THE INFLUENCE OF GROWTH HORMONE ON HYPOCOTYLS OF HELIANTHUS AND THE STRUCTURE OF THEIR CELL WALLS

by

Miss J. M. DIEHL, Miss C. J. GORTER,
G. VAN ITERSON JR. and Miss A. KLEINHOONTE.

CONTENTS

INTRODUCTION

In the summer of 1935 we began experiments on the influence of indole-3-acetic acid (hetero-auxin), using this growth hormone dissolved in lanoline according to F. LAIBACH [1], to learn above all something more on the *Pisum*-test of WENT [2].

We soon learned that the original explanation of WENT regarding the inward curvatures of the slips of the longitudinal split internodes of *Pisum*, when they were put into a solution of hetero-auxin, could not be correct. WENT attributed this curvature to the influence of the growth hormone on the cells of the cut surface. Although the application of lanolin with hetero-auxin on the intact outside of the slips caused beautiful curvatures, treatment on the cut inside of the slips with the same paste had no effect.

We soon observed that we could as well make the experiments with the slips of hypocotyls of *Helianthus annuus*, and the further experiments have been made especially with this plant. We have elsewhere already reported some of our results, e.g. Miss A. KLEINHOONTE of this laboratory read a paper at the growth-hormone symposium of the Netherlands Botanical Society at Utrecht in 1938, specially treating the successive positive, and negative curvatures of seedlings after the application of hetero-auxin on one side of the hypocotyl. (See § 5).

In 1936 L. JOST and E. REISS [3] published a paper showing that the above mentioned "Wentreaktion" can also be observed on the stems of other plants and giving data on the measurements of such curved slips and proving that the inward curvature is the result of a higher increase in the length of the cells of the outer layers than that of the cells of the inner layers.

They came to the conclusion that this was due to the fact that the cortical parenchyma has a lack of auxin, but is still capable of elongation, whereas the pith is no longer capable of the same growth

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1 Über die Auslösung von Kallus- und Wurzelbildung durch β-Indolylessigsäure. Ber. Deutsch. bot. Ges. 53, 359—364, 1935.

2 On the pea test method for auxin, the plant hormone. Proc. Kon. Ned. Akad. Wet. Amsterdam 37, 547—555, 1934.

3 Zur Physiologie der Wuchsstoffe II, Z. f. Botanik 31, 335—375, 1936.

and therefore cannot elongate to the same extent even after the further application of growth hormone.

In 1937 J. VAN OVERBEEK and F. W. WENT [1] admitted that they could not maintain the original explanation of WENT regarding the inward curvatures. By careful and interesting measurements of the elongations on the various sides of the slips after the application of growth-hormone, they concluded — as was also done by JOST and REISS — that there exists more increase in length on the epidermal than on the pith-side of the slips. However, their explanation of this fact is different from that of JOST and REISS; VAN OVERBEEK and WENT assume that the growth hormone cannot penetrate into the wounded side in contradistinction from what would happen on the epidermal side, where it would enter very easily. After infiltrating the slips with a solution of growth-hormone they measured the same elongation on the pith-side as on the epidermal side of the slips. Therefore, after such an infiltration the slips curved as much outward as after infiltration with water.

This explanation of the inward curvatures has, however, been refuted in two recent papers.

In October 1938 a paper was published by K. V. THIMANN and CH. L. SCHNEIDER [2]. These authors also confirm that the primary cause of the inward curvatures must be ascribed to the differences in growth in length between the outer and the inner layers of the tissues of the *Pisum* internodium, but they assume that this unequal increase in length must be due to difference in the rate of reaction of the outer and the inner layers to growth-hormone, and does not depend on the difference in the rate of penetration into the tissues. A plant tissue should always react with an increase in growth after treatment with even very small quantities of growth hormone; the growth of the outer layers would however be increased by small quantities to a lesser degree than that of the inner layers, the outer-layers requiring more auxin to reach a maximum of growth. Under normal conditions the growth hormone would only be present in such a small concentration that the outer layer would be deficient in it, and tissue-tension would be the result. After the application of growth hormone, however, as given when slips are put into a solution of growth-hormone of not too low a concentration, the better supply of growth-hormone only would cause a small increase of the growth for the inner layers, but the outer layers would obtain a much higher increase of growth in length. This would cause the

[1] Mechanism and quantitative Application of the Pea-test, Botanical Gazette **99**, 22—41, 1937.
[2] Differential Growth in Plant tissues. Amer. J. Bot. **25**, 627—641, 1938.

inward curvatures of the slips of the *Pisum*-internodes after such an application. THIMANN and SCHNEIDER assume even a better penetration through the wound surface than through the epidermis.

This explanation of the "Went-reaktion" could be substantiated by the researchers in several ways. They were for instance able to prove that growth-hormone solution in extremely high dilution may increase the outward curvatures. When they isolated cylinders of pith of different diameters and cortical cylinders by boring in a *Pisum*-internodium and put them into solutions of low concentrations they found that the thin cylinders of pith showed no increase of growth in length, but that the thicker cylinders did. This latter increase is, however, much less than that in the case of cylinders consisting of outer layers, which show a considerable increase in length in a solution of growth hormone as compared with such cylinders placed in water. The same difference could be found between thick slices of the outer side of the internodes and of the internal tissues after treatment with such solutions. The outer layers proved to have their optimal increase in length at a considerably higher concentration of the growth-hormone than the inner tissues.

THIMANN and SCHNEIDER failed to observe the outward curvature of the slips of the hypocotyl after infiltrating them with growth hormone solution, which was regarded as so important by VAN OVERBEEK and WENT.

L. JOST [1] also published a paper objecting to the explanation given by VAN OVERBEEK and WENT on the differences in growth shown by the outer and the inner layers of the slips in the *Pisum*-test. His arguments, though given independently of those of THIMANN and SCHNEIDER, are the same as the ones put forward by these authors, as is also his explanation of the mechanism of the *Pisum*-test. To a certain degree the arguments of JOST are even more convincing, for whereas he as a rule used growth-hormone paste, THIMANN and SCHNEIDER did so only in a few experiments, all others being made with solutions of growth-hormone. JOST rightly observes that in water or in watery solutions the tissues may increase more in volume than would have been the case under natural conditions, and this increase in volume may have caused an increase in tissue-tension, making the effect of the influence of the growth-hormone less obvious. Of course this can never be the case when growth-hormone paste is applied.

JOST also explains the WENT-reaction by assuming that the

[1] Zur Physiologie der Wuchsstoffe IV, Z. f. Botanik 33, 193—215, 1938.

growth stimulating effect on the pith will be surpassed even with a low concentration of the growth-hormone, a concentration that does not yet reach the optimum for stimulating the growth of the cortical parenchyma. JOST, however, concluded only indirectly from his experiments that there was an optimal concentration, whereas THIMANN and SCHNEIDER definitely proved the existence of such an optimum. JOST could not give an explanation of the infiltration-experiments of VAN OVERBEEK and WENT.

Our own experiments, some of which were practically identical with those of JOST, brought us to conclusions in agreement with those of THIMANN and SCHNEIDER, and with those of JOST. Therefore we first hesitated whether, after the publication of the papers of these authors, we were still justified in publishing the results of our experiments.

However, it will become clear that we drew other conclusions in respect of some points, which we deem of a certain importance. In anticipation of what follows we may say here a few words about the difference in our opinions.

The above mentioned experimentors speak of a different optimum for the *growth of pith and cortex;* in our opinion, however, the total *growth of the walls* of the pith or the cortical cells is stimulated by various concentrations in the same way. THIMANN and SCHNEIDER as well as JOST believe in the existence of rather pronounced different optimal concentrations because they only studied growth in the length of *tissues* and not of *cell-walls.*

Secondly we shall try to make clear that the tensions in the cell-wall (which determine the micellar structure and the direction of growth of those walls) determine whether growth in length or in thickness (of course we mean only primary growth in thickness) of the hypocotyls will result.

In the Experimental Part we shall describe some experiments, also carried out by the above mentioned experimentors. As a rule, however, they made their experiments in a different way, sometimes with other plants, and even though our results were in some cases the same as theirs we thought it worth while to publish them here.

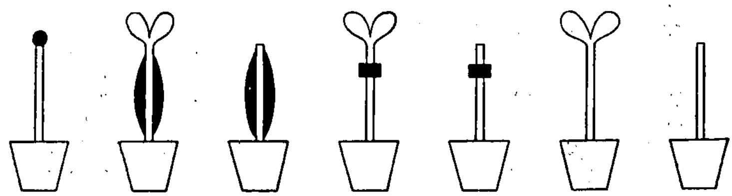
For the greater part we made our experiments with seedlings of *Helianthus annuus*, the most important results, however, were also obtained with several other plants and therefore we believe that our conclusions have more general importance than might be concluded from the name of this paper.

A. EXPERIMENTAL PART.

1. *Growth in length and thickness after the application of growth hormone paste to the cut surface of decapitated seedlings, or all around the hypocotyl of decapitated or normal seedlings.*

When growth paste in various concentrations is applied to the cut surface of decapitated seedlings of *Helianthus annuus*, longitudinal growth is the invariable result.

TABLE 1. *Growth in length and thickness in percentages of the original length and thickness for hypocotyls of Helianthus after a treatment with growth pastes of different strength during 1, 2 or 3 days in the way indicated at the head of the columns and the controls.*



		A		B		C		D		E		F		G	
Concentration of paste	days after begin	l.	th.	l.	th.	l.	th.	l.	th.	l.	th.	l.	th.	l.	th.
1 : 1	1	30	—	29	—	53	—	27	—	38	—	65	—	6	—
	2	35	24	49	48	53	43	38	48	41	20	223	0.7	5	3
	3	38	24	51	100	56	54	57	108	47	40	313	0	6	4
1 : 2	1	46	—	33	—	57	—	27	—	45	—				
	2	61	26	47	63	68	36	45	43	48	31				
	3	71	23	57	112	78	34	47	111	52	42				
1 : 10	1	55	—	57	—	57	—	33	—	37	—				
	2	66	17	85	47	63	21	62	48	55	23				
	3	70	19	125	57	71	12?	89	80	62	32				
1 : 100	1	15	—	111	—	89	—	58	—	54	—				
	2	20	10	230	18	119	21	132	22	81	9				
	3	30	17	363	20	121	20	278	26	94	13				
1 : 1000	1	7	—	82	—	27	—	58	—	25	—				
	2	12	15	203	20	44	16	129	7	35	24				
	3	25	23	325	24	53	19	309	3	39	32				

Table I column A gives the increase in length after 24, 48 and 72 hours (the paste being renewed once for this case). The 1 : 1 paste was prepared by rubbing a solution of 20 mg. of hetero-auxin in 10 cc. of distilled water thoroughly with 10 g. of pure wool grease. The other concentrations were obtained by diluting the 1 : 1 paste with lanoline (an emulsion of wool grease in water). We measured the increase in length by checking the length over a marked distance of originally 1.5 cm.

The growth in length is, to a small extent, caused by the auxin still present after decapitation. This auxin, however, causes only an elongation of about 6 % after 24 hours, as is shown in Table 1 column G.

When we compare these figures with those obtained after application of the growth hormone paste all round the hypocotyls (Table 1 column C) especially when the concentration is low, we see that even the greatest growth obtained by treatment with paste on the cut surface of the decapitated plant is not very important. When the hypocotyls are treated with a small ring of paste, close below the cut surface (compare Table 1 column E) the lower concentrations show better longitudinal growth than after application to the cut surface, but as a whole the increase falls short of that after all round treatment.

Though we do not agree with VAN OVERBEEK and WENT, who assume that growth hormone cannot penetrate a wound, the facts mentioned bear strong evidence that penetration of the intact epidermis is easier than of a wound. We are sure that this difference must be of some importance in the explanation of the *Pisum*-test.

When growth-paste in a low concentration (e.g. 1 : 100 paste) is applied all round a normal hypocotyl (compare Table 1 column B) we find a better growth in length than in the case of the untreated plant (compare Table 1 column F). Growth hormone paste in concentration 1 : 1 and 1 : 2 however causes a decrease in longitudinal growth but an increase in growth in thickness.

To demonstrate this we give Figure 1, showing seedlings of originally the same length treated in this way. The photograph was taken after 3 days (the plants were placed on the klinostat in a room of constant temperature).

We think it highly important to draw attention to this increase in longitudinal growth, caused by treatment with growth hormone paste in low concentration, as both A. TH. CZAJA [1] and U. RUGE [2]

[1] Polarität und Wuchsstoff, Ber. d. bot. Ges. 53, 197—220, 1935.
[2] Unters. über den Einfluss des Hetero-Auxins auf das Streckungswachstum des Hypocotyls von Helianthus annuus, Z. f. Botanik 31, 1—56, 1937, see especially p. 11.

concluded there was a growth in thickness when the hormone penetrates in a horizontal direction. RUGE writes: „Die Streckung

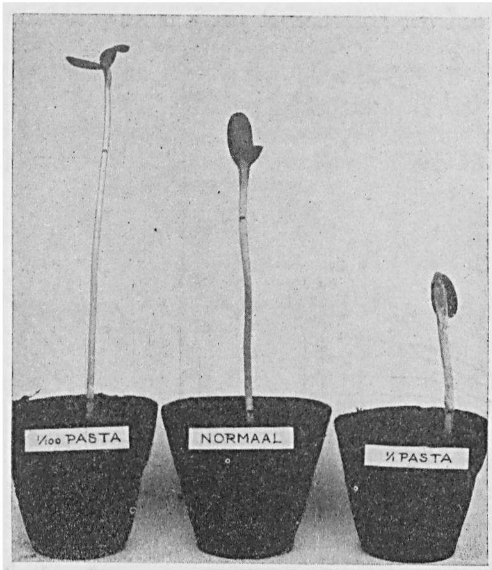


Fig. 1. Seedlings of *Helianthus* originally of the same length and thickness, after three days rotating on the horizontal disc of the klinostat at room temperature, *a*. treated all round the hypocotyl with paste 1 : 100, *b*. not treated, *c*. treated all round the hypocotyl with paste 1 : 1.

erfolgt also allein in der Diffusionsrichtung des Wuchsstoffes".

From column B we got the impression that there exists a correlation between the growth in length of the plant and the increase in thickness, the total growth being almost the same. When we treated, for instance, plants of 2 cm. length with paste 1 : 1, 1 : 10 and 1 : 100 we measured a length of 3.4, 6.2 and 12 cm. and a corresponding thickness of 3.5, 2.7 and 1.8 mm. respectively, and though we are convinced that the way of measuring is far from exact, we may say that the volumes of the plants are about the same. The same holds good for the application of a ring of growth paste.

We also compared the *distribution* of the growth in length for plants treated in the above cited way with that of normal untreated plants (see Figure 2). We used hypocotyls of 2 cm. long, marked with ink at intervals of 2 mm. For this experiment we used vaseline into which indene-acetic-acid had been dissolved. In contrast with lanoline this solution is transparent, and therefore we could observe the growth without removing the growth paste.

It is clear from the figure that paste in low concentration causes

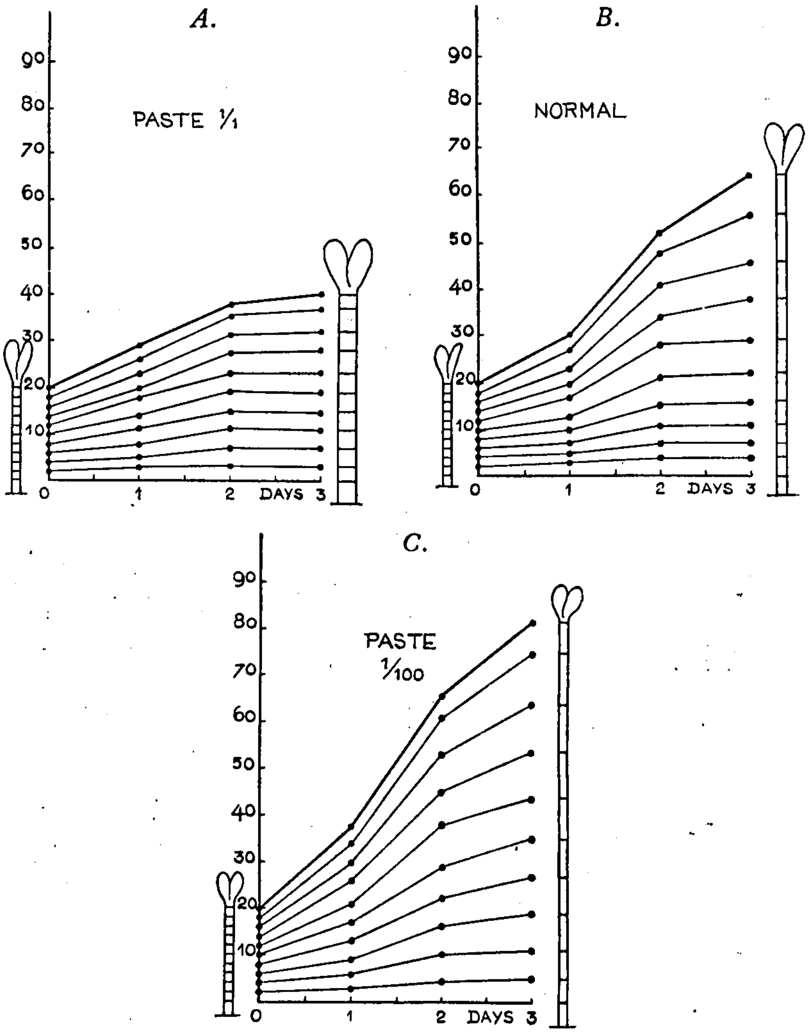


Fig. 2. Distribution of the growth in length for seedlings of *Helianthus* originally of the same length, after 1, 2 and 3 days, *A*. treated all round with paste 1 : 1, *B*. not treated, *C*. treated all round with paste 1 : 100.

an increase in the longitudinal growth of nearly all parts of the hypocotyl, but that the increase, as would be expected, is greatest where normal plants show the most active growth. High concentration causes a decrease of the growth in length and this too is greatest also in the afore-mentioned places. Growth in the thickness of the hypocotyls is about the same all over the hypocotyl.

Table I shows the important fact, that laterally applied growth-hormone even in the highest concentration cannot entirely check longitudinal growth, as might be concluded from the experiments of RUGE.

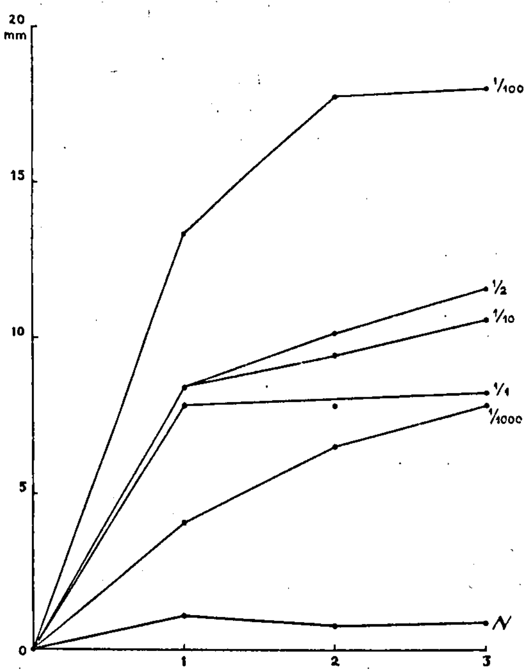


Fig. 3. The growth of decapitated hypocotyls of *Helianthus* in mm., after rotating on the horizontal disc of the klinostat for 1, 2 and 3 days, treated all round with growth pastes of different concentrations and their control (N) (compare Table I column C and G).

Still more clearly is this shown in figure 3 (where we used decapitated plants that were treated 48 hours after decapitation when their own growth hormone was entirely used up).

We also measured the growth distribution in hypocotyls that had been treated with a ring of concentrated growth hormone paste. The seedlings were 2.8 cm. long and divided into zones 2 mm. long; the ring of concentration 1 : 1 was applied on zone 6 and 7 reckoning from the top.

TABLE 2. *Elongation (in per cents of the original length) after 2 days of the parts above the 6th and below the 7th zone for untreated hypocotyls and for hypocotyls treated on these zones with growth-hormone paste 1 : 1.*

	untreated	treated
Elongations of the part above the 6th. zone	266 %	55 %
Elongations of the part below the 7th. zone	139 %	62 %

Table 2 shows that decrease in elongation occurs both above and below the ring, this decrease is however greater above the ring than below it. On measuring the growth below and above zones 6 and 7 (the numbers begin at the apex) we find in the case of the untreated plants 100 and 108 mm. and in the case of the treated plants 68 and 36 mm. respectively, which means a decrease of 32 % below and 70 % above the ring respectively.

BARBARA LE FANU [1] found the same for the internodes of *Pisum* and R. POHL [2] for the coleoptiles of *Avena.*

As for the application of a ring of strong paste: CZAJA [3] was the first to demonstrate the formation of thick swollen places, which become visible after 2 or 3 days. He obtained the best results when the ring was applied in the upper part of the hypocotyls, as we did in the experiments mentioned above. When the ring is applied to the lower zones, the swelling is much less evident.

Later on we shall again refer to the influence of rings of paste in high concentrations.

2. *Curvatures, caused by the unilateral application of growth-hormone on the hypocotyls of normal and of decapitated seedlings.*

Shortly after the publication of the paper by LAIBACH [4] on curvatures caused by the unilateral application of growth-hormone on *Avena*-coleoptiles, we made similar experiments with *Helianthus*

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[1] Auxin and Correlative Inhibition, The New Phytologist 35, 205—220, 1936.

[2] Das zonale Wachstum der Avena-Koleoptile unter dem Einfluss von künstlich zugeführtem Wuchsstoff, Ber. d. Bot. Ges. 55, 342—353, 1937. See also R. POHL, Über die durch Wuchsstoff induzierte Wachstumshemmung der Koleoptile, Ber. d. Bot. Ges. 56, 368—379, 1938.

[3] l.c.

[4] l.c.

seedlings. A. TH. CZAJA, L. JOST, K. V. THIMANN and CH. L. SCHNEIDER have also reported on such experiments with the same plant.

The influence of the unilateral application of growth hormone paste is very clearly demonstrated in figure 4, which shows that

Fig. 4. Curving of a hypocotyl of *Helianthus*, originally straight, after the unilateral application of growth paste, when the curving is checked by a piece of wood.

the plant succeeds in curving, though this has been made difficult by placing a small piece of wood close to the erect plant at the beginning of the experiment.

It is evident that important forces are the result of the unilateral application of the growth hormone. These forces can be caused by the active growth of the convex side of the seedling, or by a relative contraction of the concave side. Which of these two possibilities is in this case the active one, will be shown by the results of the further experiments.

We have not only made experiments with indole-3-acetic acid, but also used various other substances that give the *Avena*-, or the *Pisum*-test, and found them all active. Curvatures of hypocotyls of *Helianthus* were also caused by applying lanoline into which we had diffused auxin from a number of coleoptile-tips of *Avena*.

As a rule we found stronger curvatures by applying the growth hormone on the hypocotyl directly below one of the cotyledons, than by doing so on the other sides as is shown in figure 5.

