ON FACTORS DETERMINING THE AUXIN CONTENT OF THE ROOT TIP

by

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CHAPTER I.
Introduction.

§ 1. *Statement of the Problem.*

BOYSEN JENSEN discovered in 1933 that cut root tips put on agar containing 10% of glucose delivered more auxin than those put on plain agar. This statement of BOYSEN JENSEN was chosen as a starting point for the research reported in this paper.

Generally speaking one may say that auxin mostly is present in young organs and not, or in distinctly smaller quantities, in adult tissues. In some cases auxin is regularly spread all over the organ concerned, e.g. in the hypocotyl of *Lupinus* (DIJKMAN, 1934) and in the epicotyl of *Vicia Faba* (VAN DER LAAN, 1934). In other cases a part of the organ is richer in auxin content

than the other parts; the auxin diffuses from this „auxin-centrum" towards the other cells of the young organ. The best known case of this is the coleoptile of the *Gramineae,* where auxin is continually produced in the tip and is transported from the tip to the basal regions.

The question may rise, why the auxin content is so much higher in the cells of the tip of the coleoptile than in the other cells. One may explain the lower auxin content in the region of growth by assuming that the auxin is consumed in the growth process (BONNER and THIMANN, 1935). This, however, does not hold good for the cells below this region, which have stopped growing. The fact, that the auxin content of these cells is also lower than that of cells of the tip, indicates that their metabolism, in its broadest sense, is different.

Which metabolic processes do determine the auxin content of the cell? By which processes the auxin is produced and — apart from transport to elsewhere — by which processes does auxin disappear?

This is not only a problem in the coleoptile of the *Gramineae,* but it presents itself everywhere auxin occurs. It can only be solved by comparing the metabolism of two cells with different auxin contents. Of course those cells, which differ only in their auxin content and as far as possible not in other characters, would be most suitable for such a comparison. In that case the probability would be greatest that differences eventually stated in the metabolism of these cells really may correlate with a difference in auxin content.

This equality in other characters will be realized as good as possible in that cases, where the auxin content of one and the same cell is rapidly changed under the influence of an external factor. Such cases, however, rarely occur.

An example of it is the so called regeneration of the physiological tip, as discovered by ROTHERT (1896). DOLK (1926) has investigated this phenomenon more thoroughly. He cut off the upper zone of decapitated coleoptiles and determined the amount of auxin, delivered by this small cylinder. He found that the upper cylinder produced no or only little auxin, if the coleoptiles were decapitated shortly before. If, however, the decapitation preceded a considerable time before, the upper cylinders proved to deliver auxin in appreciable amounts. This really is a case, in which the auxin content of certain cells increases rather rapidly and that, therefore, is suitable for a research into the problems mentioned above. This phenomenon thus has been

investigated by several authors. SÖDING (1929) e.g. stated that the amount of auxin produced by the regenerated tip was independent from the length of the decapitated end, which varied from 1 to 5-6 mm. TSI TSUNG LI (1931) studied the influence of temperature upon the velocity of the regeneration of the physiological tip. SKOOG (1937) succeeded in suppressing the regeneration of the physiological tip by removing one day before the endosperm from the seedlings. Such „deseeded" plants were used as test plants in the following experiment. Cylinders of normal coleoptiles were placed with their *apical* cut surface upon agar slides for some time. Blocks of this agar then were placed unilaterally upon deseeded coleoptiles. In the beginning these plants behaved as if no auxin was present in the agar. This is in full agreement with VAN DER WEY'S (1932) experiments, who stated that no auxin is tranported in apical direction. Therefore no auxin could diffuse into the agar from an apical cut surface. Two hours after placing the agar blocks on the deseeded coleoptiles, however, a curvature started, which gradually increased. The agar thus proved to contain a substance, which gradually is transformed into auxin. In this way SKOOG showed that auxin is produced from a „precursor", which is transported from the seed to the apex. These investigations on the regeneration of the physiological tip have yielded important data on the origin of auxin in the coleoptile.

Another case in which cells rather quickly change their auxin content is the effect of glucose upon the auxin content of root tips mentioned above. Research on this subject is still scanty.

BOYSEN JENSEN (1933a), discovering this phenomenon, supposed in the beginning that the normal root tip was too poor in plastic material to be able to produce auxin. Stating later on, that mannite had the same effect as glucose, he gave up this idea since mannite probably could not be converted by the cells of the root tip. He then ascribed the increase of the auxin delivery to some physical influence, e.g. an improved contact between root tip and agar, if the latter contained some hygroscopic substance like glucose or mannite.

Also THIMANN (1934) ascribes the influence of glucose on the auxin production to some „physic (osmotic) action."

CHOLODNY (1934) on the other hand, believes that glucose acts as a nutrient, since root tips with glucose continue to deliver auxin for a longer time than without.

The mechanism of the glucose effect on the auxin production has only been studied occasionally by these three authors.

Although BOYSEN JENSEN's discovery has been of great importance for a better understanding of the auxin function in the root, till now it has not been applied to elucidate the processes, which determine the auxin content of the cells. That was the aim when starting the investigations reported in the present paper.

§ 2. *Literature.*

It seems appropriate to resume briefly what was known about auxin in the root before BOYSEN JENSEN's discovery. For a more detailed survey of the literature must be referred to GORTER (1932). and BOYSEN JENSEN (1935).

The literature on growth and tropisms of roots of the last ten years — since 1926 — is reigned by discussions on CHOLODNYS' theory (1924; 1926). According to this theory the positive geotropism of the root is explained as follows. In the root tip the same hormone would be produced as in the tip of the aerial organs. This hormon accelerating the growth of the cells in the latter, would retard the growth in the root. By placing the root horizontally, the hormon, as in the stem, would shift to the lower side, which consequently would be more retarded in growth than the upper side. This would result into a positive curvature.

The presence of auxin in aerial organs and the inequal distribution of this substance under the unilateral influence of gravity has been proved by the work of WENT (1928) and DOLK (1930). The proof of their experiments is chiefly based on the method devised by WENT to test quantitatively the amount of auxin in tissues. To this purpose the tissue to be tested is placed for a certain time on a slice of agar, which afterwards is divided up into small agar blocks, which are placed unilaterally upon a decapitated coleoptile of *Avena*. This curves negatively under the influence of the auxin present in the agar. The magnitude of the curvature, is, within certain limits, a measure for the amount of auxin in the agar block. By means of this method the presence of auxin in any organ can be stated directly. Before WENT applied this method already theories were formulated, explaining tropistic movements by means of chemical substances (e.g. BOYSEN JENSEN (1911); PAÁL (1919). The data endorsing these theories, however, were obtained only indirectly and were therefore more liable to criticism than the experiments of WENT and DOLK, who really isolated the auxin from the coleoptile of *Avena*.

It was obvious to try to judge the correctness of CHOLODNYS' theory on the action of auxin in the root by means of the same direct method. This proved, however, to meet with great difficulties. HAWKER (1932) succeeded in recovering auxin in this way from root tips. In her experiments geotropically stimulated root tips of *Vicia Faba* were split and the two halves were put on gelatine for some time. Then the blocks of gelatin were placed unilaterally upon the stumps of decapitated roots. A curvature resulted in the direction of the gelatin block, the curvature being larger with gelatin blocks, on which the lower half of the root tips had been placed, than with those, on which the upper halves had stood.

Contrasting to these experiments are those of GORTER (1932). She tried to isolate auxin from root tips of *Pisum* and *Zea* by placing pieces of roots on agar or sand. In the latter case the sand was washed with water and ether; this extract was mixed with agar and its auxin content tested with *Avena* coleoptiles. Both methods failed to show the presence of auxin in roots.

The results of these effords to isolate auxin from roots are contradictory. The same applies for the data obtained with indirect methods.

CHOLODNY himself (1924, 1926) based his theory on the following experimental results. Decapitated roots of *Lupinus* did not curve geotropically; the ability to curve was restored by placing a tip of a coleoptile of corn on the cut surface. Decapitated roots of corn grew under water and in humid air markedly quicker than intact roots. By sticking a coleoptile tip on the cut surface the growth rate was slackened again. A new proof was given in 1928: a decapitated root of corn was placed horizontally. A tip of a coleoptile of corn was fixed on it by means of gelatin in the normal (vertical) position. Although the coleoptile tip was not stimulated geotropically, the root curved positively geotropically. This proves in the mean time that the curvature is not to be ascribed to specific geotropic stimulating agents. Coleoptiles of *Avena* were decapitated and the tips replaced by those of roots of corn. The phototropic and geotropic responses proved to be much larger than without these root tips.

SNOW (1923) also had discovered already that decapitated roots of *Vicia Faba* regained their geotropic sensitivity by replacing their tips.

CHOLODNYS' theory was chiefly criticized by BÜNNING and GORTER.

BÜNNING (1928) found in a number of plants that after decapitation the root shows a retardation of growth or even a con-

traction first, but that the rate of growth afterwards increases to a higher value than that of the normal root. He ascribes these phenomena to wound growth reactions and does not believe that the root tip produces growth accelerating or -retarding substances. On the other hand he also states that the rate of growth of decapitated roots of *Lupinus* is decreased by replacing their tips on them. The same effect, however, is produced by placing, in stead of tips, any other section of a root on the cut surface.

CHOLODNY (1929) repeats BÜNNING's experiments, but he finds that tips of roots placed on decapitated roots decrease the growth rate and that cylinders from other sections of the root have no effect upon the growth rate. BÜNNING's results are due by CHOLODNY to deficiency of his material.

GORTER (1932) did not find that decapitation affects the rate of growth of roots of *Pisum*. Further she stated that the geotropic response of roots decapitated at only 1 mm from the root tip equalled that of normal roots. Root tips of *Pisum*, unilaterally placed on decapitated coleoptiles of *Avena* did not produce a curvature. The geotropic reactivity of decapitated coleoptiles was not increased either by placing root tips on them.

Also on this criticism CHOLODNY replied (1933). He deduced from GORTER's growth curves that after decapitation indeed an acceleration of the growth of the roots did occur. Moreover in her experiments the growth rate showed a constant decrease, even in not decapitated roots, a fact, which CHOLODNY ascribes to lack of humidity in the milieu. Also the fact, that root tips failed to produce curvatures in decapitated and horizontally placed coleoptiles, is ascribed by CHOLODNY to too dry experimental conditions. It is rather amusing that on the contrary GORTER explains the positive result of CHOLODNY in the latter experiment to exsiccation of the decapitated coleoptiles, on which no root tip was placed, so that they could not curve.

From these discussions it will be clear, how difficult it is to prove the presence of auxin in the root by indirect methods. BOYSEN JENSEN's discovery (1933 a), mentioned above, however, took away all uncertainty on this point. He placed root tips of corn on agar to which 10% of glucose was added. The tips delivered considerable amounts of auxin to this agar. The results were still better with agar + 10% of glucose + 0,1% of $Ca(NO_3)_2$ + 0,025% of K_2HPO_4 + 0,025% of $MgSO_4$ + a trace of $FeCl_3$. A root tip of corn delivered more auxin to this agar than a tip of a coleoptile of *Avena*.

BOYSEN JENSEN (1933 b) also with the aid of glucose agar investigated the influence of gravity on the distribution of auxin in horizontally placed root tips. To this purpose a geotropically stimulated root tip was placed on two separated agar blocks. In one block the auxin from the upper half, in the other that of the lower half of the root tip was received. He found that the lower half delivered more auxin than the upper half.

By these experiments of BOYSEN JENSEN the presence of auxin in the root tip as well as the unequal distribution by geotropic stimulus was proved. Since KÖGL, HAAGEN SMIT and ERXLEBEN (1934) had shown that auxin inhibits the growth of roots, CHOLODNY's theory on the function of auxin in the root was completely confirmed. Undecided, however, remained the question, whether auxin really is *produced* in the tip of the root. The literature on this point will be discussed in chapter III.

§ 3. *Methods.*

a. *The analysis of agar blocks containing auxin.*

For the estimation of the auxin content of the agar blocks the *„Avena"* test was used as described by WENT (1928), later on improved in some details by VAN DER WEY (1931). For the test the pure line of oats, „Siegeshafer" was used from Svalöv, kindly supplied by Prof. Dr. A. ÅKERMAN, ass. director of the Experiment Station of the „Svensk Utsädes Förening".

The coleoptiles were decapitated three times, with intervals of 1½ hours. Immediately after the third decapitation the agar blocks were placed upon the stumps. These agar blocks were obtained by deviding up an agar slice of 8 × 6 × 0,9 mm into 12 exactly equal parts. In this paper an agar *slice* means a layer of agar of the given dimensions, an agar *block* is 1/12 part of it and therefore has a volume of 3.6 mm^3.

