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PROEFSCHRIFT

TER VERKRIJGING VAN DEN GRAAD VAN DOCTOR IN DE WIS- EN NATUURKUNDE AAN DE RIJKSUNIVERSITEIT TE UTRECHT, OP GEZAG VAN DEN WAARNEMENDEN RECTOR-MAGNIFICUS L. VAN VUUREN, HOOGLEERAAR IN DE FACULTEIT DER LETTEREN EN WIJSBEGEERTE, VOLGENS BESLUIT VAN DEN SENAAT DER UNIVERSITEIT TE VERDEDIGEN TEGEN DE BEDENKINGEN VAN DE FACULTEIT DER WIS- EN NATUURKUNDE OP MAANDAG 15 DECEMBER 1941, DES NAMIDDAGS TE 3 UUR

DOOR

WILLEM FREDERIK FLORUS OPPENOORTH Jr.
GEBOREN TE SOEKABOEMI

N.V. DRUKKERIJ v/h KOCH & KNUTTEL — GOUDA

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AAN MIJN VROUW

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ON THE ROLE OF AUXIN IN PHOTOTROPISM AND LIGHT-GROWTHREACTIONS OF AVENA-COLEOPTILES

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CHAPTER I.

INTRODUCTION AND STATEMENT OF THE PROBLEM.

§ 1. Introduction.

In 1909 BLAAUW, discovering — simultaneously with FRÖSCHEL (1908) — the stimulus quantity law, tried to explain phototropism as well as changes in phototonus in terms of photochemical reactions. Negative phototropism of normally positive phototropic organs, occurring after the exposure to high amounts of light energy, were compared to the solarization of the photographic plate. BLAAUW thus advocated a unity of the mechanism of both the positive and the negative reaction.

A few years later (1914, 1915, 1918) BLAAUW stated that all-round illumination causes light-growthreactions, their magnitude largely depending on the light quantities. Since at unilateral illumination both halves of the phototropic organ are irradiated with different amounts of light energy, this illumination would cause an unequal change in the growth rate of the light-(L) and of the dark-(D) side of the organ. Phototropism after unilateral illumination therefore would represent a special case of the light-growthreactions after all-round illumination, the latter being the primary phenomenon (1915, page 187): „Die Lichtwachstumsreaktion ist die primäre, der Phototropismus die secundäre Erscheinung, welche notwendig aus ihr erfolgt, wenn durch örtlich ungleiche Belichtung örtlich ungleiche Wachstumsreaktionen entstehen."

Meanwhile ARISZ (1914) had extended the validity of the stimulus quantity law for *Avena*-coleoptiles. Up to a light quantity of 100 M.C.S. the phototropic curvature proved to be proportional to the light energy. Further the stimulus quantity law proved to be valid for the lower threshold value of the negative phototropic curvature of the *Avena*-coleoptile too, provided that the light energy (ca 4000 M.S.C.) be administered within a certain time limit (20 minutes). Finally ARISZ made it probable that changes in phototonus are not due to changes in sensitivity of the organs, but to an interaction of different phototropic responses of the L- and D-side. The latter assumption urged ARISZ to advocate that the phototropic curvature is the primary reaction upon unilateral illumination, all-round illumination causing complicated secondary effects.

The apparently minor controverse between BLAAUW's and ARISZ's views in fact since has been the most disputed item in the problem of phototropism and of light-growthreactions.

In a number of accurate investigations on growth and light-growthreactions BLAAUW's theory has been carefully checked, the majority of them endorsing its correctness a.o. V. J. KONINGSBERGER (1922), BRAUNER (1922), VAN DILLEWIJN (1927).

Especially VAN DILLEWIJN (1927) gave a thorough analysis of the light-growthreactions. In coleoptiles of *Avena* he found:

1) a long lasting light-growthreaction after illumination of the tip, even with light energies below 25 M.C.S. (tip reaction). This growth-reaction attains its peak after 1—2 hours. At light quantities below 8000 M.C.S. a slowing down of the growth rate was found, for light quantities above 8000 M.C.S. an acceleration.

2) a short lasting light-growthreaction after illumination of the base and — at large light quantities — also after that of the tip (base reaction). This reaction always consists of a retardation of the growth rate, which reaches its maximum after ca 30 minutes. For the base reactions relatively high amounts of light energy (> 800 M.C.S.) are required.

