Botany. — *Coleoptile Growth as Affected by Auxin, Aging and Food.* By F. W. WENT. (WILLIAM G. KERCKHOFF Laboratories, California Institute of Technology, Pasadena).

(Communicated at the meeting of June 29, 1935).

1. *The original theory.*

The conception of growth in Avena originally advocated in my dissertation (WENT 1928) was that auxin is formed in the tip of the coleoptile, transported downwards, and while producing growth is being used up, so that an auxin deficiency in the basal regions causes their growth to drop and eventually to stop. The decrease in growth rate of the regions nearest the tip had to be explained in a different way, and a number of facts led me to draw the conclusion that this is due to limitation by another factor, which has to cooperate with auxin to cause growth, and which comes from the seeds or roots. This factor was called "Zellstreckungs Material", Z.S.M., and will be called in this paper "food factor". Eight years ago this seemed to be an adequate explanation of the facts known, but since then our knowledge of growth has considerably increased and a number of facts have been discovered not fitting into this scheme. The object of the present paper is to reconsider the problem, especially experimentally.

2. *Objections to the outlined theory.*

The maximum angle, that is the maximum curvature which can be obtained within 110 minutes by placing agarblocks with auxin unilaterally on coleoptile stumps, was considered to be due to limitation of the growth rate of the side supplied with auxin by the food factor (WENT 1928). However, DU BUY and NUERNBERGK (1930) proved that this maximum angle was caused by a sideways "leaking" of the auxin after a certain concentration was reached. Later, however, THIMANN and BONNER (1933) were able to show that in the case of the effect of auxin on straight growth the latter is limited in the same way as the auxin curvature so that beyond a certain amount of auxin no increase in growth can be obtained. Here the food factor, ruled out by DU BUY and NUERNBERGK, again becomes apparent.

WEBER (1931) in experiments with geotropic curvatures in which especially the reactions of the various zones are investigated also comes to the conclusion that growth cannot be explained by my two factor scheme.

In 1933 DU BUY published a number of experiments throwing more light on the interrelation of the factors determining growth in the coleoptile

of Avena. No new evidence in regard to the postulated food factor was obtained, but a new factor, the aging of the cells, was recognised. He was able to show that the growth rate, irrespective of the auxin concentration, will decrease when the cells become older. The old cells of the base of the coleoptile hardly respond at all to a given auxin supply, whereas the cells of the rapidly elongating region near the tip will do so. This aging can be produced experimentally by limiting the supply of auxin to the cells. It seems that the stopping of growth of the lower zones of coleoptiles is due to aging rather than to a depletion of the auxin; this aging, however, can be caused by a relative decrease in the concentration of the auxin in those lower zones. All these facts are fully discussed by DU BUY.

According to JOST (1935) the final disproval of my theory of the limitation of the growth of the lower zones by auxin was given by the determinations of THIMANN (1934), which show that the auxin concentration in the lower zones does not fall to zero, but that it is present there, even in not growing zones, in appreciable amounts. Still THIMANN and BONNER (1935), like previous authors, come to the conclusion that auxin is being used up during growth.

Finally, if we consider that in specially designed experiments (unpublished) neither DOLK nor myself could get any direct evidence for the existence of the food factor, it must seem that my growth theory as outlined above, even with the amendment of DU BUY, has failed completely. This negative state of affairs cannot be considered satisfactory, and therefore the present paper represents an attempt to bring as many facts as possible together and to reconsider the problem.

3. *Experimental procedure.*

All following experiments have been carried out in a darkroom kept at 25° C. and 85 % humidity, red or orange light being used for the observations. Avena seedlings, germinated and raised under standard conditions in the glass holders, are marked with tiny dots of india ink, the length of the marked zones depending on the type of experiment. As the growth of each zone has to be followed over a number of successive periods, and the growth rate is changing continuously, no importance was attached to marking zones of constant length. The growth rate has been marked on the graphs with the actual distance from the tip at the beginning of each growthperiod as abscissa. If the growth of two coleoptiles has to be compared, it is first recorded graphically and then the two graphs are compared. This can be done graphically by interpolation of the growth rates at distances 4, 8, 12, 16, 20, 24, 28 and 32 mm from the tip. In the graphs the growth rate is recorded as per cent increase in length per hour, as a point in the geometrical centre of the zone.

In all cases the length of the zones has been measured with a horizontal microscope with a magnification of 10 times linear and an accuracy of one

hundredth of a millimeter. The obtained accuracy is far above the one obtainable with a film camera (NUERNBERGK and DU BUY 1930).

Auxin was applied in all cases in the form of a paste of β-indolyl acetic acid (hetero-auxin). The original paste contained about 10 mg crystalline hetero-auxin per gram pure lanolin (paste 1) and a number of dilutions were made ($^1/_4$, $^1/_{16}$, $^1/_{64}$, $^1/_{256}$, $^1/_{1024}$), all being kept in the ice box without loss of activity. The diffusion out of lanolin is exceedingly slow, so that these enormous concentrations have to be used in order to obtain an excess of auxin inside the coleoptile. Dr. VAN OVERBEEK was kind enough to inform me, that an auxin paste $^1/_{64}$ increases the amount of auxin transported inside a plant about 4 times. The application always is by adding approx. 1 mg of paste to the extreme tip of the coleoptile. There is no reason to assume that the heteroauxin acts differently from the auxin a present in the coleoptile under normal conditions.