Reactions are highly dependent on temperature; as a rule we got the best resuls at about 21—23° C. Unilateral application did not give any reaction at ± 3° C., unless the plants were left after the application for one hour at room temperature.

The best results are obtained by placing the plants on the vertical disc of the klinostat; no anti-reactions of gravity then take place.

First we shall describe experiments such as we generally made with normal plants in an upright position on the horizontal disc of the klinostat to avoid phototropic curvatures. Growth hormone paste was applied in equal quantities of about 30 mg. over a section of 20 mm. long and 3 mm. broad, near the top of the hypocotyls. Of course we always used plants of equal length.

We found hetero-auxin active in this way up to a paste of 1 : 5000. With a concentration of 1 : 10 000 hardly any curvatures were obtained. Figure 6 shows clearly that the rate of curvature varies with the concentration of growth hormone. The photographs were made after 5 hours.

To learn something about the concentration causing the highest degree of curvature (as was studied for *Avena* by B. SCHEER [1]) we measured the curvatures with a protractor. In table 3 we give the degrees of curvature reached after 2 and 24 hours, measured on 4 plants; moreover figure 7 shows the relation between the degree of curvature and the concentration of the growth-hormone in two graphs.

TABLE 3. *Curvatures of seedlings, unilaterally treated with paste in various concentrations* (Series A).

Concentration	After 2 hours	After 24 hours
1 : 2000	3°	0°
1 : 1000	2°	4°
1 : 500	4°	0°
1 : 200	16°	17°
1 : 100	27°	22°
1 : 50	35°	30°
1 : 20	31°	65°
1 : 10	28°	66°
1 : 5	32°	101°
1 : 1	10°	68°

[1] Straight Growth of the Avena Coleoptile in Relation to Different Concentrations of Certain Organic Acids and their Potassium Salts, Am. J. Bot. 24, 559—565, 1937.

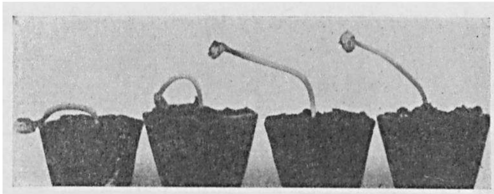


Fig. 5. Seedlings of *Helianthus* treated with growth paste. For the two plants on the left the paste was applied unilaterally under one of the cotyledons, for the two plants on the right the paste was applied unilaterally under one of the groves between the cotyledons.

A.

B.

C.

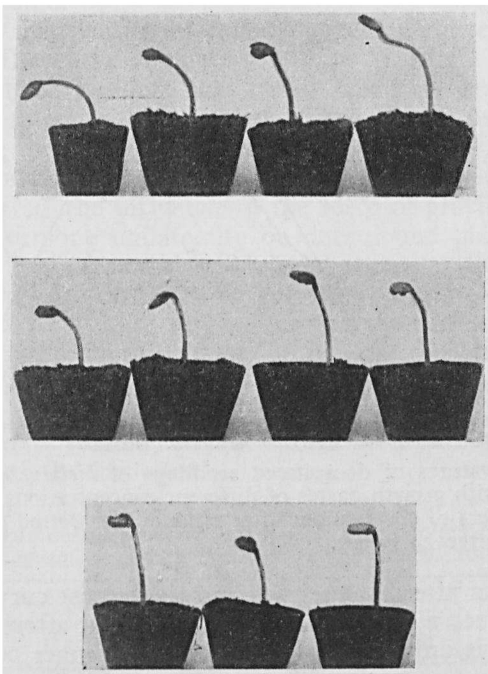


Fig. 6. Seedlings of *Helianthus* 5 hours after the unilateral application on the hypocotyl of growth paste of five different concentrations (1 : 100, 1 : 1000, 1 : 2000, 1 : 5000 and 1 : 10 000) each applied on two seedlings; the plant on the right in *C* is the control with lanoline.

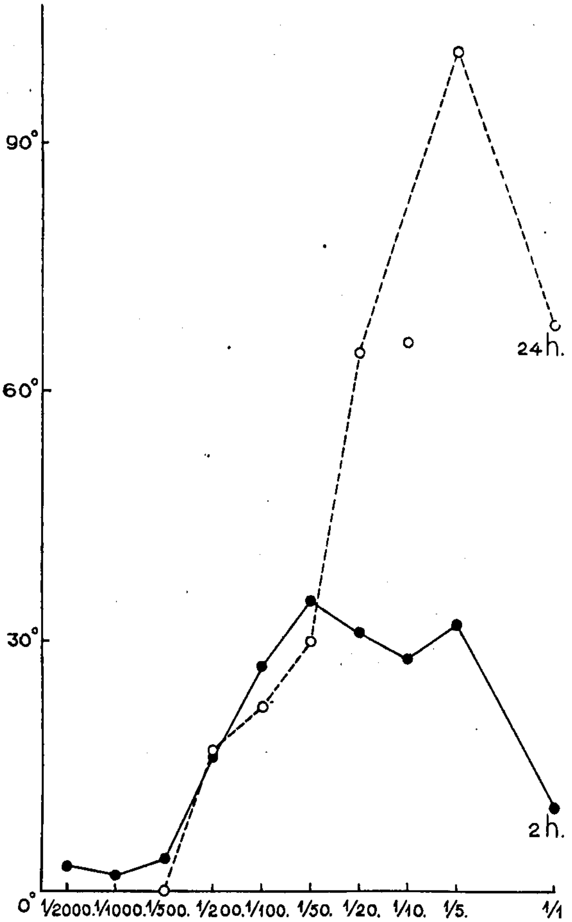


Fig. 7. The curvatures of decapitated seedlings of *Helianthus*, unilaterally treated with growth pastes of different concentrations; the unbroken curve refers to observations after 2 hours, the dotted curve to observations after 24 hours.

It is clear that after 2 hours we find the largest curvatures when paste 1 : 5, 1 : 10, 1 : 20 and 1 : 50 is used; but after 24 hours the highest degree is only reached when much stronger concentrations are used.

As the auxin of the plant itself might in the same way influence the curvatures of the treated plants, we made the same experiments with decapitated plants which after 72 hours turning on the klinostat could be regarded as auxin-free.

Table 4 gives the results of this experiment after 4 hours and after 24.

TABLE 4. *Curvatures of decapitated, auxin-free seedlings, unilaterally treated with paste in various concentrations* (Series B).

Concentrations	After 4 hours	After 24 hours
1 : 2000	9°	8°
1 : 1000	9°	10°
1 : 200	12°	11°
1 : 100	16°	17°
1 : 20	29°	72°
1 : 10	46°	123°
1 : 5	50°	132°
1 : 1	32°	63°

Though after 24 hours the curvatures reach a much higher degree than after 4 hours, the concentration which gives the optimum angle is the same.

There may be 3 different factors to which the existence of an optimum in the curve is due.

a. It might be possible that, when a curvature is caused by applying a high concentration, part of this growth hormone is conveyed by the force of gravity to the concave part of the hypocotyl, and that it causes an increase of growth in length of that side.

We eliminated the influence of the force of gravity by applying the growth hormone unilaterally on decapitated plants (after they had become free of auxin) and placed them on the vertical disc of the klinostat. In Figure 8 we give three groups of plants, with 1 :1, 1: 2 and 1 : 5 paste resp., after they had been rotated for 20 hours. There is no doubt that also in this case the curvature is more evident for the 1 : 2 paste than for 1 : 1 and 1 : 5.

Table 5 gives the average curvatures of five plants; half of the plants were rotated on the horizontal, half of them on the vertical disc of the klinostat.

TABLE 5. *Curvatures of decapitated auxin-free seedlings with unilaterally applied growth hormone of various concentrations, half of which were rotated on the horizontal, and half of them on the vertical disc of the klinostat.*

Concentration		Time in hours	Curvatures on horizontal disc	Curvatures on vertical disc
Series C	1 : 1	5	15° ± 6°	29° ± 4°
	1 : 1	24	30° ± 3°	45° ± 8°
Series D	1 : 10	3	67° ± 3°	60° ± 5°
	1 : 10	6	81° ± 6°	96° ± 2°
	1 : 10	24	123° ± 6°	154° ± 5°

This table shows that stronger curvatures are obtained when the force of gravity is eliminated, though there is no doubt that even in these circumstances an optimum angle concentration exists.

b. Part of the hetero-auxin in high concentration might be conveyed through the hypocotyl to the other side and might there cause an increased growth, thereby occasioning a decrease in the degree of

A.

B.

C.

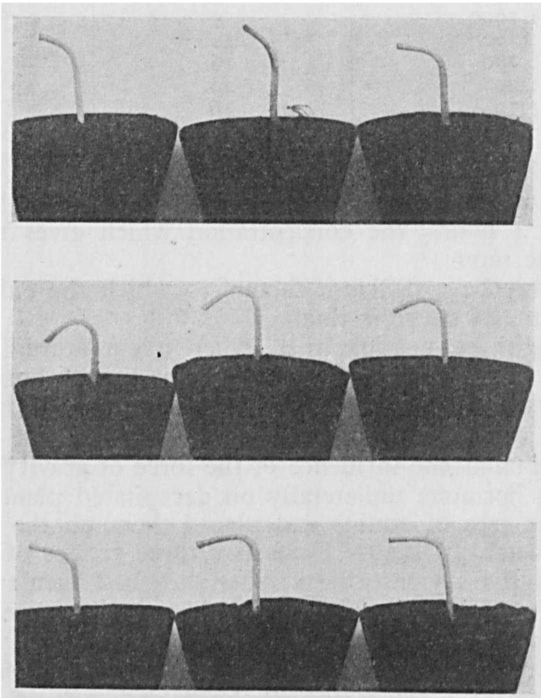


Fig. 8. Decapitated seedlings of *Helianthus* freed from auxin and treated unilaterally with growth pastes of three different concentrations, after 20 hours *A.* with paste 1 : 1, *B.* with paste 1 : 2, *C.* with paste 1 : 5.

curvature on similar lines as was demonstrated to be the case under the influence of the force of gravity. In § 3 we shall show that such a movement really exists.

c. Thirdly, hetero-auxin in high concentration might actuate the growth in length less, or it might even be harmful to the plant. To ascertain this possibility we limited the action of the hetero-auxin to one side of the hypocotyl by placing a blade of triacel (a film of cellulose acetate containing some nitrate groups in the cellulose acetate molecule) in a longitudinal cut through the plant and fixed the two halves of the plant close together by thin threads. The experiments were made on the vertical disc of the klinostat, and as Table 6 shows, curvatures are greater when paste 1 : 10 is applied than when one applies paste 1 : 1.

TABLE 6. *Curvatures of decapitated, auxin-free, plants, unilaterally treated with growth hormone paste; transverse transport prevented by an impermeable blade of triacel.*

Series E	Time in hours after application	Curvatures with paste 1 : 1	Curvatures with paste 1 : 10
	5	24° ± 5°	32° ± 5°
	24	37° ± 5°	56° ± 8°

This result is in agreement with our experiment reported in Table 1, that shows that growth hormone, when applied all round the hypocotyl shows a smaller effect on growth in length when used in the highest concentration.

We may conclude that there exists an optimum in the concentration curve, partly because the transport of growth hormone to the opposite side of the hypocotyl takes place when higher concentrations are used, but especially because a real optimum concentration exists for the growth in length of the treated side.

To demonstrate the influence of the force of gravity on the distribution of the *innate* growth substance, we give the results of the following experiments.

A number of normal plants (series F) or of decapitated plants (series G) unilaterally applied with paste 1 : 10 and 1 : 20, were placed on the horizontal, and a number of similar plants were placed on the vertical disc of the klinostat. As is shown in Table 7 there is more difference in the curvatures than was shown in Table 5 (Series D) where decapitated, auxin-free plants were treated in the same way.

TABLE 7. *Curvatures of seedlings, not auxin-free, unilaterally treated with growth hormone paste and rotated on the horizontal or on the vertical disc of the klinostat.*

Concentration			Hours after application	Curvature on horiz. disc.	Curvature on vert. disc.
Series F	No. 1	1 : 10	3	36° ± 3°	62° ± 6°
	No. 2	1 : 10	18	60° ± 8°	95° ± 7°
Series G	No. 1	1 : 20	4	37° ± 3°	73° ± 1°
	No. 2	1 : 20	24	60° ± 7°	160° ± 8°

Figure 9 shows some of these experiments. The upper four were all unilaterally treated with paste 1 : 10, the middle ones were decapitated but not auxin free, but immediately treated with paste 1: 20. The lower ones were decapitated, made auxin free and thereupon

A.

B.

C.

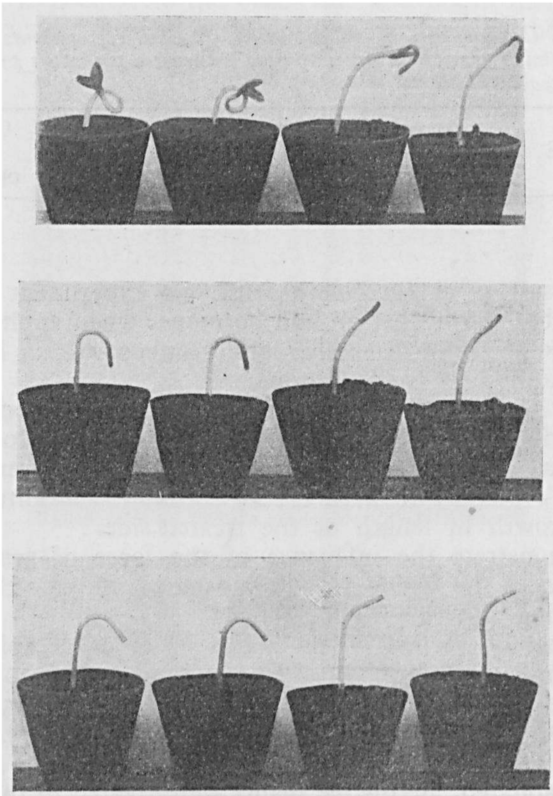


Fig. 9. Four normal and eight decapitated seedlings of *Helianthus* treated unilaterally with growth pastes, *A.* with paste 1 : 10, *B.* four seedlings containing still auxin treated with paste 1 : 20, *C.* four seedlings freed from auxin treated with paste 1 : 20. The two plants on the left of each row rotated on the vertical disc of the klinostat, the two on the right on the horizontal disc.

unilaterally treated with paste 1 : 20. Two of the plants in each group of 4 (the ones on the left) were rotated on the vertical, the other 2 (on the right) on the horizontal disc. The influence of the gravity is more pronounced when the plants have some auxin of their own.

3. *The influence of unilaterally applied growth-hormone paste on hypocotyls, entirely or unilaterally without epidermis.*

When we scrape off the epidermis of the entire hypocotyl of normal seedlings and then apply growth-hormone paste unilaterally, negative curvatures occur (negative means the treated side turning into the convex one). We found this with concentrations 1 : 1000, 1 : 500, 1 : 100, 1 : 10, 1 : 2 and 1 : 1. The series of plants A_2, B_2 and C_2 in Figure 12 (which figure will be explained below) show the same result when the experiment is made with decapitated auxin free plants.

However the degree of curvature is less than would have been the case if we had used normal plants. Owing to the possibility of growth-hormone to penetrate easily through a wound, we were soon convinced that we had to assume for the epidermis a higher increase in growth in length with the same concentration of hetero-auxin than for the sub-layers.

This experiment is much more interesting when the epidermis is removed only unilaterally. Of course we have to wait some time before the application of the growth-hormone paste in order to give the seedling an opportunity to regain its upright position when a negative curvature has been caused by the wound.

After the application to the wound of growth-hormone paste in low concentrations (1 : 10 or lower) none or only very small reactions will result. Figure 10 demonstrates this.

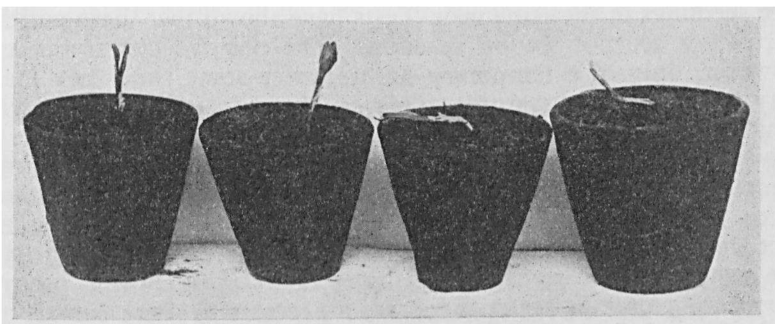


Fig. 10. The different reaction of seedlings of *Helianthus* to the unilateral application of moderately strong growth paste when the epidermis is taken away at the side where the paste is applied (the two plants on the left) and when the epidermis is taken away at the other side and the paste applied on the intact side (the two plants on the right).

Entirely different however is the reaction after growth-hormone paste in high concentrations has been applied to the wound.

In this case *positive* curvatures result (the treated wounded side turning into the concave one)[1]. The result is most striking when we compare them with the negative curvatures of plants of which the epidermis has not been removed. Fig. 11 shows the result of

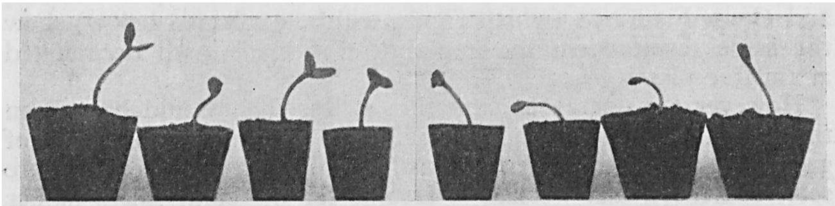
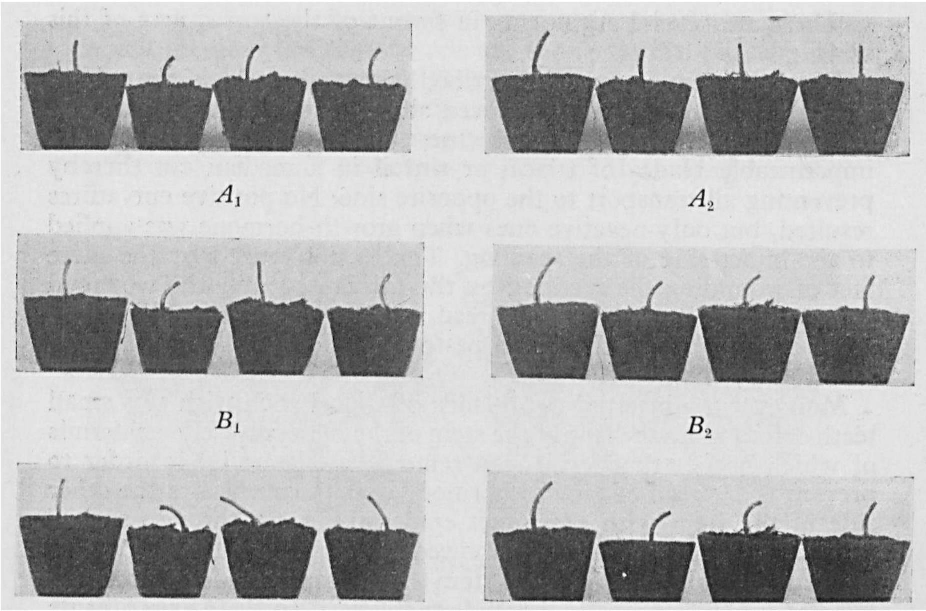


Fig. 11. The different reaction of seedlings of *Helianthus* to the unilateral application of strong growth paste when the epidermis is taken away at the side where the paste is applied (the four plants on the left with positive curvatures) and when the epidermis is intact (the four plants on the right with negative curvatures). The photo was taken 5 hours after the application of the paste.

treatment with paste 1 : 1 after 5 hours on plants with the epidermis removed unilaterally (left), and on normal plants (right); if observed closely the paste will be seen on the seedlings. The plants to the left bend in a positive, those to the right in a negative sense.

The same phenomenon results after treating decapitated auxin free seedlings in the same way. Tests have shown that these positive curvatures are not wound reactions. The positive curvatures are, however, only of a temporary nature; after some time they make way for negative curvatures. This can be seen in Figure 12 with which figure one can moreover compare the reactions of plants to growth paste 1 : 1 when the epidermis is unilaterally or entirely removed. We repeated these experiments also with decapitated auxin free plants with the same results. We draw attention to the fact that the plants with the epidermis unilaterally removed, have the wound on the right side. After 5 hours they showed positive curvatures, after 24 hours there was a decrease in these curvatures, and after 48 hours the plants showed negative curvatures. The series of plants, with all around removed epidermis, were treated on the left side with growth-hormone paste; they always showed negative curvatures after 5 as well as after 24 and 48 hours.

[1] The same phenomenon can be obtained with seedlings of *Raphanus sativus*.



C_1 C_2

Fig. 12. The result of the unilateral application of growth paste 1 : 1 on decapitated seedlings of *Helianthus*, A_1 and A_2 after 5 h., B_1 and B_2 after 24 h., C_1 and C_2 after 48 h.; in the case of A_1, B_1 and C_1 the epidermis was taken away on one side and growth paste applied on the wound; in the case of A_2, B_2 and C_2, the epidermis is taken away all around the hypocotyl and the paste applied unilaterally on the left side.

As was explained by Miss A. KLEINHOONTE in a paper read at Utrecht, there is no doubt that the positive curvatures must be the result of the activity of the growth-hormone, which penetrates from the wound surface through the tissues of the stem to the epidermis on the other side and causes there an increase in the length of the cells. We must thus assume that the increase in the length of these epidermal cells, caused by the hetero-auxin must be greater than that of the subepidermal layers and later on we will prove that this indeed is the case.

The fact that the positive curvatures are after some time superseded by negative curvatures can only be due to the subsequent reaction of the subepidermal tissue under the wound to the strong growth-hormone paste in the same way as the epidermis would have reacted. The reaction of the subepidermal layers must be slower.

There are several arguments in favour of the correctness of this view.

Firstly the experiment described above, in which the plants with the entire epidermis removed showed no positive curvatures.

Secondly we proved the correctness of our view by placing small impermeable blades of triacel or tinfoil in a median cut thereby preventing all transport to the opposite side. No positive curvatures resulted, but only negative ones when growth-hormone was applied to the intact side of the seedling. This is not caused by the mere fact of wounding the seedling by the median cut, for the wounded plant wrapped round with a thread without an impermeable blade treated unilaterally with growth paste showed the positive curvature followed by the negative one.

Moreover we inserted two blades of triacel each with two small teeth entirely into the side of the stem of the hypocotyl (the epidermis of which had at that level been removed unilaterally) in order to prevent the spread of growth-hormone over the surface to the other side of the stem with the intact epidermis; a cut through such a seedling after the experiment showed that as a rule the triacel had been pressed so deeply into the stem that transport had been prevented also through the outer subepidermal layers. In these experiments the unilateral application of growth-hormone paste to the side without epidermis showed the positive curvatures; therefore it is certain that the positive curvatures of these seedlings are caused by the growth hormone that has been transported through the inner part of the stem to the opposite epidermis and not by transport over the surface or through the subepidermal layers only.

