Botany. — *Further Experiments on the Mechanism of Growth.* By A. N. J. HEYN. (Communicated by Prof. F. A. F. C. WENT.)

(Communicated at the meeting of March 28, 1931.)

In my experiments communicated before (Proceedings Vol. XXXIII, N°. 9, D. p. 1045) it appeared that the extensibility of cut-off coleoptiles, as it can be measured after plasmolysis, is about the same in normal and in decapitated specimens a few hours after decapitation. It appeared at the same time that the extensibility of both was much less than that of plants growing normally. On the strength of this the conclusion that growth-substance does not exert any influence on the extensibility, was not yet justified. For in the first place the production of growth-substance may be greatly diminished or arrested in cut-off coleoptiles; in the second place the transport can greatly decrease. Further the possibility exists that the action of growth-substance can only take place, if at the same time also lengthening is possible. Finally the variation in the extensibility of the cell wall may disappear by plasmolysis or wilting.

G. I have, therefore, examined in the first place whether a difference of extensibility exists in cut-off coleoptiles, on which agar blocks with, or agar blocks without growth-substance were placed. The following table gives a survey of all the experiments.

Number of plants	Extensibility without growth-substance	with growth-substance	Normal coleoptiles	Hours after decapitation	Action on the plant	Time of plasmolysis
3—3	12.7	18.7		0	24 hours	21 hours
6—5	10.9	18.5		0	18 „	4½ „
8—8	17.6	18.6		0	10 „	13 „
6—8	17.5	18.4	20.2	0	2 „	5½ „
7—8	17.4	18.4	24.2	0	24 „	24 „
9—9	16.9	17.7		0	17 „	2 „
8—10	19.8	21.1		0	13 „	24 „
7—8	11.1	14.6		0	18 „	2 „
8—8	11.0	12.6		0	19 „	25 „
8—8	19.7	21.2	24.0	2	7 „	
Average	**16.5**	**18.0**	**22.8**			
	Difference	1.5 unit				

Accordingly there is a slightly greater extensibility in the coleoptiles with growth-substance than in those without, in both the extensibility is less than in normal coleoptiles comparable with them, quite in accordance with what was found for cut-off coleoptiles decapitated or not decapitated.

In order to obtain perfect certainty that the differences found are real, and not owing to errors of observation, I have finally compared the extensibility in cut-off normal and decapitated coleoptiles by means of an apparatus with which, quite objectively and very accurately, the variation of the distance between the marks was determined in units of 11 μ.

Number of plants	Extensibility		Time after decapitation
	normal	decapitated	
2—3	58.3	56.3	$1^1/_2$
5—5	55.4	54.6	$1^1/_2$

I have shown in my previous communications that the growth of coleoptiles under the influence of the concentration of growth-substance always used in these experiments, is equal to, if not greater, than of normal coleoptiles. If it is taken into consideration that the difference in extensibility between cut-off coleoptiles with and without growth-substance is on an average 1.5 units, and that between normally growing decapitated coleoptiles and normal ones, e.g. in *B* p. 1052, is 6.6 on an average, it is very improbable, indeed, that the small difference in extensibility which we found in cut-off coleoptiles, would be the cause of the difference in growth.

Shortly I intend to show this also by direct calculation. In what follows I will also demonstrate that *the decrease of the extensibility* as I described this in my previous communications, which takes place with cut-off coleoptiles, and of which I will show that it is identical with the similar process as this is found after decapitation, may be attended with an *increase of the power of growth.*

In my previous communication *F.* p. 1057, I showed at the same time that it is very probable that the difference in extensibility is the *result* of the growth. It actually appeared now that the cut-off coleoptiles with growth-substance had grown a little during the time that they were under the action of the top.

While it has been made exceedingly inplausible that the difference in growth can be accounted for by the difference in extensibility, as this can be measured after plasmolysis, it will be demonstrated in what follows that *in the cut-off coleoptiles considered the action, the transport, and the production of growth-substance continues all the same.*

H. For this purpose I have examined whether in cut-off coleoptiles

actually a difference in cell extension could be detected in normal and decapitated plants (some hours after decapitation), when they were placed in water.