3) a ,,dark" growthreaction after long lasting illumination. This reaction, being a weak reflection of the base reaction, consists of a short lasting acceleration of the growth rate.

From these three types of responses, respectively the tip-, the base- and the dark reactions, the different types of phototropic responses were derived. The so called first positive, the negative and the second positive curvature would result from the interaction between different competing light-growthreactions.

At the time, that BLAAUW's theory seemed so well corroborated, it was thoroughly shaken by the growth substance theory of CHOLODNY-WENT (1928). Since BOYSEN JENSEN (1910, 1911, 1913) ascribed phototropism to a phototropic stimulus substance, this view was held by a number of scientists (PURDY (1921), STARK & DRECHSEL (1922), SNOW (1924), BOYSEN JENSEN & NIELSEN (1926) and STARK (1927)).

PAÁL (1914, 1919) believed that this substance is always produced in the coleoptile tip as a growth promoting substance, that would unequally be distributed by unilateral illumination. The definite proof on the correctness of the latter conception of the substance has been brought by WENT (1928).

Trapping separately in agar blocks the auxin diffusing from L- and D-side of unilaterally illuminated coleoptile tips (1000 M.C.S.), he found much more auxin delivered by the D-side (57 %)

than by the L-side (27 %), the total of diffused auxin amounting to 84 % of that of the dark controls. Originally WENT attributed these results to:

1) a partial inactivation of the auxin by the light,

2) a „redistribution" of the auxin as a consequence of a lateral shift of the auxin transport to the D-side by the illumination.

Later investigations, especially those of VAN OVERBEEK (1933), brought further evidence of a transversal diversion of the auxin by the light. Since then this redistribution is the main item in the CHOLODNY-WENT theory on phototropism, as it had already been formulated by CHOLODNY (1928 p. 134): „Die Ursache dieser Erscheinung (the phototropic curvature) ist vielmehr darin zu suchen, dass die aus der Spitze diffundierende Wuchshormone sich zwischen den verschiedenen Seiten des wachsendes Organs ungleichmässig verteilen."

The mechanism of the lateral transport still completely remaining obscure, the eventual effect of light upon the permeability of the protoplasm, as earlier advocated by LEPESCHKIN (1909) and TRÖNDLE (1910), got a new interest. Especially BRAUNER (1922, 1924) tried to explain phototropism and light-growthreactions by light-induced changes of the permeability and also VAN DILLEWIJN (1927) gave some further evidence of the probability of such changes. If they really occur, they could offer a means to explain the lateral transport of auxin and also that of other substances.

According to the CHOLODNY-WENT theory phototropism cannot directly depend on light-growthreactions. The latter are induced by all-round illuminations, which, of course, cannot yield any transversal diversion of auxin. Therefore this theory is incompatible with that of BLAAUW. This hold true after the effort by VAN OVERBEEK (1933) to bridge the gap by assuming that phototropism would be caused by a lateral transport of auxin and light-growthreactions by a changed reactivity on auxin of the cell wall.

On the other hand WENT (1928) himself had found also a consistent decrease of the auxin quantities diffusing from coleoptile tips that had been illuminated (1000 M.C.S. from the top side) table 20, p. 92:

				dark controls:	amount	of	auxin		100%
first	half	hour	after	illumination:	,,	,,	,,		72%
second	,,	,,	,,	,, :	,,	,,	,,		88%
third	,,	,,	,,	,, :	,,	,,	,,		87%

This can only be explained by a partial inactivation of the auxin by the light.

Further VAN OVERBEEK (1936, a,b,c,) found a quite different be-

haviour against light in decapitated coleoptiles provided with auxin and with indole-3-acetic acid. When illuminated all-sidedly during the time wanted for the auxin curvatures, these curvatures with auxin proved to be much smaller than in the dark controls. With indole-3-acetic acid the difference was much smaller or almost zero. He explains these results by a destruction of the auxin by the light, whilst indole-3-acetic acid would almost not be affected by the light.

Meanwhile in further chemical investigations on the auto-inactivation of auxin-a KÖGL, C. KONINGSBERGER & ERXLEBEN (1936) stated the photo-inactivation of auxin-a-lactone by ultra-violet radiation.