Another, but more indirect, proof is obtained when we compare the curvatures of seedlings whose epidermis has been removed unilaterally and to which growth-hormone paste has been applied on the intact side, with those of normal plants unilaterally treated with growth-hormone. The curvatures, which of course are all negative ones, are much more pronounced in the plants with no epidermis on the opposite side than in the case of normal plants, as is clearly shown in Figure 13.

We have therefore to assume that also under normal circumstances part of the growth-hormone, especially when a high concentration is used, is transported to the opposite side and there decreases the effect of the negative curvature. Moreover the results of our experiments confirm the correctness of one of the explanations given in § 2 regarding the existence of an optimum in the graphs demonstrating the relation between angle of curvature and rate of concentration of unilaterally applied growth-hormone.

The two following experiments once again reveal that the way in which the growth-hormone reaches the epidermal tissue is quite immaterial for the increase of growth.

Firstly we made such a deep cavity in one side of a seedling as to leave hardly any underlying layers under the opposite epidermis. Growth-hormone paste was applied to the inner side of the

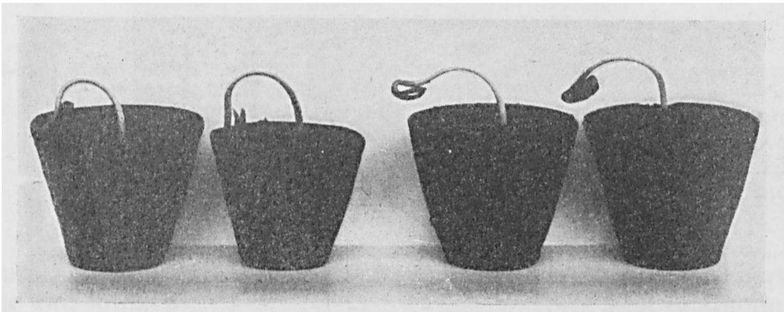


Fig. 13. Seedlings of *Helianthus* treated unilaterally with growth paste 1 : 2; on the left two plants unilaterally stripped off the epidermis, but with the paste applied on the intact side of the hypocotyl, on the right two plants wholly intact.

epidermis. Normal curvatures resulted then, that is to say the same as when the paste had been applied to the outer surface of the epidermis. The same curvatures resulted when tissue was taken away on two opposite sides of the hypocotyls up to the pith and growth-hormone applied to two corresponding parts of the two wounds in such a way that from both sides the same part of the epidermis was reached by the diffusing hormone; we took care that no growth-hormone paste came in direct contact with this part of the epidermis.

4. *The influence of permanent or temporary application of a ring of growth hormone paste on curvatures of hypocotyls.*

In § 1 we reported the results of experiments, showing that application of a ring of growth-hormone paste in high concentration decreases growth in length below and above this ring — though it does not stop this growth entirely — and that this decrease in our experiments is stronger above than below the ring.

The permanent or temporary application of such a ring has however some more interesting results. We will illustrate this with some of the many experiments we have made.

When we applied such a ring and allowed it to remain for two hours and after removal of the ring we unilaterally applied growth-hormone paste e.g. 1 : 200, no reactions were visible after 5 hours, though the controls to which a ring of lanoline had been applied and left for 2 hours and which were then treated in the same way, showed distinct curvatures. Compare Figure 14.

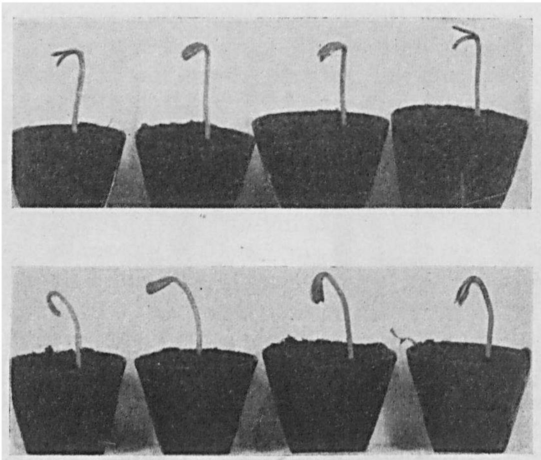


Fig. 14. Experiment showing that the temporary application of a ring of strong paste on seedlings of *Helianthus* makes them less sensitive to the unilateral application of growth paste. Above: four plants which were rotated for 2 hours on the klinostat with a ring of paste 1 : 1, which was then removed, after which paste 1 : 200 was applied unilaterally and left for 5 hours. Below: four plants treated with a ring of lanoline instead of the ring of paste 1 : 1, but otherwise in the same way.

We got the same results when growth paste in concentration 1 : 1 was unilaterally applied after a temporary application of a ring of paste 1 : 1. After 6 hours no curvatures were to be observed. Only after 24 hours were curvatures visible though to a far less extent than in the case of the control. We draw attention to the fact that the curvature was most pronounced below the place where the ring was applied. See Figure 15.

A temporary or permanent ring of growth hormone also influences the capacity of the plant to react to the force of gravity. Figure 16 shows seedlings that were treated with a ring of growth-hormone paste 1 : 1 for 2 hours and then placed horizontally. The photographs to the left show the plants that had been in that position for 3

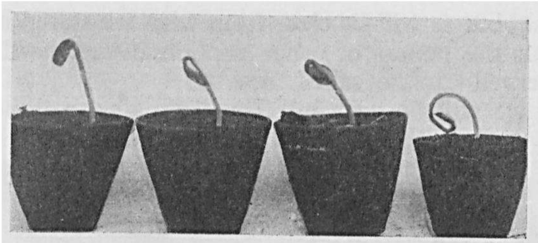


Fig. 15. Experiment showing that a temporary ring of strong growth hormone paste on seedlings of *Helianthus* makes them less sensitive to the unilateral application of growth hormone. The three plants on the left were treated with a ring of growth paste 1 : 1, moreover growth paste 1 : 1 was applied unilaterally, then they were left for 24 hours. The single plant on the right was only treated unilaterally with growth paste 1 : 1 and left for 24 hours.

hours, the right hand photographs the same plants after 22 hours. The two lower plants are the controls on which a ring of lanoline had been applied instead of a ring of growth hormone paste. After 3 hours the plants treated with a ring of growth-hormone paste show practically no reaction to the force of gravity, and only after some time is a recovery to be observed.

Some provisional experiments showed that a ring of growth-hormone paste in high concentration can also prevent phototropic curvatures; an application left on for only a very short time is sufficient to demonstrate this and the plant recovers only slowly after the removal of the ring.

We still wish to draw attention to the fact that the influence of the rings in all the experiments we described is evident long before an increase in thickness of the hypocotyl is visible.

We have not tried to give a complete explanation of these curious phenomena. We would only suggest that paste in high concentration causes in the first place: a certain disturbance of the original arrangement of the micelles in the cell walls and as a consequence a cessation of the normal reactivity of these walls to small amounts of growth-hormone. The normal reactivity will then only return after the walls have had an opportunity of restoring, to a certain degree, the original arrangement of the micelles.

5. *Curvatures of the halves of split hypocotyls.*

We first of all paid much attention to the curvatures of the halves of split hypocotyls, because we expected them to throw more light on the *Pisum*-test of Went. As a matter of fact this proved to be the case, but as will be clear from what we stated in our introduction about the papers of other experimentors, hardly anything that we observed can be called new.

Therefore we will be very brief in dealing with this part of our

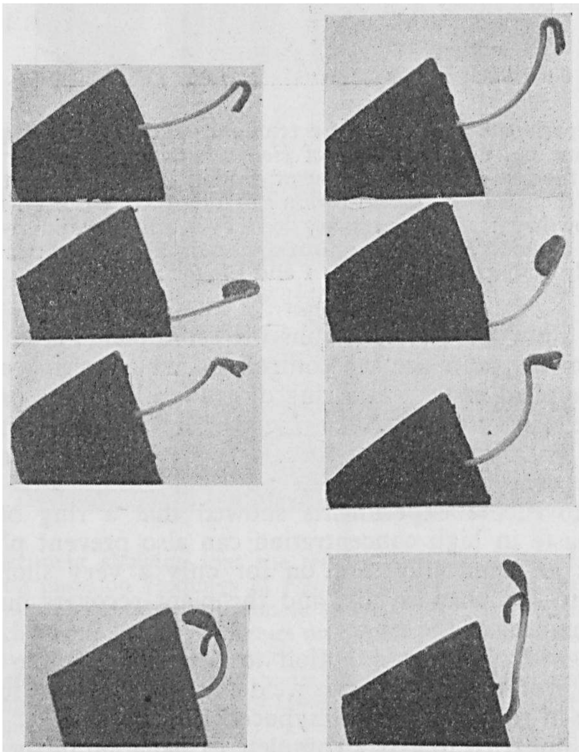


Fig. 16. Experiments to demonstrate that a temporary treatment of seedlings of *Helianthus* with a ring of growth hormone paste makes them less sensitive to react to the force of gravity. The plants, with the exception of the two below (the controls with a ring of lanoline), were treated for 2 hours with a ring of growth paste 1 : 1. Then the plants were placed horizontally. Left: the plants as they were after 3 hours, right: the same plants after 22 hours.

experiments. We decapitated the plants and split the stems by making an apical medium cut 2 cm. long. The experiments were carried out in a small room at constant temperature and in a highly humid atmosphere, because it was found that the degree of the curvature is highly dependent on the humidity of the air.

In Figure 17 we give the results of experiments made with various concentrations of growth hormone paste, applied either on the inside (for the plants on the left in the figure) or on the outside of the halves (for the plants on the right of the figure).

A.

B.

C.

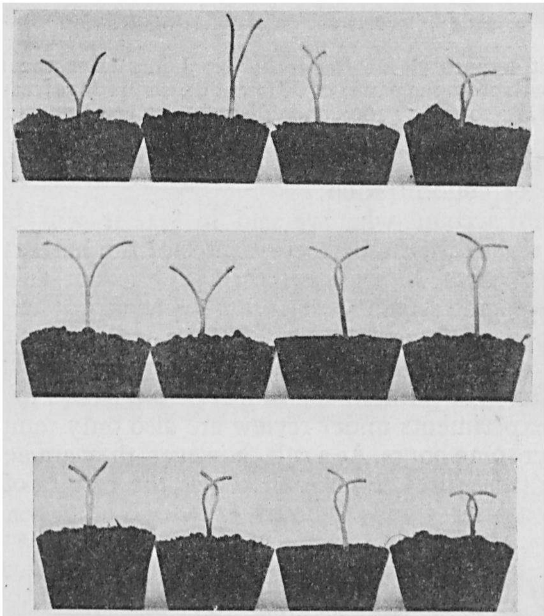


Fig. 17. Experiments to demonstrate that growth hormone paste applied to the epidermis on slips of split hypocotyls of *Helianthus* gives a stronger reaction than when applied to the inner side of the slips; *A.* treated with growth hormone paste 1 : 100, *B.* with paste 1 : 10, *C.* with paste 1 : 2. In the case of the plants on the left the paste was applied on the inner side of the split hypocotyls, in the case of the plants at the right side the paste was applied on the epidermis, that is on the outer side of the split halves. The photos were taken 5 hours after the application of the paste.

Growth hormone pastes in concentration of 1 : 10 or weaker are inactive when applied to the inside, whilst high concentrations (e.g. 1 : 1 or 1 : 2) cause positive curvatures. Applied to the outside both weak and high concentrations cause more pronounced (negative) curvatures than the same concentrations applied to the inside.

Figure 18 shows a series of plants treated on the inside with different concentrations decreasing from left to right; the plant at

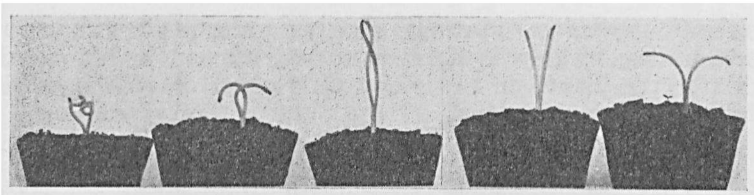


Fig. 18. Split hypocotyls of *Helianthus* 29 hours after the application of growth hormone pastes of different concentrations (respectively 1 : 1, 1 : 2, 1 : 10 and 1 : 100), and of lanoline on the inner sides of the slips.

the end is the control with lanoline. The reactions are dependent on the rate of concentration.

Taking into account what we said in § 3, it will be clear that we have to regard the positive curvatures of the halves as the result of the transport of heteroauxin through the cut surface and the tissues of the split stem to the intact epidermis.

In common with the positive curvatures of plants with the epidermis removed unilaterally and the wound treated with growth hormone paste, the positive curvatures of the halves of the hypocotyls in the experiments under review are also only temporary; they decrease after some hours. As a rule, however, they are never followed by negative curvatures. Figure 19 shows the results of application of paste 1 : 2 after 5 and 24 hours. [1]

A.

B.

Fig. 19. Split hypocotyls of *Helianthus* treated on the inner sides of the slips with growth hormone paste 1 : 2; *A.* the plants 5 hours, *B.* the same plants about 24 hours after the application.

[1] The experiments of Figure 18 and 19 were made under slightly different circumstances; this explains why the second plant of Figure 18 still shows the positive curvature.

Without any doubt the decrease of the curvature must be the result of an active elongation of the pith.

The next experiment shows what happens when growth hormone paste has been applied to the apical cut surfaces of the halves; the curvatures which were first outward now change to inward. Figure 20 shows three plants treated with 1 : 1, one with lanoline after 3 days. Of course the result may be accounted for in the same way.

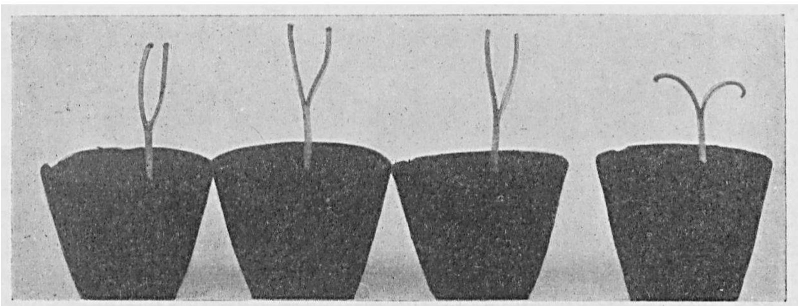


Fig. 20. Three split hypocotyls of *Helianthus* (on the left) treated with growth hormone paste on the apical cut surfaces of the halves and one split hypocotyl (on the right) treated on those surfaces with lanoline.

We made similar experiments with plants from which the epidermis had been removed. After the application of growth hormone paste to the inside of the halves, positive curvatures did indeed result, but formed much slower; after 5 hours they were hardly visible, after 24 hours they were distinct, but no decrease was observed afterwards. This is shown in Figure 21.

Just before the publication of the last paper by JOST, we made one of the experiments described therein, and this strongly indicated the existence of active growth in the length of the epidermis and of the underlying cortical layers (by active growth in length we mean growth in length of this tissue independent of the elongation caused by tissue tension). We think this experiment sufficiently important to give details of it here.

Decapitated plants were coated with growth hormone paste 1 : 10 or 1 : 20 on all sides. We left them for some hours in this condition (during which time the seedlings considerably increased in length), and then made an apical medium slit in each of them. As the slips remained straight, it was proved that no tissue tension existed after this treatment. When, however, such seedlings were split in the same way immediately after the application of the paste, inward curvatures resulted after the same course of time. Of course, controls that were not treated with paste after the same lapse of time showed the normal outward curvatures.

We varied this experiment by fixing the two halves together with a small strip of gauze and then applying the paste to the outside. After some hours the strip was removed and it was found that

A.

B.

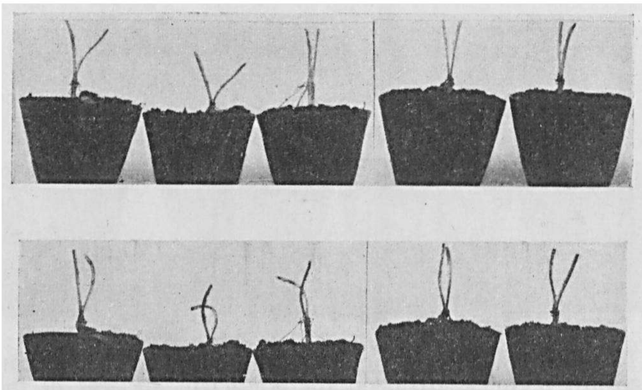


Fig. 21. Hypocotyls of *Helianthus* that were entirely stripped off the epidermis and after being split were treated with growth hormone paste 1 : 2 on the inner sides of the halves; *A*. after 5 hours, *B*. after 24 hours.

now no curvatures had resulted. This means that also in this case the tissue tension had disappeared.

We again draw attention to the fact that VAN OVERBEEK and WENT were the first to prove by measurements that in the *Pisum*-test the inside curvature under the influence of a solution of growth hormone is the result of more increased growth of the outside than of the inside of the halves, and that JOST showed that this was probably also the case with the corresponding curvatures of *Helianthus*-hypocotyls. The most important feature proved by this is, in our opinion, that the epidermis and the underlying layers are capable of growth in length, independent of tissue tension.

That epidermis and probably also underlying layers do possess this capacity can moreover be demonstrated by the following simple experiment.

When we cut off one halve of a median split hypocotyl and hang it by means of a thread over a bowl of water in such a way that the basal cut surface just reaches the water, a highly pronounced curvature caused by the tissue tension results, the epidermis being on the concave side. The same thing occurs when paste 1 : 10 is applied to the epidermis. When, however, the pith of such a slip is removed by means of a small hollow spatula, only the epidermis with some underlying layers remaining, no curvature will result, unless paste is applied to the epidermis, but then the curvature is such that the epidermis is on the convex side.

The only way to account for this fact is to presume an active elongation of the epidermis, unless one should wish to take it for granted that there is a decrease in the length of the few remaining subepidermal collenchymatic layers, but for this there is hardly any reason.

The next experiment proves that the capacity for active elongation is not only limited to the epidermis, but is also characteristic of the underlying layers. When the stem of a hypocotyl of *Helianthus* is cut by a median split, after the epidermis has been removed, and the halves are then hung above a bowl of water, the basal surfaces reaching the water, only a small curvature results (the outside being concave). If, however, the outer surface is coated with growth hormone paste 1 : 20 the outside will become convex when the curvature occurs.

6. *Elastic extension, elastic extensibility and plastic extensibility of longitudinal walls of the cortical parenchyma of Helianthus hypocotyls.*

In the theoretical part we shall endeavour to prove that the current ideas regarding elastic and plastic extension and extensibility of cell-walls call for revision, but in this experimental part we will follow the usually accepted views of these features. We use the expressions "elastic extension", "elastic extensibility" and "plastic extensibility" as is done by U. RUGE (l.c.). "Elastic extension" is therefore the reversible elongation which the cell-wall undergoes under the influence of the turgor pressure which exists in the intact tissue of the plant. "Elastic extensibility" is the reversible elongation of this wall when the cell is put in water, and "plastic extensibility" is the irreversible elongation which the wall undergoes in these circumstances. RUGE rightly observes that we have to distinguish between the elastic properties of longitudinal walls lengthwise and horizontally.

RUGE made numerous observations in respect of the behaviour of the cortical parenchymous tissue of hypocotyls of *Helianthus* to determine the three above mentioned mechanical properties of the longitudinal cell-walls in both directions and he did this with untreated seedlings as well as with seedlings after the application of growth hormone. He applied the growth hormone in two ways; either the decapitated hypocotyl was treated laterally with paste 1 : 1 or the cut surface with paste 1 : 8.

As he found in these two cases different results of the application of the growth hormone, RUGE thought this to be the consequence of the fact that in the first case the growth hormone penetrated in a horizontal, in the second case in a vertical direction.

From what we wrote in § 1 it will be clear that we consider the explanation wrong. The difference found by RUGE is the consequence of the fact that, when applying the growth paste laterally, he took a higher concentration than was used on the cut surface. Even if he had used the same concentrations he would have found differences; he has enhanced this difference by using 1 : 1 in the first case and 1 : 8 in the second.

In our Figure 22 we give part of „Figur 12" of RUGE's paper, viz. two graphs demonstrating the elastic extension on several successive days as determined with decapitated seedlings treated on the apical cut surface with growth hormone paste 1 : 8. The amounts mentioned are the decrease in length in percentages of the original dimension observed in the two directions, after the plasmolysis of the cells which had first been measured in paraffin-oil.

We draw attention to the fact that RUGE demonstrated that the osmotic value of cells treated with growth hormone paste soon decreases; this decrease is evident after a single day. The graph

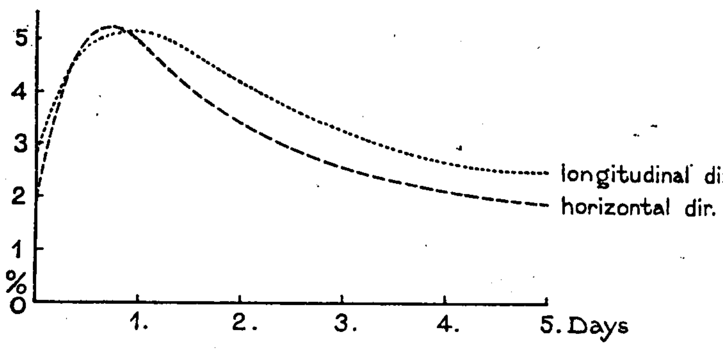


Fig. 22. The elastic extension in percentages of the cell walls in the longitudinal direction and in the horizontal direction on several succeeding days in the case of cells of the cortical layers of decapitated seedlings of *Helianthus* which were treated with growth hormone paste 1 : 8 on the apical cut surfaces (after RUGE).

therefore shows that maximum elastic extension occurs when there is a decrease in osmotic value.

We wish to point out that these graphs tend to show that elastic extension under the influence of the turgor pressure in the non-treated plants (see the values after 0 days) is less in horizontal direction than in longitudinal direction. In the theoretical part of this paper we will show that the tension in the wall caused by turgor pressure is considerably higher in horizontal direction than that in longitudinal direction. When we take this into consideration we find that the elastic *extensibility* of the cell wall, as caused by the turgor pressure in the non treated tissue, must be much less in horizontal than in longitudinal direction. This will surprise no one who reads our further statements regarding the submicroscopial structure of the cell wall.

According to RUGE the increase in the "elastic extension" resulting from the application of growth-hormone which, as the above graphs show, reaches a maximum after about 18 or 24 hours, is due to the decrease in the thickness of the cell walls („Die erste Phase beruht also allein auf eine Streckung"). He comes to this conclusion because he finds that the double refraction of the cell-wall in that period decreases by 22 %, whilst at the same time the total surface of the cell wall enlarges to the same extent. He assumes both features to be a consequence of a process of stretching in the cell-wall. Assuming that the "elastic modulus" does not change, RUGE points out that the thinner the wall the greater the elastic extension must be.

Later on we shall demonstrate why we consider that RUGE is wrong in assuming the decrease in double refraction to be an indication of the decrease in the thickness of the cell-walls. Such a decrease might just as well be the result of a change in the degree of statistical preference of the micelles for one special direction, and such a change may be expected in this case.