The coleoptiles were marked in the same way as in the determinations of extensibility. The distance between the marks, which was again about 12 mm., was measured by means of an apparatus which is to be described more closely. The numbers represent units of ± 11 μ. The accuracy was one unit. Then the coleoptiles were placed in water at room temperature, and measured again after some time. Always alternately a normal coleoptile was measured, and one that had been decapitated. Before being measured and placed in water, the coleoptile was cut away close under and above the two marks, so that the power of imbibition of water by the planes of section was the same in the two groups of coleoptiles.

The following table shows the results of the investigation :

Number of plants	Increase of length		Time after cutting off and decapitation	Time in water
	Normal	Decapitated		
a. 6—7	**37.3**		1 hour	
7—8	36.1	27.3	2.5 hours	
7—8	32.3	24.0	3 hours, 10 min.	2 hours
b. 5—6	31.4	24.2	1/2 hour	
7—4	**37.0**	24.5	1 „	
5—7	34.0	31.8	3 hours	
c. 5—6	**47.1**	28.3	1 hour	
5	33.4		3 1/2 hours	2 1/2 hours
2—2	26.2	28.5	5 „	

With this should be compared the extension that appears when the plants are placed into water immediately after having been cut.

Normal and decapitated plants will have to behave in the same way in this respect. Comparision of these two series only supplies a datum on the limit of errors of the method. The results can be seen from the table on page 477.

It appears, therefore, that the greatest increase of length is found in normal coleoptiles, when they are placed in water one hour after having been cut off.

This increase of length is greater than in freshly cut coleoptiles, and diminishes again when the coleoptiles are left cut off for a longer time, before being put in the water.

In coleoptiles that have been decapitated, the increase of length remains

the same as that of freshly cut coleoptiles. Not until they have been cut off for 3 hours or longer, do they show a greater extension (regeneration of the power of producing growth-substance?). At this moment there is no longer any difference between normal and decapitated plants.

	Number of plants	Mean norm.	Increase of length decapitated	Time after cutting and decapitation	Time in water
a.	6—4	23.0	23.1	0	2 hours
	5—5	46.6	27.6	1	
	the same plants kept in water for a longer time:				
	6—4	35.3	37.0	0	4 hours
	5—5	63.3	35.9	1	3 ,,
b.	3—3	17.7	18.8	0	1½ ,,
	5—5	31.2	22.6	1	1½ ,,

I showed in my previous communication that when the plants had been cut off, the extensibility as measured after plasmolysis always decreased. *Evidently this decrease has no influence on the possible further extension, neither in positive nor in negative sense,* as follows from the behaviour of the decapitated plants. I wish to point out this fact very emphatically, and I shall revert to it later on.

For the greater certainty I have repeated the experiments described so-that the quantity of growth-substance present was varied by placing agar with or without growth-substance on decapitated coleoptiles. If after the decapitation also some time is waited before the plants are cut off, the advantage is reached that before the experiment begins, the minimal quantity of growth-substance is present in the coleoptiles, which was not the case with the first arrangement of the experiment. In the coleoptiles which were not provided with growth-substance, a smaller increase in water must be expected than was found in the earlier experiments, which actually appeared to be the case.

To give an idea of the limit of errors, I give in this case all the measurements.

Plants cut off ½ hour after decapitation. Agar with or without growth-substance placed on them. Time of action 2 hours, mean increase of length of the 12 mm. zone after remaining two hours in water after the removal of the agar blocks.

With growth-substance: 26, 32, 43, 28, 24, 55, 34, 30, mean **34.0**

Without ,, ,, 11, 19, 75, 15, 13, 14, 18, 11, ,, **13.4**

Thus it has been shown that the difference in lengthening in normal coleoptiles which have been cut off for some time, and those which were

besides decapitated during the same time, rests on the action of the growth-substance. At the same time this lengthening appeared (see above) to be independent of the fact whether the process of increase of the extensibility of the cell wall, which was already shown in my previous communication to be probably the process of opposition or intussusception, can take place.

I. It was now of the greatest importance to examine whether the increase in length, as it was described under *H*, is due to an increase of the *extension*, which would then increase in a much higher degree in coleoptiles with top than in those which had been decapitated.

If growth-substance acts on the extensibility (either as it can be measured after plasmolysis, or as it is present in fresh, not plasmolysed coleoptiles, for so far as these two are not identical), on the osmotic value of the cell juice, or on permeability or turgorpressure of the protoplasm, in the first place the *extension* will have to increase when coleoptiles are lengthened as the result of the action of the growth-substance.