4. *Considerations about aging.*

First we will consider the aging factor as far as we can draw the facts from the literature.

The explanation of the aging is given by the experiments of HEYN (1931, 1933, 1934) and of BONNER (1934). HEYN proved that the final effect of auxin is on the cell wall, which is made more plastic by it. However, in addition the elasticity is somewhat lowered (HEYN and VAN OVERBEEK 1931). Nevertheless the main changes in elasticity after the action of auxin are considered to be the result of growth rather than the cause of it because it only occurs when cells are allowed to elongate under the influence of auxin. When cells are not growing their elasticity decreases, unless they are kept at 0° C. At that temperature auxin will cause an increased plasticity without changing the elasticity. Thus plasticity and elasticity are proved to be independent characteristics of the cell wall. The following mental picture explains the observed phenomena. Growth is a dual phenomenon: in the first place we find a continuous stretching of the cell wall due to sliding of the pre-existent cell wall particles along each other; this phenomenon is influenced by auxin, causing the cell walls to become thinner and weaker. At the same time new layers of cell wall are laid down inside the outer walls. Both processes go on at the same time inside the cell wall.

BONNER (1934) brought other experimental evidence that we have to distinguish between the two types of cell wall growth: 1 the growth in length and 2 the growth in weight. At 25° C. with adequate auxin supply the elongation and increase in weight of the cell walls of coleoptile sections are balanced, both going on at the same rate. If those sections are placed at 1° C. the elongation proceeds while the increase in weight is stopped. On the other hand it is possible to increase the weight beyond the rate of elongation. SÖDING (1934), basing himself on rather scant experimental evidence, comes to a completely different conclusion: growth in length of the cell wall is caused by active intussusception of new particles, and this

process is influenced by auxin. The very few experiments of SÖDING not in accordance with the theory outlined above, are not able to offset the interesting paradox: growth in weight (in dry matter) is opposed to growth in length. This completely explains aging: if cell wall formation and elongation keep each other in equilibrium, the growth rate will remain constant. If, due to lack of auxin, elongation is checked, cell wall formation will go on, making the cell wall stiffer and less reactive to auxin. Or in one word, the cells age.

5. *Why do the basal zones stop their growth?*

As we know that auxin is used up in growth (THIMANN and BONNER 1935) and that the auxin concentration really falls towards the base of a coleoptile (THIMANN 1934) it is clear that beyond a certain distance from the tip the aging of the cells must start. Only when the coleoptiles are very short, so that the decrease in auxin concentration towards the base is insignificant, the aging does not occur (curve *a*, figure 10 in WENT 1928), but starting from a given length aging of the lower cells will set in, and once it starts it goes on at an increased rate. In the zones where aging occurs the growth rate x of a given zone in a given hour will depend on the growth y of the same zone in the previous hour as follows

$$x = -0.50 + 0.96 \, . \, y \quad \text{(expt 25 V 35)}$$

if x and y are expressed in % elongation per hour.

The auxin concentration in the lower zones will drop still further towards the base, but the aging gets ahead and so the lower zones stop growing before the auxin concentration has become 0. What will happen if we increase the auxin concentration in those not growing zones? I published earlier an experiment (WENT 1928, page 69) in which auxin was applied to those not growing lower zones. They immediately resumed growth, though slowly. In view of the above mentioned criticisms the experiment has been repeated with more plants and longer periods of observation. Table I shows the result of the most striking experiment. The growth of a few basal zones of 3—6 mm is being followed over two periods of 8 hours, and one of 2 hours. After that period the growing upper parts are cut off (or left in a few controls) and auxin is applied to part of them. The resulting growth is measured in four consecutive periods of 12 hours each. In all cases there is a slight shortening of the measured zone during the 2-hour period, which always occurs at the end of the growth period of each cell (see WENT 1928, p. 88). The division of the plants into four groups is completely at random. Group I is left intact. Group II is decapitated just above the not growing zones and the primary leaf is pulled out. Group III is decapitated as group II, the primary leaf pulled out and auxin paste 1 is put on the cut surface. Group IV is the same as group III except for the fact that the primary leaf is not pulled out. The result is very convincing. Groups I and II are almost alike: no growth occurs once it has stopped. The shortening even goes on. This means that the stopping of growth

really means the end of the growth period in the normal or the decapitated plant. In group III growth is resumed due to the auxin application, proving that there exists auxin hunger in the not growing cells. Still more interesting is the fact that this growth does not drop off rapidly, but instead increases and remains constant for almost a day, and finally drops off again. If we

TABLE 1.

Expt 20 V 35. Growth rate in % per 10 hours of the zone which just has stopped its growth after the second period of observation. Auxin paste 1 applied to group III and IV at end of 2-hour period.