However, we shall also report about *direct* observations which convinced us of the fact that the first phases of longitudinal growth of the cell walls mechanically lead to decrease in thickness. We consider, however, that the conformity between the calculation of this decrease in thickness from the decrease in double refraction and from the enlargement of the cell wall surface, as was found by RUGE, is a mere coincidence. Moreover his supposition that the "elastic-modulus" remains constant during the stretching of the cell-wall is not correct.

7. *Elastic extension, elastic extensibility and plastic extensibility of longitudinal walls of epidermal cells.*

As we considered that the epidermis plays an important part in respect of the phenomena described, we thought it important to make experiments with this tissue similar to those made by RUGE with the cortical parenchyma. Seeing, however, that we always found that the phenomena were most characteristic within few hours after the application of the growth hormone, we took measurements in the main some hours after the beginning of the experiment. In some cases we used plants growing upright under influence of growth hormone paste, in others plants bending under the influence of unilaterally applied growth hormone paste. The experiments were made at 21° and 23° C., as the results are more evident at these than at lower temperatures.

We decapitated plants of 3 cm. length and applied paste 1 : 10 all round. The paste was removed after 1, 2, 3 or 4 hours, and we then determined the mechanical properties of the walls of the epidermal cells. The same was done with decapitated untreated plants. Moreover with some treated plants we took measurements after 16 hours.

We soon learned that it is no use taking measurements with strips of epidermis torn from the stem; the cells of such tissue proved to be very sensitive and for the greater part died when placed in the solutions. Therefore we always used epidermal tissue cut from the hypocotyl with a razor. We obtained our figures by measuring the total length of whole series of cells.

In common with RUGE we took our measurements for the tissues:

a. in paraffin-oil (we call this value N),

b. after a stay of one hour in glucose solution, in a concentration 0.2 mol. higher than the limit concentration determined in a preliminary experiment (this value is called P_1),

c. after a subsequent stay of half an hour in water (this value is called W),

d. after a further stay of half an hour in the above mentioned glucose solution (this value is called P_2).

Therefore in agreement with RUGE:

$$N - P_1 = \text{elastic extension,}$$
$$W - P_2 = \text{elastic extensibility, and}$$
$$P_2 - P_1 = \text{plastic extensibility.}$$

Reckoned as percentages of the quantity P_1, these values are indicated with the figures R, E and P.

Though hundreds of measurements were made, we only give here the data of three experiments made on different days. The figures in the table below are in each case the mean of 3 determinations.

TABLE 8. *Values (percentages of the length of the plasmolized cell) of elastic extension (R), elastic extensibility (E) and plastic extensibility (P) of the longitudinal walls of epidermal cells of decapitated hypocotyls of Helianthus, treated all round with growth hormone paste 1 : 10.*

	after 1 hour	after 2 hours	after 3 hours	after 4 hours
R	2.7	3.3	2.0	1.8
E	5.5	6.9	5.3	3.7
P	2.0	2.0	2.5	1.2
R	2.6	—	—	2.3
E	3.8	8.5	—	5.9
P	1.9	1.6	—	0.4
R	1.9	2.9	1.5	2.9
E	6.8	6.1	7.3	5.4
P	1.2	1.1	1.6	1.4

The following table gives the means of all our measurements (including those mentioned in Table 8) together with their standard error. We added the values for plants that were not treated with growth hormone paste.

TABLE 9.

	plants not treated	after 1 hour	after 2 hours	after 3 hours	after 4 hours
R	2.5 ± 0.2	2.4 ± 0.4	3.1 ± 0.5	1.8 ± 0.4	2.3 ± 0.4
E	3.6 ± 0.3	5.4 ± 0.7	7.2 ± 0.4	6.3 ± 0.5	5.0 ± 0.5
P	1.0 ± 0.2	1.7 ± 0.2	1.6 ± 0.3	2.1 ± 0.2	1.0 ± 0.2

Figure 23 moreover is a graph in which the average errors are shown by vertical lines.

It is clear from our measurements that for our plants there was only an optimum for *E*. For *R* and *P* an optimum can hardly be defended, the standard error being too great.

By a comparison of Figure 23 and 24 (see page 748) one can conclude that the amounts of *E* for decapitated plants treated with growth hormone paste are always higher — even after passing the optimum — than for decapitated non treated plants. The same might perhaps be said, though with less certainty, for the amounts *R* and *P*.

From this we may conclude that one hour after application of growth hormone paste the mechanical properties of the cell walls undergo a change, which reaches the culminating point after 2 hours, but remains distinct for several hours thereafter. This change is especially noticeable in the elastic extensibility of the walls.

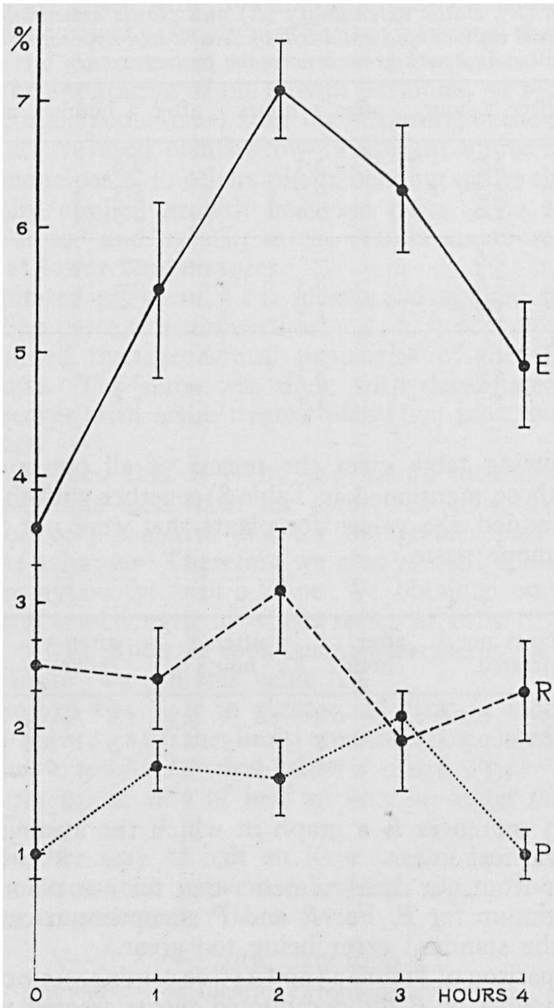


Fig. 23. The elastic extension (*R*), the elastic extensibility (*E*) and the plastic extensibility (*P*) of the longitudinal walls in the lengthwise direction in the case of epidermal cells of decapitated hypocotyls of *Helianthus*, treated on all sides with growth hormone paste 1 : 10; the vertical lines indicate the standard errors.

There is no reason to draw from the above figures the conclusion that changes in plastic extensibility are the primary effects of the influence of growth hormone in the cell walls of the epidermal cells. In our opinion these figures show that during the first few hours after the application of the growth hormone there is an increase in the elastic extensibility of the cell wall. The enlargement of the wall (in longitudinal direction) during the application of growth hormone must therefore be attributed more to elastic than to plastic extension. However, later on we shall show that one must be very careful in drawing such conclusions.

We have also made determinations of *R*, *E* and *P* with decapitated seedlings, not treated with growth hormone paste, at different times after decapitation. The changes in the values *R*, *E* and *P* were only slight, and as no optimum was to be expected in the first few hours, we made no further determinations during these hours, but only after 16, 24 and 48 hours. Table 10 gives the average values of 3 measurements.

TABLE 10. *Values of R, E and P of the longitudinal walls of epidermal cells of normal and of decapitated hypocotyls of Helianthus ascertained at different times after decapitation.*

	normal plant	16 hours after decapitation	24 hours after decapitation	48 hours after decapitation
R	2.5	2.0	1.0	0.9
E	3.6	2.2	1.5	0.7
P	1.0	0.5	0.2	0.0

Moreover the figures are given for normal (not decapitated) seedlings, being the averages of 5 values, each in its turn being the average of 3 measurements.

Figure 24 shows the amounts for the decapitated plants in the graphs. On the horizontal axis time is given in small units; the slopes of the lines in this figure cannot therefore be compared with the slope of the lines in the figure 23.

It is clear from the graphs that the amounts of *R*, *E* and *P* after 16, 24 and 48 hours decrease, this is most evident for *E*.

It should be borne in mind that the osmotic value of cells which lack growth hormone is higher than that of cells which increased in dimensions under the influence of a supply of this substance. RUGE showed this in the case of cortical parenchymous cells, whilst we found the same in epidermal cells; e.g. we found a value for the osmotic value (O_g according to G. BLUM and A. URSPRUNG, which nomenclature is also used by RUGE) of decapitated plants that received no growth hormone of 0.48 mol. and of 0.31 mol. for decapitated plants some days after they were supplied with that substance.

In connection with the fact that *R*, *E* and *P* are: extensions caused by turgor pressure (which depends on the osmotic value), there

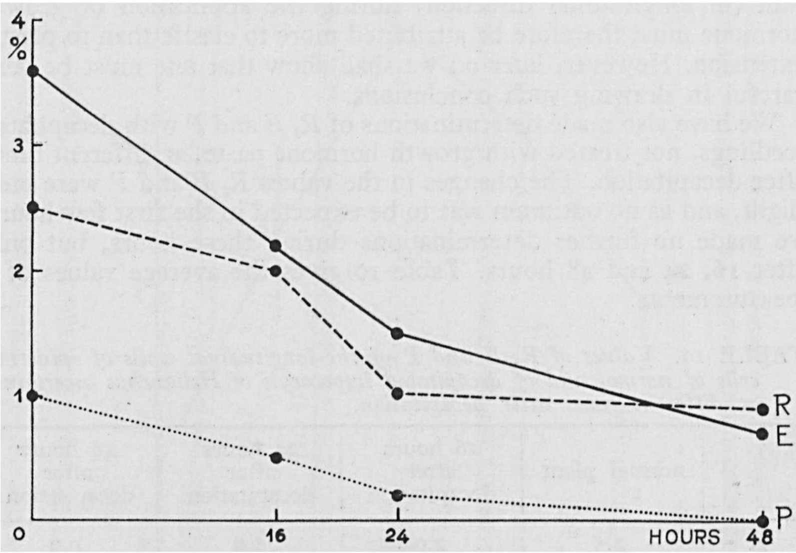


Fig. 24. The elastic extension (*R*), the elastic extensibility (*E*) and the plastic extensibility (*P*) of the longitudinal walls in the lengthwise direction in the case of epidermal cells of decapitated non-treated hypocotyls of *Helianthus* at different times after the decapitation.

is no doubt that longitudinal walls of epidermal cells of decapitated plants decrease their extensibility in length. Considering this, it is very striking that we found in the case of epidermal cells, as RUGE had done with cortical parenchymous cells, an increased elastic extensibility under the influence of growth hormone paste.

We also found distinct differences in the osmotic value in the case of cells of the convex and concave sides of hypocotyls unilaterally treated with growth hormone paste. Table 11 shows the results and we would observe that they are genuine and of importance.

TABLE 11. *Osmotic limit concentration of epidermal cells of the convex and concave sides of decapitated seedlings, curved by unilaterally applied growth hormone-paste.*

Concentration paste	Hours after application	Osmotic limit concentration (mol.)	
		convex side	concave side
1 : 1	24	0.25	0.29
1 : 1	48	0.33	0.35
1 : 1	48	0.29	0.34
1 : 1	48	0.30	0.33
1 : 1	72	0.29	0.31
1 : 1	72	0.25	0.28

Note: these measurements were made on various dates and with plants of various length. They are not to be compared with each other.

This difference becomes particularly striking if a direct comparison is made of slices of epidermis from the concave and the convex side of curved hypocotyls in a same solution. If we consider the great differences in the volume of the cells of these two slices (we wil' treat these differences later in this paper), the differences in osmotic values are not surprising. The growth hormone increases the extensibility of the cells of the convex side and therefore a smaller osmotic value may have caused a greater extension of the cell-walls of the convex side than of the concave side, where growth hormone will only arrive after some time, and at first in a low concentration.

8. *Some experiments to determine whether tissue tension affects the growth of hypocotyls and curvatures.*

Almost at the outset we asked ourselves whether the growth in the length of the cortex and especially of the epidermis could be due to extension caused by tissue tension alone. One might in fact imagine that the pith is active in growth in length, and thereby causes a passive growth of the cortex. In these circumstances we should have to take it for granted that in the cell walls of the epidermis and of the cortex deposition of cell-wall material, as a secondary process, takes place.

To find an answer to this question we pierced decapitated plants with a narrow metal drill, a kind of "hollow-punch" or "corkdrill". Leaving the drill in the pith we removed the cortex after cutting a horizontal and some vertical slits in it, only the pith inside the drill remaining (Fig. 25). The drill was then extracted and care was taken that the pith column did not desiccate. After 2 days it showed a growth in length of 32 %, partly by the release of the tissue tension, partly probably on account of the auxin in the pith. However, when we treated the column with growth hormone paste 1 : 1 we measured an elongation of 62.5 %.

In another experiment the pith was removed by means of a narrow drill across the end of which a thin platinum thread had been fixed. When the drill was pierced in the stem and then rotated, the column of pith was severed from its base and could be removed. In this way we had an isolated cortex cylinder. We then applied lanoline to the cut surface and after 2 days measured an elongation of 5 %; after treatment with growth hormone paste 1 : 1 however 32 %.

Fig. 25. Decapitated hypocotyl of *Helianthus* after a part of the cortex had been cut away.

These experiments make it clear that the cortex does react to growth hormone. From the figures one might, however, conclude that the reaction on the pith is a little stronger than the reaction on the cortex. This is by no means the rule, as was proved by the following experiment.

By means of a drill the pith and the cortex were isolated from each other, but both remained connected to the rest of the stem. The release of tissue tension very soon causes an elongation of the pith.

Some of the plants were treated with growth-hormone paste on the cut surface, some with lanoline, the elongation of both tissues was measured as soon as this elongation had ceased.

We found:

Elongation	of pith	with	growth-hormone	48.3%
”	” cortex	”	”	36.7%
”	” pith	”	lanoline	22 %
”	” cortex	”	”	0 %

At the end of the experiment the pith was still a little longer than the cortex. It will however be seen that, as regards elongation, the growth hormone has a greater influence on the cortex (*viz.*, 36.7 per cent.) than on the pith (*viz.*, 48.3 minus 22 or 26.3 per cent.).

We must therefore conclude that the cortex is capable of active elongation, as was also found by JOST [1], whose experiments were

[1] We refer to his last publication.

slightly different from ours. He cut the pith to the level of the cortex after the elongation of the pith, that was due to the release of the tissue tension, had stopped. Then he laid the hypocotyls in a solution of hetero-auxin.

We repeated our experiment, but with a further slight variation compared with JOST's. In this instance we applied growth-hormone 1 : 10 laterally to the cortex and found that after one day the cortex had elongated so much more than the pith as to make the latter disappear between the walls of the cortex-cylinder, as is shown in fig. 26.

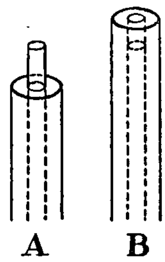


Fig. 26. A decapitated hypocotyl of *Helianthus* pierced with a drill so as to isolate the cortex of the pith, the latter expanding by tissue tension; then the outer parts were treated with growth hormone paste 1 : 10, *A*. the hypocotyl immediately after the isolation of the pith, *B*. the plant after one day.

We have to draw attention to the fact that the cortex cylinders in the above experiments without doubt contained cortical parenchyma and we shall later on demonstrate that this tissue reacts, at least in its inmost layers, exactly like the pith. If we bear in mind that by applying growth-hormone to the pith and cortex this latter tissue shows more elongation than the first, it will be realized that these cortical layers do not cause the active elongation of the cortical cylinders, and therefore the outer layers of the cortex must react more actively than the inner layers.

However, we too are convinced of the fact that, next to the active growth under the influence of growth hormone, tissue tension is of importance for growth; we will treat this when we report on the curvatures caused by growth-hormone paste on isolated pith-columns and isolated cortex-cylinders. Firstly, however, we must draw attention to the influence of wounds on growth in length, because this influence has to be taken into account in considering the above experiments.

When we controlled the growth in length of hypocotyls of which the epidermis was removed unilaterally over some distance, we obtained remarkable results. We used 2 series of each 6 seedlings, originally 4 cm. long and controlled the growth in length over a marked distance of 25 mm. reckoned from the top. After 24 hours this distance in both series had increased to 37 mm. in average and we therefore were sure that both series showed the same velocity of growth. We scraped off the epidermis of the plants of one series on one side over a distance of 2 cm. reckoned from the top and

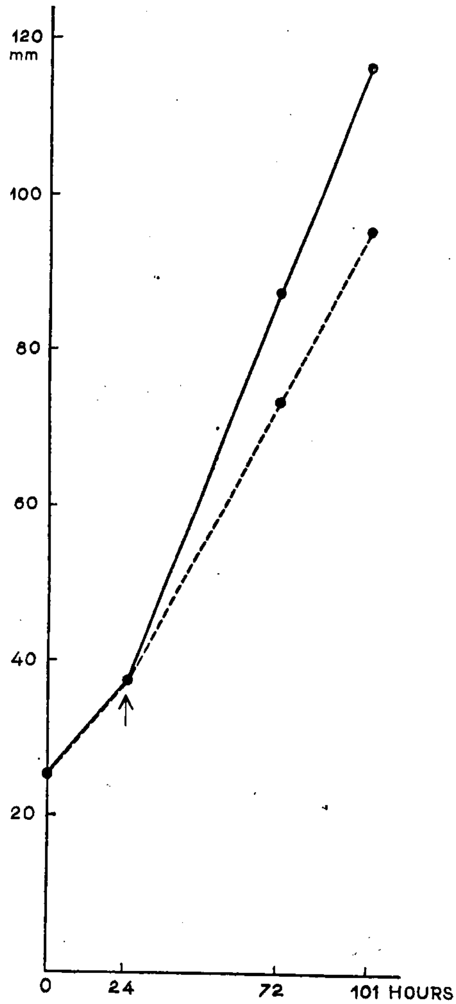


Fig. 27. The decrease of growth of seedlings of *Helianthus* when the epidermis has been partly and unilaterally removed; the unbroken curve: the growth of an intact seedling, the dotted curve up to the arrow, the growth of an intact seedling, furtheron the growth of this seedling after the unilateral removal of the epidermis.

then measured the originally marked distance of now 37 mm. after 48 and 76 hours.

TABLE 12. *Increase in length in mm. and in percentages of a marked distance of (originally 4 cm. long) hypocotyls of normal seedlings and of seedlings of which the epidermis had been removed unilaterally.*

	Increase in length in mm. and in percentages	
Time after begin of experiment	Normal plants	Unilaterally scraped off plants
48 hours	50 mm. (135 %)	36 mm. (97 %)
76 hours	79 mm. (213 %)	58 mm. (157 %)

As Figure 27 shows the growth of the unilaterally wounded plants is less than that of the normal plants.

Some further experiments showed that the decrease in growth in length after the unilateral removal of the epidermis over a distance of 2 cm. is far greater when the scraped section is in the middle than when it is in the apical part of the stem, and — as might be expected — still greater when the epidermis is removed over 2 cm. than when 1 cm. is scraped. Probably the unilateral removal is most harmful in the zone of greatest growth in length.

The results of these experiments make it clear why the increase of length (expressed in percentages of the original length) of isolated tissues is less than that of normal; differences in growth that are distinct in experiments of others as well as in those reported here.

We now come to curvatures that may result from the unilateral treatment of isolated pith or of cortex with growth hormone paste.

We could observe no clear curvatures after the unilateral application of growth paste on isolated pith columns. In no instance was there a connection between the curvatures of such pith columns and the place of the unilateral application of paste in higher or lower concentration. Unilateral treatment of cortex cylinders without pith, however, caused distinctly negative curvatures, as can be seen from Figure 28.

We repeated the last experiment, but this time we left the column of loosened pith in the cylinder of the cortex. We refer here to our Figure 29. The plants on the left are normal plants, those on the right have their pith loosened but not removed. Growth-hormone paste 1 : 10 was applied to all plants. The curvatures of the normal plants are more pronounced than of those with the loosened pith cylinders.

The following experiment shows that the greater curvature of the normal plants is caused by the action of tissue tension in the pith.

To the decapitated seedlings we attached a narrow india-rubber tube containing a small glass-bar by means of which we pressed the pith back into the cortex-cylinder. After such a seedling with

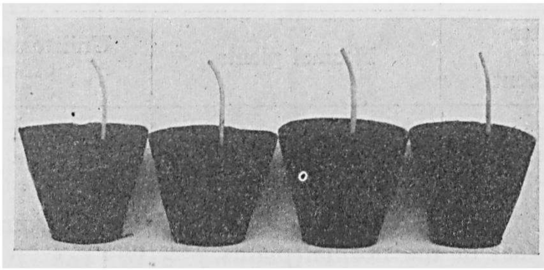


Fig. 28. Decapitated seedlings of *Helianthus* from which the pith cylinder had been removed (at the top), treated unilaterally (on the right) on the isolated (now hollow) cortex cylinder with growth paste 1 : 10; photo taken 5 hours after the application.

repressed pith had been treated unilaterally with growth-hormone paste 1 : 10, curvatures resulted as great as those of normal plants. See Figure 30. The pith of the plants to the left was not loosened, the pith of the middle ones was loosened but not repressed, and in the case of those to the right it was loosened and repressed. There is no difference to be seen in the curvatures of plants on the extreme right and left [1].

The artificial restoration of tissue tension in our experiment leads to an increase of the curvature.

Finally we wish to report on some features which we have observed but for which we cannot fully account. We can however state that these features indicate that there are rather complicated mechanical factors active in growth-processes.

We found that when a narrow band of non elastic material e.g. of gauze or of raffia is wound spirally round the hypocotyl there is a distinct decrease in growth in length, but that there is no such decrease when elastic material, e.g. strips of non-vulcanized rubber, is used.

Considering that the presence of the bands might possibly have

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[1] Special experiments showed that the weight of the glass-bar was of no influence.

A.

B.

C.