To arrive at some conclusion about this, the coleoptiles, after they had lengthened in water for some time, and had been measured, were plasmolysed. Then the contraction that had taken place, was measured.

Number of plants	Lengthening in water		Time after cutting off and decapitation	Time in water	Decrease in length on plasmolysis	
	normal	decapitated			Normal	Decapitated
7—6	40.0	28.3	1 hour	2 hours	106.5	101.0

In the first series the shortening of coleoptiles, plasmolysed *before* the lengthening in water, was 86.5 mean of 4 plants (under *L* I will show that the contraction of normal and decapitated, cut-off coleoptiles is about the same for some hours after they have been cut off).

It appears from the table, that, though there is a distinct difference in lengthening, the difference in extension is insignificant in normal and decapitated plants.

While in the first series e.g. the ratio in lengthening is as 28 : 40, the ratio in the extension is at most as 101 : 106.

If the extension determines the growth, as is supposed in SACHS' mechanic theory of growth, it is certainly not to be expected, that there will be so little proportionality between extension and growth !

We considered, however, just now the total lengthening after two hours stay in water. If only the permanent lengthening is considered, we arrive at the same result. For this is : $86.5 + 40.0 - 106.5$ for normal plants, and $86.5 + 28.3 - 101.0$ for the decapitated plants. Hence the ratio is 27 : 40.

Even the absolute difference in extension between normal and decapitated plants (5.5) is much smaller than the absolute difference in increase of length (21.7).

By what precedes I think I have made it exceedingly probable, if it has not been proved, *that growth-substance does not exert a direct influence on osmotic value, permeability, for so far as these would increase the extension of the wall, on elastic extensibility of the cell wall or on any other factor increasing the extension,* as a consequence of which a stronger growth would be found, according to SACHS' mechanic theory of growth, (because only by extension deposition of new particles in the cell wall would become possible (SACHS)).

With the following experiments I have been able to prove this still much more completely.

In my experiments on the reaction of growth-substance on growth it had appeared, that already immediately after agar blocks with growth-substance had been placed on coleoptiles which had been decapitated for 2½ hours, and which had an exceedingly small growth, if any, a marked acceleration of growth sets in. After 1½ to 2 hours the growth has again become equal to that of normal plants, if not greater.

The following table gives some values, which express here again the growth in the successive half hours in units of 40 μ.

Normal	Decapitated		Normal	Decapitated		Normal	Decapitated	
—	6	5	—	8	9	11	4	4
9	3	3	10	2	3	13	2	1
11	3	3	11	0	1	13	0	2
9	1	1				13	2	0
8	3	2	11	0	1	12	3	3
8	2	2	11	2	6			
8	2	2	11	1	8	10	5	G. 5
			11	3	10	10	3	P. 8
8	2	G. 3	12	2	11	11	2	S. 9
9	3	P. 8	12	2	10			
6	1	S. 8	12	4	G. 10			
7	0	10	13	3	P. 11			
			13	5	S. 12			
			11	3	14			
			12	4	13			
			14	4	15			
			11	3	14			
			11	5	11			

The line always indicates the moment after which agar with growth substance was placed on the decapitated plant given in each third column (marked G. P. S. = growth promoting substance) and pure agar on the other decapitated plant.

I have now also investigated what is the process of extension, when $2\frac{1}{2}$ hours after decapitation growth-substance is applied in the same way.

The extension was measured after the contraction on plasmolysis.

The following table gives the result :

Number of plants	Contraction on plasmolysis		Time after decapitation	Contraction after G. P. S. action	Time of G. P. S. action
	normal	decapitated			
a. 7—8—5	123.0	110.4	2 hours	107.2	1 hour
b. 3—7—6	125.3	97.0	$2\frac{1}{4}$	104.1	2 hours
c. 5—5—3	119.0	95.4	$2\frac{1}{2}$	96.0	$1\frac{1}{2}$ „

Of part of the plants of series c. the growth was determined at the same time in order to be able to make a perfectly trustworthy comparison.

The table gives the growth in the same way an in the foregoing, the values for the coleoptiles with growth-substance in agar are the means of 3 plants. those of the normal control plants the means of 2.

Growth-substance plants ; 15 minutes after decapitation
5.7, 3.3, 2.3, 0.3, G. P. S. — 2.0, 5.0, 9.0, 9.0, 9.3.
Normal control plants
12, 13, 16.5, 14, 13.5, 13.5, 12.5, 12.5, 13.5, 10.5.