Successive periods of		8 hrs	8 hrs	2 hrs	12 hrs	12 hrs	12 hrs	12 hrs
	Treatment							
I	Control + leaves not decapitated	27.4	3.9	—0.25	0.05	0	—0.3	0.05
II	Control — leaves decapitated	33.5	7.0	—0.8	0.1	—0.1	—0.4	0
III	No leaves, with auxin, decapitated	29.7	7.2	—0.1	0.54	1.30	1.1	0.33
IV	With leaves, with auxin, decapitated	37.2	9.6	—0.5	0.2	0.2	0	0.2

only consider the first 36 hours after decapitation (perhaps afterwards the auxin supply from the paste drops off), we can draw the conclusion, that no further aging takes place as long as the auxin supply is excessive. This gives further support to DU BUY's theory that aging is due to inadequate auxin supply, and that it is not primarily caused by the time factor itself. We even observe the opposite phenomenon, rejuvenation, for the growth rate is increasing during the action of the excess auxin for a period over 12 hours long. Nevertheless the growth rate of normal young cells is by no means reached. It is interesting to note that these cells have been growing two days longer than they would have done under normal conditions.

Group IV does show some growth, but very little compared with III. In this case the leaf is present and apparently inhibits the growth of the coleoptile. As we will see later this probably is due to a competition for food. The case seems comparable with the thickening of the coleoptile when decapitated, if the primary leaf is pulled out (DU BUY 1933). This also has been ascribed to better nutrition.

In the experiments on the growth of the basal zones it is clear that no other known factor will limit it except auxin and aging. As the growth is so exceedingly slow the food factor does not come into the picture, except where the leaf is present. How is this in the zones nearer the tip?

6. *Reaction of an intact plant to excess of auxin.*

As the condition of each cell in the coleoptile is different as far as aging, auxin and food factor is concerned, we must differentiate as far as possible, which makes the picture rather complicated. A three dimensional diagram is the nearest possible approximation.

A coleoptile is marked into zones of 3—4 mm length by means of india ink droplets. The elongation of each zone is followed by measuring its length every hour. In this way not only the distribution of the growth rate over the whole length of the coleoptile, but also its change with time is recorded. Thus the effect of application of excess auxin to the tip can be followed, as seen in figure 1. The curves in the plane of the paper

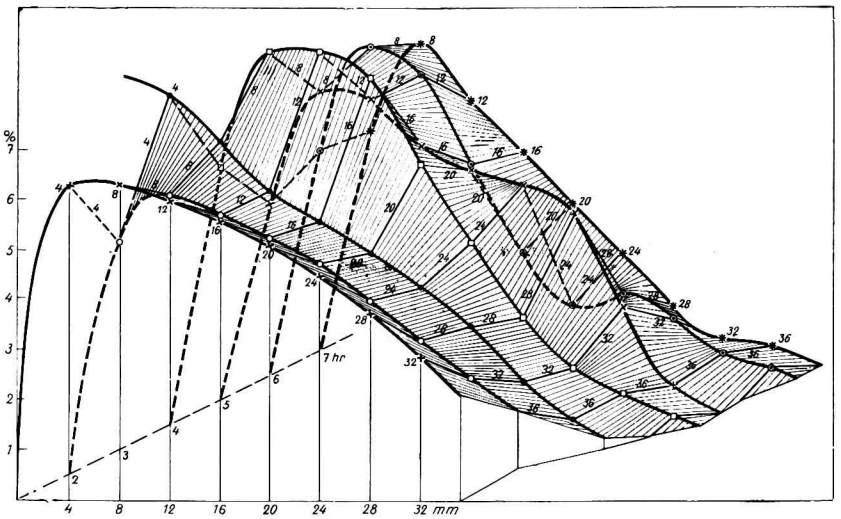

Fig. 1. Change of growth distribution in a coleoptile after application of auxin after 2nd hour. Ordinate: growth rate in % per hour. Abscissa: zones in mm from the tip. Third axis: time in hours.

represent the growth distribution in the coleoptile in a given hour. The ordinate shows the growth rate in % elongation per hour, the abscissa the zones at 4 mm distance each, the tip always at the zero point. The time factor is represented on the third axis perpendicular to the plane of the paper and the growth distribution curves for the consecutive hours are spaced along that axis. The points on these curves corresponding with the 4, 8, 12 etc. mm zones are connected by lines in the plane of the time axis and ordinate, representing the change in growth rate of each zone with time.

The first two hourly periods represent the normal growth, which is falling off equally in all zones during the second hour. At the end of the second period heteroauxin paste 1 is put on the tip of the intact plant. In the next hour the upper 10 mm of the coleoptile has considerably increased its growth rate, compared with the second hour, but the lower

zones are still growing at their normal rate. In the 4th hour the growth of the highest 5 mm drops again to normal, but all lower zones down to about 25 mm increase their growth rate. In the 5th hour the wave of increased growth moves further down. In the 6th hour the growth wave can still be distinguished at 25—40 mm from the tip, while in the 7th hour the growth distribution seems normal again, except for the fact that all zones are growing faster than in normal coleoptiles.