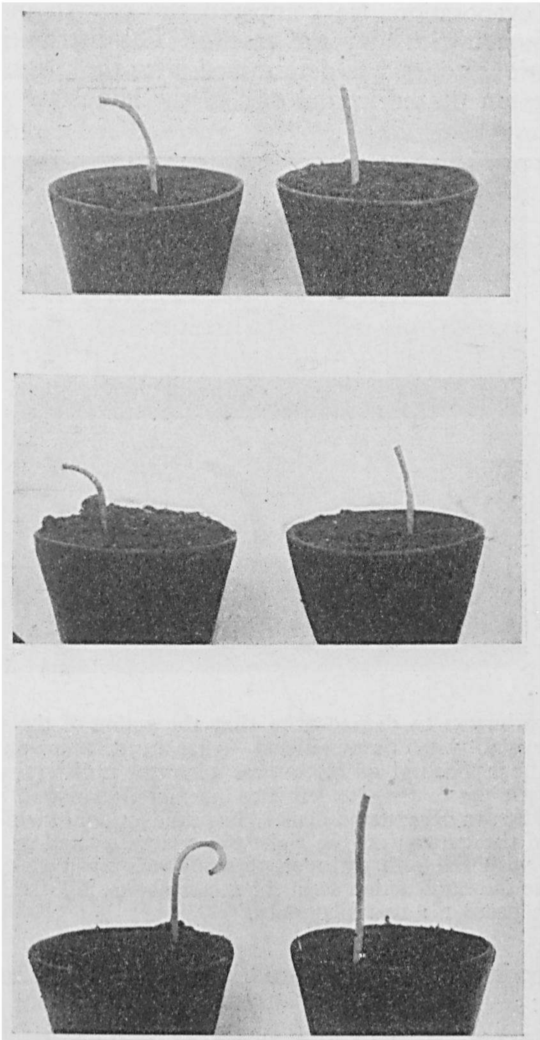

Fig. 29. Experiments demonstrating how the reaction to unilateral coating of growth-hormone paste 1 : 10 is affected when part of the cortex is loosened from the pith by drilling in decapitated seedlings of *Helianthus; A.* and *B.* photos taken 4 hours after the application, *C.* photo taken after 24 hours (in the latter case the paste is present at the opposite side compared with the first cases). On the left normal decapitated plants, on the right decapitated plants with the pith loosened at the upper part, but not removed.

influenced evaporation, we compared the growth in length with seedlings covered with a layer of vaseline. The distribution of growth over the various zones was determined after the plants had rotated for 72 hours on the horizontal disc of the klinostat.

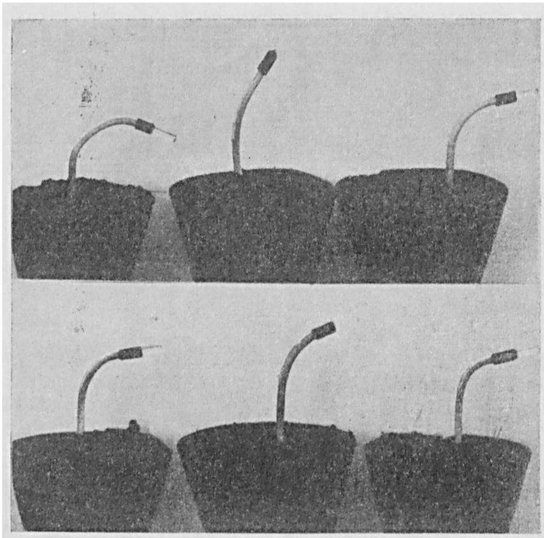

Fig. 30. Experiments to demonstrate that the action of the pith — which is caused by the tissue tension — has an influence on the curvature of the hypocotyl of *Helianthus* after the unilateral application of growth paste. On the left two normal decapitated plants; in the middle two decapitated plants, the pith cylinder of which is loosened from the cortex; on the right two plants treated in the same way but with the pith cylinder pressed back by a glass bar so as to cause the same action as in the intact plants. All plants were treated with paste 1 : 10 unilaterally.

The following percentages are the means of the measurements of 4 plants:

normal plants	140 %,
plants with vaseline	150 %,
with rubber bands	150 %,
with gauze bands	97 %,
with raffia bands	55 %,

A non-elastic band therefore causes a decrease in longitudinal growth.

9. *Anatomical observations.*

If we view the surface of the epidermis of very young seedlings (with a hypocotyl about 8 mm. in length) we find that the epidermal cells are only a little higher than they are broad. When the stems reach a length of 2 cm. however, the cells in the middle are distinctly stretched. The cells in the middle of plants about 8 cm. high show a considerable increase in length, but also an increase in breadth (see Fig. 31).

The same differences are also to be found in the epidermal cells of a seedling of 8 cm., when one first examines the cells close beneath

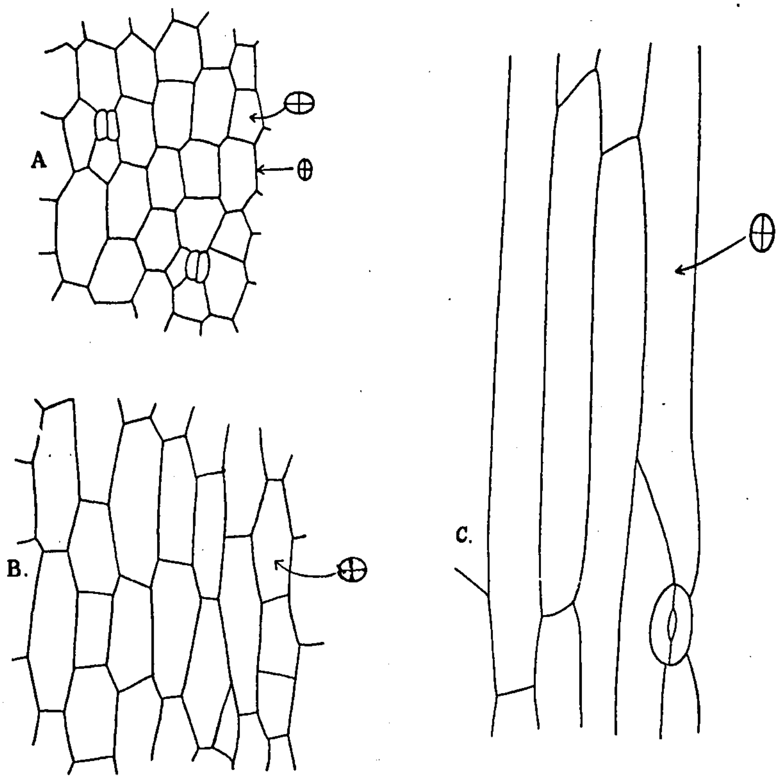

Fig. 31. Tangential sections of the epidermis in the middle of the hypocotyl of *Helianthus* seedlings of different length; in *A*. the hypocotyl was about 8 mm., in *B*. 2 cm., and in *C*. 8 cm. long. The small ellipses indicate the positions of the major and the minor refractive index as active in the cell wall in surface view or in transverse section.

the cotyledons, then those at a distance of about 1.5 cm. and afterwards those 4 cm. from the cotyledons.

In a surface view however there is not much variation to be seen in the thickness of the (radial) cell walls of the epidermes. When however transverse slides are made, it becomes clear that there is much difference in the thickness of the outer walls of the epidermal cells. In the upper part of the hypocotyl they are thickest, those at 2 or 4 cm. distance are distinctly thinner. A similar difference in thickness is also to be observed in most of the cell walls of the

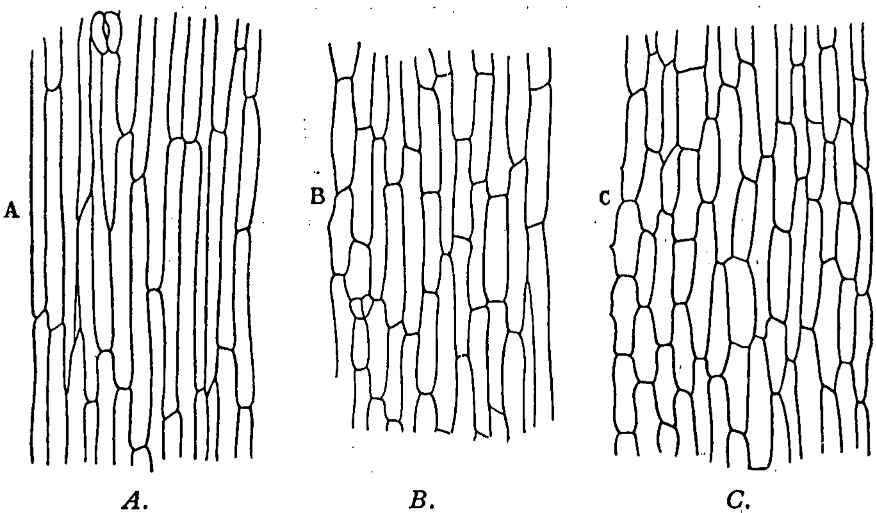

Fig. 32. Tangential sections of the epidermis of the middle part of hypocotyls of *Helianthus* which were treated all around with growth-hormone paste; the sections were made 2 days after the application; *A.* concentration of the paste 1 : 100, *B.* concentration 1 : 10, *C.* concentration 1 : 1.

cortical parenchyma and of the pith at different distances from the cotyledons.

From these observations it may be concluded that during growth in length a decrease in thickness of the cell walls takes place.

Moreover, when we compared the wall-thickness on transverse sections of normal plants with those of plants that had been treated during 24 hours with growth hormone paste in a low concentration, a similar decrease in thickness could be seen; this time it was the result of the increase in growth in length caused by the hetero-auxin.

We also took seedlings 3 cm. long which had been treated all round with growth hormone paste of the concentrations 1 : 100, 1 : 10, and 1 : 1 and compared the epidermal cells of the middle part of the stems after the plants had been turning for 2 days on the horizontal disc of the klinostat (at 23° C.). They had then reached a length of 12, 6.2 and 3.4 cm. Figure 32 shows that with an increase of concentration cell length decreases and cell width increases. As a rule the cells of normal plants and of plants treated with growth hormone in low concentrations are of almost the same breadth, those of plants treated with growth hormone in high concentrations are very unequal, one can find broad and narrow cells in one and the same slice.

Transverse sections of the plants after the treatment as shown in fig. 33 are very remarkable. The epidermal cells of the most elongated hypocotyls (A) are on an average narrower and have a thinner outer wall than those of hypocotyls treated with paste 1 : 1 (C); the latter aie broader and have thicker outer walls. Some of these thick cells protrude and thereby oppress the adjacent narrow ones. Without doubt this accounts for the above-mentioned inequality of the surface of the epidermis (and also for the changing direction of the long axis of the refraction-index ellipse, which will be treated in the next paragraph). As regards structure the epidermis of seedlings treated with 1 : 10 paste may be graded between the two other types.

In these transverse sections there is no clear difference in the thickness of the radial cell walls of the various plants. However, as a rule we found that after two days' treatment with growth hormone all cell walls showed an increase in thickness, especially after the application of high concentrations. Ruge also observed this.

From the foregoing it would appear that we have drawn the same conclusion as Ruge, though on much surer grounds, viz., that the decrease in the thickness of the cell walls is the first phase of the growth in length under the influence of growth paste, and it may be regarded as very probable that this is due to a mechanical elongation of the walls, which have apparently become more extensible.

In this connection the observations of Fr. Overbeck are worthy of mention. Overbeck found that the elongation of all the cells of the seta of *Pellia epiphylla* is accompanied by a decrease in the thickness of the walls. The total quantity of cell wall material however considerably increased though not sufficiently to keep pace with the decrease in the thickness of the walls. Overbeck

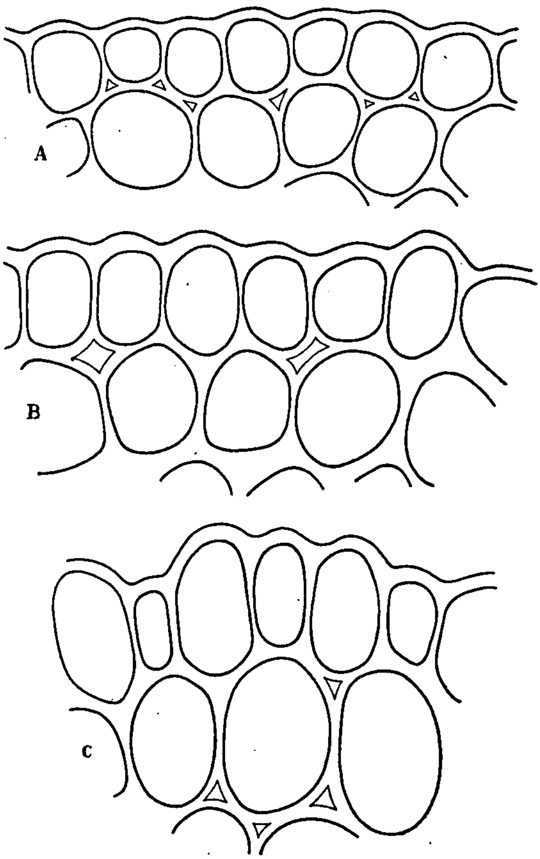

Fig. 33. Transverse sections of the outer layers of 3 seedlings of *Helianthus*, treated all around with growth-hormone paste, after rotating for three days on the horizontal disc of a klinostat. Concentration of the paste for *A.* 1 : 100, *B.* 1 :10, *C.* 1:1.

could not find any auxin in the setae. The decrease in the wall thickness of cells of *Helianthus*-hypocotyls is slight and the increase that follows in the second phase of the process had probably commenced at the very outset.

RUGE has reported on the differences in shape in the cells of the cortex caused by the application of hetero-auxin in a low and in a high concentration. Practically speaking, the diluted paste caused elongation only, whereas the strong paste merely caused a broadening of the cells. RUGE is, however, wrong in supposing that the differences were to be attributed to the fact that in the first instance the growth hormone was applied longitudinally and in the second horizontally. He would have found the same difference if he had applied the pastes in one and the same way in both cases.

When seedlings have been treated unilaterally with growth hormone paste, examination of the curved plants shows that a difference exists in the length of the epidermal cells of the convex and the concave side of the hypocotyl. Table 13 gives the amounts, and it is clear, that as a rule not only an increase in length is the result, but also a small increase in the breadth of the cells.

TABLE 13. *Average length and breadth of epidermal cells of convex and concave side of decapitated seedlings curved after unilateral application of growth hormone.*

number of the experiment	concentration of paste	hours after application	average length of epidermis cells		average breadth of epidermis cells	
			convex	concave	convex	concave
1	1 : 4	20	49	29	5.1	4.8
2	1 : 4	48	42	27	4.9	4.1
3	1 : 4	96	41	21	6.4	6.5
4	1 : 1	20	42	26	5.4	4.3
5	1 : 1	24	46	23	4.2	5.4
6	1 : 1	48	25	13	6.2	4.5
7	1 : 1	48	56	21	4.8	4.8
8	1 : 1	48	49	28	5.4	4.5
9	1 : 1	72	57	37	4.7	4.4
10	1 : 1	72	37	20	5.8	4.6

The experiments were of various dates, therefore they are not comparable the one with the other.

Figure 34 shows the difference between the epidermis of the convex and concave sides of decapitated seedlings as seen from the surface after the unilateral application of growth hormone paste 1 : 1 (the convex side was treated with paste).

The drawings A_1, A_2, A_3, B_1, B_2 and B_3 show the cells as seen after 1, 2 and 3 hours. They clearly show that after 1 hour no differences were to be seen, after 2 hours they became slightly visible, after 3 hours they were distinct. These drawings are once more a direct proof that curvatures of unilaterally treated seedlings are actually the result of the increased growth in the length of the cells, though they also show some increase in growth in breadth.

The drawings A_4 and B_4 give the surface views of the epidermis of such plants 145 hours after the unilateral application of growth-

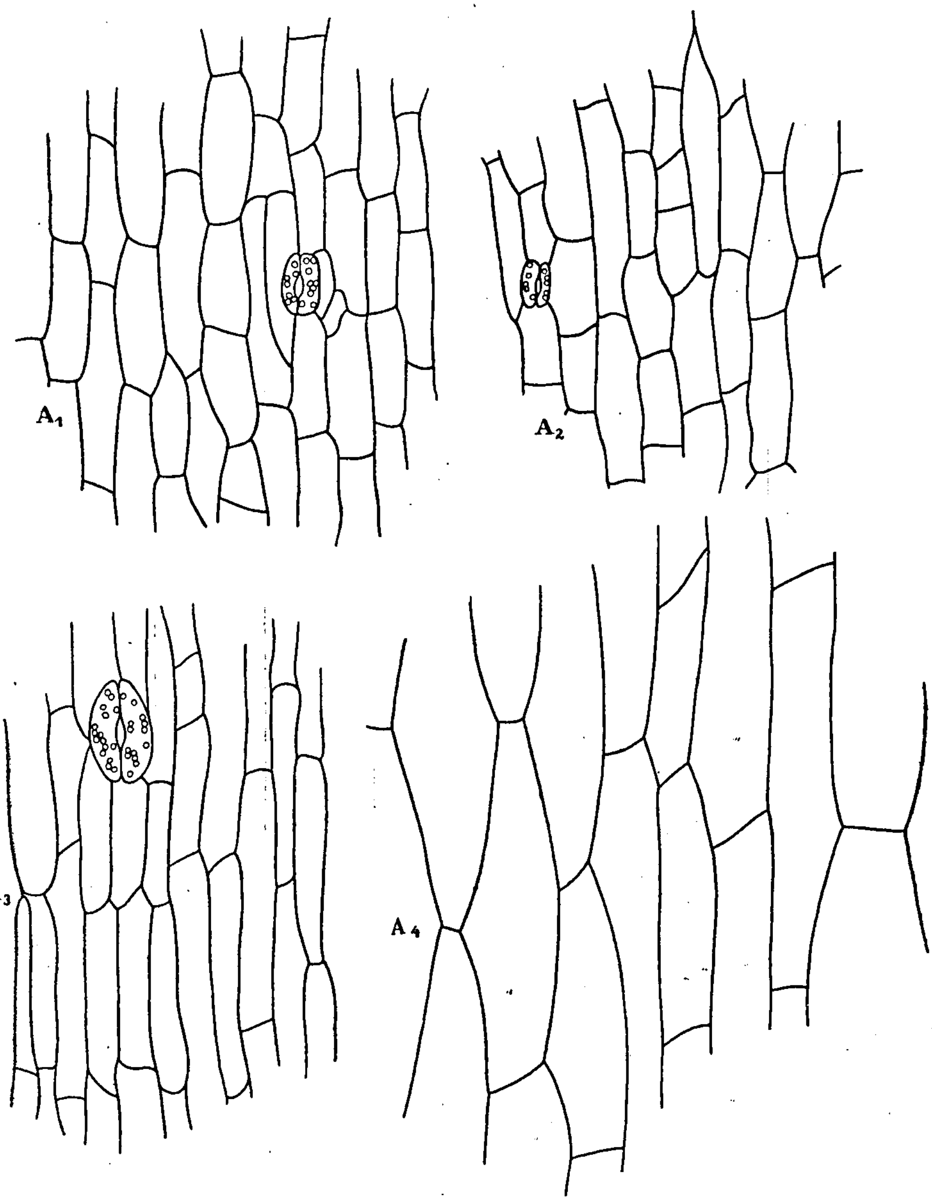

Fig. 34 (I). — See also page 763.

Fig. 34. Differences in the dimensions of the cells on the convex and the concave sides of seedlings of *Helianthus* hypocotyls unilaterally treated with growth-hormone paste 1 :1, the place of the application becoming the convex one; A_1, A_2, A_3, A_4 tangential sections from the convex sides 1, 2, 3 and 145 hours after the application, B_1, B_2, B_3 and B_4 tangential sections of the concave sides at the same moments. All magnifications are the same.

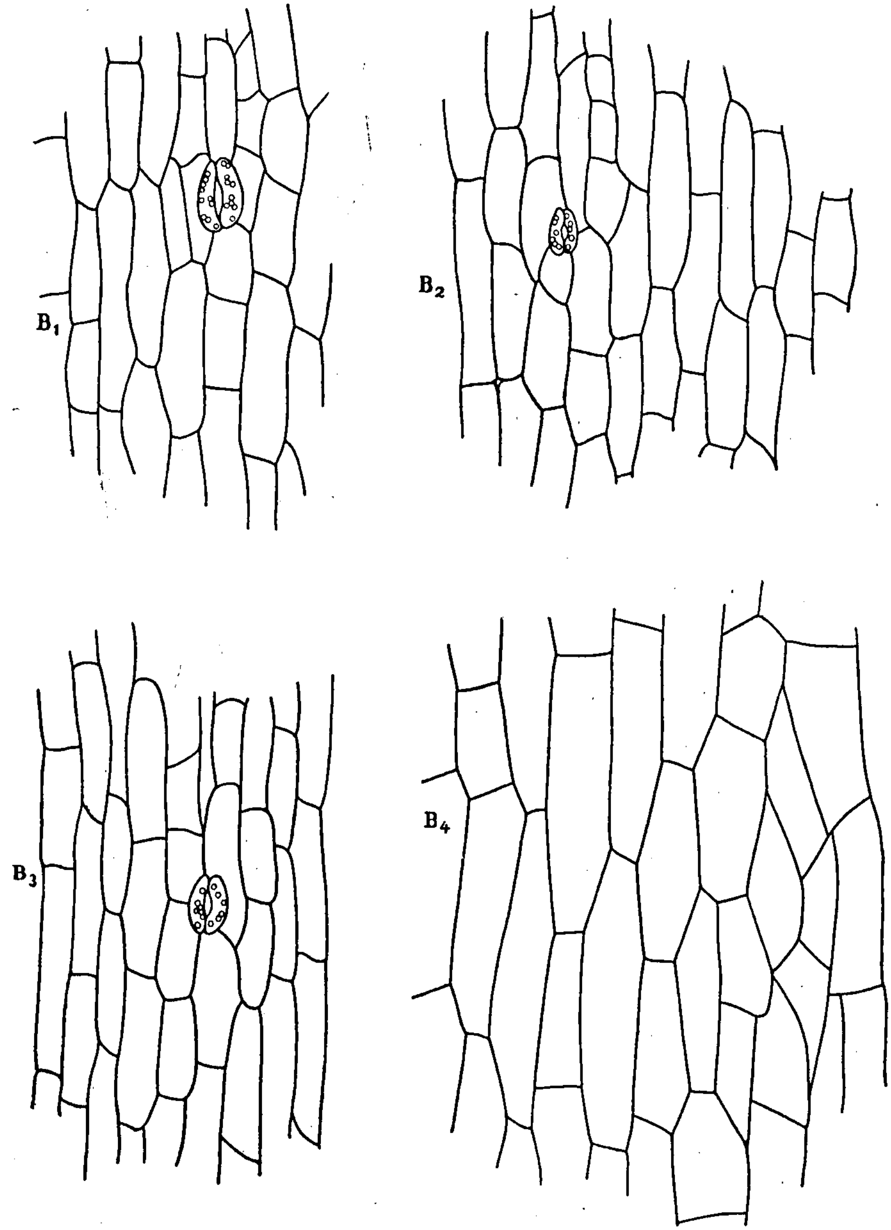

Fig. 34 (II).