Accordingly while the growth rapidly increases when agar with growth-substance is placed on decapitated plants $2\frac{1}{2}$ hours after decapitation, the extension after 1 hour is still smaller, it is equal after $1\frac{1}{2}$ hours and even exceedingly little greater after as much as 2 hours, than immediately before the growth-substance agar was applied, and much smaller than of normal plants. By this it is excluded that the growth-substance directly influences any of the factors which, on increase, enhance the extension of the cell wall, as might be expected from the osmotic value of the cell juice, the permeability, or the pressure of imbibition of the protoplasm, or the elastic extensibility of the cell wall.

There is therefore no other possibility left, than that the direct influence of the growth-substance changes some condition or other of the cell wall which is not identical with elastic extensibility.

Accordingly SACHS *mechanic theory of growth, according to which the extension of the wall is the primary variable factor in the growth, is no longer tenable.*

If it is, therefore, assumed that the growth-substance acts on the cell wall, the following possibilities present themselves :

K. Among others URSPRUNG and BLUM advocate the view that the primary factor in the process of growth is the active growth of the cell wall. By this the existing extension is fixed, and further extension is rendered possible. In the same sense SÖDING recently expressed the supposition that growth-substance can have influence on the growth by intussusception of the cell wall. The cell wall would be made more extensible by it. For after the appearence of my paper: "On the Relation between Growth and Extensibility of the Cell Wall" SÖDING [1]) published an investigation in which he also finds, independently of me, that two hours after decapitation a difference in extensibility can be shown in coleoptiles. He cannot ascertain whether this difference in extensibility is cause or result of the growth, but thinks himself justified in assuming that it is the cause, and than he advances the above mentioned hypothesis.

I have, therefore, tried to examine this fixation process mere closely. For this purpose I compared the contraction on plasmolysis in 50 % potassium nitrate in freshly cut off coleoptiles with that, which takes place in the same coleoptiles, when they are first left standing in the room of 90 % humidity for some time after having been cut off.

Number of plants	Contraction		Time after cutting	Time of plasmolysis
	growing	cut off		
4—6	158±5.5	116±6.0	2 hours	2 hours
4—6	105	63	3 hours	3¾ hours

After the plants had been kept in the damp room for this time, the change in length was in the first series +18 units, in the second —14 units. The phenomenon is, therefore, not owing to dessication!

Hence, the extension decreases in a cut off coleoptile.

It is now exceedingly important, to examine, whether the phenomena of *fixation of extension* and *increase of extensibility*, as described in my previous communication (of which the probability was shown that it is the process of intussusception or apposition and for which supposition shortly I shall give further arguments) are causally connected.

As I already stated in my previous communication, low temperature inhibits the last mentioned process. It was now examined, whether low temperature also inhibits this fixation process in the same way.

At the same time I investigated the progress in time of the fixation process. The table at the top of page 482 gives the results.

The change of length of these coleoptiles during the time they were kept in the damp room was + 19.

Accordingly low temperature inhibits the process of fixation of the extension in the same way as that of the increase of extensibility.

[1]) Jahrb. f. wiss. Bot. Bd. LXXIV, H. 1, p. 127.

Number of plants	Contraction on plasmolysis		Time after cutting	Time of plasmolysis
	0° C.	23° C.		
8		109	0 hour	
8		85	1 hour	4 hours
9—8	87	55	5 hours	

Preliminary experiments seem to show that growth substance has no influence on the process of fixation of the extension.

For this purpose I compared the contraction on plasmolysis or wilting of cut off coleoptiles which had been decapitated for some time, with that of coleoptiles of which the top had not been removed. Before plasmolysis or wilting the tops were also removed from these.

Number of plants	Contraction		Time after cutting off decapitation	Time of plasmolysis or wilting
	Normal	Decapitated		
12—12	77.2	72.2	2 hours	3 hours wilting
12—11	91.7	91.4	$2^1/_4$ „	$2^1/_2$ „ „
6— 6	71.3	63.3	$1^1/_2$ „	$2^1/_4$ „ plasmol.

In this last series the contraction on plasmolysis of normal growing plants (which had not been cut off) was 102, number of plants 6.

This experiment was repeated so, that pure agar or agar with growth substance was placed on decapitated coleoptiles.