The test plants were photographed 1 h 50 min after placing the agar blocks upon the stumps. The curvatures were measured by means of a protractor. In the tables the auxin quantities always are expressed by the curvatures (in degrees) of the test plants. These figures are the averages of 10-20 plants, the mean error being calculated from the formula

$$m = \sqrt{\frac{\Sigma\, p\, v^2}{n\,(n-1)}}$$

b. *The isolation of auxin from the root tips.*

The root tips, used in my experiments, were all of *Vicia Faba* L. The variety used was „Origineele Mansholt's Wierboonen"

from the firm Dr. R. J. MANSHOLT at Westpolder (Groningen).

The seeds were soaked in water for 24 hours and than planted in saw dust. For the experiments the root tips of 5 days old plants were used. The beans were taken from the saw dust and thoroughly washed under the tap; then the tips were cut at a length of 5 mm. To this purpose the roots were placed over a glass slide and a piece of calibrated (in mm) paper. A razor blade was used to cut off the tips.

Two methods were used for the isolation of auxin from the root tips:

1) A number of root tips (mostly 7) were placed for some time on an agar slice; then the auxin content of the agar was determined. When the influence of glucose on the delivery of auxin was investigated glucose Ph. Ned. Ed. 5 was used. In order to obtain a certain concentration of glucose, in the beginning the amount of glucose wanted was added when preparing the agar (3%). Later on the agar slices simply were soaked in solutions of glucose of the desired concentration. The latter procedure also was followed, when the influence of salts on the delivery of auxin was studied. In this way it is not certain that the total concentration in the agar slice is the same as that in the solution, but it is probable that the free fluid, not adsorbed to the colloidal agar particles, indeed has the same concentration.

2) A number of root tips were extracted with ether, according to prescriptions by DOLK and THIMANN (1932), KÖGL, HAAGEN SMIT and ERXLEBEN (1933) and THIMANN (1934). Briefly the method may be described as follows: the root tips are ground with a little acid and ether, free from peroxides. When the tips had been suspended in a fluid as was the case in the experiments on respiration, also the fluid was acidified and washed with ether. The ether was decanted and the extraction repeated twice with new ether. Finally all the ether was evaporated and the residu solved in 0,2 cm^3 of a buffer solution. Two agar slices remained over night in the refrigerator in this solution. On the next day their auxin content was determined.

The ether was freed from peroxide by destilling it shortly before the extraction over $FeSO_4$ and CaO.

For extraction the pulp of the root tips was acidified in order to liberate the auxin, which eventually was bound to alkali. In the experiments of chapter III this was done by adding a few drops of hydrochloric acid. Then the extract was placed over night in the refrigerator over ignited Na_2SO_4. This method proved to be not entirely reliable; some times the auxin had

disappeared from the extract. To eliminate this objection in the other experiments a few drops of 0,5 n sulphuric acid was used instead of hydrochloric acid [1]). Since this acid is not volatile, it could not be removed from the ether by evaporation. For that reason the extract was washed twice with water, acidified with sulphuric acid to the conversion point of congo red. Although in this way most of the sulphuric acid was removed from the ether, still the danger remained that a last trace of this acid would destroy the auxin after the evaporation of the ether.

In order to prevent this, the method of evaporation was changed a little. The extract was not entirely evaporated, but only so far that the volume was about 5 cm^3. This rest was put into a small tube and 0,2 cm^3 of the buffer solution was added. This tube was kept in a beaker with warm water and the evaporating ether was blown away by means of an air current [2]). In this way the auxin was dissolved in the buffer solution without evaporation of the extract to dry. In order to remove the water insoluble substances as far as possible, the preparation was washed with petrolether, which afterwards was decanted (according to KÖGL, HAAGEN SMIT and ERXLEBEN (1933), auxin is insoluble in petrolether).

The buffer, in which the auxin was solved, served to eliminate the eventual last traces of acid. It was a diluted buffer after McILVAINE, a solution of 0,04 mol citric acid and 0,02 mol Na_2HPO_4; its pH was ± 5,4.

I often succeeded in extracting by means of the described methods a substance from root tips, which shows auxin activity. From investigations of recent years it is, however, known, that a number of chemically widely different substances show a positive result in the *Avena* test (e.g. HAAGEN SMIT and WENT, 1935). Therefore it is not quite certain that the growth substance from the root really is identical with auxin. This is, however, made highly probable by the investigations by HEYN (1935), who estimated the molecular weight of the growth substance from the root by means of its diffusion rate through agar. He found as an average for the diffusion coefficient the value $D = 0{,}391$, which corresponds to a molecular weight of ± 330.

[1]) Perhaps it would be advisable to add in future in experiments of this kind diluted acetic acid.

[2]) The blowing off of the ether vapour by means of an air current and the application of petrolether were suggested by Dr. J. MACPHERSON ROBERTSON, to whom I feel much indebted for his valuable advice.

The real molecular weight of auxin a = 328, that of auxin b = 310.

CHAPTER II.
Preliminary Experiments.

§ 1. *The influence of different glucose concentrations on the delivery of auxin by root tips.*

BOYSEN JENSEN (1933 a), when discovering that the delivery of auxin by root tips could be increased by the addition of glucose to the agar, applied only one concentration of glucose, i.e. of 10%. He did not mention whether this really is the optimal concentration.

In an earlier paper some results of experiments on this subject have been published (VAN RAALTE, 1936); fig. 1 represents

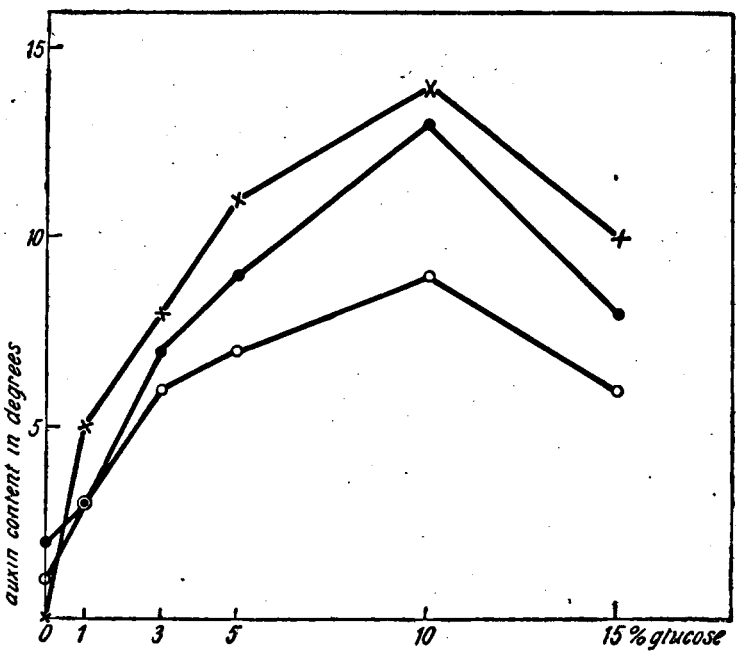

Fig. 1. (See text).

these results once more. In these experiments root tips were placed on agar slices containing different concentrations of glucose. Ten tips stood for two hours on each slice; then the auxin content of the agar was determined. In the graph the glucose content of the agar is plotted on the abcissa, the amount of auxin on the ordinates (in degrees of the curvature of the test plants). The optimal concentration proves to be 10% of glucose. The fact, that agar with 15% of glucose induces smaller curvatures in the test plants, does not necessarily indicate that this agar contains less auxin than that with 10% of glucose. This smaller curvature may be caused also by an adverse influence of the high glucose concentration on the test plants. Such an influence was apparent in control tests. Unilateral application of agar blocks, containing 15% of glucose but no auxin, often induced positive curvatures in the test plants. Such curvatures would partly counterbalance eventual curvatures induced by auxin.

§ 2. *The influence of glucose on the auxin production by Avena-coleoptiles.*

In order to isolate a well measurable amount of auxin from the root tip of *Vicia,* one must add glucose to the agar. In this regard the root tip behaves differently from other organs, e.g. from the tip of the coleoptile of the *Gramineae;* the latter also delivers auxin to the agar without addition of glucose. The question rises what may be the reason of this difference.

Possibly the synthesis of auxin in the coleoptile proceeds in quite a different way than in the root, the presence of glucose not being necessary in the former. Another possibility is, that glucose (or another reducing sugar) is required for the synthesis of auxin in the coleoptile as well as in the root tip, the coleoptile, however, containing a sufficient amount of these substances of itself, the addition of extra quantities being superfluous.

A simple FEHLINGS' test already shows the different sugar contents of the coleoptile and the root tip. When one crushes some tips of *Avena*-coleoptiles on a slide, adds a few drops of FEHLINGS' solution and heats, a strongly positive reaction appears. The same experiment with root tips of *Vicia* gives a much weaker reaction; it often is questionable, whether any reaction occurs. Apparently root tips contain considerably less reducing sugars than tips of coleoptiles.

It still remains open, whether the difference in sugar content is large enough to eliminate the influence of an extra supply of

sugar on the delivery of auxin by coleoptile tips. The next experiment had to discriminate this question. Equal numbers of coleoptile tips of *Avena* stood for four hours on plain agar and on agar, containing 10% of glucose. Then the auxin content of the agar was determined (table 1).

TABLE 1. (16-1-'34). Amount of auxin delivered by a coleoptile tip of *Avena* during 4 hours.

On plain agar	14,0° ± 1,6 (average of 11 plants)
On agar + 10% glucose	5,4° ± 0,8 (average of 18 plants)

The table shows that no favourable effect of glucose on the delivery of auxin occurs. The result points to the contrary; it has, however, not been investigated, why less auxin had been delivered to the glucose agar.

In any case the former experiment shows that in the coleoptile tip the content of reducing sugars is not a limiting factor in the production of auxin. In order to endorse this conclusion, it was tried to reduce this content to such a degree, that the production of auxin would decrease. If this could be obtained, the addition of glucose should increase the delivery of auxin.

In order to obtain plants with a lower content of nutrients, I proceeded as follows. Two days after laying out the seeds for germination the endosperm was removed from the seedling. The latter was planted into saw dust. The plants treated in this way grew well; the coleoptiles, however, being thinner than those of normal plants; moreover they often remained a little shorter than those. At an age of 5 days the coleoptile tips were cut. Part of them were put on plain agar, part on agar in which certain substances were solved. After a certain time the tips were removed and the auxin content of the agar determined (tables 2, 3, 4 and 5).

TABLE 2. (30-1-'34). Effect of glucose on the amount of auxin delivered by tips of deseeded *) *Avena* coleoptiles.

Amount of auxin delivered by 10 tips to one agar slice during:

	1 hour	3 hours	5 hours
agar + 5% glucose	1,6° ± 0,2	1,5° ± 0,5	2,1° ± 0,5
plain agar	0,5° ± 0,3	3,4° ± 0,9	3,4° ± 0,6

*) These „deseeded" plants are not to be confused with the deseeded plants of Skoog (1937), as the seed was taken away in a different stage of their development.

TABLE 3. (31-1-'34).Effect of glucose and fructose on the amount of auxin delivered by tips of deseeded *Avena* coleoptiles.
Amount of auxin delivered by 15 tips to one agar slice, during 4 hours.

plain agar	10,0° ± 2,0	agar + 5% glucose	7,2° ± 1,2
plain agar	4,2° ± 1,5	agar + 5% fructose	5,6° ± 0,8

TABLE 4. (26-1-'34). Effect of glucose and fructose on the amount of auxin delivered by tips of deseeded *Avena* coleoptiles.
Amount of auxin delivered by 12 tips to one agar slice, during:

	1½ hours	1 hour
plain agar	5,2° ± 0,6	7,2° ± 0,7
agar + 5% glucose	7,2° ± 1,0	—
agar + 10% glucose	3,0° ± 0,8	—
agar + 5% fructose	3,3° ± 0,3	4,7° ± 1,2
agar + 10% fructose	4,1° ± 0,9	—

TABLE 5. (5-2-'34). Effect of glucose on the amount of auxin delivered by tips of deseeded *Avena* coleoptiles.
Amount of auxin delivered by 15 tips to one agar slice during 3 hours.

plain agar	3,3° ± 0,9	agar + 5% glucose	4,4° ± 0,8
plain agar	2,7° ± 0,7	agar + 5% glucose	2,8° ± 0,6
plain agar	2,1° ± 0,7	agar + 5% glucose	3,6° ± 0,7

When looking through the tables it is evident, that the amount of auxin, delivered to the plain agar is, as a rule, considerably less than the amount delivered by normal tips. Under the conditions prevailing in these experiments, a coleoptile tip delivers, as an average, an amount of auxin agreeing with a curvature of 10° in the test plants.

When comparing the delivery of auxin by tips from agar containing glucose or fructose with that by tips from plain agar, never a distinct effect of the sugar can be stated. We therefore did not succeed in getting the sugar content of the tip limiting factor for the auxin production.