A similar reaction is obtained when an auxin paste of lower concentration ($^1/_4$, $^1/_{16}$) is applied. The growth wave moves downward at approximately the same rate. As the description takes much space and nothing materially new is encountered, we can draw conclusions from the described reaction without fear of considering an exceptional case.

The first conclusion to be drawn is: in the intact plant the auxin moves at almost exactly the same rate as VAN DER WEY (1932) calculates for the auxin transport in cut cylinders of coleoptiles.

Secondly it seems that under normal conditions the auxin concentration limits the growth rate over practically the whole length of the coleoptile, because all zones are responding to an excess of auxin with a growth increase. It seems moreover that the percentage growth increase is approximately the same for all zones, indicating that the other growth factors, in this case the food factor, is present everywhere in about the same excess. In this respect it is perhaps still more interesting that the growth increase, ranging from 30—70 %, is followed by a growth decrease before it reaches a more or less constant level. This is typical for a growth response. E.g. a light growth response in Avena is generally a growth decrease followed by an increase. According to some authors, e.g. VAN DILLEWYN (1927), the first growth decrease is due to the change darkness-light whereas the increase is caused by the transition light-darkness, so that the combination of growth decrease and increase is not organic, but each of them a definite reaction. In the case of the growth response to excess auxin, described above, we know that but one factor has been changed, and that the growth increase as well as the subsequent decrease both are due to the high auxin concentration. Thus my conclusion is, that a growth acceleration inevitably is followed by, because it causes, a growth retardation, acceleration and subsequent retardation being one single growth response.

For an explanation of this behavior I offer essentially the same as DOLK (1930) gave for the autotropism: the total available amount of the food factor is limited. The excess of auxin uses up all there is for the moment, leaving a shortage, which causes the growth to drop below the original level. To me it seems that this typical growth response is another proof of the existence of a second growth factor, not identical with aging, which never could explain a growth wave but only demonstrates itself as an increased growth resistance, and on which growth responses may be superimposed.

7. *Comparison of normal and "excess auxin" growth.*

In the preceding presentation the growth response to auxin has been considered in respect to the growth of the same plant previous to the administration of the paste. A completely different picture arises when we compare the growth of the treated plants with not treated ones during comparable periods. For this purpose we will compare figure 2 and 3. In each figure the growth distribution in consecutive periods of 2, 4 or 10 hours over the zones of one single plant are assembled. In the control plants (fig. 2) we observe a gradual drop of the growth rate of all zones, most rapid though in the lower ones, where the growth drops to zero. The upper 3 curves represent the growth during the first three consecutive periods of two hours each. The 4th and 5th curve are from four-hour periods, and consequently are further apart, whereas the last one is over a ten-hour period. Figure 3 gives the data about a plant of the same lot as figure 2; the only difference is that after the second two-hour period auxin paste $^1/_4$ has been applied. It represents an extreme case, but illustrates the effect of auxin application in a most effective way. The intermediate forms of response will be treated afterwards.

To begin with, the growth distribution is comparable with that in figure 2. In the first two periods the growth rate differs somewhat, but the mean of both exactly overlaps the mean of the same two of figure 2.

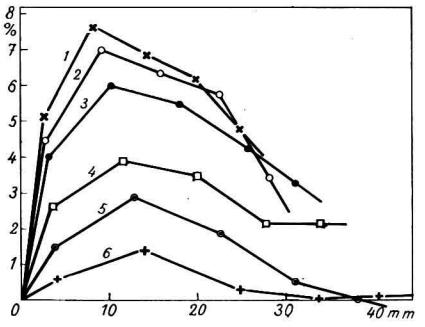

Fig. 2. Growth distribution in an untreated coleoptile in successive periods, 1, 2, and 3 each 2 hours, 4 and 5 each 4 hours, and 6 10 hours. Ordinate: growth rate in % per hour, Abscissa: zones in mm from the tip.

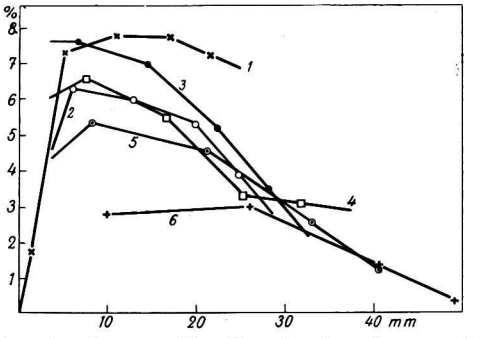

Fig. 3. Same as Fig. 2, only after the second period auxin paste ¼ is applied.

The third period shows the effect of auxin: there is a marked increase in the growth of the highest zones, whereas the lower ones keep their original rate. In the successive periods the growth rate of the higher zones (the upper 20 mm) drops off with time just as we will see in par. 8. But the growth rate of the lower zones remains constant. In all zones the supply of auxin is excessive (at least 20 times normal) and now we observe that *no aging* takes place in the lower zones, even not after 20 hours. It is clear

then that the drop in growth rate of the higher zones cannot be explained as a result of aging, but that food is becoming limiting. Most of the food factor is being used in the lower zones during its upward transport, and therefore less reaches the zones near the tip. In this case the relationship between auxin, aging and food factor is extremely simple: excess auxin completely prevents further aging, the cells go on growing at approximately the same rate, dependent on their state of aging before the administration of auxin. In those lower zones the food factor is not limiting, but higher up we only have to do with limitation of the growth rate by the food factor. An interesting point is that apparently the total amount of food factor available is greater in the plants with extra auxin than in the controls. In par. 11 this will be more closely considered.