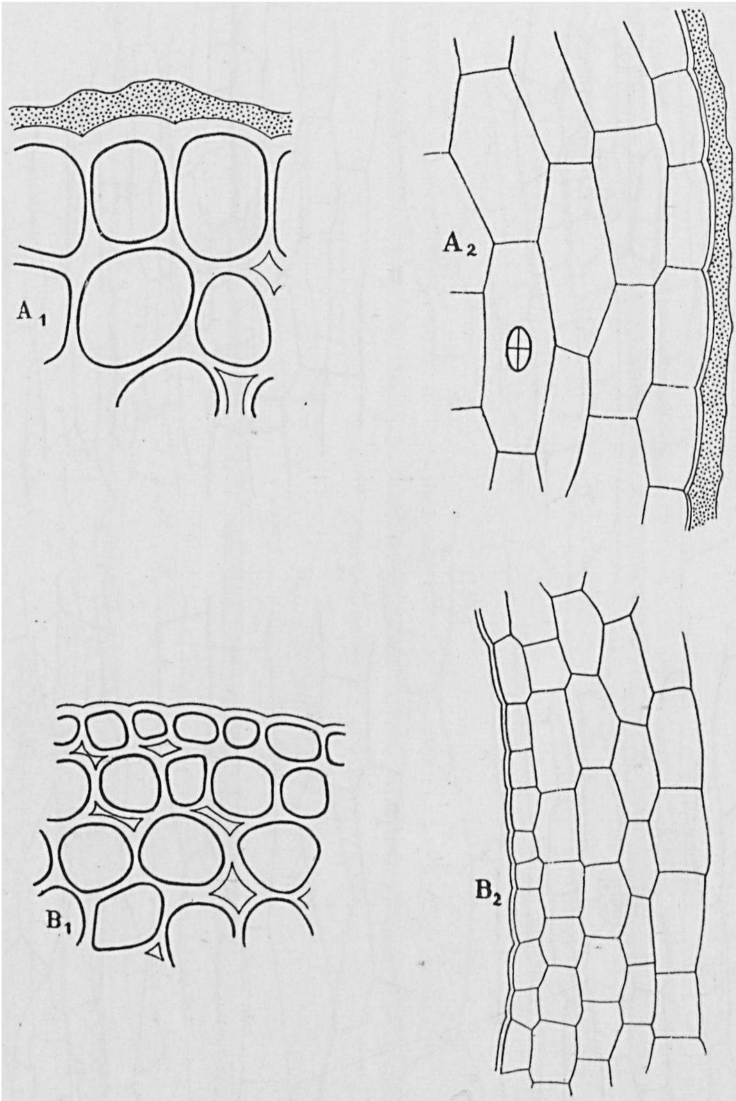

Fig. 35. Differences in the dimensions of the cells on the convex and concave sides of seedlings of *Helianthus* unilaterally treated with growth-hormone paste 1 : 1; the paste is indicated by the dotted area; A_1 and A_2 are a transverse and a radial section of the convex side, B_1 and B_2 are a transverse and a radial section of the concave side. The magnification of A_1 and B_1 is higher than that of A_2 and B_2.

hormone paste in high concentration. Differences have now become very pronounced.

In figure 35 we give transverse and radial sections of the cortex of the convex and concave sides of a curved seedling after some days of treatment with growth hormone. It is evident that the cells have not only become longer and broader on the treated side, but that also the quantity of cell wall material has increased.

We also examined the structure of the small tubers which formed 4—5 days after growth hormone paste in a high concentration had been applied as a ring round the hypocotyl. This fact was first observed by CZAJA [1]. In the two upper figures of Figure 36 we give transverse sections of a small part of the pith and of the cortex of a normal seedling, in the figures at the bottom of Figure 36 those of a treated seedling. Both plants were of the same age. It is evident that especially the cells of the cortical parenchyma have increased in volume, but that this is also the case with the cells of the pith.

Finally we wish to report on a phenomenon observed in examining the epidermis in the upper and lower parts of greatly elongated hypocotyls. We often found that the cuticula of cells in the most elongated part of the stems becomes detached from the epidermis. We were able to demonstrate this fact after an examination of the shape of the water meniscus at different levels of the hypocotyl in the same way as had been done in this laboratory by Miss J. M. DIEHL [2] to prove the presence or the absence of the cuticle on the epidermis of the leaves of *Helodea canadensis*. As might be expected, when the upper part of the hypocotyl was placed in a tube with water, the meniscus was depressed, and when this was done with the lower part of the hypocotyl, the meniscus bent upwards, so as to form a hollow meniscus (see Fig. 37 *A* and *B*).

10. *Observations on the submicroscopical structure of the cell walls of the hypocotyl of Helianthus annuus.*

RUGE was the first to establish the transverse position of the longest axis of the active refractive index-ellipse in the longitudinal walls of the cortical parenchymatous cells of the hypocotyl of *Helianthus*. He concluded that the cellulose micelles in the longitudinal walls show a statistical preference for the transverse direction. Using the nomenclature of FREY-WYSSLING one may say that these cells have a "Röhrenstruktur". According to RUGE

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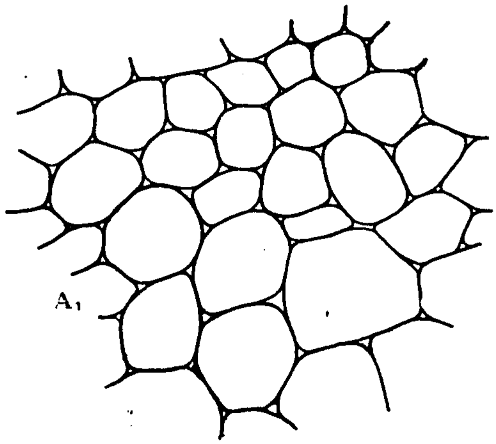
[1] Polarität und Wuchsstoff, Ber. Deutsch. bot. Ges. 53, 197—220, 1935.
[2] Einige Beobachtungen über die Aufnahme von Farbstoffen durch die Blätter von Helodea densa und Helodea canadensis, Recueil travaux bot. néerl. 23, 502—508, 1936.



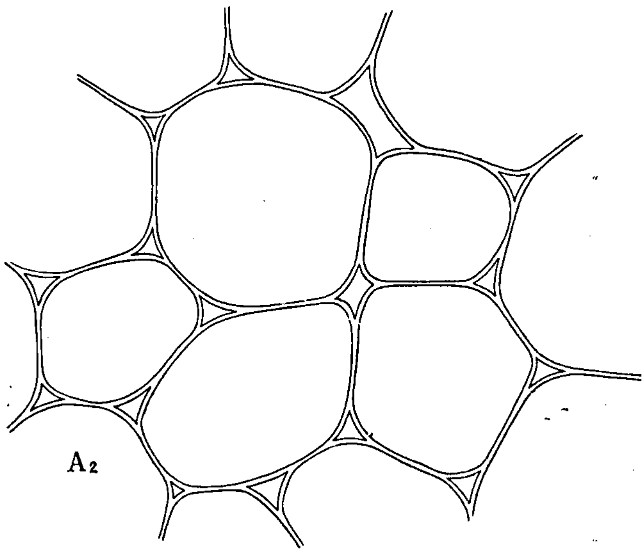


Fig. 36 (I). — See also page 767.

Fig. 36. The enlargement of the cells when small tubers are formed after some days on the hypocotyls of *Helianthus* through the application of strong growth hormone paste; A_1 and B_1 parts of the cortex and the pith of normal seedlings, A_2 and B_2 parts of the same tissues in the tubers. The magnification is the same for all the figures.

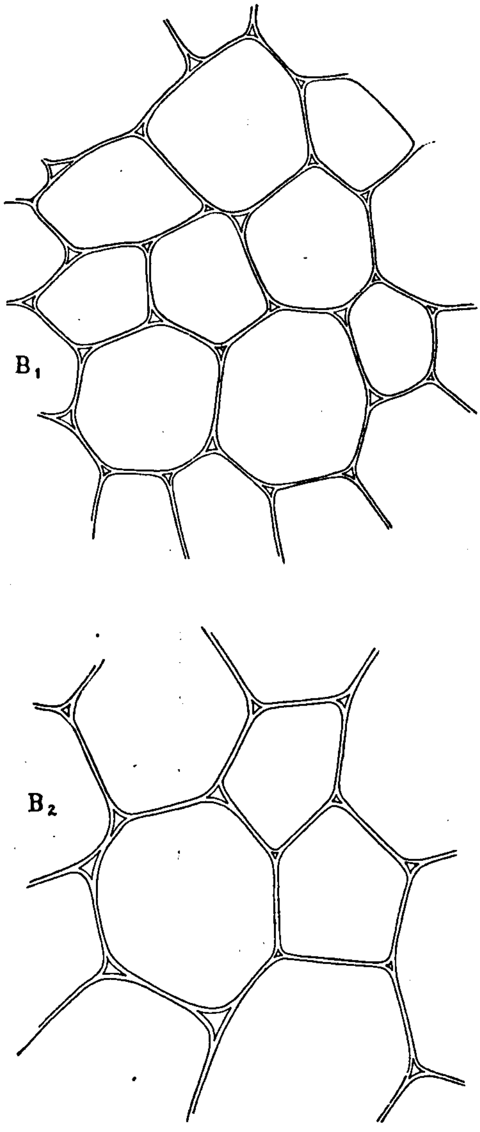


Fig. 36 (II).

this structure remains unchanged during the growth in length of the cortical parenchyma. The transverse walls of this tissue, however, were found by RUGE to be isotropic in surface view, this means

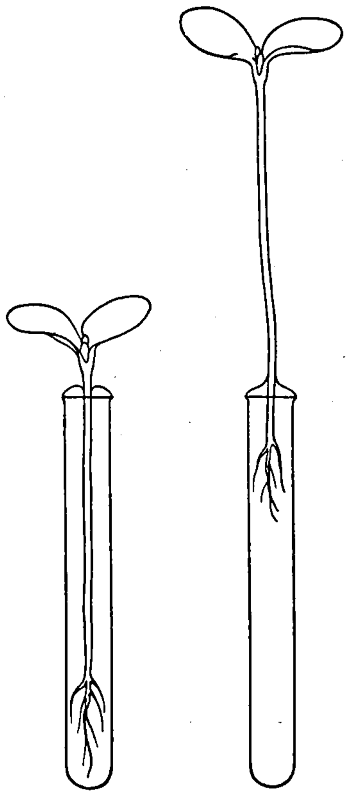


Fig. 37. Demonstration of the fact that in the younger part of the hypocotyl of *Helianthus* the cuticle is present, and in the older parts it is absent; *A*. the water forms a convex meniscus when the seedling is immersed deeply into the water, *B*. the water forms a hollow meniscus when the seedling is only immersed for a small part.

that in the plane of these walls the micelles showed no preference for a special direction.

We made similar observations. We found the above-mentioned structure in the case of the walls of the cortical parenchyma of hypocotyls which, after treatment with growth-hormone paste in rather high concentrations over the whole surface, have shown an increased growth in thickness, as well as in the case of the walls of the cortical parenchyma of hypocotyls which have swollen to tubers after being treated with a ring of strong growth-hormone paste. Therefore it is sure that cortical cells growing more in transverse than in longitudinal direction do not change their submicroscopical structure.

The optical properties of pith cells are the same as those of the cortical parenchyma, they too have „Röhrenstruktur", irrespective of whether the pith has grown more in longitudinal or more in transverse direction.

A still more interesting feature however is the structure of the outer cell wall of the epidermis. In young cells the longest axis of the refractive index ellipse in this wall is horizontal, but in slightly older cells the walls are isotropic or almost so, whereas in still somewhat older cells the axis is vertical. In the drawings of such sections (Fig. 31 on page 757) we have indicated the different optical behaviour by means of small refractive index ellipses. One may conclude from these observations that the micelles of epidermal cells very soon show statistical preference for the longitudinal direction.

The same submicroscopical structure has been established by A. N. J. Heyn [1] for the outer epidermal cell walls of the *Avena* coleoptile (for the cells of the parenchyma of this object H. Söding [2] found a tubular structure, but Preston [3] some time ago stated that these cells had a spiral structure). J. Bonner was the first to describe the above mentioned structure for the epidermis of the *Helianthus*-hypocotyl. As a matter of fact this structure is often to be observed in the outer walls of epidermal cells; several of the older investigators were acquainted with this structure.

Söding has asked whether the difference in orientation of the micelles in parenchyma and in the outer wall of the epidermis of the *Avena*-coleoptile might be caused by tissue-tension. One might very well suppose that this tension causes an orientation of the micelles of the outer cell wall. We shall treat this problem in the theoretical part of this paper.

There is no doubt whatever that turgor pressure also stretches

[1] A. N. J. Heyn, Further investigations on the mechanism of cell elongation etc. I, Protoplasma **19**, 78—96, 1933.

[2] H. Söding, Über die Wachstumsmechanik der Haferkoleoptile, Jahrb. wiss. Bot. **79**, 231—255, 1934.

[3] R. D. Preston, The structure of the walls of parenchyma in *Avena*-coleoptiles, Proc. Roy. Soc. London B. **125**, 372—386, 1938.

the epidermal outer wall much more in a longitudinal than in a horizontal direction. We have to draw this conclusion from a structural characteristic which has already been observed by C. NÄGELI [1] and by CORRENS [2] for some of the epidermal outer walls and which has also been described for this wall of the *Avena*-coleoptile.

In this instance we have to deal with the occurrence of small wrinkles on the inner side of these walls. SÖDING observed that this wrinkling is only visible when the cells have been plasmolized or when they are dead, so that one has to consider it as a result of the puckering of the inner side of the wall, which was first stretched through turgor pressure.

If the outer wall had been stretched more in a horizontal than in a longitudinal direction, vertical wrinkles would have resulted.

We observed this structural peculiarity in the case of the epidermal cells and also in the case of the underlying cells of the hypocotyl of *Helianthus*.

If one should ask how there can be a greater extensibility in the outer wall in longitudinal than in horizontal direction, although the fact that the micelles show statistical preference for the longitudinal direction might induce one to expect the opposite, we would refer to the theoretical part of this paper. There is however another structural peculiarity, perhaps closely related to this question. Nevertheless we must admit that this peculiarity is possibly more of a secondary than of a primary nature.

B. J. D. MEEUSE [3] has demonstrated in this laboratory that, after treatment with a solution of chloride of zinc and iodine of a special concentration or with colouring materials (with some objects no treatment at all is necessary, with others observation in polarized light is sufficient), the outer wall of cells of various epidermal tissues shows signs of very delicate transverse striae, which are not however a consequence of the above mentioned puckering.

NÄGELI (l.c.) and CORRENS (l.c.) were the first to observe this phenomenon in the epidermis of *Hyacinthus orientalis* and the latter observed this also in some other epidermes. HEYN (l.c.) observed these striae in the epidermis of the *Avena*-coleoptile. MEEUSE has furnished a drawing of this structure, for the outer cell wall of the epidermis of the *Helianthus*-hypocotyl.

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[1] C. NÄGELI, Über den inneren Bau der vegetabilischen Zellmembranen, Sitz. ber. München, 7 Mai 1864.

[2] C. CORRENS, Zur Kenntnis der inneren Struktur der vegetabilischen Zellmembranen, Jahrb. wiss. Bot. **23**, 254—338, 1892.

[3] Some observations on special structure in the cell-walls of plants. Proc. Kon. Akad. Wet. Amsterdam **41**, 965—975, 1938.

Very careful observations brought the latter experimentor to the conclusion that these striae are caused by the occurrence of alternating layers of two kinds of bands, perpendicular to the surface of the cell-wall and transversely arranged in this wall. It would seem that some of these bands have a regular and dense arrangement of the cellulose-micelles in the longitudinal direction of the wall, whilst in the others the micelles are not ordered at all or only slightly so.

It is of course quite possible that the presence of the latter bands causes a greater extensibility in length of the outer cell walls.

As regards the submicroscopical structure of the radial cell walls and of the inner walls of the epidermal cells of *Helianthus*, these walls, provided there is no collenchymatic thickening, are almost isotropic in surface view; sometimes there might perhaps exist a slight statistical preference of the micelles for the longitudinal direction but more often a preference for the horizontal is to be observed. It is however very difficult to examine these walls under the microscope.

What we have said regarding the radial and inner walls of the epidermal cells applies also in the case of the thinner parts of the collenchymatic cells, which lie under the epidermis.

Probably the micelles in the thicker parts of these collenchymatic cells show a statistical preference for the longitudinal direction though it is very difficult to observe. This is due to the fact that the shortest axis of the refractive-index ellipsoid is always perpendicular to the wall surface, so that in considering "optical sections" of a wall, a longer axis of the active index ellipse always lies in the longitudinal direction of that wall section.

We wish to confirm the foregoing exposition on the submicroscopical structure of the cell walls of the hypocotyl of *Helianthus annuus* by reporting the results of some swelling experiments with the cells of the hypocotyl.

Former experimentors, to wit C. NÄGELI (1864), R. VON HÖHNEL (1882 and 1884), A. ZIMMERMANN (1883), A. ZIMMERMANN and G. EICHHOLZ (1886), S. SCHWENDENER (1887) and especially C. STEINBRINCK (1888 and later) gave several proofs from which may be concluded that cell walls of cellulose, after treatment with swelling agents in low concentration, show the highest degree of swelling in the direction of the shortest axis of the refractive-index ellipsoid (perpendicular to the wall surface) and the lowest in the direction of the longest axis, whereas the swelling is intermediate in the direction of the middle axis. When high concentrations are used there is even shrinkage in both of the last mentioned directions, and the greatest shrinkage will be found in the direction of the longest axis of the refractive-index ellipse.

VAN ITERSON [1] has drawn attention to the fact that this thesis can be applied to advantage in the study of the submicroscopical structure of cell walls.

We have adopted the same principle also in studying the cell

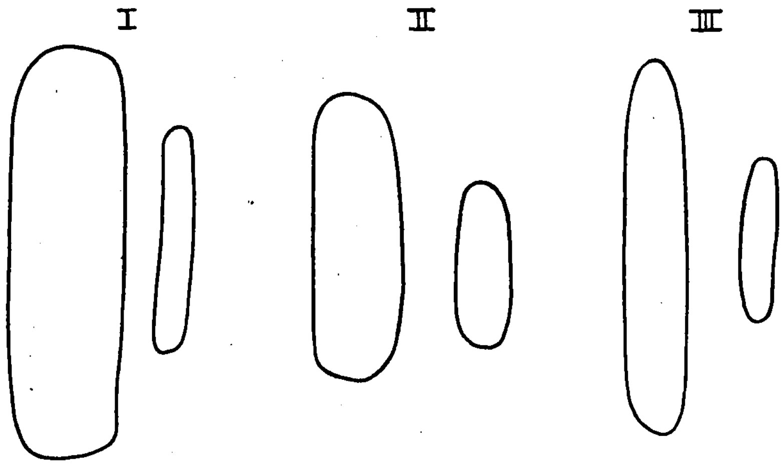

Fig. 38. Examples of shrinkage of cells, isolated by maceration, after treatment with a 50 per cent. solution of sodium hydroxyde; the original cells on the left, the cells after the shrinkage on the right; I. a cell of the pith of a *Helianthus* seedling, II. a cell of the cortex, and III. a cell of the epidermis.

walls of the hypocotyl. We accordingly put sections in a solution of sodium hydroxide (50 %), but we also treated in this way cells that had been isolated by means of maceration with hydrogen-peroxide (according to KISSER). Before and after treatment with the swelling agent we took the dimensions of the cells.

In Figure 38 I we give a pith cell of a 12 cm. long hypocotyl and the same cell after treatment with sodium hydroxide of 50 per cent. Figure 38 II shows the same for a cell of the cortical parenchyma and Figure 38 III for an epidermal cell.

The average shrinkage, which was determined for a number of cells of the same kind, amounted to:

[1] A few Observations on the Hairs of the Stamens of Tradescantia virginica, Protoplasma 27, 190—211, 1937.

30 per cent. for pith cells in vertical direction,
54 „ „ „ „ „ „ transverse „ ,
41 „ „ „ cortical parenchyma in vertical direction,
44 „ „ „ „ „ „ transverse „ ,
49 „ „ „ epidermal cells in vertical direction,
44 „ „ „ „ „ „ transverse „ .

In some other cases we found still more pronounced differences, for instance, for pith cells of seedlings resp. 4.7 and 10 cm. long, we found shrinkage in vertical and in horizontal direction of resp. 35 and 55 per cent., 21 and 68 per cent., 21 and 60 per cent. For the epidermal cells of another seedling 10 cm. long, shrinkage in both directions of 54 and 45 per cent. We are however fully aware that our experiments were not numerous enough to yield accurate results and we have no doubt that a more thorough study might reveal further interesting aspects.

These results of the swelling tests in any case confirm the views of RUGE regarding the submicroscopical structure of the walls of the cortical parenchyma and our views of this structure in respect of the walls of the pith cells and the epidermal cells, based on optical observations.

11. *Summary of the Experimental Part.*

1. Growth-hormone paste applied to the cut surface of decapitated hypocotyls, hardly growing in length, increases this growth in length to an extent dependent on the concentration of the growth hormone; this increase however is less than when the paste is applied all round the decapitated hypocotyls.
2. Growth-hormone paste in low concentration applied all over the surface of normal or of decapitated hypocotyls causes an increase or even a renewal of the growth in length; paste in high concentration on the other hand, when similarly applied, retards growth, or in the case of decapitated seedlings causes only a slight renewal of growth in length, but always increases at the same time the growth in thickness. This increase is however less in the case of decapitated plants.
3. When normal seedlings are treated with a ring of strong growth-hormone paste near the top, or unilaterally treated with a strip of strong growth-hormone paste, there will be a decrease of growth in length and moreover an increase in growth in thickness; the same treatment of decapitated plants causes a renewal of growth in length and at the same time an increase in growth in thickness.

4. The degree of the growth in the length and in the width does not depend on the direction in which growth hormone is applied, but on the strength of the concentration used.
5. In agreement with the results of other workers with the *Avena*-coleoptile, we found that if a ring of strong growth-hormone paste is applied to the *Helianthus*-hypocotyl in the zone of the greatest growth in length it causes more decrease in growth in length above the ring than below it.
6. Curvature will occur in the hypocotyl of *Helianthus* under influence of unilaterally applied growth hormone even though this may call for an exertion on the part of the stem.
7. Curvatures caused by the unilateral application of growth-hormone paste to normal or to decapitated seedlings of *Helianthus* show an optimum at a certain concentration; this optimum changes distinctly in the first instance and slightly in the second case when the reaction proceeds.
8. This optimum may be partly accounted for by the fact that part of the growth hormone is transported by the force of gravity through the curved hypocotyl to the opposite side and there increases growth in length, which causes considerable anti-reaction especially when pastes of high concentrations are used; therefore if during the experiment the plants are placed on the vertical disc of the klinostat, a more pronounced curvature will result than when they are placed on the horizontal disc.
9. For another part the optimum is due to the fact that growth hormone moves independently of gravitation to the opposite side of the hypocotyl, where it causes an increase of growth in length and therefore an anti-reaction, especially when high concentrations are used; therefore, when the movement of the growth hormone is prevented, a greater curvature will be obtained than when this movement is possible.
10. When we rendered the movement of growth hormone absolutely impossible there was still evidence of an optimum concentration for the curvatures.
11. The force of gravity transports the natural auxin as well as synthetic growth hormone to the opposite side of a bending hypocotyl.
12. The unilateral application of growth hormone to hypocotyls from which the epidermis has been entirely removed causes negative curvatures in all concentrations; but these curvatures are less pronounced than when normal plants are used.