After the experiments described above, some experiments were done in which also peptone or asparagine was added to the agar. Tables 6 and 7 give the results.

It is clear that also the combination of peptone with glucose does not exert a distinct influence upon the delivery of auxin by the tips. The peptone containing agar always was rich in auxin, but also the control blocks proved to exert growth-

TABLE 6. (7-2-'34). Effect of glucose + peptone and glucose + asparagine on the amount of auxin delivered by tips of deseeded *Avena* coleoptiles. Amount of auxin delivered by 15 tips to one agar slice during 4 hours

		check agar blocks without auxin
plain agar	5,6° ± 1,4	
agar + 5% glucose	6,1° ± 1,1	
agar + 5% glucose + 1% asparagine	4,2° ± 0,5	2,5° ± 0,9
agar + 5% glucose + 1% peptone Poulenc	14,1° ± 0,9	0,7° ± 0,7

TABLE 7. (9-2-'34). Effect of glucose, glucose + peptone and peptone on the amount of auxin delivered by tips of deseeded *Avena* coleoptiles. Amount of auxin delivered by 15 tips to one agar slice during 3 hours

		check agar blocks without auxin
plain agar	10,8° ± 1,4	—
agar + 5% glucose + 1% peptone Poulenc	13,2° ± 1,2	—
agar + 5% glucose + 1% peptone Poulenc	11,0° ± 0,9	2,2° ± 0,1
agar + 1% peptone Poulenc	14,5° ± 0,6	6,7° ± 0,5
agar + 1% peptone Poulenc	12,5° ± 1,1	—
agar + 5% glucose	6,5° ± 1,1	—

substance activity. This is not surprising, since experiments by THIMANN (1935) showed that peptone may contain hetero-auxin, that can originate from oxidative changes of tryptophane. Tryptophane itself uses to be an impurity in peptone.

In order to eliminate many possible impurities, an experiment was done with peptone washed in ether and dried afterwards (table 8).

TABLE 8. (6-3-'34). Effect of glucose + purified peptone on the amount of auxin delivered by tips of deseeded *Avena* coleoptiles. Amount of auxin delivered by 12 tips to one agar slice during 4 hours

		check agar block without auxin
plain agar	4,9° ± 1,1	
plain agar	5,9° ± 0,5	
agar + 5% glucose	3,2° ± 1,0	
agar + 5% glucose	6,3° ± 1,3	
agar + 5% glucose + 1% peptone	7,9° ± 0,6	
agar + 5% glucose + 1% peptone	4,8° ± 0,8	1,0° ± 0,6

Purified peptone proved indeed to exert only a weak growth substance effect; the agar in controls, on which no root tips had

stood, giving a mean curvature of only 1°,0 ± 0°,6. The difference in the amounts of auxin delivered to the different agar preparations, however, has now practically disappeared. Neither glucose or glucose combined to peptone proved therefore to be able to increase the auxin production in the coleoptile tips.

§ 3. *The influence of glucose on the delivery of auxin by cotyledons of Raphanus.*

Another object in which was tried to detect a glucose effect were the cotyledons of *Raphanus*. VAN OVERBEEK (1933) found that the cotyledons of seedlings of *Raphanus*, when kept in the dark for some time, delivered less auxin to agar blocks on which they were placed than those of seedlings normally grown in the light. If the „dark" plants were brought back into the light, the auxin content of the cotyledons increased again. VAN OVERBEEK could thus show that auxin is only formed in the cotyledons in the light. The decrease in auxin content, however, did not occur in the cotyledons of very young plants, kept in the dark. In these the cotyledons had a reserve stock of auxin, stored already in the seed. Another case, in which auxin is formed only in the light has been described by AVERY (1935) for tabacco leaves.

Nothing is still known about the way in which light affects the synthesis of auxin. It is an obvious hypothesis that auxin would be formed as an accessory product in photosynthesis.

Another possibility, however, is that in the cited cases the formation of auxin is dependent upon the content of reducing sugars as is the case in root tips. In long lasting dark periods the carbohydrate content of the green parts decreases, since there is no photosynthesis. This decrease in carbohydrate content could possibly check the synthesis of auxin. If the latter hypothesis holds true, it should be possible to increase the auxin content of plants, kept in the dark, by the supply of glucose. In order to investigate this next experiment was taken.

A number of seedlings of *Raphanus* was kept for two days in the dark; an equal number remained in the green house. The seedlings were 9 days old, the age at which, according to VAN OVERBEEK (l.c. p. 576), the cotyledons are free from reserve auxin. At the end of the second day (of darkness) the cotyledons of both series were cut and on the cut surface of each cotyledon an agar block was placed in the way described by VAN OVERBEEK (l.c. p. 566). One half of the cotyledons of each series got a block of plain agar, the other half a block of agar containing 5% of glucose. The results are given in table 9.

TABLE 9. (2-3-'34). Amount of auxin delivered by cotyledons of *Raphanus*.

	cotyledons from normal plants	cotyledons from plants in the dark room
plain agar	12,8° ± 1,2 (21)	6,0° ± 1,1 (18)
agar + 5% glucose	15,8° ± 1,0 (21)	8,3° ± 1,1 (18)

The figures between brackets indicate the number of test plants.

Glucose seems to increase perhaps the delivery of auxin a little as well in normal as in etiolated cotyledons. The addition of glucose, however, entirely fails to balance the deficiency of light. Since another experiment yielded the same result, we may conclude, that the auxin formation in the light is not caused by an increase in concentration of reducing sugars.

§ 4. *The effect of purified glucose.*

In recent years a continuously increasing number of cases has been reported in literature, in which very small amounts of certain organic compounds would affect the growth rate. Apart from auxin and physiologically related substances, the compounds of the „bios"-complex have to be mentioned, which in very small quantities strongly accelerate the growth of yeast and other fungi. It could be possible that such a substance with oligodynamical activity would occur as an impurity in glucose and would affect the metabolism in the root cells in such a way, that the latter would produce more auxin.

In order to check this possibility, the effect of the usually applied glucose „purissimum" was compared with that of carefully purified glucose. To this purpose glucose was solved in water, washed with ether and then recrystallized from an alcoholic solution. The experiment was done as follows: an equal number of root tips was placed for two hours a) on a slice of plain agar, b) on a slice of agar containing ordinary glucose and c) on a slice containing purified glucose. After removal of the tips, the auxin content of the agar was determined (table 10).

TABLE 10. (27-9-'35). Effect of purified and ordinary glucose. Amount of auxin delivered by the same number of root tips to one agar slice during 2 hours.

plain agar	2,7° ± 0,8	agar + 5% ordinary glucose	11,7° ± 0,8	agar + 10% purified glucose	11,8° ± 0,9
plain agar	3,3° ± 0,5	agar + 10% ordinary glucose	11,7° ± 1,7	agar + 10% purified glucose	13,5° ± 1,1

The action of purified glucose proves to equal that of ordinary glucose. This proves that eventual impurities of ordinary glucose, soluble in ether and alcohol, do not affect the auxin delivery by root tips.

§ 5. *Protection against inactivation in the neighbourhood of the cut surface.*

Another effect of glucose, which one could suggest, is the protection of the auxin against inactivation by enzymes. When passing from the root tip into the agar, the auxin has to travel through the cut surface, where a great number of dead and dying cells occurs. Now, it is a well known fact, that no or only very little auxin can be recovered from tissues, ground in water (WENT, 1928; THIMANN, 1934). Probably the auxin is destroyed by liberated intracellular enzymes. Recently such auxin destroying substances proceeding from dead or dying tissues actually have been detected. VAN OVERBEEK (1935, 1936) put tips of coleoptiles, of which the auxin production had ceased, and coleoptile cylinders on agar blocks, containing known amounts of auxin. After some time a part of the auxin proved to have disappeared. He made it probable, that this disappearance of auxin is to be due to a destruction of auxin by oxidative enzymes, diffusing from the tips and cylinders into the agar blocks. LARSEN (1936) in a similar way could detect an auxin inactivating substance in wounded stems and ground tissues of *Phaseolus*. The next experiment shows that such auxin inactivating substances also may arise in root cells of *Vicia Faba*. A number of root tips is ground to a pulpy mass. In this pulp slices of agar were soaked, they remained there over night at 4° C. Next day 12 tips of *Avena*-coleoptiles were placed for two hours on each slice. As a blanc the same number of tips was placed on slices of plain agar for two hours. After removing the coleoptile tips, the auxin content of the agar slices was determined (table 11).

It is evident that the agar slices from the root tip pulp contained some auxin inactivating substance. The character of this substance has not been investigated further. The substance detected by LARSEN in *Phaseolus* is thermolabile, the inactivation irreversible and therefore destructive. This points to an action of destroying enzymes, as was the case in VAN OVERBEEKS' investigations. There is some evidence, that the inactivation, stated here, is also due to a similar enzymatic action. In cut root tips these enzymes chiefly will be liberated in the cut surface and here partly destroy the passing auxin.

TABLE 11. Auxin inactivating substances in ground root tips. Amount of auxin delivered by 12 tips of *Avena* coleoptiles during 2 hours

	to a plain agar slice	to an agar slice containing substances from ground root tips
Expt. 1 (17-6-'35)	17,7° ± 0,9	8,5° ± 0,6
	16,9° ± 1,3	3,9° ± 0,9
		4,4° ± 0,7
Expt. 2 (18-6-'35)	16,7° ± 1,3	10,3° ± 1,2
	22,3° ± 1,3	11,0° ± 1,3
	17,3° ± 1,3	15,3° ± 1,1
	19,1° ± 1,3	11,1° ± 0,9

Now, it might be possible that the glucose effect actually means that the sugar prevents this destruction. If this would hold true, this action chiefly would be confined to the neighbourhood of the cut surface, i.e. there, where the tip and the agar block are in contact with each other. In that case the glucose effect should realize itself as soon as this contact is made, which can be checked in a simple way. To this purpose the root tips have only to stand on the agar as short a time as is required to get a detectable amount of auxin. If the analysis shows that even then the auxin content of glucose agar is higher, i.e. that the glucose effect is realized in such a short time, there would be given evidence, that the glucose acts in the neighbourhood of the boundary surface. If however, the sugar has to travel along a certain distance in the root tip in order to exert its influence, a certain lapse of time will be wanted, until the sugar has covered that distance. In the latter case during a short time after placing the root tips on the agar no or only little effect of the glucose will be noticeable. Table 12 gives the results of an experiment on this subject. In this experiment 10 root tips were placed on each agar slice. After one hour already these were removed and the auxin content of the agar tested.

TABLE 12. (13-4-'34). Amount of auxin delivered by 10 root tips to one agar slice during the first hour after decapitation.

Glucose concentration in the agar	Amount of auxin given off
0	2,9° ± 1,0
1%	2,1° ± 0,4
3%	3,5° ± 0,6
5%	3,4° ± 0,5
10%	4,9° ± 0,6
15%	1,6° ± 0,4

It is clear from table 12 that the glucose has only little effect during the first hour. This is still endorsed by determining the amount of auxin delivered to the agar during the second hour. In an earlier paper (VAN RAALTE, 1936, p. 264) the results of such an experiment have been reported; they are reproduced in fig. 2. In this graph the glucose concentrations are plotted on

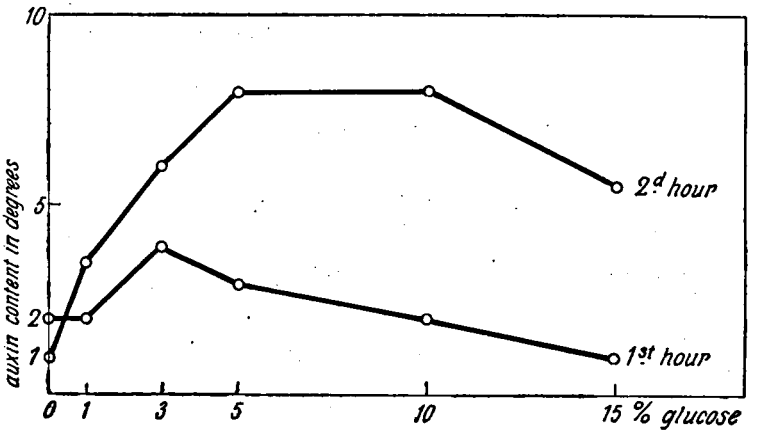

Fig. 2. (See text).

the abcissa, the amount of auxin on the ordinates. Comparison of the curves for the first and the second hour teaches that the effect of glucose mainly demonstrates itself in the second hour.

