Botany. — *On the occurrence of growth-accelerating and growth-retarding substances.* By CHRISTINE J. GORTER. (Communicated by Prof. F. A. F. C. WENT.)

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As a result of his experiments, PAÁL (1919) became convinced, that substances are formed in the top of the Avena-coleoptile, which regulate the growth. In a normal coleoptile these substances are transported basipetally, and upon reaching the growth-zone, promote the growth, which means: the coleoptile grows straight.

When interrupted (injured) or exposed to the light at one side only, an uneven distribution of growth-regulators will result, and a positive curvature develops. PAÁL believes that light affects the growth-regulators, either bij destroying or retarding their formation, or by impeding their diffusion. PAÁL therefore is the first biologist to introduce the subject of growth-substances. His conception that these same substances have a relation to the conduction of the stimulus, is contradicted by many other experimenters.

STARK (1917—1922), claims, that certain specific stimulating substances play a role.

The experiments of WENT Jr. (1926) throw more light on the subject of the substances, which are formed in the coleoptile-top and which influence the growth. He has even isolated the growth-regulators from the coleoptile-tops.

Previous to these recent experiments Miss SEUBERT (1925) had endeavoured to find the above mentioned substances. She speaks of "Wuchsenzyme" where from we may deduct that she is of the same opinion as PAÁL. To become acquainted with these substances, we can follow two methods, miss SEUBERT says: either isolate the substances from the press-juice of the coleoptiles or determine which of "the various substances existing in the plant" are able to cause a curvature.

She found early in her experiments that it was impossible to isolate the active substances from press-juice. Therefore she investigated the influence of different substances upon the growth. She mixed the substance to be examined with agar, and put small blocks of this mixture on the top of the decapitated coleoptiles at one side.

When a negative curvature ensued (the covered flank of the coleoptile becoming convex) Miss SEUBERT concluded that at this side the growth had been increased.

The remarkable result was that the kind of curvature either positive or

negative, was dependent upon the different concentrations of the same substance. Miss SEUBERT claims that the same substance either helps or retards the growth, depending upon the concentration.

My experiments have also shown that positive and negative curvatures are the result of the use of one substance in different concentrations; but that at the same time, there is quite possibly a different explanation with regard to the development of a new physiological top.

DOLK (1926) has discovered, that a decapitated coleoptile reaches a growth-minimum after 150 minutes, and that thereafter the growth increases again by reason of the formation of new growth-accelerating substances; which at the same time bring about a return of the phototropic sensibility.

Miss SEUBERT photographed the curvatures after 6 hours, "meist nach 6 Stunden wurde das Ergebnis notiert", and I repeated these experiments, keeping in mind however, the development of the new physiological top. A repetition of Miss SEUBERT's experiments does not seem to be superfluous, in as much as my results with pure agar differ from hers.

I describe below the experiments made with little blocks of unmixed agar.

The seedlings are sown in the usual way in a zinc box filled with soil, in a dark room of constant temperature (25° C.) and humidity (90 %). After decapitation and the loosening of the primary leaf, thirty minutes should elapse before the agar-blocks are placed on the tops. Only the perfectly straight seedlings were used.

It is necessary to loosen the primary leaf, because in continuing its growth, it might lift away the agar-block from the section, where it has been placed. The agar must be washed several times in both clear and distilled water, and then melted and poured on a glass plate. The mass is divided into small blocks and placed unilaterally on the top of the previously decapitated coleoptiles.

At regular periods the curvatures were observed. (See table I and II.)

During the first 2½—3 hours after decapitation the plants are still straight, but after some time a positive curvature develops, which steadily increases in intensity and after 5—6 hours the curvature is clearly visible. Three hours after decapitation more and more plants become positively curved.

It is evident, that there must be some relation between the positive curvatures and the newly formed physiological top. It is to be understood, however, that a positive curvature is not yet visible after exactly 2½ hours, because at that time the new growth-substances merely begin to form. Some considerable time must elapse before the different quantities of growth-substances at each side of the coleoptile appreciably affect the curvature. Moreover, in order to observe the first stages of the curvature, a much closer observation is needed. I have only taken in consideration curvatures that could be detected with the naked eye.

In regard to the influence of the agar-block upon the coleoptile, two conclusions may be drawn.

TABLE I (pure agar).

20 seedlings decapitated 12.30 h. Agar applied 1.15 h.

Time	Straight	Negative	Positive	Useless plants [1])
2.15 h.	20	0	0	—
2.45	20	0	0	—
3.15	18	0	2	—
3.45	12	0	7	1
4.45	7	0	12	1
5.45	4	0	14	2

TABLE II (pure agar).

22 seedlings decapitated 10 h. agar applied 10.30 h.

Time	Straight	Negative	Positive	Useless plants
11.30 h.	21	1	0	—
12.30	18	0	4	—
1.30	11	0	11	—
2.30	3	0	18	1
3.30	3	0	18	1
4.30	2	0	19	1

1. In the case of a plant which had been decapitated 3 hours before, growth substances are formed in the new top. At one side only a quantity of these substances diffuse upwards into the agar, so that less substances descend at that side, which causes a positive curvature.