13. There is no doubt that the reaction of the epidermis to all concentrations of growth hormone begins with an increase in growth in length, which is more pronounced than the increase in the length of the underlying layers.
14. Low concentrations applied to the wound surface of unilaterally scraped hypocotyls cause (after recovery from wound reaction) only weak negative curvatures or no curvatures at all.
15. High concentrations applied in such circumstances cause first positive curvatures to a degree dependent on the concentration of the paste and — when the positive curvature was not very strong — afterwards negative curvatures; the first, positive curvatures are occasioned exclusively by the movement of growth hormone to the opposite side of the hypocotyl and the consequent increase of the growth in length of the epidermis on that side.
16. To bring about an increase of growth in length of the epidermis, which is the invariable result of treatment with growth hormone in any concentration whatever, it is immaterial how the growth hormone reaches the epidermis.
17. A temporary or permanent ring of growth hormone paste of high concentration stultifies the reaction capacity of the hypocotyl to the unilateral application of growth hormone, or to the force of gravity, partly or completely, permanently or temporarily.
18. Growth-hormone paste in low concentration applied to the outside of the halves of hypocotyls (curved outward on account of tissue tension) causes an inward curvature of these halves; when however such paste is applied to the inside no change will occur in the original outward curvature.
19. Growth-hormone paste in high concentration applied to the outside of such slips will cause strong negative curvatures (the slips bending inward); when such paste is applied to the inside the resultant curvatures will primarily be positive and — when the positive curvature was not very strong — later negative; the positive curvatures are due to the growth hormone reaching the epidermis after passing through the tissue and to the fact that this tissue is in the beginning more stimulated to growth in the length than is the case with the other tissues.
20. The epidermis and underlying tissue are capable of active reaction to a supply of growth hormone by increasing in length, without the coöperation of tissue-tension, which stretches the epidermis both in intact and in split hypocotyls.
21. The differences observed by U. Ruge in the mechanical proper-

ties of longitudinal walls of cortical parenchyma of hypocotyls of *Helianthus* when these were treated *a.* with a ring of paste 1 : 1 on the outside of the decapitated hypocotyls, or *b.* on the cut surface with paste 1 : 8, are due to the differerences in the concentrations of the growth-hormone that reached the growing parenchyma and not to the moving of the growth-hormone in different directions.

22. From the observations of U. RUGE it may be concluded that the longitudinal walls of the cortical parenchymatous cells are more extensible in the longitudinal than in the transverse direction.
23. When growth-hormone paste 1 : 10 is applied all round the hypocotyl an increase in the lengthwise elastic extensibility of the longitudinal walls of epidermal cells of decapitated hypocotyls occurs after a few hours; then a decrease follows, after 4 hours this extensibility is still greater than it would have been at that time if no growth hormone had been applied.
24. After decapitated hypocotyls have been treated unilaterally with various concentrations of growth-hormone paste the osmotic limit concentration of epidermal cells is distinctly lower on the convex than on the concave side.
25. A hollow cylinder of cortical tissue with normal epidermis prepared free from the pith but still connected with the base of the hypocotyl, shows a greater increase in length after treatment with growth hormone than a cylinder of pith that has been prepared free after cutting away the cortical parenchyma. Therefore we conclude, as was also done by JOST and some other workers, that the outer cortical layers can show active growth in length (growth in length without coöperation of tissue-tension).
26. The unilateral removal of the epidermis of seedlings causes a very brief curvature away from the wound; after recovering the upright position the hypocotyl shows a pronounced decrease in growth in length..
27. The unilateral treatment of the outside of a hollow cylinder of the outer part of the stem causes distinct curvatures away from the paste; this also occurs when the cortical and the pith cylinder are loosened from each other; but in both cases the curvatures are less pronounced than after treatment of intact decapitated seedlings; after the mechanical restoration of tissue tension this difference no longer exists.
28. Spirally surrounding of a hypocotyl with an elastic narrow band has no influence on the growth in length; when however

a non elastic band is used, growth in length is highly decreased.

29. Epidermal cells of hypocotyls show an increase of growth in length when the hypocotyls are treated all round with growth-hormone paste in low concentrations (e.g. 1 : 100); there is a decrease when the growth hormone is used in a high concentration.
30. Wall thickness at first decreases, both under the influence of innate auxin and after the application of hetero-auxin, but an increase results soon afterwards; the first phase of surface growth is caused by the extension of the walls, probably accompanied with deposition of new cell-wall material; afterwards the cell walls may enlarge without decreasing in thickness, in fact there is often even an increase in thickness during the growth process.
31. The administration of growth hormone in low concentrations causes above all an elongation of cells; this elongation is less when a high concentration of growth hormone is used, but then the cells also expand in width; this applies for pith-cells and cortical parenchymatous cells as well as for epidermal cells.
32. The unilateral treatment of hypocotyls with growth-hormone causes more increase in length and width as well as in wall thickness of the cells under the paste than of those on the opposite side.
33. The epidermal cells cast their cuticula even at a fairly short distance from the top of the hypocotyl.
34. The longest axis of the active index-ellipse occurs not only transversely in the surface view of the longitudinal walls of cortical parenchymatous cells of normal hypocotyls, as was found by RUGE, but analogously in the case of seedlings which show a high increase in thickness due to a treatment with strong paste; the longitudinal walls of pith cells show the same position of the longest axis of the index-ellipse; thus all the cells mentioned here have „Röhrenstruktur".
35. In very young epidermal cells of the *Helianthus* hypocotyl the longest axis of the index elipse in the outer walls runs transversely; when these cells have grown older this axis runs longitudinally and always keeps this direction no matter how much the cells may grow in length or in width under the influence of natural auxin or under the influence of artificially applied growth-hormone.
36. The inner layers of the outer wall of the epidermal cells and the walls of the underlying cells show the same wrinkling after plasmolization and also after death as has been observed in the

epidermal cells of several other plants; this wrinkling shows that the outer wall has been stretched by turgor pressure more in longitudinal than in transverse direction; this greater extension may perhaps show some connection with a structural peculiarity of these walls, which was incidentally observed by other researchers; B. J. D. MEEUSE showed that this peculiarity is widely spread and is very distinct in the *Helianthus* hypocotyl.

37. The above mentioned conceptions regarding the submicroscopical structure of cells of epidermis, of cortical parenchyma and of the pith, based on optical observations, are confirmed by the degree to which these tissues decrease their dimensions in different directions after treatment with strong swelling agents.

B. THEORETICAL PART.

12. *Tangential tensions in the walls of cylindrical cells under the influence of turgor pressure.*

Wrong conceptions are frequently expressed regarding tensions in the walls of cells inflated by turgor pressure. When these cells are globular the uniform transmission of hydrostatical pressure in all directions (law of PASCAL) will lead to the same tangential stress in all parts of each unit of section in every direction in these walls. When the wall is of uniform thickness the tension per square centimetre in the section of the wall will also be everywhere the same. If however the cells are not globular in shape the tangential tensions in the walls will no longer be everywhere the same and will moreover differ in varicus directions.

In a paper published in 1937 [1] attention was drawn to this fact and it was announced that a separate paper on this question would appear later. In the meantime we give a photographic reproduction il-

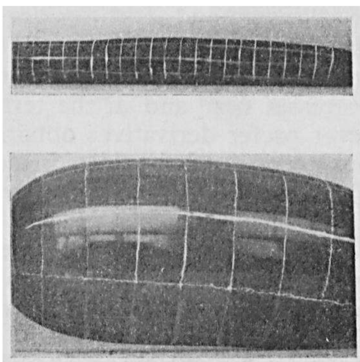

Fig. 39. Experiment to demonstrate that in an oblong cylindrical cell with walls of uniform thickness the turgor causes in the wall a greater strain in the transverse than in the longitudinal direction. In the upper figure a rubber balloon without pressure with squares on the surface; in the figure at the bottom the same balloon inflated.

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[1] G. VAN ITERSON JR., A few Observations on the Hairs of the Stamens of Tradescantia virginica, Protoplasma 27, 190—211, 1937.

lustrating a simple experiment by which this fact can very easily be demonstrated (Figure 39).

On an oblong cylindrical balloon of caoutchouc with uniform wall thickness we have drawn, before inflation, small squares with one side parallel to the longitudinal axis of the balloon. When such a balloon is inflated these squares become transformed into oblongs, the longer sides being in the transverse direction, the shorter sides in the longitudinal direction of the balloon. From this it may be concluded that in the first mentioned direction the tangential stresses are greater than in the longitudinal direction. The above fact can be easily verified by means of statics especially if applied after some simplifying suppositions; in this connection we would refer to E. CASTLE [1]. In publications on elasticity one may find more exact demonstrations [2], whilst an account of an experimental study, which is of great importance in connection with our further considerations, is given by E. G. COKER and L. N. G. FILON [3]. In their experiment COKER and FILON determined the distribution of the tangential stresses in the walls of cylinders of nitrate-cellulose and of glass cylinders and measured them optically. Internal gas-pressures made the walls of such cylinders, at least at some distance from the top and the bottom, to a high degree anisotropic — the axes of the refractive index ellipse were located in the surface view of the wall (*a*) the minor axis: in line with the axis of the cylinder; and (*b*) the major axis of the ellipse: perpendicular to this direction.

In another paper we hope to give further details regarding the distribution of the stresses near and in the terminal planes of the cylinders. We however prefer derivatives obtained in experimental studies with balloons to derivatives given in accordance with the theory of elasticity, because this theory takes only small deformations into consideration.

Such distribution of stresses as may occur in the walls of cylindrical cells with a circular transverse section may also occur in elongated cells with rectangular transverse section and which may be regarded as an elongated prism, or as a combination of such a prism with rounded segments as terminal walls. Conditions are changed however

[1] Almost simultaneously with VAN ITERSON E. S. CASTLE (Membrane Tension and Orientation of Structure in the Plant Cell Wall. J. Cellular and Comparative Physiology **10**, 113—121, 1937) drew attention to this effect; in an "addendum" he cites the paper by VAN ITERSON, which reached him while his publication was in the press.

[2] See i.a. F. GRASHOF, Theorie der Elasticität und Festigkeit, 2. Aufl., Berlin 1878, p. 316—329.

[3] A Treatise on Photo-Elasticity, Cambridge 1931, p. 303—304.

when the breadth of the cell is greater than the height. In this case the distribution of stress at the equator is exactly the opposite of that given in the above example. In the equator of a rotation-ellipsoid with the rotation-axis as the shortest axis, the greatest tangential stress, for instance will be in the direction of the axis and the shortest in the direction of the equator.

We wish to point out here that when we have a series of cells whose rounded outer walls form a continuous cylinder and whose common walls may be regarded as transverse walls (partitions in the cylinder) assuming that the transverse walls offer a low resistance to extension, the distribution of tension in the outer wall must be the same as for a single long cylindrical cell. This also applies when the separate cells arranged in the above way are less high than broad.

When however we inflate a long cylindrical cell with a longitudinal wall which is not of uniform thickness the tension per unit of wall

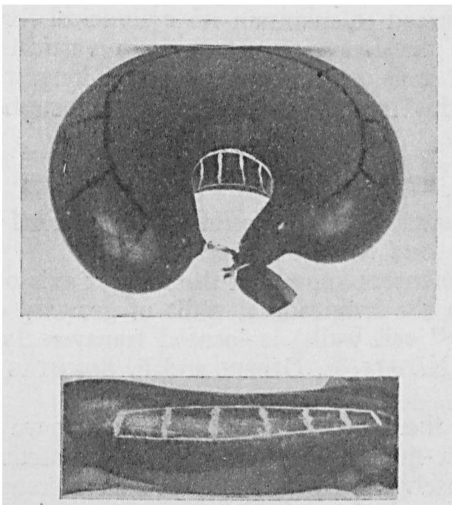

Fig. 40. In the upper figure: a cylindrical balloon of rubber the wall of which was unilaterally thickened with a strip of rubber, was expanded by means of water pressure; the balloon, curved in a high degree with the strip on the concave side; the rectangles to be seen on the concave side with the shorter sides in the longitudinal direction of the balloon were originally squares. In the figure at the bottom: a similar balloon treated in the same way but forced by hand back into a cylindrical form; the squares on the surface of the balloon now show rectangles with the long sides in the longitudinal direction of the balloon.

section must be much greater in the thinner parts than in the thicker parts.

In highly extensible cylinders, such as rubber balloons and young plant cells, whose walls are not uniform in thickness, the *distribution* of the tension in various directions of the plane of the wall also differs from that in the walls of cylinders that have walls of uniform thickness. Matters become still more complicated when we prevent curvatures of such cylinders during the process of inflation.

We would draw attention to the explanation given by S. SCHWENDENER [1] regarding the mechanism of opening and closing of stomata; he demonstrated this by means of a unilaterally thickened rubber balloon, which bent when inflated. When we prevent the curvature or when we straighten the curved balloon, the distribution of the tension in the thicker part of the wall will be different from that in the curved balloon.

In a unilaterally thickened cylindrical cell, inflated by turgor pressure and forced to maintain its cylindrical form, the greatest tangential wall tension may occur in a vertical direction, the least tangential tension transverse to the longest axis. We have demonstrated this in an experiment with a rubber balloon (see Figure 40).

13. *The orientation of the micelles in longitudinal walls of growing cylindrical cells and the growth process.*

Older experimentors knew that the longest axis of the refractive-index ellipse in the longitudinal walls of growing cylindrical cells (with "primary" cell walls) is located transversely [2] in the plane of the wall. C. NÄGELI, L. DIPPEL and P. SONNTAG give very good examples.

Regarded in the light of the micelle-theory we have to accept that the cellulose-micelles in such walls show a statistical preference to arrange themselves with their longest axis in transverse direction.

FREY-WYSSLING has termed this structure: „Röhrenstruktur" (tubular structure). At the 6th Botanical Congress, held at Amster-

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[1] Über Bau und Mechanik der Spaltöffnungen, Monatsberichte 1881.

[2] We have made no mention here of the cases where this axis has a slight slope; we shall treat this subject elsewhere, in connection with the views of R. D. PRESTON (The structure of the walls of Parenchyma in *Avena*-coleoptiles, Proc. Roy. Soc. Lond. B 125, 372—386, 1938) regarding the structure of the parenchyma of the *Avena*-coleoptile.

dam in 1935 this experimentor [1] and also G. VAN ITERSON [2] gave some new examples of growing cells with this structure, and during the same year J. BONNER [3] drew attention to this structure as being the characteristic one for cells growing in length. We give here as another characteristic example, viz., for the cylindrical cells of *Hydrodictyon reticulatum*, which during growth considerably increase in length and less in width, during their development the longest axis of the refraction index ellipse is always located transversally in the wall (see Figure 41).

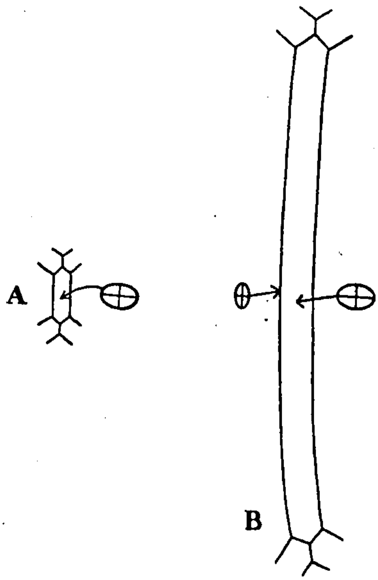

Fig. 41. Cells of *Hydrodictyon reticulatum* and their connection with other cells of the net, *A*. a young cell, *B*. an older cell at the same magnification. The ellipses demonstrate the situation of the refractive index ellipse in the surface view of the single cell wall; this situation remains the same.

In view of what has been said in the foregoing paragraph regarding the distribution of tension in the plane of the longitudinal cylindrical

[1] Über die optische Erschliessung der submikroskopischen Zellwand-Struktur, Proc. 6th Int. Bot. Congr. II, 294—296, 1935.
[2] G. VAN ITERSON JR., Submicroscopical structure of the cell wall, Idem 291—293, 1935.
[3] Zum Mechanismus der Zellstreckung auf Grund der Micellarlehre, Pringsh. Jahrb. 82, 377—409, 1935.

cell walls of uniform thickness where pressure exists one must suppose (as was done by CASTLE and by VAN ITERSON in 1937 independently of each other) that the micelles in the above mentioned cell walls are directed by the tension in these walls. This accounts for the statistical preference for the transverse direction and moreover makes it clear why this preference is not absolute (the longitudinal sides of the squares on the rubber balloon are stretched during the process of inflation, albeit to a less degree than the transverse sides).

We must however give the growth process of the walls of these cylindrical cells further consideration. BONNER (l.c.) rightly pointed out that the wall of a cell with „Röhrenstruktur" spread out free must be more easily stretched in the longitudinal than in the transverse direction. This may be concluded, for example, from the observations of P. SONTAG [1] on ductile fibres and from those of P. JACCARD and A. FREY [2] on the structure of wood grown under pressure or strain, as well as from the results obtained in this laboratory by H. EBBINGE [3], who stretched artificial films in which the micelles were more or less orientated.

BONNER suggests that we have to assume there is indeed a mechanical extension of the longitudinall walls of the cells during growth in length and, since he finds that the micelles during this process keep their transverse orientation, he comes to the following conclusion: "Beim Wachstum bleibt der 'Richtungseffekt' der Zellwandmicelle teilweise oder vollkommen aus" (during the growing process the directing effect of the micelles in the cell wall is at most only partial). The afore-mentioned distribution of the tensions in the wall with which BONNER was not acquainted remains unchanged even when there is a considerable growth in length of this wall and this implies that the „Richtungseffekt" (directing effect) must be in action in the plane of the wall, not in longitudinal direction of the cell but transversally to that direction; it has therefore *not* been eliminated. Although — as the reader will realise — we do not agree with the above-mentioned standpoint of BONNER, or share his view when he speaks of: „Einlagerung neuer Micelle zur Kompensation des Richtungseffektes" (depositing new micelles as a compensation for the directing effect), we do assume in common

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[1] Die duktielen Pflanzenfasern; der Bau ihrer mechanischen Zellen und die etwaigen Ursachen der Duktilität, Flora **59**, 203—259, 1909.

[2] Einfluss von mech. Beanspruchung auf die Micellar Struktur u.s.w., Pringsh. Jahrb. **68**, 844—866, 1928.

[3] Eenige onderzoekingen over doorzichtige verpakkingsmaterialen, Chemisch Weekblad **29**, 167—172, 1932.

with him that the longitudinal growth of cylindrical cell walls must be the result of interposition of micelles (perhaps it would be more correct to speak of long cellulose-molecules) between the ones already present.

However, one need not necessarily in this case think of *new* micelles. One might very well imagine that those micelles which are interposed in transverse direction, between the existing ones, originate from the wall itself. Such surface growth of the cell wall would cause a decrease in thickness.

To elucidate this conception we draw attention to the remarkable phenomena which have been described by P. MARTENS [1] in respect of the inner layer of the hairs on the stamens of *Tradescantia virginica*, a description which was confirmed and supplemented by VAN ITERSON (l.c. Protoplasma 1937). Under certain conditions one can observe elongation of this part of the cell wall sometimes to more than double its original length, the width only increasing slightly. This process happens within a few minutes. In the stretched cell-wall the „Röhrenstruktur" is not lost at all, probably it is even improved. In this case the interposition of new micelles is impossible, so that we have to assume that those micelles found at the expense of the thickness of the wall are interposed between other micelles of the wall.

Our observations respecting the rapid increase in the thickness of the cell walls of *Helianthus* under the influence of growth hormone have however convinced us that with a normal growth process *new* micelles are as a rule interposed between the others during the longitudinal growth of cylindrical cells. In view of the observations made by OVERBECK on the setae of *Pellia* (see also § 9) we are also inclined to accept this theory for some cases where the cell wall greatly decreases in thickness during the longitudinal growth. OVERBECK observed a considerable increase in the total cell wall material present after the growth in length of the longitudinal walls, which had noticeably decreased in thickness.

We observed that the longest axis of the refractive-index ellipse in the walls of all the cells in the seta of *Pellia* before and after the elongation (this elongation amounted within a few days without cell division to sometimes more than 40 times the original length) was transversely orientated in the plane of the wall. The same can be said of the outer wall of the epidermal cells of the seta, which becomes very thin after the elongation.

[1] Phénomènes cuticulaires et phénomènes osmotiques dans les poils staminaux de Tradescantia, La Cellule 41, 17—48, 1932—33.

After what has been said about the high extensibility of isolated walls of cells with „Röhrenstruktur" in longitudinal direction, one is justified in assuming that entirely new cell wall material or cell wall material coming from elsewhere in growing cells with such a structure is preferably laid between the transversely arranged micelles. It must be much easier for new material to overcome the slight resistance in this case than the strong resistance which exists between the ends of the micelles.

In this way it is easily understandable how long cylindrical cells with walls of uniform thickness grow in length. Before dealing with the growth in length of cylindrical cells with non-uniform wall thickness, we would once again treat the submicroscopical structure of the cell wall to show the relation between the above-mentioned unequal extensibility of the wall in various directions and the special orientation of the micelles therein.

It is curious that the views of NÄGELI regarding the structure of gels in general and of the cell walls of plants in particular are far from generally known. In this connection we especially have in mind his final conclusions — the result of 20 years research — which he laid down in the supplement of his work „Die Theorie der Gärung" (1879). In this publication NÄGELI writes among other things that „nicht bloss die einzelnen Micelle wie Crystalle durch Auflagerung wachsen, sondern dass auch mehrere oder viele sich mit einander vereinigen und durch Verwachsung zusammengesetzte Micelle bilden" (the growing process not only causes apposition of new material to the micelles, but also connections between micelles, thus leading to complex micelles) and „dass die Micelle sich in Ketten an einander anhängen und ein Gerüste von Balken mit weiten Maschen bilden, in welchen das Wasser eingeschlossen ist" (the micelles are linked together and form a lattice-work with wide meshes in which water is enclosed). The same views form the basis of various modern theories concerning the structure of gels and cell walls, although the various conceptors were not aware that similar ideas had already been published by NÄGELI.

In 1927 H. G. BUNGENBERG DE JONG [1] assumed a net-structure for gels and in 1932 he gave together with J. P. HENNEBERG [2] an augmentation of his theory, assuming the meshes of the net to be

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[1] Über die Gelatinierung lyophiler Sole und die Struktur lyophiler Gele, Zft. physik. Chemie **130**, 205—216, 1927.

[2] Zur Kenntnis der yophilen Kolloide XIII. Eine Methodik zur Messung kleiner elastischer Deformationen an hydrophilen Gelen u.s.w., Kolloid-Z. **35**, 441—475, 1932. Kapillar elektr. Ladung u. Hydratation als Zustandsvariable d. hydroph. Gele, Idem **36**, 123—177, 1932.

elastically extensible and the junctions being elastically deformable. W. ABITZ, O. GERNGROSS and K. HERRMANN [1] in 1930 expounded the "Fransentheorie" (the fringe theory) for the structure of gelatine, a theory closely resembling the conclusions of NÄGELI; in 1932 O. GERNGROSS, K. HERRMANN and R. LINDEMANN [2] applied this theory also to other gels and especially to caoutchouc. At the 6th Botanical Congress VAN ITERSON drew attention to the special importance of this theory to account for the growth processes in the cell wall.