These results indicate that diffusion should proceed for some time, before the glucose reacts. One can imagine that first a sufficient amount of glucose has to travel upward in the root tip, but also that first a sufficient amount of substances (precursor?) has to diffuse from the tip into the agar, where they would have to be transformed into auxin. The latter idea, however, does not agree with the facts, as could be shown in the following way.

A number of root tips of *Vicia* stood for two hours on a slice of pure agar. Then they were removed and for another two hours the agar slice was covered by a second agar slice, containing 10% of glucose. All kinds of substances from the root tip were present in the first slice. If these substances could react with the glucose from the second slice, auxin had to be formed in

the agar. The analysis, however, showed that this is not the case. This proves that the glucose effect is not realized in the agar. It seems therefore probable that the glucose has to travel upward in the root tip to exert its effect upon the auxin content of it.

This view is endorsed to some degree by the fact that one also can make diffuse the glucose previously into the root tip and catch the auxin afterwards in a slice of plain agar. This experiment was taken as follows: 12 root tips were soaked for two hours in a 10% solution of glucose, then they stood for another two hours on a slice of plain agar. For comparison another set of 12 root tips was placed immediately after cutting on plain agar, also for two hours, a third set for the same time on agar containing 10% of glucose (table 13).

TABLE 13. (8-12-'33).Amount of auxin delivered to plain agar by root tips to which glucose had been applied previously.

plain agar no glucose	10% glucose agar	plain agar glucose previously applied
1,7° ± 0,5	5,9° ± 1,1	8,2° ± 0,9

Resuming we may conclude that probably the glucose does not exert its influence upon the delivery of auxin in the agar and neither in the boundary between agar and root tip.

CHAPTER III.

The Production of Auxin in the Root Tip.

In the preceding chapters the terms „production" and „delivery" of auxin have been intermingled. In fact this was not correct. If one discusses the fate of auxin in the root tip, it is necessary to discriminate between the symptoms observed and the supposed internal processes.

Above the delivery of auxin meant the transit of auxin from root tips into the agar. The glucose effect showed itself in an increased delivery: the agar slices with glucose received more auxin from the root tips than those without glucose.

What can be the cause of this increased delivery? The answer depends upon the question whether or not auxin can be synthetisized in the root tip from other substances. This process, the synthesis of auxin from other substances, further will be called auxin production.

By auxin production in the tip the auxin concentration would

increase. In that case probably there would be also one or more processes, which make the auxin concentration decrease. Processes of this kind will be indicated as auxin inactivation. It will, however, not be tried to discriminate whether 1) auxin is really inactivated or destructed, or 2) it is consumed in some growth process, or 3) the reaction precursor → auxin is accompanied by an opposite reaction: auxin → precursor.

Now, the glucose effect can be due to different possible causes. These possibilities are surveyed below:

I. Auxin production does never occur in the root tip.	II. Auxin production can occur in the root tip.
The glucose effect may be caused by:	
1. translocation of auxin from the tip to the glucose agar, 2. a decrease of auxin inactivation under the influence of glucose.	1. translocation of auxin from the tip to the glucose agar, 2. a decrease of auxin inactivation under the influence of glucose, 3. an increase of auxin production under the influence of glucose, 4. a cooperation of the factors 2 and 3.

This survey shows, that it is of evident importance to know, whether the root tip can produce auxin or not. In the latter case the factors II 3 and II 4 can be excluded and only the factors 1 and 2 remain open to further research.

The possibility of auxin production in the root tip for years has been a much discussed problem. A priori there is no reason to exclude this possibility. Since the root tip, however, does give off very little, if any auxin to plain agar, it was impossible to demonstrate auxin in the root directly, until BOYSEN JENSEN (1933 a) showed, that the auxin delivery is increased by glucose. The indirect methods, earlier applied, yielded contradictory results.

Two theories were given on the topographic origin of auxin, eventually present in the root tip. The older one by CHOLODNY (1926), who stated that a decapitated root grows faster than a normal one. This increase in growth rate was stopped by placing a tip of a coleoptile of corn on the cut surface. CHOLODNY concluded, that the root tip produces auxin in the same way as the tip of the coleoptile does. This hormone, causing an increase of

the growth in the coleoptile, would, however, decrease the growth rate in the root. In a root, declining from the vertical position, the lower half would show a higher auxin concentration than the upper half. The growth in the lower half would be retarded more than in the upper half, which would result into a downward curvature of the root tip. CHOLODNY thus assumes auxin production in the root tip.

WENT (1932), however, gives quite a different theory on the origin of auxin in the root. He concluded from the literature and from own experiments, that there would exist in the plant an electric potential difference between the tip of the stem and the tip of the roots, the potential of the root tip being positive, that of the stem tip negative. Under the influence of this P.D. positive and negative ions would travel cataphoretically in opposite directions. The kations would move towards the region of growth in the stem, the anions towards that of the roots. Auxin bearing an acid character, WENT assumes, that the (negative) auxin ions move continuously from the upper parts of the stem to the zone of growth in the root. According to this conception no auxin is formed in the root tip and, if once present there, the auxin cannot move away.

This theory seemed to be endorsed by investigations of THIMANN (1934). This author extracted root tips by means of chloroform. The quantities of auxin, obtained in this way, were compared with those delivered by the same number of root tips to glucose agar. The quantities delivered to the agar never exceeded those obtained by extraction with chloroform. From this THIMANN concluded, that auxin is not produced in the root tip, but that it diffuses from the seed and accumulates in the tip. The glucose effect was explained by the osmotic force of the glucose, which would — as has been mentioned above — extract the auxin from the root tip against the original polar direction of its transport.

THIMANN suggests still another possible explanation of his experiments. One can imagine, that auxin is formed in the root tip, but that the stock of „precursor" is so small, that it is consumed quickly after cutting off the tip. So THIMANN actually considers the possibility of auxin production in the root tip. He stresses, however, his first hypothesis so much, that this one is often cited in literature as *the* conception of THIMANN.

Meanwhile the polarity theory of WENT met readily with opposition.

CHOLODNY in 1934 critisized it thoroughly. The following ex-

periment is the most conclusive one: roots of corn are detipped and then a cylinder of 8 mm long is cut from the apical end. These cylinders are placed horizontally. A tip of a corn coleoptile is placed either on the apical or on the basal cut surface, an agar block on the opposite surface. After some time the agar blocks on the basal surface do contain auxin, those on the apical surface do not. This proves that in the root of corn auxin is only transported from the tip to the base, that means: the polarity is just opposite to that postulated by WENT's theory. No auxin can reach the root tip from without; it can only be synthetisized from other substances on the spot.

BOYSEN JENSEN (1936) repeats THIMANN's experiments on *Avena* roots with roots of *Vicia*. He states, that the quantity of auxin delivered by a root tip during 20 hours is 21 times as large as that, obtained by extraction with chloroform. Also in this case auxin must be produced in the root tip.

NAGAO (1935) isolated from roots of *Vicia* small cylinders, 2—6 mm from the tip. Those cylinders are placed on glucose agar, either with the basal or with the apical cut surface. Much more auxin diffuses from the basal surface than from the apical one. Also in *Vicia* the polarity of auxin transport runs from the tip to the base of the root.

NAGAO also repeats THIMANN's experiments with *Avena* roots. His results with *Avena* are quite different from those of THIMANN. He states that the quantity of auxin, delivered by the root tip in one hour, is twice as large as that extracted with chloroform. Moreover a tip on agar continued for at least 6 hours to deliver auxin to the agar. Being obtained with the same object, this result directly contradicts THIMANNS'.

CHOLODNYS' theory is opposed by a recent paper of FIEDLER (1936), who cultivated excised roots in pure culture. The roots developed very well, they also showed a geotropic response, but the extraction of auxin by means of THIMANNS' chloroform method failed. It was proved that in corn and pea the auxin disappeared from the roots within 24 hours after their excision. FIEDLER explains this disappearance by a diffusion of the auxin to the cut surface, where it is destructed by oxydases. The fact, that no auxin is regenerated, proves, according to FIEDLER, that the root tip has no ability to produce auxin.

It is clear, that the data in the literature on the problem of auxin production in the root tip are not conclusive. Therefore all possibilities, mentioned on p. 299, must be considered for an explanation of the glucose effect. For that reason the first

possibility was investigated: is the glucose effect caused by a translocation of auxin from the tip to the agar, without any change in the total amount of auxin? (i.e. THIMANNS' prevailing explanation of the glucose effect; see p. 300).

This being true, root tip and agar slice may be compared with communicating vessels: an increase of the auxin content of the slice can only coincide with a decrease in the tip. Since more auxin is delivered to glucose agar than to plain agar, the auxin content of root tips from glucose agar should be smaller than that of root tips from plain agar.

It has been investigated, in the following experiments whether this holds true or not. Different numbers of root tips were placed during a certain time on slices of plain agar or of agar containing 10% of glucose. Afterwards the tips were ground and extracted with ether and the obtained quantity of auxin tested. Table 14 gives a survey of the data obtained. [1])

TABLE 14. Amount of auxin present in root tips from glucose containing agar and from plain agar.

Experiment	tips from	number of tips extracted	curvature of test plants	ratio
1. (21-11-'35)	glucose agar	10	5,7° ± 0,8	
	plain agar	50	6,9° ± 0,8	4,1
2. (10-12-'35)	glucose agar	15	6,4° ± 0,6	
	plain agar	25	3,5° ± 0,6	3,0
3. (9-12-'35)	glucose agar	30	10,8° ± 0,7	
	plain agar	50	8,0° ± 0,7	2,3
4. (2-12-'35)	glucose agar	30	4,0° ± 0,4	
	plain agar	75	2,7° ± 0,4	3,7
5. (3-12-'35)	glucose agar	15	3,2° ± 0,6	
	plain agar	75	8,1° ± 0,9	2,0
6. (19-12-'35)	glucose agar	15	2,7° ± 0,5	
	plain agar	50	1,9° ± 0,4	4,5

The last column of table 14 gives the ratios of the mean auxin content of *one* root tip from glucose agar to that of *one* tip from plain agar (the tips stood on the agar during the same time). Not always the same numbers of tips were extracted. This is due to the fact, that only limited concentrations of auxin can be determined quantatively in the *Avena* test. The curvature of the test plants, caused by this concentration, was called by WENT

[1]) These results have already been published in an earlier paper: VAN RAALTE 1936.

the „limit-angle" („Grenzwinkel"). The size of this angle varies from day to day and cannot be predicted. By extracting different numbers of root tips almost always a quantity of auxin could be obtained, which yielded a smaller curvature than the limit angle. For this reason it was not always feasible to compare equal numbers of tips from glucose agar and from plain agar.

Column 5 of table 14 shows, that root tips from glucose agar contain 2—4,5 times as much auxin as those from plain agar. The glucose effect therefore is not restricted to a higher delivery of auxin to the glucose agar, but it also increases the auxin concentration in the tips themselves. It cannot be a mere translocation of auxin from the tip to the agar.

In the preceding experiment, however, the higher auxin content of tips from glucose agar was only relative, in comparison with that of tips from plain agar. It remains open, whether this must be ascribed to a real increase of the quantity of auxin on glucose agar, or to an inactivation of auxin on plain agar, which would be prevented by glucose. In order to investigate this question, next experiment was made. 60 root tips were extracted with ether immediately after cutting. Another set of 60 tips first remained during two hours on agar, containing 10% glucose, and then were extracted with ether, together with the agar on which they had stood. (Table 15).

TABLE 15. (12-5-'36). Increase of total amount of auxin in root tips on glucose agar.

Amount of auxin in 60 root tips immediately after cutting	Amount of auxin in 60 root tips and the agar on which they had stood
6,6° ± 0,6	11,0° ± 0,6

From this experiment can be concluded, that the glucose effect consists in an increase of the quantity of auxin in the root tip. *This increase of the quantity of auxin in a root tip, isolated from the plant, can only be explained by the assumption, that in the root tip production of auxin, i.e. synthesis from other substances, has taken place.*

This result completely agrees with the experiments of Cholodny, Boysen Jensen and Nagao. It is hard to explain Thimann's different results. Du Buy and Nuernbergk (1935, p. 347) suppose, that Thimann did not change the agar slices, on which the roots were standing, frequently enough. This could have caused such a high auxin concentration in the agar, that the auxin production

was hampered, the equilibrium precursor ⇄ auxin being shifted to the left. With greater evidence NAGAO suggested, that the auxin would be consumed or inactivated during the transport in the root tips, which, in THIMANNS' case, were 10 mm long. In fact, this author did not succeed either in isolating auxin by diffusion into agar from root tips of 10 mm length, but it was readily isolated from 2 mm tips.