C. KONINGSBERGER (1936) showed that the absorption bands, immediately found with solutions of auxin-a-lactone in the ultra-violet spectrum, are not due to the lactone itself, but to its ready conversion by the radiation into a physiologically inactive product, called lumi-auxin-a-lactone. In slightly acid solutions in vitro auxin-a is in equilibrium with its lactone. Accounting for the possibility that such an equilibrium would also occur within the living tissue, KÖGL c.s. (1936) wrote (p. 274): „Dieser Stoff (auxin-a-lactone) wird überraschenderweise auch durch B e s t r a h l u n g physiologisch inaktiv, eine Erscheinung, die vermutlich von grosser Wichtigkeit sein wird für die Deutung der phototropischen Krümmungen".

This interest preliminarily has been shown by V. J. KONINGSBERGER & VERKAAIK (1938). They used deseeded and decapitated coleoptiles of *Avena*, according to SKOOG's method (1937). Such coleoptiles practically are free from auxin and „show phototropic curvatures (base response) if auxin-a is supplied as growth substance, and not with indole-3-acetic acid". This base response (the 2nd type of light-growthreactions after VAN DILLEWIJN) was ascribed by the authors to the partial photo-inactivation of the auxin-a $\rightleftharpoons$ auxin-a-lactone system; in their experiments there was no evidence of any "redistribution" in the base of the coleoptile.

Since the auto-inactivation of auxin-a-lactone in vitro occurs only by ultra-violet radiation, the authors assume the presence of a sensibilizer in the growing cells of the coleoptile. Referring to the statement of WALD & DU BUY (1937) for *Avena* coleoptiles, and of BÜNNING (1937) for *Phycomyces* sporangiophores, that carotinoids occur in these phototropic organs, they linked up the auxin theory with BÜNNING's (1937) carotene theory of phototropism by assuming that carotinoids may act as sensibilizers in the inactivation of auxin-a-lactone by light of visible wave lengths.

This hypothesis gained considerably in probability by the investigations of SCHURINGA (1941), who stated that in vitro auxin-a-

lactone is readily inactivated indeed by light of visible wave lengths in the presence of α- and β-carotene and lycopin and turns into the inactive lumi-auxin-a-lactone. The maximum of the photo-inactivation in the presence of carotinoids shifts from the ultra-violet (ca 3300 Å) towards the maximum of the absorption band of the carotinoid; i.c. for β-carotene to about 4500 Å, that is exactly the peak of the light sensitivity of the *Avena* coleoptile.

§ 2. **Statement of the Problem.**

There was therefore more than one reason to investigate once more the role of the auxin in phototropism and in the light-growth-reactions.

The photo-inactivation of auxin-a by the visible light in the presence of carotinoids can lead to a decrease of the auxin content after illumination according to the reaction:

auxin-a ⇄ auxin-a-lactone
(active, photo-stable) (active, photo-labile)
↓ carotinoids + light
lumi-auxin-a-lactone
(inactive)

The possibility remains open that this process actually plays a part in phototropism (i.c. also in the tip reaction) and in the light-growth-reaction. In that case possibly the lateral shift of the auxin transport, as postulated by the CHOLODNY-WENT theory, has been shammed by an auxin-inactivation. If this proved to hold true, the explanation of photo-tropism and light-growthreactions would greatly be linked up again with BLAAUW's theory and this would match with a modified growth substance theory on phototropism.

First it had to be discriminated whether the inactivation of auxin-a-lactone actually plays a part in phototropism and in the light-growthreactions in general, apart from its probable effect in the s.c. base response.

On the other hand it might be possible, that the light also affects the synthesis of auxin in the tip. By using the diffusion method such a synthesis easily would be masked by a real or seeming lateral shift of the auxin transport.

Besides, the distribution of auxin after unilateral illumination had to be examined again. It was not to be excluded, that the earlier data on the auxin distribution at the L- and D-sides of the coleoptile had erroneously been interpreted. In the first place because the photo-inactivation had been ignored. Secondly, all data had been gained by means of the diffusion method. This however, does not

give a right estimation of the auxin content at a certain moment, since the auxin needs a relative long time to diffuse from the tip into the agar slice. During this diffusion time the production (respectively the inactivation) of auxin in the tip might be liable to considerable alterations. This objection *a fortiori* holds true for the efforts to explain still more complicated processes, e.g. the negative phototropic curvature (ASANA, 1938) by means of data obtained with the diffusion method.

