag{10}$$

It might have been foreseen that, since θ is proportional to x, and since successive θ's decrease by 15%, therefore successive x's decrease by 15%, and hence the amount passing into the plant, which is responsible for the curvature, is proportional to 15% of x.

The amount of growth substance in blocks having the concentration above, but volume v_0/n, is $x'_1 = x_0/n$, and the angle obtained at the first application is given by the equation

$$\theta'_1 = \frac{\theta_0}{0.150n}\left(1 - \frac{1}{\text{antilog } Kn/v_0}\right). \tag{11}$$

Similarly the angle for a second application can be obtained:

$$\theta'_2 = \frac{\theta_0}{0.150n \cdot \text{antilog } Kn/v_0}\left(1 - \frac{1}{\text{antilog } Kn/v_0}\right). \tag{12}$$

With the aid of equations (11) and (12), the curvatures θ'_1 and θ'_2 caused by blocks having a volume any fraction $1/n$ of our standard blocks, may be compared with the curvature θ_0 caused by blocks of the standard volume and of the same initial concentration. Table 4 shows the values of θ'_1 and θ'_2 as a factor of θ_0.

TABLE 4

COMPUTED RATIOS FOR THE CURVATURES OBTAINED WITH FRACTIONAL BLOCKS TO THOSE OBTAINED WITH WHOLE BLOCKS. VALUE OF K/v TAKEN FROM (8)

RATIO OF BLOCK SIZE, n	ACTUAL VOL. OF BLOCK, MM.^{3}	ANTILOG $\frac{Kn}{v_0}$	$\frac{\theta'_1}{\theta_0}$	$\frac{\theta'_2}{\theta_0}$
1	10.7	1.18	1	0.85
2	5.35	1.39	0.915	0.66
4	2.68	1.93	0.790	0.41
8	1.34	3.73	0.598	0.16
9	1.19	4.43	0.573	0.129
10	1.07	5.23	0.539	0.103
12	0.89	7.29	0.479	0.066

For experimental determinations of these curvatures, the 12 blocks made by the special cutter, each of the volume 10.7 mm.^{3}, were further subdivided into 2, 4 and 8. This was done by hand, using the cutter blades without the socket, and to allow for inequalities in the resulting blocks, as many as possible of them were used and the curvatures averaged. Thus, if one of the originally equal blocks were divided into two unequal halves, both halves would be used and the inequality balanced out.

With these small blocks the risk of drying out during the test is much greater. This may lead to anomalous results, as pointed out by Went (1928). Special precautions were therefore taken, and a high humidity was successfully maintained without guttation.

The collected results are given in table 5, together with the expected curvatures calculated from the ratios in table 4. Considering that the curvatures are only measured to a degree, and that the fractions are obtained by averaging a number of plants (in general from 12 to 24), we consider the agreement between observed and calculated values to be fairly satisfactory.

It is thus interesting to observe that a relationship derived from experiments with repeated application of the same block can be used to predict the curvatures obtained from a single application of fractional blocks.

TABLE 5

CURVATURES OBTAINED WITH FIRST AND SECOND APPLICATIONS OF FRACTIONAL BLOCKS

EXP. NO.	CURVATURE WITH STANDARD BLOCK, θ_0	SIZE OF FRACTIONAL BLOCK	CURVATURE WITH FRACTIONAL BLOCK FIRST APPLICATION OBSERVED θ_1'	FIRST APPLICATION CALCULATED θ_1'	SECOND APPLICATION OBSERVED θ_2'	SECOND APPLICATION CALCULATED θ_2'
4	9.6°	Half	9.2°	8.8°	..	..
5	13.8°	Half	12.6°	12.6°	..	..
	13.8°	Quarter	10.6°	10.9°	..	..
	13.8°	Eighth	9.1°	8.2°	..	..
6	14.4°	Half	12.9°	13.0°	9.0°	9.5°
	14.4°	Quarter	9.0°	11.4°	6.5°	5.9°
	14.4°	Eighth	7.9°	8.6°	1.5°	2.3°
7	13.5°	Eighth	9.0°	8.1°	3.2°	2.2°
8	14.4°	Half	13.2°	13.0°	9.6°	9.5°
9	15.6°	Eighth	8.5°	9.3°	2.5°	2.5°

6. *Comparison with the Results of Other Workers.*—The above considerations enable our results to be compared with those of Went. In order to find, as he did, that the curvature is proportional to the *amount* of growth substance in the block, the conditions must be such that all of the substance enters the plant. Since Went's *Avena* plants were from the same stock as ours, and since he allowed the same time (110 minutes) for the curvatures to develop, the values of K and t are the same, and hence the difference between his results and ours must be sought in a difference in the volume of the blocks. In general, the blocks of Went were much smaller than ours. His largest and smallest blocks had only 0.127 and 0.065, respectively, of the volume of our standard block (10.7 mm.^{3}).