Now in figure 4 an extreme case in the other direction is presented. The plant belongs to the same lot as figure 2 and 3, the periods of observation etc. are identical. Again the growth distribution of the first and second two hour period are the same as in figure 2. Then auxin paste $^1/_4$ is applied and just as in figure 3 we see for the 3rd and 4th period a growth increase near the tip, whereas growth remains almost constant in the lower zones. No aging becomes apparent. But suddenly in the 5th and 6th period growth drops almost to the level of the control plant. This must be ascribed to the food factor which has become exhausted, or rather whose supply has become limited. All intermediate stages between figure 3 and 4 are possible, depending on the moment that the food factor becomes limiting, and the degree of limitation.

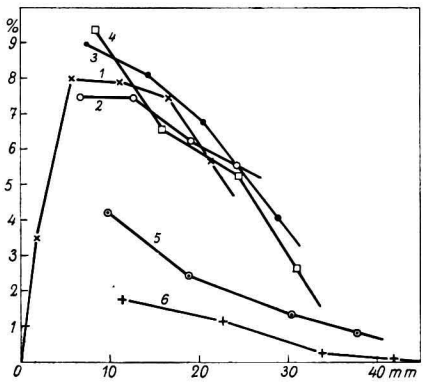

Fig. 4. Same as Fig. 2 and 3, after second period auxin paste ¼ applied.

Another set of experiments, which will not be presented here, confirms the view that the growth rate of te lower zones is limited only by the degree of aging at the moment of auxin application.

8. *Analysis of the food factor in young and old plants.*

It should be possible to analyse the food factor by increasing the auxin concentration and measuring the growth of the upper zones in which the aging has not yet occurred. Thus in the following descriptions the growth of only the most rapidly elongating zones is considered.

In the next experiment 7 groups of 6 plants each have been measured and the growth of the upper 10—15 mm is recorded. Two of the groups are kept as controls and are not supplied with extra hormone, only one of them (I) being represented in figure 5. Their growth shows something

like the aging effect, that is, it falls with time, first slowly, then more rapidly, and flattens out at last. To find out to what extent auxin, aging or the food factor are causing this drop auxin paste $^1/_4$ is applied to the tips of the plants of the remaining groups, in each group at a different moment, at two-hour intervals, so that the whole period of the rapid drop in growth rate is covered. The result is given in figure 5. The growth rate

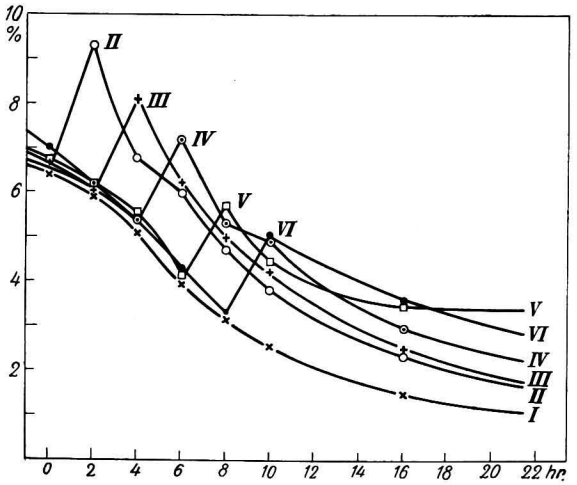

Fig. 5. Change in growth rate of highest zones after application of auxin paste ¼ to groups II—VI in successive 2-hour intervals. Ordinate: growth rate in % per hour. Abscissa: time in hours.

of each group before auxin is applied corresponds almost exactly with the controls, so that the material must be considered as very homogeneous. As soon as auxin is administered, the growth rate in the successive groups jumps up to: 143, 134, 134, 137 and 149 % of the value in the preceding period of two hours, and falls off again in the following periods. So it seems that the increase of the growth rate due to excess auxin is a more or less constant value.

Now does the food factor or the aging limit the growth rate of the treated plants after the first two hours of application of the auxin? Of course, considering the excess auxin, it is unlikely that aging plays a part in the subsequent growth decrease. The final length of the coleoptiles at the end of the experiment tells the story. In the two control groups it is 192 and 198 % of the original length, and in the five other groups: 248, 246, 253, 251 and 244 %. This means that it is immaterial whether the auxin application begins early or 8 hours later, — the final length is the same. In the first period the growth rate is just twice the rate in the 5th period, so the original growth rate at the moment of auxin application does not influence the final length at all; this means that aging has in fact nothing to do with it (auxin is in excess). The total amount of the food factor, however, is the same in all seeds. Normally only part of this is

used for growth of the coleoptile, but upon application of auxin paste the total amount of the food factor becomes available and will be used, whether in 12 or in 20 hours.