THIMANN's results being probably to be ascribed to his method, this does not hold for FIEDLER's conception. He also believes that no auxin can be produced in the root tip and says literally: „Durch die dargelegten Versuche ist wohl bewiesen, dass die Wurzelspitze als ein ständig Wuchsstoff produzierendes Organ nicht in Frage kommen kann". (l.c. p. 426). I do not believe that this conclusion from FIEDLER's experiments is justified. As all other organs, which do not contain chlorophyll (the coleoptile of the *Gramineae* included), the root depends for the production of its organic material upon the substances supplied by the seed or by the green parts. After an interruption of this supply at some time the stock of these substances in the root must become exhausted and the normal production of organic material will be checked. This holds as well for auxin as for other substances, e.g. cellulose. The statement „dass der Wurzel nicht als ein ständig Zellulose produzierendes Organ in Frage kommt" can be considered as superfluous. The problem is not, whether the root is able to produce auxin, cellulose etc. without the aid of the green organs or the seed. The question in consideration is, whether these substances are delivered as such to the root, or synthetisized in the root from other substances, supplied by different parts of the plant to the root. In the latter case it would be allowed to say, that auxin, cellulose etc. are produced in the root.

In FIEDLER's experiments, the only organic substance supplied to the root is glucose. In his case no auxin could be recovered from roots 24 hours after their excision. That only means that the root with glucose as its only carbon source failed to produce auxin so quickly, that the production surpassed the consumption or inactivation. It seems improbable that only glucose would be transported from the seed or from the green parts to the roots; the transport of a large number of substances is probable. The possibility that the synthesis of auxin proceeds quicker from one of the other substances than from glucose is great; according to THIMANN this substance can be indicated as the „precursor".

According to this view, the production of auxin in the root is not fundamentally different from that of other organic substances. This also holds if glucose is a more suitable precursor of many other materials than of auxin. Also the fact, that apparently the stock of the precursor of auxin is quickly exhausted, cannot be an objection to the term „auxin production" in the root tip. Also excised tips of *Avena* coleoptiles rather soonly stop to deliver auxin (VAN OVERBEEK, 1935).

The next experiment shows, that the delivery of auxin by the root tips is continued indeed for several hours after cutting: two sets of 8 root tips were placed on an agar slice, containing 5% of glucose. From time to time the agar slice was replaced by a fresh one, the former being kept in the refrigerator. At the end of the experiment the agar slices were tested on their auxin content (table 16).

TABLE 16. (8-11-'34). Auxin delivery by two sets of 8 root tips on agar + 5% glucose.

	set 1	set 2
delivery from 10 h. 10—11 h. 10	6,1 ± 1,2	4,0 ± 0,5
„ „ 11 h. 10—12 h. 10	9,7 ± 1,2	7,6 ± 1,3
„ „ 12 h. 10—13 h. 10	10,6 ± 1,1	13,7 ± 0,9
„ „ 13 h. 10—15 h. 40	10,2 ± 0,8	10,0 ± 0,8
„ „ 15 h. 40—17 h. 10	5,2 ± 0,8	9,1 ± 1,5

Table 16 shows, that the maximum of the delivery of auxin is reached in the third hour; later on it decreases, but it continues during 7 hours after cutting the tips.

Resuming we may conclude:

1) the glucose effect is not only due to a translocation of auxin from the tip to the agar,

2) the root tip has the capacity to produce auxin.

CHAPTER IV.

The Relation Between Glucose Content, Respiration and Auxin Production of the Root Tip.

In the preceding chapter has been shown, that auxin is produced in the tip of the roots of *Vicia Faba* and that the auxin content increases under the influence of glucose. Considering the possibilities, resumed on p. 299, it is clear that this influence of glucose may be caused by 1) an increased production of

auxin, 2) a decreased inactivation of auxin or 3) a combination of 2) and 3).

It is very probable that both of these two processes depend upon other metabolic processes in the root tip. It therefore seemed reasonable to investigate, whether also some other metabolic process is influenced by adding glucose.

For this purpose the respiration has been chosen, being the best known and most accessible process. In the experiments, referred in this chapter, the following questions have been investigated:

1) whether the addition of glucose affects the respiration of the root tip,

2) whether the same effect on the respiration could be produced by some other agent than glucose,

3) whether in the latter case the auxin content of the tip also is changed.

§ 1. *Method.*

The respiration of root tips was estimated by means of the manometrical method after WARBURG (1928 b).

Open manometers were used, one of them acting as a blanc control for temperature and barometer alterations. BRODIE's fluid was used for the manometer readings. The volumes of the vessels, determined by wheighing their mercury capacity, varied from 16,89 to 22,18 cm^3.

During the experiments the vessels were placed in a waterbath of constant temperature, the fluctuations in temperature as a rule not exceeding 0,03° C. Most of the experiments were made at 23° C.

The manometers were shaken at a high rate (200 times per minute), the amplitude of the oscillation, however, being small.

For the determination of the oxygen consumption 0,2 cm^3 of a 20% KOH solution was put in a small central well in the vessel for the absorption of the carbon dioxide developed.

The carbon dioxide was estimated by expelling it by means of sulphuric acid. Three vessels containing equal numbers of root tips were treated as follows. Two of the vessels contained 0,2 cm^3 0,5 n sulphuric acid in their side bulb, the third one KOH in its central well. Immediately after the first manometer reading the amount of carbon dioxide, present in the first vessel at the start of the experiment was determined in the usual way. At the end of the experiment the carbon dioxide in the second vessel was estimated. The consumed quantity of oxygen is known from the alteration in pressure in the third vessel.

The manometer readings were estimated to an accuracy of 0,1 mm. The error in the readings cannot exceed 0,3 mm, that means an error in the estimation of the volumes of 0,6 mm^3 at most.

Equal numbers of root tips were used in each experiment; they were suspended in 2 cm^3 of fluid. This fluid was either destilled water (destilled from glass over glass) or a solution of the substance, the influence on the respiration of which was

tested. Buffer solutions soonly proved to affect the auxin production; for that reason the root tips could not be suspended in such a solution.

In order to determine the auxin content of the tips, they were taken from the vessels at the end of the experiment, ground and extracted with ether. The fluid from the vessels was also washed with ether.

The respiration figures were not calculated upon units of fresh or dry weight. In the tables the total respiration is given in mm^3 per hour of the total mass of root tips, present in each vessel. The root tips were cut as accurately as possible over millimeter paper at a length of 5 mm from the tips. Since it was impossible to apply in each experiment root tips in exactly the same stage of development, the intensities of respiration of the different sets of experiments cannot be compared with each other.

It was still questionable, whether a number of root tips of the same age and from the same culture shows the same respiration. This depends on the question, whether the number of root tips is large enough to eliminate the influence of individual variability. In order to estimate the influence of this variability some experiments were done, in which the respiration of equal numbers of root tips was compared (tables 17, 18 and 19).

TABLE 17. Oxygen consumption of 5 lots of 30 tips. Liquid medium 2 cc. phosphate buffer, concentration $\frac{1}{75}$ N, p.H. 6,8.

	lot no.:	1	2	3	4	5
mm^3 oxygen during	first 30 minutes	85,2	83,3	84,6	88,3	84,9
„ „ „	second 30 min.	82,5	84,2	81,8	89,6	84,2
„ „ „	third 30 minutes	74,7	74,0	75,9	80,7	76,4
total respiration in 1½ hours		242,4	241,5	242,3	258,6	245,4

largest difference: 258,6 — 241,5 = 17,1 mm^3 = ± 7%.

TABLE 18. Oxygen consumption of 5 lots of 20 tips. Liquid medium 2 cc. phosphate buffer, concentration $\frac{1}{75}$ N, p.H. 6,8.

	lot no.:	1	2	3	4	5
mm^3 oxygen during	first 30 minutes	53,5	52,6	57,9	58,0	52,3
„ „ „	second 30 min.	55,7	55,7	56,0	65,0	57,0
„ „ „	third 30 minutes	54,1	53,2	55,7	56,0	50,5
„ „ „	fourth 30 min.	48,0	48,5	50,9	59,5	52,8
total respiration in 2 hours		211,3	210,0	220,5	238,5	212,6

largest difference: 238,5 — 210,0 = 28,5 mm^3 = ± 14%.

TABLE 19. Oxygen consumption of 5 lots of 10 tips. Liquid medium 2 cc. phosphate buffer, concentration $\frac{1}{75}$ N, p.H. 6,8.

	1	2	3	4	5
total respiration in 2½ hours, mm³	177,3	176,0	186,6	197,8	186,5

largest difference: 197,8 — 176,0 = 21,8 = 12,5%.

The tables show that the values obtained for vessels, containing equal numbers of root tips, differ rather much from each other. For that reason always, if possible, 30 tips per vessel were used. With this number the error due to variability can be estimated on 10%. If only the oxygen consumption and not the carbon dioxide production had to be determined, the determinations could run in two or three parallel readings, the average figures thus obtained having a greater probability and a smaller error than 10%.

A serious impediment in these experiments is the relatively large diameter of the root tips (± 2 mm at the base), by which the gaseous exchange in the central cells is seriously hampered. WARBURG (1928 b, p. 105) especially emphasized the objections against experiments on respiration with objects of considerable diameter. The centrally located cells are subjected to oxygen deficiency and respire in a different way from the cells in the periphery. If in such a case changes in the respiration are stated one cannot discriminate, whether the respiration of all cells has been changed or whether the number of normally respiring cells has been changed. This objection could be eliminated by slicing the root tips into thin slices. In our case, however, this would be inadequate, since probably the auxin production seriously would be affected by the large number of deteriorating cells. The objection of a hampered diffusion could therefore in our case not be eliminated. This will be taken into consideration, when discussing the results of the experiments.

Another possible source of error was the influence of bacteria. The seeds were not desinfected and also the saw dust, in which the roots grew, was not sterile. The possibility of the development of a considerable quantity of bacteria in the vessels during the experiment is not to be excluded, so that their metabolism could interfere with the respiration of the root tips. In order to investigate this possible influence, 20 root tips were put in each vessel with 2 cm³ of water or of a 7% glucose solution. During two hours the respiration of the tips was measured in the thermostat, then the tips were removed and the vessels with the residual fluid were placed again in the thermostat. It then was

controlled whether still oxygen consumption or carbon dioxide production occurred, which had to be ascribed to bacteria, developed in the fluid. The result was negative. Since most of the experiments did not last for a longer time than 2½ hours, one may conclude that no disturbing effect of bacteria occurred.

§ 2. *The influence of glucose on respiration and auxin production of the root tips.*

It has already been shown (p. 294), that the glucose effect is not due to impurities present in the glucose, Still the glucose powder, used in the experiments described in this chapter, was treated with alcohol and ether to get it free from adhering impurities.

Table 20 represents the results of three experiments, in which the effect of different glucose concentrations on the oxygen consumption of root tips was determined. They show that glucose gives a depression of the respiration.

TABLE 20. The effect of glucose on the oxygen consumption of root tips.

	Expt. 1. (17-4-'36)		Expt. 2. (20-4-'36)		Expt. 3. (22-4-'36)	
mm³ oxygen / hours of 30 tips:	no glucose	5% glucose	no glucose	10% glucose	no glucose	7% glucose
	161,7 (100%)	150,9 (93,3%)	134,9 (100%)	94,6 (70,1%)	131,1 (100%)	90,7 (72,2%)

It is clear that the root tips, during the estimation of their respiration, are conditioned quite differently from those on agar during the delivery of auxin. In the latter case the tips are almost all-round surrounded by the air, but during the experiments on respiration they float in a fluid. By shaking the apparatus the fluid may be kept air saturated, but it remains possible that the auxin production proceeds in a way, different from that of root tips on agar. Therefore it was necessary to determine also the auxin content of the same tips of which the respiration had been estimated. The experiment on respiration being finished, the root tips with the surrounding liquid were removed from the vessels and extracted with ether. The results of these experiments are given in table 21.

As in the former experiments table 21 shows the depressing effect of glucose on the respiration of root tips. The last column shows that glucose increases the auxin content of root tips, also under these totally different conditions. Both phenomena, how-

TABLE 21. Effect of glucose on the oxygen consumption and the auxin production of root tips.

Experiment	tips in	number of of tips	oxygen consumption $\frac{mm^3}{hours}$	amount of auxin
1. (11-5-'36)				
	water	60	310,0 (100%)	7,1° (100%)
	10% of glucose	60	192,5 (62%)	12,3° (173%)
2. (13-5-'36)				
	water	60	343,2 (100%)	7,5° (100%)
	10% of glucose	60	191,8 (73%)	28,8° * (384%)
3. (14-5-'36)				
	water	60	292,5 (100%)	3,4° (100%)
	10% of glucose	60	195,9 (67%)	17,4° * (512%)
4. (15-5-'36)				
	water	60	311,6 (100%)	5,1° (100%)
	10% of glucose	60	222,6 (71%)	10,2° (200%)

The figures marked by an * have been determined with a diluted solution; the curvatures which where really obtained remained within the limit angle.

ever, do apparently not correlate in a simple quantitative way. The depression of the respiration in all four experiments of table 21 being of the same order of magnitude, the increase in auxin content is widely different (from 100 to 400%).