The only means correctly to estimate the auxin content of L- and D-sides at arbitrary moments is offered by the use of the extraction method. The course of the auxin content only can be investigated by extracting successive sets of equally treated coleoptiles at successive time intervals after the exposure to light.

Since photochemical reactions in the sense of BLAAUW (1909) could be expected to occur in the coleoptile, in most experiments monochromatic light has been used to avoid possible complications due to some interaction of different processes.

It was hoped that the data collected in this way would enable to elucidate the controverse between the theory of BLAAUW on one hand and those of ARISZ and of CHOLODNY-WENT on the other hand. It will be discussed at the end of this paper how far this aim has been approached at.

The experiments, reported in this paper, were started in the early spring 1938. It soon turned out that each auxin determination should be repeated a number of times in order to get reliable mean values, while each experiment costed a lot of time. It therefore was clear that I had to restrict myself to a few selected light quantities, mainly of the most promising short wave lengths. Unfortunately, this work had to be interrupted for a long period by the mobilisation and the subsequent war. This forced me to abandon a part of my program. It is hoped to continue it in the near future.

CHAPTER II.

DISCUSSION OF THE LITERATURE.

According to the statement of our problem, we will only discuss that part of the extensive literature, which deals with questions related to the auxin theory of phototropism. The earlier literature on this subject has been surveyed several times, e.g. by VAN DILLEWIJN (1927), WENT (1928), du BUY & NUERNBERGK (1932, 1934, 1935). In the first two paragraphs the function of the coleoptile tip and

factors, which may be involved in a lateral shift of the auxin transport, will be discussed. These factors can only play a part in phototropism proper. The factors discussed in the other paragraphs are of interest for phototropism as well as for light-growthreactions. It may be mentioned once for all that in the latter case one should read for phototropism: phototropism a n d l i g h t-g r o w t h r e a c t i o n s. A few own experiments, in which it had been tried to elucidate discrepancies in the literature, are reported in this chapter too.

§ 1. **The function of the coleoptile tip in phototropism.**

Since long, especially since the classic work of ROTHERT (1894), it is known that the coleoptile tip is many times more sensitive to light than the lower zones. More recently this sensitivity has been studied by SIERP & SEYBOLD (1926) and by LANGE (1927). As light cannot act unless it be absorbed, it often has been tried to estimate the light absorption in the coleoptile. The gradient has been estimated by a number of scientists, but the values found differ very much and run from 10—50 at the L-side against 1 for the D-side. Curiously enough these estimations were done for the hollow sections of the coleoptile only and never for the solid, light sensitive tip. On the other hand the value of this absorption gradient does not seem so very important, since LUNDEGÅRDH (1922) did not find any difference in the course of the phototropic curvature in hollow coleoptiles and in those filled up with the primary leaf. BERGANN (1930) filled isolated coleoptiles with dye solutions (blue green, blue or black). He did not find differences in their phototropic response as compared to water filled coleoptiles.

The highest sensitivity is located in the very upper part of the solid tip. After LANGE (1927) this part is only a section of 50 μ (see his fig. 9). According to his fig. 10, were a longitudinal section of the coleoptile has been reproduced, this means that the highest phototropic sensitivity is confined to only three epidermic cells. Here the lightabsorption and therefore the gradient must be much smaller than in the lower zones.

Apart from this, the solid tip must be greatly responsible for a redistribution of auxin. The solid region being not more than 200—300 μ high, the polarity of the cells of this tip region must be changed by light to make possible a lateral shift of the auxin transport. It therefore is worth while to discuss the means by which such a shift might be initiated.

The most direct evidence for a lateral transport in the tip has been given by BOYSEN JENSEN (1928). He placed a piece of a cover glass in a longitudinally split tip of the coleoptile of *Avena*. Illumi-

nation perpendicular to the cover glass practically did not yield any curvature, while a normal curvature occurred after illumination in the plane of the glass plate.

§ 2. Photo-effects, which could cause a lateral transport of auxin.