In all curves we also observe the phenomenon described in par. 6, viz. that after the growth acceleration by auxin in the first period the growth rate in the following one drops below its subsequent rate. If the growth rate of the experimental plants is expressed in percent of the growth rate of the controls in the same period, this effect is very pronounced.

9. *The food factor in artificially aged plants.*

Six groups of 6 plants each are marked 7 and 16 mm below the tip, the enclosed zone of most rapid growth being measured every 2 or 3 hours. The first group of plants is left intact; after the first 3 hour period the other groups are decapitated and the primary leaf pulled out, and those not supplied with auxin are re-decapitated every two hours to prevent the regeneration of auxin. On the second group is put auxin paste $^1/_4$ directly after decapitation, on the third the same is put after 2 hours, on the fourth after 4 and on the fifth after 6 hours, while the sixth does not recieve auxin at all. Figure 6 represents the results. The curve of group I, the controls, is typical and directly comparable with the similar curve of the controls in figure 5. The curve of group VI is typical too: after decapitation the growth falls rapidly and decreases still further in the next periods until growth almost completely stops due both to decreased auxin content and to aging. In group II we do not observe the typical increased growth rate in the first period after application of auxin, but in the second period the growth rate already exceeds that of the controls and remains slightly faster. In group III, IV and V we observe an increase in the growth rate immediately after auxin application; the most interesting fact, however, is that the maximal growth rate is not reached immediately, but only after 4 to 8 hours. The explanation follows from two facts: **1.** The available amount of food factor is great, as growth has been artificially kept down by decapitation. In this respect we can compare them with the plants of par. 5. **2.** The cells are artificially aged, because

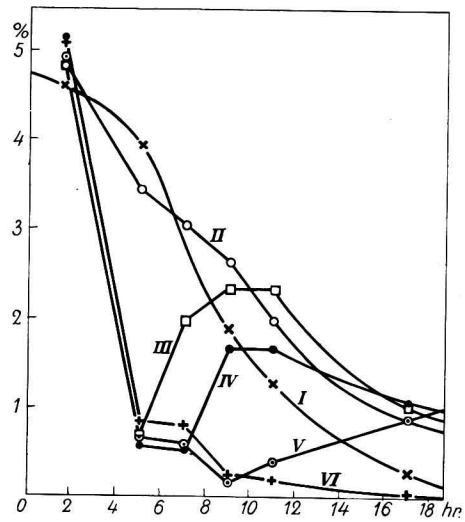

Fig. 6. Change in growth rate of most rapidly growing zones. After first 3 hours groups II—VI are decapitated and immediately or after 2, 4 or 6 hours auxin paste ¼ is applied to groups II—V. Ordinate: growth rate in % per hour. Abscissa: time in hours.

they have been prevented from growing, in this respect differing from those of par. 5. We have cells in different stages of aging, but all with the same slow growth at the moment of application of auxin.

As the food factor is far in excess, the application of auxin can induce rejuvenation. This is a slow process, and only becomes apparent when the growth rate is not limited by any growth factor except aging. Here, especially in the fifth group we have a perfect example of this rejuvenation.

10. *Deseeding experiments.*

As the food factor is considered to come from the seed, it is only natural to try to influence it by cutting the seed off and using deseeded plants. The decreased auxin formation in them (see SKOOG 1935) can be offset by addition of auxin paste. The food factor will be lowered in those plants, but at the same time we have to consider the probability that the cell wall formation, dependent on nutritive factors (see par. 4), is decreased too.

The growth distribution in respect to zones and time has been followed in 16 deseeded plants. This is not enough to obtain a detailed quantitative picture of the whole phenomenon, but the results as far as they seem significant will be discussed.

What is the effect of deseeding on the growth rate? This immediately drops to 70 or 75 % of the rate of normal plants, and the same relationship between deseeded and normal plants holds for a long period afterwards (see groups I and II of figure 7). The growth distribution in the deseeded coleoptile changes relatively little compared with normal ones, only the growing zone is somewhat shorter.

In general the effect of excess of auxin is the same in deseeded as in normal plants. This may be concluded from figure 7 in which 5 groups

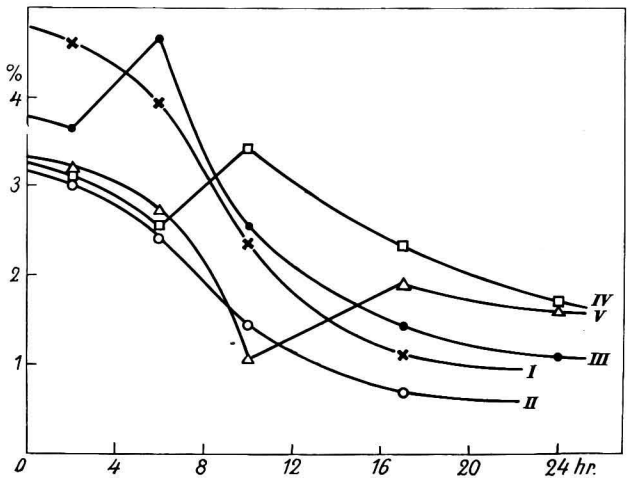

Fig. 7. Change in growth rate of highest zones of deseeded coleoptiles after application of auxin paste 1 to groups III—V. Group I not deseeded. Ordinate: growth rate in % per hour. Abscissa: time in hours.