The table gives the contraction :

Number of plants	Contraction		Time of action on the plant	Time of plasmolysis
	With G. P. S.	Without G. P. S.		
6—5	84	87	1 hour	3 hours

In G. I also showed that growth substance has no influence on the increase of the extensibility, as this can be measured after plasmolysis. This, too, is in harmony with the supposition that the two processes mentioned are connected causally.

Accordingly, if growth substance does not affect these processes, it cannot be expected either that the fixation process and the parale! process

of decrease of extensibility will be attended with a power of greater lengthening, as this is found in growth-substance.

Now it may be seen from the data under *H* that actually this power does not exist. For it appeared here that the lengthening when decapitated coleoptiles, which had been cut off for 0, 1, 2 or 3 hours, were put in water, remains the same, whereas the extensibility and extension continually considerably decrease with the time.

L. The remaining possibility, nearly proved by the preceding experiments, is that the growth-substance influences the plasticity or rather the fluidity of the cell wall.

In the first place my preliminary experiments show that the normal turgor-pressure will cause a transgression of the elasticity limit of the cell wall and that the growth promoting substance acts on the rate of this process. To show this we repeated in the first place the experiments described under *G.* at a temperature of 1° C. by which temperature active growth of the cell wall and other physiological processes are inhibited. It can be seen that a permanent increase in length also appears if the coleoptiles (after or without treatment with growth substance) are brought in water of 1° C. It also appears that the growth promoting substance determines the rate of this process.

Number of plants	Elongation in water of 1° C.		Time after decapitation	Time of action G. P. S.	Contraction on plasmolysis before elongation in water of 1° C.			
	with G. P. S.	without G. P. S.			with G. P. S.	without G. P. S.	with G. P. S.	without G. P. S.
a. 8—7	14.7	9.6	2 hours	2 hours				
b. 4—4	8.0	2.8	3 hours	1½ hours			73.4	72.0
c. 8—8	9.3	3.4	3 hours	2 hours	96.8	81.3	91.7	79.4

a. 8 plants with tip, 7 without tip.

b. and *c.* Plants provided with agar with and plants provided with agar without G. P. S.

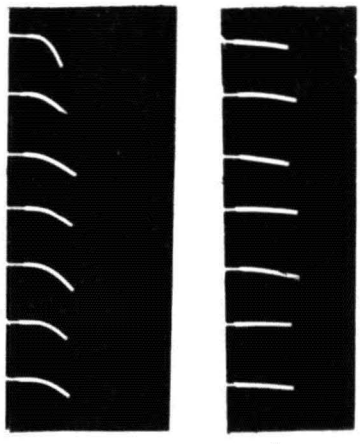
In *c.* the permanent increase in length amounts 96.8 — 91.7 + 9.3 = 14.4 for plants with and 81.3 — 79.4 + 3.4 = 5.3 for plants without G. P. S.

If we take the average of all data under *G.* it appears that the quotient of elongation between normal and decapitated plants is 1.45. At 1° C. this quotient is 1.53.

The quotient of plants provided with agar with- and without G. P. S. in series *G.* is 2.5. At 1° C. it is 2.9.

The quotient of the elongation is therefore independent of temperature, perhaps the quotient even increases at lower temperature.

In the second place, it could be demonstrated, that growth promoting substance influences the fluidity of the cell wall.



a b

Growth-promoting substance and plasticity of the cell-wall.

a. Coleoptiles provided with G. P. S.
b. Coleoptiles without G. P. S.

Riders of the same weight were placed on the free end of cut, non-growing coleoptiles which were placed in a horizontal position. The photographs show the extent of the resulting permanent curvature, after the riders are removed.

Cut off and decapitated coleoptiles were provided with blocks of agar with and without growth promoting substance. The G. P. S. is allowed to work for two hours. The coleoptiles were than placed in a horizontal position, fixed at the base. Throughout the experiment they remained in an atmosphere of 90 % humidity. Metal riders of equal weight of 0.25 grams were placed on the coleoptiles at a distance of 2 mm. from the free end. After two hours it was perceived, that the curvature of the coleoptiles with G. P. S. had increased markedly. When the riders were removed it appeared that the permanent curvature of plants without growth promoting substance is negligible, while plants which had been provided with G. P. S. showed a marked permanent curvature. The accompanying photograph illustrates these results.

The foregoing experiments prove that the Growth promoting substance influences the fluidity (plasticity) of the cell wall.

These experiments will be more fully described elsewhere.

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