In 1935 O. KRATKY [3] made some interesting experiments regarding the extensibility of wet films of cellophane; these experiments make it highly probable that this material has a net-structure and a high degree of mobility in the junctions. The view of A. FREY-WYSSLING regarding the structure of the cell wall as expressed during the International Botanical Congress in 1935, which was further elaborated by J. BONNER (l.c.) in a paper of the same year is based on the supposition of the existence of a „netzartig" (net like) continuous „Zellgerüst" (lattice-work) with meshes filled with water or with „Fremdsubstanzen" (foreign matter). BONNER assumes — as was also done by BUNGENBERG DE JONG — that there is an easy mobility of the rods in the junctions of the net and he supposes that these rods in the junctions may be loosened unter the influence of growth hormones.

The theory compounded by P. H. HERMANS [4] (1937 and 1938) partly in the light of experiments made in this laboratory by A. J. DE LEEUW in connection with the structure of threads of cellulose-xanthogenate and of cellulosehydrate also corresponds in certain respects with the conclusions of NÄGELI [5].

Some time ago G. VAN ITERSON JR. and K. E. C. BUYN [6] reasoned that a certain extension of the views of NÄGELI, especially as they are expounded in the above mentioned "Fransentheorie" [7], is necessary

[1] Über den Feinbau des Gelatinemicells, Biochem. Zeitschr. **228**, 409—425, 1930. Zur röntgenogr. Strukturforschung des Gelatinemicells, Zft. physik. Ch. B. **10**, 371—394, 1930.
[2] Über die reversibele Sol-Gel-Umwandlung, die „Kristallisation der Gelatine und den Feinbau elastischer Gele u.s.w., Kolloid-Z. **60**, 276—288, 1932.
[3] Über den mizellaren Aufbau und die Deformationsvorgänge bei Faserstoffen, Kolloid-Z. **70**, 14—19, 1935.
[4] In four papers in Kolloid-Z. **81** (1937) and **82** (1938).
[5] HERMANS has rightly drawn attention to this correspondence.
[6] Über einige bei einem Polystyren-Film beobachtete Erscheinungen und die daraus gefolgerten Betrachtungen, Kolloid-Z. **85**, 60—70, 1938.
[7] We kow that A. KÜNTZEL and F. PRAKKE in 1933 have raised objections to the fringe theory. Later on we shall try to make clear that although these

at least for gels in which one has to assume the presence of micelles. According to these authors it must be presumed that the fringes which link the micelles together may, if not prevented from doing so, perform thermal motions and wind themselves into complex curves, which may undergo a change of form. In gels under stress these curved fringes should be stretched. Unilateral stretching might cause them to arrange themselves together with other micelles in parallel groups with a crystalline structure; VAN DER WAALS' forces would fix them in this position.

This improved fringe-theory is eminently suited to account for the phenomena, which attend the violent swelling of gels, that have been irreversibly stretched beforehand. When a stretched gel with the above mentioned structure swells to a low degree, one may expect that the meshes of the net will expand on all sides through the absorption of water; when however the gel swells to a high degree the fringes become hydrated and will no longer be held in a fixed position by the attraction sphere of neighbouring fringes; the fringes will then contract into complex curves as a consequence of their thermal motion. The result of this must be shrinkage of the wall in directions opposite to those in which the wall was first stretched, but at the same time swelling takes place in directions in which the wall was contracted during the extension. In this way we can account for the phenomena described on page 771—773 [1].

This conception of the structure of gels induces one to assume that considerable *irreversible* deformations may occur without breaking the junctions in the network, or without causing micelles or molecules to glide along each other. In fact, one can often observe that swelling — and often also heating — of apparently irreversibly mechanically deformed gels, restores the *original* shape of the object. To illustrate this, we draw attention to the observations of TH. W. ENGELMANN (1893) on the contraction of stretched strings when swelling occurs, to those of F. C. VAN HEURN (1916) and those of W. DE VISSER (1925) on the contraction of raw rubber after heating and when swelling occurs, to those of R. HOUWINK (1934) on the contraction of deformed artificial resins when heated and

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objections render some complementary remarks necessary, they may not induce one to reject this theory.

[1] This conception of the swelling process of gels is quite different from what BONNER (l.c. 1935) expounded; he assumes that swelling causes ruptures in the cellulose-chains, a theory that offers no explanation of the shrinkage-phenomena which may be so noticeable when the cell wall swells to a high degree.

to those of VAN ITERSON and BUYN (1938) on the contraction of stretched polystyrene-films [1] when heated.

Let us now take the above developed, improved fringe-theory as a starting point to elucidate the growing process of cell walls.

It must be presumed that the meshes of the net of micelles in the wall of a cylindrical cell are widened when this cell is inflated by turgor pressure and that thereby the fringes are stretched out to a degree dependent on the rate of the active tensions. Some of these fringes will be fixed in this position by neighbouring fringes or micelles, some of them will remain free and will tend to contract themselves into irregular figures, trying to decrease the wall surface elastically. As there exists a greater tension in transverse direction, the meshes of the net will be stretched more in this direction of the wall than in longitudinal direction. An increase of the tensions will enlarge the meshes more and more, part of this enlargement will be irreversible, without greatly dislocating the junctions. Such loosening must, however, occur here and there, but new junctions will be formed; these new junctions will perhaps be even stronger than those that have been loosened.

When the tensions in the wall of a cylindrical cell become still greater, many junctions will be loosened and the result will be a really irreversible deformation of the wall (this means that the deformation of the wall persists after treatment with swelling materials in high concentration). As new junctions are formed between the fringes, there is no necessity whatever for the wall to become weaker. A deformation of this kind may be the means of perfecting the „Röhrenstruktur" of these cells. Such a perfection is, in fact, furthered by the unilateral stretching of the frame structure during the first phases of the inflation of the cell. It must be borne in mind that there is always a certain tension in vertical direction in the wall and we may not expect an absolute preference of the micelles for the transverse direction.

In the foregoing considerations we have only taken into account what might be expected in the longitudinal walls of cylindrical cells with walls of uniform thickness (in § 15 we shall show that a considerable extension of the cell walls must lead to a tendency to equalize the thickness of the cell walls). Assuming that the theory expounded above is correct we still have to ascertain what will be the result when cylindrical cells with unilaterally thickened or collenchymatic walls are inflated by the turgor pressure; we suppose

[1] It has to be borne in mind that these films contain no micelles but only long molecules.

that these cells must retain their cylindrical shape owing to their fixed places within the cell tissue.

After what has been said in § 12 regarding the distribution of the tensions in the thickened parts of the wall it is clear that when the thickened part of the wall has a homogeneous structure the meshes of the frame of the micelles in this section must be stretched more in longitudinal than in transverse direction. For the rest the above consideration holds good. When however the thickened part is not homogeneous, or becomes heterogeneous through ruptures (see § 10) the weakest places will be extended most; but as strengthening results from such an extension (as we shall show later on), the distribution of tension in the wall will after some time be about the same as in the case of a homogeneous part.

14. *Suppositions regarding the effect of growth hormone on the growth of the cell walls.*

We would *first of all* suggest that the primary effect of growth hormone is to lead to an increase of the swelling capacity of the cell wall. We share the view of O. RUGE [1], who sees in this swelling of the intermicellary material the cause of the growing process [2], but we do not wish to enter into details here. For our considerations it is sufficient to suppose that the contents of the meshes of the network try to increase their volume by taking up more water molecules by means of attractive forces [3].

The first effect of growth hormone on a primary cell wall in growing condition must then result in an elongation of the cell. Whether it decreases or increases its width in the meantime depends on the degree in which the fringes had already been stretched before.

Secondly we suppose that soon after the first phase of the process new cell wall material will be deposited in the meshes of the network, material that may have been found at the expense of the thickness of the wall, but which may also have been newly formed. As a result of the directing action of the tension in the wall these newly supplied

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[1] Zur Charakteristik einer für die Physiologie der Zellstreckung wichtigen Intermicellarsubstanz pflanzlicher Membranen, Biochem. Z. **295**, 29—43, 1937.

[2] One may also assume a kind of procellulose as suggested by A. N. J. HEYN (Weitere Untersuchungen üb. den Mechan. der Zellstreckung und die Eigensch. der Zellmembranen II, Protoplasma **21**, 299—305, 1934).

[3] Such a supposition was made by H. G. BUNGENBERG DE JONG in 1935 to account for the swelling phenomena of the threads of cellulose-xanthogenate and is also the basis of the consideration expounded by P. H. HERMANS in 1937 and 1938.

or newly formed micelles — or long molecules — will show preference for the transverse direction in the wall, the same as the micelles already present.

Thirdly we suppose that the next effect of growth hormone is a weakening of the junctions of the network.

Probably this is merely a simple diminishing of VAN DER WAALS' forces, as was resumed by BONNER. Perhaps we must also assume the cooperation of crystallisation forces of newly deposited micelles which disintegrate the material already present; it is known that such forces may be very considerable (the crystallisation of salts may cause rocks to fall asunder).

We however suppose that the weakening action on the junctions in this phase is not pronounced and in the main amounts to making way for new material. This new material attracts water and causes swelling, this swelling as well as the first swelling taking place especially in the longitudinal direction of the cell.

In this third phase of the growth we thus assume an element of the explanation given by J. SACHS [1] of the surface growth of the cell wall, *viz.*, the imbibition, which is a consequence of the deposit of new material. SACHS describes this process as follows: „dass durch Einlagerung fester Substanz in bestimmter Form die durch Imbibition und Turgor hervorgerufenen Spannungen teilweise ausgeglichen werden; allein zu einer wirklichen Ausgleichung kommt es nicht, da nach der Einschiebung neuer Partikel der Turgor wieder wächst und die Imbibition sich ändert, wodurch abermals neue Spannungen hervorgerufen werden" (the tensions in the cell wall caused by imbibition and by turgor are partly nullified by the deposit of new solid material, but this does not lead to a complete annihilation, as the turgor rises after the deposit of new material and imbibition increases by it, so that new tensions arise). Although in our opinion, as we have pointed out in the foregoing paragraph, turgor acts otherwise than was assumed by SACHS, we think this part of his conception rightly describes one of the elements active in normal surface growth.

For the growth of cell walls under the influence of their growth hormones in natural circumstances the action of the hormone is — in our opinion — limited to the processes mentioned above.

It is necessary to assume that growth hormone when supplied in higher concentrations than are present under normal conditions, still acts in another way; it loosens the junctions of the network in a higher degree, so that the fringes are now allowed to glide easily

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[1] See his "Lehrbuch" 4th edition, Leipzig 1873, page 762.

along each other. Furthermore it may be assumed that greater imbibition and stronger crystallisation forces of newly deposited micelles then cooperate more and more in disintegrating the junctions. Owing to such a weakening the wall of a cylindrical cell with uniform wall thickness must stretch under the influence of turgor chiefly in horizontal direction because tension is greatest in this direction and the resultant extension must for the greater part be irreversible.

When sufficient growth hormone is supplied and sufficient new material is present, this extension will also be accompanied by a deposit of new cell wall material, the weakening of the wall being checked in this way.

Proceeding from this standpoint we can easily explain why cortical parenchymatous cells — and to a lesser degree also the pith cells of the hypocotyls — of *Helianthus* show an increased growth in length after treatment with low concentrations of growth hormone, and why an increase in width sets in after treatment with higher ones. Moreover it explains why in both cases the „Röhrenstruktur" of the cells is maintained. Assuming that the transverse walls in the series of cells which are to be found in cortex and pith and which form long cylinders offer little resistance to extension, it is clear why this „Röhrenstruktur" remains unchanged, even when these cells under the influence of much growth hormone have increased more in breadth than in height.

Only with some hesitation do we illustrate what effect growth hormone will have on the thicker parts of unilaterally or collenchymatically thickened cylindrical walls. If it be borne in mind that the meshes of the network have been stretched in longitudinal direction in the thicker parts of such walls, one will expect, in view of what has been told of cylindrical cells with uniform wall thickness, that the effect of a low concentration of growth hormone will be not only a slight increase in length, but above all an increase in the width of the cells. A preponderant increase in length could, accordingly only be caused by very high concentrations of growth hormone. Experience has however taught us that even low concentrations of growth hormone cause longitudinal growth of the epidermis cells and of the collenchym.

One should not however forget that in these walls — and this is especially the case in the thickened epidermal outer wall — an extra tension exists in the longitudinal direction, partly caused by tissue tension in "active" tissues (active in the sense defined by SACHS) partly due to the fact that unequally thickened cells owing to their fixed places in the plant organ are prevented from curving as they would have done if isolated. These considerations enable one to understand why these thicker parts of the walls react by increasing in growth in length after treatment, even with pastes of low concentrations of growth hormone, notwithstanding the fact that the orientation of the micelles in these parts of the wall had suggested another result.

The existence of such an "extra-tension" in the longitudinal direction in the thick epidermal outer walls (even when tissue tension in the neighbouring tissue — e.g. after the removal of this tissue — is no longer active) accounts for the active growth in length of isolated cortical cylinders under the influence of high concentrations of growth hormone. One might even expect such a growth in length in the light of these considerations, after treatment with pastes of low concentration of growth hormone.

After these considerations it is hardly necessary for us to explain the results of unilateral treatment of the hypocotyls with growth hormone or of slips such as are obtained after splitting the hypocotyl as was described in the experimental part; they agree with our conceptions of the effect of growth hormone. However we must admit that many elements of this theory call for further tests, though it will be evident that the theory in the given form in itself throws new light on the explanation of the effect of growth hormone on roots and on other tissues.

15. *Some remarks on "elastic" and "plastic"* deformations.

Extension-experiments with films of lyophile gels, in which the micelles (or the chain-molecules) are distributed without preference for a special direction in the plane of the film, give us stress-strain diagrams consisting of three parts. This may be elucidated by means of a diagram (see Figure 42), taken from a paper of S. L. BASS and T. A. KAUPI [1], who reproduce several of such curves obtained by the extension of films which had been made by pouring out solutions of the gels on a glass-plate [2]. The micelles (or the chain-molecules) in such a film are arranged so that one of the three axes of the refraction-index ellipsoid in the film shows a high preference for the direction perpendicular to the plane of the film but so that there is no preference for the direction of the two

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[1] Evaluation of Ethylcellulose by Load-Elongation Curves, Ind. Eng. Chem. **29** I. 678—686, 1937.

[2] The greater part of these films were made of ethylcellulose, but sometimes „esters" were used; the results are, however, the same also for other films, which appear isotropic when looking at the surface.

other axes (which are situated in the plane of the film). Such a poured-out film therefore has, to use the nomenclature of FREY-WYSSLING, a „Folienstruktur" (a foil-structure), *viz.*, the same submicroscopical structure as is found in a cell wall that is optical isotropic when looking at the surface.

The first rising part of the curve corresponds to an elastic, i.e.

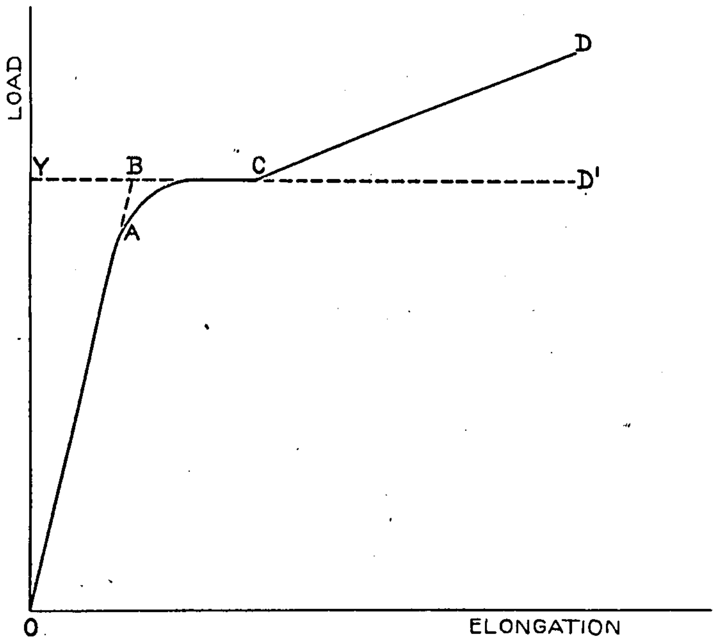

Fig. 42. Load-elongation curve of a film obtained by evaporating a solution of a lyophile gel poured out on a glass plate (after S. L. BASS and T. A. KAUPI). Similar curves are obtained from cellophane, and would be obtained in the case of an isolated isotropic cell wall, if it were possible to make extension-experiments with such a wall.

reversible, deformation, but the higher part of the rising graph corresponds to an irreversible deformation; this is especially the case where there are great deviations from the straight line. The horizontal part of the curve (this part may also be slightly inclined, or declined) corresponds for the greater part to irreversible, plastic deformation of the film. However one should be aware that this irreversibility is only apparent; when there is a violent swelling of such deformed gels they return for the greater part to their original shape.

The third part of the graph is particularly noteworthy. This part shows that greatly — for the greater part irreversibly — extended material may once more assume the mechanical properties of elastic material.

In many cases this part of the graph is not straight, it may even bend upwards (becoming concave towards the load-axis); this indicates that it becomes more and more difficult to stretch the film further, which means that the film has become stronger.

It must be clear that the shape of the stress-strain curves described here (this is the normal type for films, built up of greatly elongated molecules, primarily arranged without preference in the plane of the film) can be easily explained by the "improved fringe theory". The third part of the graph corresponds to the attachment of stretched fringes to other micelles.

We now wish to emphasize that the definition of the words "plastic" and "elastic" deformation of cell walls, which are current in the modern theories of the growth of these walls, in connection with the facts described here, must be amended before one applies them. It is not generally known that an excessive extension of plastically deformable material may convert this into an elastic material, without any sign of plasticity. We have already mentioned that one way to decide whether a gel is in such a state or not, is to make it swell. When all the dimensions increase, there has been no deformation; when some of them show a decrease the gel has been extended. In the light of these facts the phenomena we observed by treating isolated cells with swelling agents (see § 10) must strike every one who considers the plasticity and the elasticity of the cell wall.

In the paper by Bass and Kaupi cited above we can find very important observations regarding the influence of plastificators on the shape of the stress-strain curve. The addition of such a plastificator gives stress-strain curves that are situated much lower in the diagram than the curve obtained without such an addition. However, after the addition of a fairly large amount of the plastificator the phenomenon of "strengthening" still exists. Only the addition of large quantities of plastificator causes the third part in the curve to disappear. It proves that an addition of a plastificator does not in the first instance cause a dislocation of the junctions of the net. According to the developed theory referred to above on the structure of gels this might be expected.

In any case it must now be clear that the methods which according to Blum and Ursprung have been applied to determine the elastic extension, the elastic extensibility and the plastic extensibility of cell walls (see § 6) may only be used with care, because one does not know to which part of the curve the deformations of the cell wall resulting from the change of the ambient medium correspond.

The course of the stress-strain curve as described above also explains the phenomenon that when a cylindrical balloon of non-vulcanized rubber with a wall that differs widely in thickness in various places is inflated, the wall becomes uniform in thickness[1].

In parts of the wall that have been stretched more than other parts the wall must be strengthened locally and although decreased in thickness, must offer more resistance to further inflation than those places in which the wall is less enlarged. It is now the turn of these latter places to be inflated.

This peculiarity is immediately understandable from the submicroscopical structure of gels, as was assumed by us, and from the changes that must be caused by stretching them.

This also accounts for the fact — mentioned in § 9 — that many cells in the grown up stages show a uniform thickness of their walls. Now one can understand for instance why the collenchymatic thicker parts of the cell wall of the young seta of *Pellia* disappear when they are elongated by the growth process — a phenomenon that so greatly attracted the attention of OVERBECK. This disappearance must be considered as a mechanical consequence of the high extension of a wall with gel-structure.

16. *Summary of the Theoretical Part.*

1. In the longitudinal walls of cylindrical cells that have been inflated by internal liquid pressure there will exist (with exception of the parts close to the terminal surfaces) — according to the laws of hydrostatics — tensions that are greater in transverse than in longitudinal direction.
2. In a series of cells whose outer walls form a continuous cylinder and whose transverse walls offer but low resistance to extension, one will find the same distribution of the tensions in the cylindrical outer walls after a uniform inflation of those cells.
3. When we inflate a cylindrical unilaterally thickened cell which is prevented from curving, the distribution of the tensions will be the same as in the above case in the thinner parts of the

[1] It is on this peculiarity that a process is based which was applied on some rubber estates some 15 years ago for drying rubber; in this process the raw coagulum was inflated to large balloons with thin walls.

wall; in the thicker parts, however, the greatest tension will be found in the longitudinal direction, the least in the transverse direction.

4. The „Röhrenstruktur" (tubular-structure) of longitudinal walls of growing, long, cylindrical cells can easily be accounted for by assuming that the micelles (or the chain-molecules) become orientated under the influence of the tensions existing in the cell wall.
5. Growth in length of long, cylindrical cells of uniform thickness, may be the result of deposit of micelles (or chain-molecules) that are already present in the wall between the others; such growth in length takes place at the expense of the thickness of the wall.
6. As a rule growth in length of long cylindrical cells of uniform thickness takes place by arranging newly formed micelles (or new chain-molecules) between the older ones; this however does not preclude the possibility of a simultaneous decrease in thickness of the wall.
7. An improved fringe-theory concerning the structure of gels, closely related to the suppositons of NÄGELI, may give a better insight into what happens when cell walls are extended under the influence of turgor pressure, and also of the shrinkage-phenomena of these walls caused by strong swelling agents.
8. The statistical preference shown by the longest dimensions of the micelles for the longitudinal direction in the thicker parts of growing, cylindrical, unilaterally thickened cell walls may be accounted for as being the result of the orientation effect by the tensions in these parts of the wall which are a consequence of the impossibility of these cells to curve while they are still connected to other cells of the organ; one need not assume that a stretching effect is caused by tissue tension, although this effect may of course be active in the same direction.
9. Assuming *a.* that growth hormone in a low concentration increases the swelling power of the intermicellary material; *b.* that the growth hormone in a little higher concentration weakens the junctions (the connection places of the fringes in the network of cellulose micelles) here and there, *c.* that a still higher concentration weakens many of these junctions, then it is easily understandable *1.* why cylindrical cells with walls of uniform thickness grow especially in length, the walls being thereby extended and showing decrease in thickness but no change in their „Röhrenstruktur"; *2.* why these cells also can grow in

length increasing at the same time the thickness of their walls without any change of the tubular structure; *3*. why such cells under the influence of growth hormone in a higher concentration grow more in width, and *4*. why a supply of growth hormone in a very high concentration causes a strong preference of the growth for this latter direction.

10. The same suppositions make it clearer why epidermal cells with thick outer walls, and collenchymatically thickened cortex-cells, under the influence of growth hormone show above all growth in length, and why cortical cylinders isolated from the pith, when treated with a paste of growth hormone of a high concentration show an active growth in length.
11. The apparently plastically deformed isotropic gels with micelles (or with chain-molecules) may change into elastic gels after a high degree of deformation; therefore one has to be critical in drawing conclusions about the plastic and elastic deformability of cell walls.
12. The increasing resistance to extensibility that can be found for gels with long micelles (or chain-molecules) as a result of a high degree of deformation explains why unequally thickened cell walls may show a uniform wall thickness after intense growth.