In the preceding experiments the respiration was estimated as oxygen consumption. The question rises whether the carbon dioxide production is shifted in the same way by glucose, i.e. whether the respiratory quotient remains unchanged. Table 22 gives the results of some experiments on this subject.

The second column shows that, in agreement with the results of the former experiments, the oxygen consumption of root tips is lower in glucose than in water. Column 3, however, shows that the carbon dioxide production decreases much less than the oxygen consumption; as an average the production of carbon dioxide remains about the same. Consequently the respiratory quotient increases.

In the literature only a few cases are mentioned, in which the respiration was reduced by higher concentrations of the substrate. In most cases the respiration is increased by the addition of glucose. A good survey of the literature is given by GEIGER—

TABLE 22. The effect of glucose on the oxygen consumption, carbon dioxyde output and the respiratory quotient of root tips. (30 tips in each vessel).

	$\frac{mm^3O_2}{hours}$	$\frac{mm^3CO_2}{hours}$	R.Q. $\left(\frac{CO_2}{O_2}\right)$
Expt. 1. (16-11-'36)			
water	168,8 (100%)	183,3	1,09
7% of glucose	131,4 (77,8%)	246,2	1,87
Expt. 2. (17-11-'36)			
water	166,0 (100%)	150,3	0,91
7% of glucose	115,3 (69,5%)	144,7	1,26
Expt. 3. (23-11-'36)			
water	169,5 (100%)	173,1	1,02
10% of glucose	85,6 (50,6%)	167,0	1,97
Expt. 4. (24-11-'36)			
water	185,7 (100%)	217,1	1,17
10% of glucose	98,4 (53,0%)	184,7	1,88

HUBER (1935). PALLADIN and KOMLEFF (1902) determined the carbon dioxide delivery by pieces of leaves of *Vicia Faba,* floating on sugar solutions of different concentrations. The respiration proved to be at its maximum at a concentration of 5%; at higher concentrations (up to 50%) the respiration was lower.

HOPKINS (1924) studied the influence of low temperatures upon the delivery of carbon dioxide by potatoes. He found, that at 0° C the respiration increased, then being greater than at 4,5° C. This increase continued till a maximum was reached, from this point the respiration decreased again. HOPKINS ascribes this decrease to the sugar concentration, which increases continually under the influence of the low temperature. This suggestion of HOPKINS, however, is opposed to by BARKER (1933). BARKER believes, that the curve, representing for the potatoe the relation between sugar concentration and respiration, has the shape of a rectangular hyperbola: the respiration first would rapidly increase at increasing concentrations of sugar, at higher concentrations, however, further increase in sugar concentrations would not affect the respiration any more. BARKER, however, finds a lower respiration than could be expected from the relation supposed by himself. He explains this by accepting a factor, that would accumulate within the cells and inhibit respiration. This factor would exert its influence mainly at higher temperatures.

MAIGE and NICOLAS (1910) found an increase in the respiration

of seedlings of *Vicia* and embryos of *Phaseolus* at increasing sugar concentrations till an optimum was reached. At higher concentrations a decrease set in.

MEYERHOF (1925) stated that the respiration rate of yeast was greater in solutions of 0.3 and 0.5% of glucose than in 10 × higher concentrations. This was confirmed by GEIGER—HUBER (1935), who also found a lower respiration at higher sugar concentrations (3%).

The reason of this inhibiting effect of higher sugar concentrations upon respiration is not known. It seems obvious to ascribe this phenomenon to the dehydratation by concentrated solutions. In the literature also data are found on the decrease of the respiration under the influence of high salt concentrations. KOSINSKY (1902) found that the respiration of *Aspergillus niger* was decreased for 13%, if the fungus was cultivated in a nutrient solution with 8% of NaCl. INMAN (1921) stated that the respiration of *Laminaria Agardhii* was reduced in concentrations higher as well as lower than that of sea water. These data, however, are too scanty to be conclusive; the reason of the glucose effect on the respiration must be left undefined.

The decrease in respiration in the experiments of table 22 was accompanied by an increase of the R.Q. That means that the root tips produce a quantity of carbon dioxide in excess to that due to respiration. It is already known from the investigations by PASTEUR (1872) and PFEFFER (1878, 1885) that, like in yeast, also in higher plants in certain cases the fermentation increases proportionately if the respiration decreases. In the absolute absence of oxygen this aerobic fermentation changes into an anaerobic one. Reversely the aerobic fermentation decreases again if the respiration increases.

The inhibition of fermentation was called „PASTEUR-reaction" by WARBURG (1926). Also in our case the increase of the R.Q. should be ascribed to an increase of the aerobic fermentation.

We therefore may conclude upon two effects of the addition of glucose to root tips: 1) the auxin content is increased, 2) the respiration is reduced while the aerobic fermentation is increased. The next question to be investigated is whether these two phenomena depend upon each other.

If a correlation can be found, this may be either: 1) the higher auxin content causes a lower respiration or 2) — reversely — the decrease in respiration causes a higher auxin content. The first possibility is not very probable. Investigations by VAN HULSSEN (1936) and BONNER (1936) have shown that auxin has no

effect upon the respiration of coleoptiles of *Avena*. There is no reason to postulate such an effect in the root tips of *Vicia*.

There are two procedures to investigate the second possibility. One can try to induce the same effect on respiration by an agent other than glucose and see afterwards, whether also in that case the auxin content is increased. It is also possible to proceed in the reverse way: by inducing an increase in the respiration the auxin content should be decreased. A few experiments on this subject are described in the next paragraphs.

§ 3. *The effect of KCN on respiration and auxin content of root tips.*

Especially WARBURG's work has focussed the attention upon the specific inhibition of the respiration by HCN. By this agent respiration is inhibited and fermentation is brought about. The relation between HCN concentration and inhibition of respiration recently has been thoroughly investigated by HOOGERHEIDE (1935). Working with yeast, he found that at increasing concentrations of cyanic acid the fermentation is increased up to an HCN concentration of $\frac{1}{30\ 000}$ ($= 51 \times 10^{-5}$ mol/1). At this concentration the respiration was decreased to about 22% of the original value. At still higher concentrations of HCN the respiration sank to an extreme low value, but also the fermentation was decreased. Up to concentrations of $\frac{1}{30\ 000}$ the HCN effect was absolutely specific.

The effect of HCN on the respiration of green plants has e.g. been investigated for *Chlorella*. WARBURG (1919) found that HCN has only little influence upon the respiration of this alga. EMERSON (1927), however, stated that this holds only partially: the respiration of autotrophically living *Chlorella* not being affected by HCN. When cultivated in a medium containing 1% of glucose, however, the respiration of the algae was much higher. If in the latter case HCN was added, the respiration was decreased and brought on the same level as that of *Chlorella* without the addition of glucose. GÉNÉVOIS (1928) found the same for etiolated seedlings of *Lathyrus*. Also in this case the respiration was increased by the addition of glucose and the increase could be suppressed by means of HCN. Always a residual respiration remained, however, which could not be checked by HCN. The decrease of the respiration by HCN in seedlings of *Lathyrus* was

accompanied likewise by an increase in fermentation. Also in this object the PASTEUR-reaction is present. It is, however, remarkable that the PASTEUR-reaction is inhibited by HCN in the experiments of GÉNÉVOIS. At certain HCN concentrations the fermentation was already increased, whilst the respiration was not yet affected.

Experiments. The cyanic acid was supplied by adding KCN to the twice destilled water in which the root tips were suspended. Table 23 shows the effect of the KCN on the respiration:

TABLE 23. The effect of KCN on the respiration of root tips.

	concentration of KCN	number of tips	$\frac{mm^3}{hours}$ O_2	$\frac{mm^3}{hours}$ CO_2	R.Q.
Expl. 1.					
(20-7-'36)	0	20	102,8 (100%)	—	—
	77×10^{-6} mol	20	89,2 (86,7%)	—	—
	31×10^{-5} mol	20	64,4 (62,1%)	—	—
	154×10^{-5} mol	20	40,6 (39,4%)	—	—
Expl. 2.					
(5-10-'36)	0	30	158,6 (100%)	261,2	1,65
	51×10^{-5} mol	30	91,6 (57,8%)	326,4	3,56
Expt. 3.					
(6-10-'36)	0	20	187,9 (100%)	206,6	1,10
	51×10^{-5} mol	20	134,3 (65,1%)	354,8	2,64

From table 23 it is clear that the respiration is decreased by KCN and the fermentation increased. The inhibition of the respiration by KCN being of the same order of magnitude as that caused by glucose (see table 22), the R.Q. is remarkably higher in KCN than in glucose. This points to a stronger increase of the aerobic fermentation in KCN at an equal inhibition of the respiration. One may explain this by the assumption that HCN inhibits the PASTEUR-reaction in *Vicia* as GÉNÉVOIS found in *Lathyrus*.

For the determination of the effect of HCN on the auxin content the root tips were ground after the end of the experiment and extracted with ether. The liquid from the WARBURG vessels was washed with ether. Table 24 gives the result of the experiments.

For each experiment is indicated in the table how long the root tips have been in the respiration vessels, that means how long they have been exposed to the action of the KCN. The results

TABLE 24. The effect of KCN on the respiration and the auxin content of root tips.

	concentration of KCN	number of tips	time in respiration vessel	$\frac{mm^3}{hours}$ O_2	auxin content
Expt. 1. (21-7-'36)	0	60	2 hours	249,8 (100%)	7,0° ± 0,7
	20×10^{-5} mol	60	2 „	181,7 (72,8%)	7,9° ± 0,6
Expt. 2. (23-7-'36)	0	60	2 hours	300,4 (100%)	4,8° ± 0,4
	30×10^{-5} mol	60	2 „	182,2 (60,0%)	5,5° ± 0,2
Expt. 3. (24-7-'36)	0	60	2½ hours	315,1 (100%)	6,5° ± 0,4
	25×10^{-5} mol	60	2½ „	224,1 (71,0%)	9,9° ± 0,6
Expt. 4. (30-7-'36)	0	30	¾ hours	168,6 (100%)	1,1° ± 0,4
	25×10^{-5} mol	30	¾ „	92,0 (55,0%)	3,0° ± 0,7
	0	30	2¼ „	153,8 (91,2%)	1,4° ± 0,6
	51×10^{-5} mol	30	2¼ „	89,2 (53,0%)	6,6° ± 0,9
Expt. 5. (3-8-'36)	0	30	2¾ hours	133,0 (100%)	1,1° ± 0,3
	25×10^{-5} mol	30	2¾ „	95,7 (72.0%)	2,4° ± 0,5
	51×10^{-5} mol	30	2¾ „	83,2 (62,5%)	4,0° ± 0,6
	102×10^{-5} mol	30	2¾ „	83,6 (62,8%)	4,6° ± 0,5
	205×10^{-5} mol	30	2¾ „	66,4 (50,0%)	3,3° ± 0,7
Expt. 6. (4-8-'36)	0	60	2¼ hours	291,6 (100%)	3,3° ± 0,3
	51×10^{-5} mol	60	2¼ „	154,4 (53,0%)	8,1° ± 0,8
	51×10^{-5} mol	30	2¼ „	76,4 (52,4%)	3,3° ± 0,5

of the experiments are rather irregular; they all agree in one point: the auxin content was never decreased by KCN. In the two experiments at the top of the table 24 there was no influence of KCN on the auxin content; the small differences being within the experimental error. In the other experiments, however, the auxin content of the tips in KCN was distinctly higher than that of normal tips. From this we may conclude that the auxin content of root tips can be increased under the influence of KCN.

§ 4. *The influence of increased oxygen concentrations on the respiration and the auxin content of root tips.*

The results of the experiments of the preceding paragraph point to an increase of the auxin content when the oxygen consumption is reduced. It was now to be investigated, whether an increase in respiration was also accompanied by a decrease in auxin content.

The increase in respiration was obtained by replacing the air in the WARBURG vessels by pure oxygen. In small organisms the respiration seems to be independent from the oxygen tension within wide limits. (e.g. KUBOWITZ und WARBURG 1929; SHOUP,

1930). In higher plants the relation between oxygen tension and respiration has also been investigated, as a rule however the respiration was estimated, only as carbon dioxide production. In this case this method is not applicable, since the carbon dioxide is produced partly by respiration, partly by aerobic fermentation. Since the latter increases if respiration decreases, the production of carbon dioxide is an inadequate measure. This method often yields complications.