Normally the transport of auxin is strictly polarized; it only moves from the tip to the base. VAN DER WEY (1932), who especially studied this transport, confirmed WENT's (1928) statement that it is a vital phenomenon. It cannot be a mere diffusion, since its rate is too high (ca. 8 mm/h) and it shows an optimum curve in its dependence on temperature. WENT (1932) tried to explain the moving agent in terms of a cataphoretic transport, but this idea has been rejected for different reasons (see HELLINGA 1937, THOMAS 1938). Vapour of ethylene causes an abnormal growth. BORGSTRÖM (1939) pretends that under the influence of ethylene the auxin would move transversally. In fact VAN DER LAAN (1934) found that geotropism in *Vicia Faba* was disturbed by ethylene vapour since the lateral transport of auxin was hampered. It is, however, not clear how this statement can be applied upon a vertically growing plant surrounded by ethylene vapour.

The change in the direction of transport must be preceded by a change in polarization. I therefore will briefly discuss those factors, which might initiate a change of the polarization under influence of unilateral illumination.

a. *Photo-effects on permeability.*

The data in the literature on the effect of light on the permeability are incomplete and contradictory. Even the results obtained with experiments on artificial membranes are far from uniform (cf. BECKING & GREGERSON (1924), CALABEK (1927), PINCUSSEN (1930), PYRKOSCH (1936), L. & M. BRAUNER (1937)). A number of authors, working with the most different biological objects, pleads in favour of a change of the permeability of the protoplasm by light (f.i. BLACKMAN & PAINE (1918), PACKARD (1925), BROOKS (1926), HOFFMANN (1927), LEPESCHKIN (1908, 1909 a and b, 1930), MEINDL (1934), HEILBRUNN & DAUGHERTI (1933), KAHO (1937)), but even their results are often contradictory. On the other hand such an effect is denied by many others.

Only a few investigations on this subject had a direct bearing on phototropism. Working with leaf-cells of *Tilia* and *Buxus*, TRÖNDLE (1910) stated a decrease of the permeability by illumination. With increasing light quantities, however, the permeability increased, to

decrease again at still higher light quantities. TRÖNDLE wrote in his theoretical considerations (p. 223): „Die Erklärung des Umschlagens von positiver in negativer Reaktion ist auch von BLAAUW (1909 p. 81) versucht worden durch die Annahme einer positiven und einer negativen Reaktion ebenso von der Zufuhr einer bestimmten Lichtmenge abhängt, wie der positiven. Ich sehe deshalb die Bedeutung meiner obigen theoretischen Ausführungen nicht darin, aufs neue auf das Vorhandensein zweier entgegengesetzter ungleich schnell verlaufender Erregungen hingewiesen zu haben, sondern ich wollte hauptsächlich zeigen, wie man in einem speziellen Fall die Wirkung der beiden Erregungen im einzeln verstehen kann". TRÖNDLE therefore, applying his results upon phototropism, believes that the decrease of the permeability (positive reaction) gradually reaches its maximum, which causes an increase of the permeability (negative reaction).

L. BRAUNER (1922, 1924), working with coleoptiles of *Avena* found changes in the permeability after illumination. The applied light quantities were large; 50.000 M.C.S. or permanent illumination with 100 M.C. His conclusion (p. 131, 1924) was: „Bei gleicher Lichtmenge verlauft die Kurve dieser Permeabilitätszunahme annäherend parallel mit der phototropische Krümmungsgeschwindigkeit. Dies liegt den Gedanken an einer Zusammenhang dieser beiden Reaktionen nahe". Unfortunately BRAUNER later abandoned this object to study the parenchyma-cells of *Daucus carota*. In light the permeability for sugars of the parenchyma-cells is decreased and that for water is increased (L. & M. BRAUNER, 1936). They explained this as follows: The electric charge of the surface layer of the plasm would be decreased by the light and so the swelling. By decreased swelling the permeability for water would be increased, but that for sugar decreased. In 1937 they gave the results of experiments with *Elodea*-leaves and models. The light would cause a double effect on the permeability:

1. a loss of potential of negatively charged membranes and consequently a decrease of the motility of the cations and an increase of that of the anions („primärer Photoeffekt").
2. a condensation of the disperse system, due to a decrease of the E.M.F., the pores becoming smaller, which does not hamper the cations but strongly inhibits the motility of the anions („sekundärer Photoeffekt").

They used vellum paper as model of the membrane. If this was coloured a potential difference was found after illumination. The strongest photo-electric effect was obtained, when it was blackened

with graphite. The intensity of the light on the membrane was 1000 Lux.