(each the mean of 2 plants) are represented. The growth of their upper zones (10 mm) has been measured every 4 hours. The graph is directly comparable with figure 6, where the same thing is measured for normal plants over 2-hour periods. Group I is not deseeded; its growth rate drops exactly as in other experiments. The plants of group II are deseeded just before starting the measurements and are not treated with auxin. Their growth rate falls off just like that in the normal plants, only at a lower level; for the successive periods it is 66, 62, 62, 63 % of normal plants. In group III auxin paste 1 is applied at the end of the first 4-hour period, in group IV after 8 and in group V after 12 hours. In all three cases we get an increase of the growth rate, just as in normal plants. Thus we have to conclude that all three factors: auxin, aging and food drop in approximately the same ratio after deseeding.

By analysing the growth distribution after application of auxin in deseeded plants at different periods after deseeding we arrive at the same conclusion. Figure 8 shows the re-calculation of the data at hand. We compare the relative growth increase upon auxin application as the growth distribution is the same in normal and deseeded plants. Curve I represents the growth increase caused by excess auxin in normal plants during the first 4 hours, curve II for the second 4-hour period. For still later periods the effect may be over 1000 %.

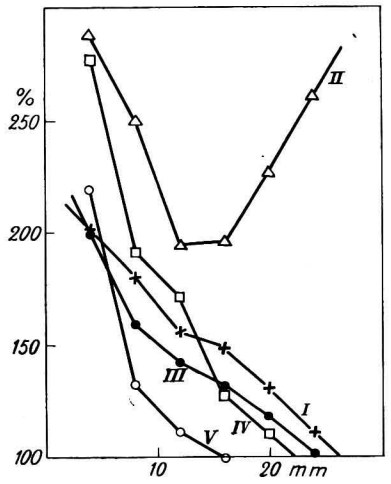

Fig. 8. Growth increase in the different zones due to application of auxin if compared with the growth of not treated plants, in the first period of 4 hours after application (curve II in second period of 4 hours). Curve III: plants deseeded 4 hours beforehand, curve IV: 8 hours deseeded, curve V: 22 hours deseeded. Ordinate: growth rate in % of normal growth. Abscissa: zones in mm from tip.

Now the excess auxin-growth of the deseeded plants in the first period of 4 hours (curve III) is essentially the same, only the effect does not reach down so far: the lowest zones, which also are affected most by aging, do not respond to auxin as in the normal plants, and the longer beforehand the deseeding takes place, the more the effect is localised in the upper zones (curves III, IV and V). But in those upper zones it is the same as in normal plants. However, the great difference between these and the normal plants is, that in the following periods there is no increased effect of auxin on the lower zones: the latter will go on growing at the same rate as they do without additional auxin. This means that the application of auxin does not increase the amount of food factor in the lower zones, while, as we have seen, it does so in normal plants (par. 7).

11. *Discussion.*

Now we are ready to reconsider the interrelations between growth rate, auxin and food factor. From all the preceding paragraphs we are led to the inevitable conclusion, that the growth of a coleoptile cannot be explained by the effects of auxin and aging alone, but that everywhere a third factor, which has been called the food factor, comes into the picture. This factor probably comes from the seed (see par. 12). It has not been possible to determine the concentration of the food factor in all zones of a coleoptile, as the only criterion for its presence is the growth rate, and in most cases this growth rate is primarily limited by the degree of aging of the cells. Only in exceptional cases does there seem to be a great excess of food factor (par. 5 and 9); in most other cases the amount of food factor available seems to bear a certain quantitative relationship to the growth rate, though it is not limiting growth. Thus it seems that the food factor and auxin are not independant. A relationship between these two may be imagined in different ways, e.g. as follows.

It is possible, that auxin, apart from its more or less direct effect on the cell wall, influences the transport of the food factor as well, so that a high auxin concentration would mean a good supply of the food factor. In view of the other processes influenced by auxin this explanation seems very reasonable; indeed the author hopes to be able to prove this viewpoint in another paper.

In some cases we have obtained rejuvenation by application of excess auxin (par. 5 and 9) but in others we have not. The most marked rejuvenation has been obtained when the growth rate is very low or zero. In par. 9 we actually observe an increasing effect the more the cells have aged. With the higher growth rates the rejuvenation, if present at all, will be so small to be undistinguishable.

12. *Food factor and leaf growth.*

A few further experiments throw some light on the food factor. In par. 5 it was found that the leaf limits the growth of the coleoptile by using the food factor. Also DU BUY (1933) describes how the growth of the coleoptile rather suddenly stops as soon as the primary leaf breaks through the coleoptile and starts to grow at a much increased speed. As the auxin formation goes on long after growth has stopped this must be caused by a lack of the food factor.