MACK (1930) for instance found in seedlings of wheat a minimum in the production of carbon dioxide between 9,8% and 20% of oxygen, dependent upon the temperature. Also THOMAS and FIDLER (1933) found in young apples a minimum in carbon dioxide production between 3% and 5% of oxygen. At the same time they determined the alcohol formed in these apples. At low oxygen tensions the alcohol formation was rather high; it decreased when the oxygen tension increased. In young apples the alcohol formation in 3% of oxygen equalled that in air. In mature apples fermentation was higher and even in plain oxygen it did not quite disappear.

Especially these experiments clearly prove that the carbon dioxide production depends upon the intensities of respiration and fermentation both; this production is therefore inapplicable for the estimation of the respiration if the latter is subjected to considerable alterations.

Since normal root tips do contain only small amounts of auxin, it would become hard to estimate the latter, if the auxin content really decreased. For that reason tips were used, which were suspended in a 7% glucose solution; these tips have a higher auxin content than those in water. Table 21 shows that the oxygen consumption of root tips in 7% of glucose is about 30% lower than that of tips in water.

It was not a matter of course that the respiration of root tips in 7% of glucose could be increased by increasing the oxygen tension. Fortunately, however, this proved to be the case.

The increased oxygen tension was obtained by conducting for several minutes practically pure oxygen (99,5%) from a bomb through the vessels. Immediately after filling them with oxygen the manometers were placed in the thermostat and the estimation of the respiration started. Table 25 gives the results.

Firstly table 25 shows that respiration in pure oxygen has almost an intensity 2½ times as great as in air. From table 22 we may conclude that the oxygen consumption in water is about 143% of that in 7% of glucose. In pure oxygen an increase is

TABLE 25. Effect of increased oxygen tension on respiration and auxin content of root tips in 7% glucose solution.

	gas medium	number of tips	time in respiration vessel	$\frac{mm^3}{hours}$ O_2	$\frac{mm^3}{hours}$ CO_2	R.Q.	auxin content
Expt. 1. (11-8-'36)	air	30	1½ hours	101,0 (100%)	—	—	6,5° ± 0,4
	oxygen	30	1½ hours	245,7 (243%)	215,9	0,88	3,4° ± 0,4
Expt. 2. (13-8-'36)	air	40	2½ hours	156,3 (100%)	—	—	1,2° ± 0,9
	oxygen	40	2½ hours	359,3 (230%)	—	—	9,0° ± 0,9
	oxygen	40	2½ hours	389,3 (249%)	—	—	2,4° ± 0,4
Expt. 3. (14-8-'36)	air	30	2½ hours	109,9 (100%)	—	—	12,9° ± 0,7
	oxygen	30	2½ hours	268,8 (245%)	—	—	6,8° ± 0,5

TABLE 26. Effect of low oxygen tension on respiration and auxin content of root tips.

	gas medium	liquid medium	number of tips	time in respiration vessel	$\frac{mm^3}{hours}$ O_2	$\frac{mm^3}{hours}$ CO_2	R.Q.	auxin content
Expt. 1. (30-11-'36)	air	2% glucose	30	1¼ hours	194,5	283,1	1,46	—
	nitrogen	2% glucose	30	1¼ hours	21,9	311,3	14,22	—
Expt. 2. (17-8-'36)	air	water	30	1½ hours	122,7	—	—	3,0° ± 0,5
	nitrogen	water	30	1½ hours	5,8	—	—	3,9° ± 0,6
Expt. 3. (24-8-'36)	air	water	30	40 minutes	170,4	—	—	3,5° ± 0,6
	nitrogen	water	30	40 minutes	4,2	—	—	2,6° ± 0,4
Expt. 4. (25-8-'36)	air	water	40	45 minutes	—	—	—	5,0° ± 0,6
	nitrogen	water	40	45 minutes	—	—	—	5,3° ± 0,9
	air	water	40	2 hours	—	—	—	6,0° ± 1,0
	nitrogen	water	40	2 hours	—	—	—	5,1° ± 0,8
Expt. 5. (28-8-'36)	air	water	20	1½ hours	89,4	—	—	3,9° ± 0,6
	nitrogen	water	20	1½ hours	6,1	133,1	21,81	2,5° ± 0,5
Expt. 6. (27-8-'36)	air	3% glucose	28	1½ hours	124,5	—	—	5,6° ± 0,6
	nitrogen	3% glucose	28	1½ hours	10,1	211,7	29,60	2,0° ± 0,5

found up to 240%; that means that not only the inhibition of the respiration by glucose is balanced but even that the respiration is much higher than in normal conditions.

The last column of table 25 shows that the increased respiration is accompanied by a lower auxin content. Also this result indicates that the intensity of respiration affects the auxin content of the root tips.

§ 5. *The influence of low oxygen concentrations on the respiration and the auxin content of root tips.*

From the preceding paragraphs we know that the auxin content of the root tips decreases, when oxygen consumption increases and reversely, that the auxin content increases, when the oxygen consumption decreases. The decrease of the respiration was obtained by adding glucose or KCN. The inhibition obtained was at most ± 50%. The question rises, whether the auxin content will still increase if the respiration is still more inhibited. The substances used to inhibit the respiration, glucose and KCN, cannot be applied in higher concentrations, since these probably would interfere with other processes. HOOGERHEIDE (1935) showed that HCN inhibits at high concentrations as well the aerobic as the anaerobic fermentation. For that reason it was tried to obtain a greater decrease of the respiration by reducing the oxygen tension in the vessels, by replacing the air by nitrogen.

The nitrogen applied did not contain more than 0,1% of oxygen, according to the analysis by the factory. Since strictly anaerobic conditions were not strived at, these traces of oxygen were not absorbed; also the liquid in which the root tips were suspended was not freed from oxygen, so that the root tips still showed traces of respiration. Table 26 shows the results of these experiments.

Since the oxygen consumption is extremely low the „apparent respiratory quotient" is very high. The figures of the last column represent the auxin contents of the roots tips. These prove at these extremely low oxygen tensions to be about equal to those in air. In one case (exp. 6) the auxin content is even lower in low oxygen tension than in air. It seems, therefore, that the increase in auxin content does not occur if the respiration is reduced too much.

§ 6. *Discussion of the results.*

On p. 308 we discussed the drawback of the large diameter of the root tips. The question rises, how much this factor has

influenced the results obtained in the experiments described in this chapter.

The oxygen diffuses to the oxygen consuming cells through the surrounding tissue of the root. The track of the diffusion to the greater part of the cells proves to be so long, that the diffusion cannot keep pace with the want of oxygen of these cells. This follows from the increase of the respiration at increasing oxygen tensions. Only the cells in the most external layers of the root tip will receive more oxygen than wanted. One may therefore distinguish between two zones in the root tip: an internal one, where the oxygen tension acts as limiting factor on the respiration, and an external one, where this process is limited by other factors. These two zones may behave differently, when the respiration is changed.

An increase of the oxygen tension will only have an influence in the internal area, where the oxygen is limiting factor. Here the respiration of the cells will increase.

KCN will exert its action mostly and soonestly upon the cells of the periphery. By WARBURG's investigations (1928 a) has been shown, that the inhibition of the respiration by HCN exists in a blocking up of the haemin ferment, which activates the oxygen. At increasing HCN concentrations a continually increasing portion of haemin ferment will be blocked. Consequently at a certain HCN concentration the haemin ferment will become limiting factor for the respiration. This will happen firstly in those cells, where the intensity of the respiration is highest, that means in cells of the periphery. A much higher HCN concentration will be wanted to reduce the respiration in the central area, where it is limited by oxygen deficiency.

When the oxygen consumption of the root tip has been depressed by means of KCN to 50%, still oxygen and not the quantity of haemin ferment is limiting factor in the central tissue. This view is endorsed by the following experiment. Root tips were put into a 51×10^{-5} mol KCN solution. In this solution the respiration sank to 50% of that of root tips in water. The air in the vessels was replaced by pure oxygen and consequently the respiration increased with 44% again.

Thus we may conclude that the increase of the respiration at increasing oxygen tensions and the decrease after addition of KCN are partly located in different areas of the root tips. Although perhaps in these cases the reaction is located in cells of different tissues, the type of the responses indicates that the reactions have identical physiological characters: increase in

respiration results into a lower R.Q., decrease into a higher R.Q. On p. 312 it was already discussed that these changes of the R.Q. are caused by a shifting of the relation: $\frac{\text{respiration}}{\text{aerobic fermentation}}$.

By the investigations by KLUYVER and HOOGERHEIDE (1934, 1936) and HOOGERHEIDE (1935) a relation between metabolism and redox potential of the cell has been made probable. They found e.g. in *Saccharomyces cerevisiae* during the anaerobic fermentation always an Eh of about—43 millivolts. With increasing respiration also the redox potential rises in a definite way, so that a definite Eh belongs to each value of the quotient: $\frac{\text{respiration}}{\text{aerobic fermentation}}$.

Endorsed by these experiments we may assume that the alterations in respiration stated in the cells of the root tip are accompanied by changes in the redox potential. We learned that the auxin content decreased with increasing respiration and increased with decreasing respiration. This may be also formulated: the auxin content increases with sinking redox potential, it decreases with rising redox potential.

The suggestion, that the auxin content of a tissue would depend upon the redox potential has already been made by VAN OVERBEEK (1935). This investigator found that in the coleoptiles of the corn variety *nana* more auxin was inactivated than in coleoptiles of normal corn. Moreover the *nana*-coleoptiles further proved to be richer in catalase. If the catalase content of normal coleoptiles was increased artificially, the auxin inactivation increased also in these coleoptiles. This made VAN OVERBEEK conclude that the difference in auxin inactivation in normal and *nana* coleoptiles is to be due to a difference in „oxidation level (rH ?)".

A photochemical oxidation of auxin was stated by SKOOG (1935). He found that auxin solutions were inactivated by X-rays. Ordinary light proved to have the same effect, if a small quantity of eosin was added to the auxin solution. Since the presence of oxygen was wanted for the inactivation of auxin, it should be a photo-oxidation.

In our case we thus can explain the decrease of the auxin content at higher oxygen tensions by a rise of the redox potential in a number of cells, by which the oxidation of auxin is accelerated. The increase of the auxin content in the presence of KCN should, reversely, be ascribed to a sinking redox potential [1]).

[1]) It is remarkable that BONNER and THIMANN (1935) in *Avena* coleoptiles did not find any influence of HCN on the auxin content of the cells.

The mechanism of the changes in auxin content leaves room for different possibilities:

1. The oxidation of auxin can be thought to be a reversible process. In that case the oxidation product should be reduced to auxin at a sinking redox potential. No data are available in the literature on the reversibility of the auxin oxidation. Therefore this hypothesis is lacking any ground.

2. The production of auxin is greater at a low redox potential. This possibility is disproved by the resutls of the experiments of paragraph 5. Here the quotient $\frac{\text{respiration}}{\text{aerobic fermentation}}$ — and therefore also the redox potential at low oxygen pressure must have been much lower than in the experiments with KCN. It was, however, found that the auxin content did not markedly shift in comparison with that of root tips in air. That means a decline in comparison with the auxin content of tips in KCN, which cannot be ascribed to oxidation of the auxin. It seems probable that the process, by which auxin is produced, is checked if the redox potential becomes too low.

3. Finally the most probable and simpliest way to explain the increase in auxin content with decreasing respiration is the assumption that the oxidation of auxin is slackened at a reduced redox potential.

The aim of the experiments reported in this chapter was to elucidate the glucose effect. The result can be resumed as follows: a high glucose concentration reduced the respiration of the root tips. In the mean time the R.Q. increases. It seems justified to exegete this as a decrease of the redox potential. This reduces the oxidative inactivation of auxin, so that the auxin content of the root tip is increased.

CHAPTER V.

The influence of phosphate buffer solutions on the respiration and the production of auxin in the root tips.

§ 1. *The influence of pH on the curvature of the test plants.*

Auxin is an acid. According to DOLK and THIMANN (1931) salts of auxin do not cause curvatures in the *Avena* test. Therefore the pH of the agar blocks applied in this test never should exceed pH 7. BONNER (1934) showed that the auxin in the *Avena* coleoptile partly occurs as inactive salt. By soaking cylinders of coleoptiles in acid buffer solutions the auxin could be liberated

from its salt, by which liberation the growth was accelerated.

These facts are important in connection with the experiments described in the preceding chapter. It was stated that the auxin content of root tips increased, if the respiration decreased in favour of the fermentation. It is possible that in this shifting of the metabolism organic acids accumulate. In the applied method of extraction these acids perhaps might be extracted too and finally reach the agar blocks. In that case these agar blocks could obtain an extremely low pH [1]), by which the auxin could be liberated from its salt unilaterally in the coleoptile. The test plants then would show much stronger curvatures than would agree with the auxin content of the agar blocks applied.