Went presents a number of experiments designed to show that the curvature obtained was proportional to the *amount* of growth substance in the agar. In his determinations, which are summarized in table 6,

TABLE 6

COMPARISON BETWEEN WENT'S OBSERVATIONS AND OUR CALCULATIONS

DATA OF WENT: EXP. NO.	CONCN. OF GROWTH SUBSTANCE INITIALLY IN BLOCK	VOLUME OF BLOCK, MM.^{3}	ANGLE	COMPARISON: VOL. OF WENT'S BLOCK / VOL. OF OUR BLOCK	RATIO BETWEEN ANGLES OBSERVED	RATIO BETWEEN ANGLES CALCULATED
176	*a*	0.90	9.1 ± 0.4	0.083	1.52 ± 0.12	1.67
180	*a*/2	1.20	6.0 ± 0.5	0.111		
181	*b*	1.20	8.3 ± 0.6	0.111	0.91 ± 0.07	0.80
182	3*b*/2	0.90	9.1 ± 0.6	0.083		
190	*c*	0.90	8.6 ± 0.5	0.083	1.34 ± 0.14	1.21
194	*c*	0.69	6.4 ± 0.7	0.065		
333, 334	*d*	0.90	7.2 ± 0.6; 7.1 ± 1.0	0.083	1.43 ± 0.20	1.28
335	*d*	1.35	10.3 ± 0.4	0.127		

the different concentrations of growth substance were obtained by varying the number and diffusion time of coleoptile tips upon agar. For clarity we have expressed these in the table simply by the ratios of the concentrations used in each set of experiments. The first four columns give his essential data. Column 5 gives the ratio of volume between his blocks and our standard blocks, and columns 6 and 7 present the ratios between the angles, as observed by him, and as calculated by equation (11) using the factors in table 4.

It will be seen that the agreement, though not exact, is nevertheless fairly close. There is no systematic difference between the calculated and observed ratios, and since the determinations of Went were made with only 9 or 12 plants in each case, they must, however carefully carried out, have been subject to considerable variation. It is therefore clear that the results of Went, which previously had seemed to show that the curvature was proportional to the *amount* of growth substance, are equally in accord with our interpretation. The results of van der Wey, recently reported, seem to show a still smaller dependence of curvature upon block size than would be expected from our calculations. His experiments, however, were carried out using the method of double decapitation previously described by him, and under these circumstances it may well be that the numerical relations deduced above do not apply. His results, however, confirm ours qualitatively in that they show that only a small fraction of the growth substance in a block diffuses out at a single application.

Summary.—(1) An attempt has been made to provide a theoretical foundation for the process by which the growth-promoting hormone or growth substance passes from solution in agar into decapitated coleoptiles of *Avena*.

(2) By applying an agar block containing growth substance to a succession of test plants and measuring the resulting curvatures it has been found that a constant fraction of the growth substance in the block passes into the plant.

(3) Upon the assumption that the amount of growth substance entering the plant is proportional to its concentration in the agar, our data make possible a calculation of the reaction rate constant for the process. This constant is independent both of the concentration of growth substance and of the size of block.

(4) For blocks of different sizes, curvatures calculated from the value of this constant agree closely with those observed.

(5) The data of Went, which were originally believed to indicate a different behavior of the growth substance, are shown to agree, within the limits of experimental error, with the mechanism here described.

The authors take this opportunity of thanking Mrs. F. Dolk for her untiring and skilful assistance.

* It has, however, been shown by Du Buy that the curvatures obtained are reduced when higher concentrations of agar are employed. Our value of K/v applies to 1.5% agar.

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IMPULSES FROM SENSORY NERVES OF CATFISH

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Using a Matthews[1] amplifier and oscillograph in conjunction with a loud speaker, a standing wave screen and a camera, electrical responses were recorded from various sensory nerves of the catfish *Ameiurus nebulosus* (Les.) in response to mechanical, thermal and chemical stimulation of the receptors which these nerves supply. The nerves tested were lateral line nerves, spinal nerves supplying the skin of the flank, and branches of the facial nerve supplying the tactile endings and tastebuds of the lips and barbels. Some 52 nerves were examined in 32 fishes.

After severing the medulla oblongata (in some cases brain and cord were pithed), the nerve to be tested was exposed at a point as near its region of emergence from the central nervous system as was anatomically convenient, and freed from surrounding tissues for a suitable length. It was then tied proximally with a thread, cut and drawn across silver, silver chloride electrodes connected to the recording system. The preparation was generally so arranged that the receptive areas were kept immersed in water while the nerve and incision were out of water and bathed with Ringer solution.

Maps of skin areas supplied by spinal and facial nerves were made by stroking the skin with the tip of a feather and listening to the bursts of impulses from the loud speaker. In this way it was possible to ascertain the particular skin areas supplied by a branch of nerve. By means of the volume of sound, it was also possible to determine the relative distribution of tactile sensitivity of the skin.