On the other hand rapid coleoptile growth inhibits leaf growth. In the plants treated with auxin paste the coleoptile grows faster and the leaf slower, as figure 9 shows. That this inhibition is due to the increased growth of the coleoptile and not to the auxin in itself, is proven by the next experiment. From a number of plants the growing zone of the coleoptile is cut off, and on the cut surface of half of the plants is put auxin paste I. The auxin is present but does not produce any appreciable growth in the basal cells of the coleoptile, and in this case auxin does not

inhibit leaf growth. The mean values are: leaf length in controls: 75 mm; when auxin paste 1 is added: 72 mm. All preceding facts are satisfactorily explained by assuming that for the growth of both leaf and coleoptile the same food factor is necessary. Its total supply by the seed is limited and allows for the normal growth of both, but as soon as the growth of one of them is increased beyond normal and uses more food factor, the growth of the other will decrease. This explanation is completely in line with the conclusion of par. 8, that the total supply of the food factor to the coleoptile is greater after auxin is applied. Moreover it explains the fact that in normal plants there exists a positive correlation between coleoptile and leaf growth. If the coleoptile is long, the leaf is too, or both are short. This is due to a variable available amount of food factor in the seed. Here we see that the distribution of the food factor between coleoptile and leaf is a finely regulated mechanism, which is upset by excessive auxin supply.

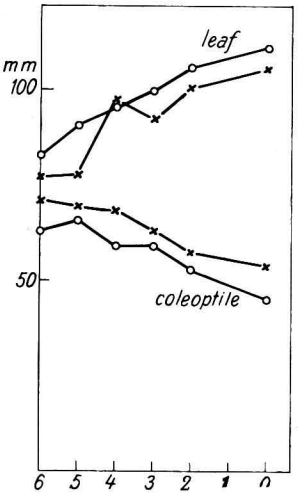

Fig. 9. Total length of coleoptile and primary leaf if treated with different auxin concentrations. Ordinate: length in mm. Abscissa: log. of auxin concentration.

Except in par. 7 no evidence has been obtained concerning my original hypothesis, that the growth of the highest zones, near the tip, is limited by the food factor. In the absence of any better explanation I provisionally maintain this view.

13. *Summary.*

By artificially increasing (10—50 times) the auxin concentration inside a coleoptile it can be shown, that in normal growth auxin limits the growth rate of practically all cells, either directly as a growth promoter or indirectly by preventing aging (par. 6). The aging is due to cell wall formation exceeding the rate of elongation, so that the cell walls become stiffer (par. 4). The reverse phenomenon, rejuvenation, has also been demonstrated (par. 5 and 9). This is a gradual increase in growth rate of old or aged cells after an excess of auxin has been applied (par. 11). Cells which have stopped growing by the combined effect of aging and auxin deficiency resume growth upon excessive auxin supply (par. 5).

A third factor limiting growth is recognised in the food factor, a nutrient stored in the seed and increasing both leaf and coleoptile growth (par. 12). Under normal conditions the food factor is in relative excess in the plant, but becomes limiting upon application of excess auxin (par. 8, 11), or after deseeding (par. 10).

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Anatomy. — *Experimentelle Untersuchungen über die basale optische Wurzel beim Meerschweinchen.* Von EUGEN FREY (Zürich). (Communicated by Prof. C. U. ARIËNS KAPPERS.)

(Communicated at the meeting of June 29, 1935).

In meinen früheren Arbeiten (Proceedings Vol. XXXVI, N°. 2 und 3, 1933) habe ich den Versuch gemacht, die basale optische Wurzel der Reptilien und Vögel auf vergleichend-anatomischer Basis einer systematischen Untersuchung zu unterziehen, bei spezieller Berücksichtigung der Beziehungen dieser Wurzel zur Commissura transversa Gudden und deren Kern. Als weiteres Ziel nahm ich mir vor, den Aufbau des Systems der basalen optischen Wurzel und ihres Kernes bei einigen Säugetieren vom gleichen Standpunkte aus zu erforschen.

Der Nachweis der basalen Optikuswurzel und ihres Kernes bei verschiedenen Säugetieren wurde von MARBURG ('03) im Zusammenhang mit dem Tractus peduncularis transversus gebracht. Dieser Tractus wurde bereits von GALL und SPURZHEIM (1810) und von INANZI und LEMOIGNE ('61) gesehen, aber erst von GUDDEN ('70, '81) genau beschrieben und als Tractus peduncularis transversus betitelt. Nach GUDDEN degeneriert dieser Tractus nach einseitiger Enukleation des Augapfels auf der gekreuzten Seite. Seine charakteristischen Eigenschaften bei verschiedenen Tieren (Kaninchen, Hund, Schaf, Katze) sind: Verlauf vom vorderen Rand der vorderen Vierhügel am Rande des Mittelhirns zur Hirnbasis zur Austrittsstelle des Nervus oculomotorius, wo dann der Tractus peduncularis transversus im Winkel zwischen dem Corpus mammillare und dem Pedunkulusrand ins Mittelhirninnere eintritt, unter Formung eines kernartigen Gebildes. Weiterhin verläuft er dorsolateralwärts zwischen dem unteren Rand des Nucleus ruber und der Substantia nigra.