It was investigated whether this possible source of error actually comes into the play. To this purpose root tips, which had stood on glucose agar, were extracted with ether. After evaporation of the ether the residu was not solved in 0,2 cm^3 of McILVAINE's buffer solution, but in the same quantity of destilled water. The pH of this solution was determined colorimetrically. This experiment was repeated for a number of times; the pH of the extract always proved to be higher than pH 4.

This shows, that if any organic acids may come into the agar block, their quantity must be extremely small. Moreover, the extract was not solved in water but in a buffer solution pH $\pm$ 5,4. There is, therefore, no reason to be afraid for an influence of organic acids on the magnitude of the curvatures of the test plants.

§ 2. *The influence of phosphate buffer solutions on the production of auxin and on the respiration of the root tips.*

Originally I intended to suspend the root tips in all experiments on respiration in buffer solutions. This proved, however, not to be applicable, since the phosphate buffer solutions used proved to affect the production of auxin in the root tips. This influence will be described below.

The method in measuring the respiration and the auxin was completely the same as in the experiments described above. The buffer solutions were prepared after KOLTHOFF (1923) from solutions of $\frac{1}{15}$ mol Na_2HPO_4 and KH_2PO_4. In experiments with glucose the latter was solved in the buffer solution. The pH was determined either colorimetrically with a HELLIGE comparator

[1]) In the case that the concentration of the acid would be so high, that it would overbalance the buffer capacity of the McILVAINE solution (see p. 287).

(table 27), or potentiometrically with the hydrogen electrode (tables 28 and 29).

TABLE 27. Effect of $\frac{1}{15}$ Mol. phosphate buffer on the respiration of root tips.

Expt. 1. (20-5-'36) 20 tips per vessel.		Expt. 2. (26-5-'36) 20 tips per vessel.	
liquid medium	$\frac{\text{m m}^3}{\text{hours}}$ O_2	liquid medium	$\frac{\text{m m}^3}{\text{hours}}$ O_2
water	107,0	water	81,8
buffer pH 5,5	96,7	water	84,0
buffer pH 5,5 + 7% glucose	75.4	buffer pH 5,5	90,0
buffer pH 7,8	98,7	buffer pH 5,5	87,4
buffer pH 7.8 + 7% glucose	72,9	buffer pH 5,5 + 7% glucose	65,8

Expt. 3. (27-5-'36) 20 tips per vessel.	
liquid medium	$\frac{\text{m m}^3}{\text{hours}}$ O_2
water	85.0
water	104,6
buffer pH 5,5	101,6
buffer pH 5,5	103,9
buffer pH 5,5 + 7% glucose	72,6

In table 27 the influence of a buffer solution of pH 5,5 on the respiration of root tips is represented. The respiration in water equals that in buffer solution. Glucose affects the respiration in the same way in buffer solution and in water; the consumption of oxygen is decreased by it.

Also the next table 28 shows that the influence of glucose on the respiration of root tips is not shifted by the presence of phosphate.

TABLE 28. Respiration and auxin content of root tips in phosphate buffer and in phosphate buffer and glucose.

	tips in	number of tips	pH	$\frac{\text{m m}^3}{\text{hours}}$ O_2	auxin content	R.Q.
Expt. 1. (15-6-'36)	buffer	60	5,42	321,4	10,0° ± 0,5	1,05
	buffer + 7% of glucose	60	5,42	247,8	13,5° ± 0,9	
Expt. 2. (8-6-'36)	buffer	60	5,50	255,9	8,4° ± 0,6	
	buffer + 7% of glucose	60	5,50	185,5	7,6° ± 0,8	

When considering the figures in the fifth column of table 28, however, one states that the auxin content of root tips in glucose + buffer solution does not differ from that of tips in buffer solutions alone. Here no effect of glucose on the auxin content can be stated!

In fact the auxin content of the root tips in table 28 is much higher than it uses to be of root tips in water. This indicates that the phosphate solution causes an increase of the auxin content of the same order of magnitude as glucose does.

For that reason the auxin contents of root tips in water and in phosphate solutions were compared; the results are given in table 29.

TABLE 29. Respiration and auxin content of root tips in water, phosphate buffer and 7% glucose.

	tips in	number of tips	pH	$\frac{mm^3}{hours} O_2$	auxin content
Expt. 1. (18-6-'36)	water	60	—	314,0	2,5° ± 0,4
	buffer	60	5,62	315,4	8,1° ± 0,7
Expt. 2. (1-9-'36)	water	30	—	136,7	1,9° ± 0,4
	buffer	30	7,91	135,8	8,0° ± 0,7
	buffer	30	6,87	154,0	7,5° ± 1,1
	buffer	30	5,63	142,8	5,9° ± 0,8
	7% glucose	30	—	114,2	6,4° ± 0,6

The auxin content of tips in phosphate solution proves indeed to be much higher than that of tips in water. The difference is of the same order of magnitude as that caused by glucose.

The described influence of a phosphate solution on the auxin content of the root tip is still completely obscure. It is possible that the buffer solution changes the pH in the root tips and that this change affects the auxin content. It is possible as well, that the influence of the phosphate solution has nothing to do with its buffering capacity. Perhaps these facts correlate with the influence of salts, reported earlier (VAN RAALTE, 1936). It was stated that the amount of auxin, delivered by root tips to agar was considerably larger, if the agar contained KCl or $BaCl_2$. According to THIMANN (1934) I used the term „osmotic action". This author namely supposed that the effect of glucose on the auxin production consisted in an „osmotic action": the auxin would be extracted from the tip into the agar by osmotic force. In a verbal discussion Prof. C. E. B. BREMEKAMP critisized this

explanation of the salt effect. He remarked that such an osmotic attraction was hard to visualize. I believe indeed, that for this phenomenon the term „osmotic" should preferably not be used, until more experimental evidence has been given to such an effect.

CHAPTER VI.

Summary and discussion of the results.

§ 1. *Summary of the results.*

1. The influence of different concentrations of glucose on the delivery of auxin by root tips has been determined (Chapter II, § 1 p. 288).

2. Glucose has no increasing effect on the delivery of auxin by the tip of the *Avena* coleoptile (Chapter II, § 2 p. 289).

3. Glucose has no increasing effect on the delivery of auxin by the cotyledons of *Raphanus* (Chapter II, § 3 p. 293).

4. The presence of auxin inactivating substances in ground root tips was proved (Chapter II, § 5 p. 295).

5. The effect of glucose on the delivery of auxin by the root tip is not due to reactions in the agar or in the boundary between agar and root tip (Chapter II, § 5 p. 296).

6. The effect of glucose on the delivery of auxin consists in an increase of the total amount of auxin, present in the root tip. In the root tip, therefore, auxin can be synthetisized from other substances (Chapter III, p. 303).

7. The oxygen consumption of root tips is smaller in 7% or 10% solutions of glucose than in water; the respiratory quotient and the auxin content is higher (Chapter IV, § 2 p. 309).

8. The decrease of the oxygen consumption of root tips by means of KCN is associated by an increase of the respiratory quotient and of the auxin content (Chapter IV, § 3 p. 313).

9. The oxygen consumption of root tips is increased, the auxin content decreased, by a high oxygen tension of the milieu (Chapter IV, § 4 p. 315).

10. If the oxygen tension is reduced very strongly (to 3—10% of that of normal air) the respiratory quotient strongly increases, the auxin content, however, remains about on the same level as in normal air (Chapter IV, § 5 p. 318).

11. In 1/15 mol phosphate solutions the oxygen consumption of root tips is the same as in water; the auxin content, however, is higher (Chapter V, § 2 p. 322).

§ 2. *Discussion of the results.*

The most important features of the results, summarized above, are:

1. The proof, that auxin can be synthetisized in the root tip of *Vicia* from other substances. The importance of this fact for our knowledge of the growth of the root and the literature on this subject have already been discussed in Chapter III (p. 299) to which may be referred here.

2. The proof of a correlation between the auxin content of the cells and their respiratory metabolism. What is the importance of the latter correlation for our knowledge of the growth process in the root?

In Chapter IV the higher auxin content, resulting from a reduced redox potential was explained as a consequence of a reduced oxidation of auxin. For this phenomenon, however, also a more indirect explanation can be found. One can suppose that the auxin content of the cells is determined by the rate of auxin production and by the rate of auxin consumption during the growth at the other hand (the inactivation of auxin is left out of consideration). When the rate of growth is reduced by some external factor and the production of auxin is not affected, or at least to a much smaller degree, the auxin content of the cells consequently will increase. In our experiments the rate of growth possibly may be reduced as a consequence of a reduced oxygen consumption or of a high salt (phosphate) concentration of the milieu. In these cases also the auxin content of the root tips increases. An exception must be made for the experiments in nitrogen. This can be explained by assuming that a too strongly reduced redox potential stops not only the auxin consumption, but also its production, so that the auxin of the cells does not shift any more. The reduction of the auxin content of root tips in pure oxygen can, according to this explanation, be ascribed to an increased rate of growth, by which the auxin consumption would increase.

All results, reported in Chapter IV and V, can be explained in this way. The whole explanation, however, is based on the hypothesis, that auxin is actually consumed in the growth process of the root cells. Our knowledge of the function of auxin in the root is still too scanty to assume such an hypothesis. Only during the last year data have been published, which point into the direction of an auxin consumption by the root. It may perhaps be useful to survey briefly the literature on this point.

KÖGL, HAAGEN SMIT and ERXLEBEN (1934) found that the growth

of roots of *Avena* was inhibited by pure auxin. NIELSEN (1930), BOYEN JENSEN (1933) and others found an inhibiting effect of hetero-auxin on the growth of roots. According to these results, the cells of roots and cells of stems react in an opposite way on these hormones.

CZAJA (1935) tried to explain this opposite reaction. He thought that his experiments allowed the conclusion that the growth of a cell is inhibited by a bipolar supply of auxin, accelerated by an unipolar supply. He tried to prove that in the root two opposite flows of auxin occur: one from the aerial parts traveling downwards and another from the root tip traveling upwards. These flows would meet in the growing region and inhibit the growth rate in this zone. In a decapitated root the latter flow would be suppressed, the cells would receive auxin only from one side and so the growth rate would be increased. As a proof for this conception CZAJA placed decapitated roots horizontal: they curved positively geotropically.

This theory of CZAJA, however, met with serious criticism. This criticism partly attacked his experiments in which inhibition of growth by opposite flows of auxin had to be proven (JOST and REISZ, 1936; LANE, 1936). At the other hand the results of CZAJA's experiments with decapitated roots could not be reproduced and proved to be inconclusive (FABER, 1936; THIMANN, 1936).

Much more evidence than CZAJA's theory has the conception, that the effect of auxin is dependent on its concentration. As early as 1925 JOST had already suggested this possibility to explain the opposite behaviour of stem and root. In the literature issued during the last year a number of data has been reported indeed, which indicate that low concentrations of (hetero-) auxin accelerate the rate of growth in roots. FABER (1936) and THIMANN (1936) derive from their experiments that hetero-auxin supplied to roots, which contain only little auxin, accelerates the growth, while the same external concentration would inhibit the growth of roots containing much auxin of their own. Also the experiments by JOST and REISZ (1936) weakly point to a growth accelerating effect in roots of low hetero-auxin concentrations. AMLONG (1936) supplied hetero-auxin unilaterally to roots of *Vicia*. A concentration of 10^{-9} mol caused a negative curvature, higher concentrations positive ones. The concentration of 10^{-9} mg therefore would accelerate the growth.

GEIGER—HUBER and BURLET (1936) cultivated under sterile conditions isolated excised roots and studied the effect of hetero-auxin on the growth. They stated that concentrations of $2.86.10^{-13}$

—$2.86.10^{-10}$ accelerate the growth, with the maximum acceleration at a concentration of $2.86.10^{-11}$. Hetero-auxin concentrations of $2.86.10^{-9}$—$2.86.10^{-5}$ inhibit the growth. The authors conclude from their experiments, that auxin is indispensable for the growth of the root. They further stated that the hetero-auxin is inactivated by the root, also in concentrations, which inhibit the growth.

From the cited literature we perhaps may conclude that auxin is indispensable for the growth of the root. The fact, that the concentration has to be extremely low for an acceleration of the growth rate, indicates that the quantity of auxin consumed by the growth process is very small. If this holds true, no considerable increase in the auxin content can be expected if the growth is checked. For this reason it seems more probable that the increase of the auxin content, reported in Chapter IV and V, should be ascribed to a decreased inactivation rather than to a decreased consumption, due to an inhibition of the growth. To discriminate between these two possibilities, however, still more should be revealed on the function of auxin in the growth process of the root.

This work has been carried out in the Botanical Institute of the State-University, Utrecht; it was started under the direction of the late Prof. Dr. F. A. F. C. WENT and continued under the direction of Prof. Dr. V. J. KONINGSBERGER.

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