2. No new top regenerates under the agar, but this is not the case at the other side : a positive curvature develops.

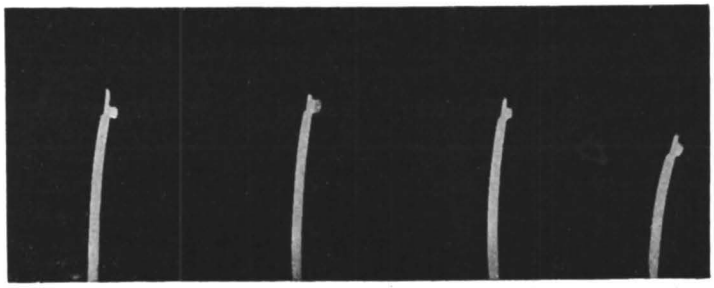
My results with unmixed agar differ from those of Miss SEUBERT. Miss SEUBERT found that the plants remained straight.

Some idea as to the intensity of the curvature, will be obtained from the picture below, which was taken by casting the reflexion of the plants on photographic paper.

These and many other experiments show that positive curvatures never develop within 3 hours after decapitation. Miss SEUBERT, investigating the influence of various substances upon the curvature, photographed the

[1]) By useless plants I mean those upon which the agar-block dryed out or otherwise fell from its place.

plantlets after 6 hours. If positive curvature resulted, one must consider that this was most likely due to the development of a new physiological top and not necessarily due to the growth-retarding influence of the substances.



Blocks of unmixed agar are placed on one side of the coleoptile. The picture, taken $5^1/_2$ hours after decapitation, shows the positive curvature quite clearly.

Therefore I experimented with many of the substances, used by Miss SEUBERT, and noted the time that elapsed before the curvatures developed.

Here again it was proven that positive curvatures never develop within 3 hours after decapitation.

A full description of my experiments would take too much space, and I will therefore merely mention that salts: KCl (concentration 10 % as well as $2\frac{1}{2}$ %) did not give a positive curvature within 3 hours, after this period positive curvatures develop.

The conclusion is warranted, that KCl neither helps nor retards growth, but that the positive curvatures referred to by Miss SEUBERT are caused by the development of the new physiological top.

Using maltose (1 %) I had the same result. Miss SEUBERT obtained also a positive curvature by the use of various sugars. She therefore concludes: "Vergleichen wir die Wirkung der einzelnen Zuckerarten unter einander, so zeigt sich, dasz alle ungefähr gleich starke positive Krümmung erregen. Die Prozentzahl und die Stärke der Krümmung entspricht im Durchschnitt ungefähr dem der untersuchten Preszsaft-konzentration und dem einer $\frac{\text{mol}}{3}$ KCl- oder NaCl-Lösung."

She also found that independent of the nature of the substance or its concentration, the intensity of the positive curvature found, is about the same.

Even this fact should enable us to conclude that the curvature is caused by the new physiological top. Particularly since we know that a positive curvature does not begin to develop until after 3 hours after decapitation.

However there are different substances which cause negative curvatures, stimulating the growth (for instance several enzymes such as saliva,

diastase, pepsine). These are the substances of which Miss SEUBERT found that they either helped or retarded growth, in accordance to the concentration.

I also experimented with these substances in different concentrations. Table III and IV show the results for diastase.

TABLE III (Diastase-agar 1 %).
14 seedlings decapitated 9.30 h. agar applied 10.30 h.

Time	Straight	Negative	Positive	Useless plants
12.— h.	0	14	0	—
1.30	0	14	0	—
2.30	0	14	0	—

TABLE IV (Diastase-agar 1 %, diluted 1 : 10).
41 seedlings decapitated 9.30 h. agar applied 10 h.

Time	Straight	Negative	Positive	Useless plants
12.— h.	31	10	0	—
1.—	18	3	16	4
3.30	23	2	12	4

The above tables show that:

1. Within 3 hours after decapitation negative curvatures develop by using diluted as well as undiluted diastase.

2. After about 3 hours positive curvatures begin to develop by using diluted diastase.

Here we find a tendency to bend in two different directions: the growth-accelerating action of the diastase will cause a negative curvature. This negative curvature becomes visible as soon as sufficient diastase has been diffused into the stump. The curvature will be more pronounced with increasing quantity of diastase diffusing into the plant. After 1½ hour these negative curvatures are very clearly visible. After 2½ hours the new physiological top begins to develop, and the plant shows an inclination to develop a positive curvature.

There are of course three posibilities:

a. The negative curvature is the strongest, completely dominating the positive curvature. This is the result of the use of great concentrations.

b. Both tendencies to curve are about the same. The plant first develops a negative curvature, and later straightens itself again.

c. The positive curvature is stronger than the negative. It sometimes happens that in the early stages the negative curvature is so slight that it

is practically invisible, and the plant appears to be quite straight. Later on, however, the plant develops a positive curvature.

Another possibility is that a slight negative curvature forms immediately, and then later develops into a positive curvature.

In this case it will be observed that the plant is first negative, then straight and finally shows a positive curvature. This result has been obtained in many instances, thereby proving that diastase invariably tends to increase growth, but that the use of lower concentrations tends to make the negative curvature so slight, that after 6 hours it is completely dominated by the positive curvature.

The same is observed in the use of saliva and pepsin.

Miss SEUBERT's explanation of the growth-retarding influence of salts, sugars and diluted enzymes is the result of more or less complicated hypotheses. Therefore the idea that certain concentrated substances either help or retard growth, is unsound; since we have shown that a positive curvature results from the formation of a new physiological top.

Summary: The substances used in my experiment all accelerate the growth. A growth-retarding substance has not been found. Its appearance should cause a positive curvature of the stump within $2\frac{1}{2}$ hours.

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