The scanty data on this subject, still interwoven with many hypothetical elements, are not conclusive. One must, however, account for the possibility that light affects the permeability of the protoplasm and that this effect decreases with increasing wave length.

Many investigators take such a change of permeability for changes of the electric charge of the protoplasm particles. If this potential changes, it is attended with a change in hydratation. A further effect of it is, that the viscosity of the protoplasm must change too.

b. *Photo-effects on protoplasmic streaming.*

Although there is no definite indication that transport of substances in the tissue is linked with protoplasmic streaming, many scientists believe that the transport largely depends on the latter process. In this connection it is important that BOTTELIER (1933) stated that the rate of protoplasmic streaming is decreased by illumination. Further he stated that the spectral sensitivity curves for phototropic curvature and for protoplasmic streaming reactions of oat coleoptiles are similar. In both cases, there is no reaction to wave lengths longer than about 5400 Å. Both curves show a maximum between 4800—4300 Å. The quantity of light needed for the first phototropic curvature (tip illumination) and for a marked decrease in protoplasmic streaming is about the same. The relation between the two phenomena still being obscure, the parallelism in their behaviour against light seems too great to be merely incidental. It may be possible that both effects primarily are due to some photochemical "master"-reaction.

In this connection it is of interest to note that THIMANN & SWEENEY (1937, 1938) found an acceleration by indole-3-acetic acid (in physiological concentrations $< 0{,}5$ mg/l) on the protoplasmic streaming. This effect, however, lasts only for a short time (30 minutes). In the presence of a suitable sugar, however, this effect was not transient, but is maintained for at least two hours (1938).

On the other hand CLARK (1938) brought some evidence that the longitudinal transport of auxin does not depend on the protoplasmic streaming. A 0,05 % solution of saponin stops the protoplasmic streaming in *Avena* without affecting the transport of indole-3-acetic acid, while sodium glycocholate, in non toxic concentrations, abolishes the transport of indole-3-acetic acid without affecting the rate of protoplasmic streaming, permeability, respiration, potential etc.

In the mean time, however, CLARK found that coleoptiles infiltrated with Na-glycocholate solutions show normal phototropism

and geotropism. He concludes therefore that the lateral transport of auxin depends on a mechanism, quite different from that of the longitudinal transport.

It therefore still is impossible to value the parallellism between the spectral photo-sensitivity of phototropism and of protoplasmic streaming.

c. *Photo-effects on viscosity of the protoplasm.*

In literature many data are given on the influence of light upon the viscosity of the protoplasm and of the cell wall, as well for zoological as for botanical objects. The results, however, can hardly be compared, as they greatly will depend on the method used for determining the viscosity. For our purpose it is of interest that GIBBS (1926) stated for *Spirogyra* that a short exposure to ultraviolet light induced a liquefaction and a longer exposure rather a stiffening of the protoplasm. Here again a typical analogy is found that the result of a short exposure has an effect opposite to that of a long one. According to STRUGGER (1934) the plasm of growing cells (stated with *Helianthus* hypocotyls) is of much higher viscosity than that of full-grown cells. Though this could not be affirmed by BORRISS (1937), it is possible that light has another, i.c. a stronger, effect upon young, still growing cells than upon older ones.

d. *Photo-effects on bio-potentials.*

In connection with our problem it is of interest briefly to survey the literature on the effect of P.D. on auxin and reversely. BRAUNER & BÜNNING (1930) claim that roots of *Vicia Faba* seedlings, placed in an electric field of 640 Volt/cm^2, grow towards the negative plate and the coleoptiles of *Avena* towards the positive plate; in the roots the deviation got clearly visible after one hour, in the coleoptile already after 20 minutes. This is explained by a lateral shift of the auxin. The positive plate induces a negative potential in the side of the plant facing it; the auxin molecules (anions) should consequently migrate by cataphoresis towards the other side. This would cause a growth acceleration in this side of the coleoptile, in the root a growth inhibition. After some time a recovery takes place; the curvature of the coleoptile decreases after two to three hours and entirely disappears after five hours. This would indicate a re-establishment of the potential balance in the plant, which, however, is hard to be understood.

RAMSHORN (1934) found a potential increase with decapitated *Avena*, when growth substance was applied (p. 750): „Die Potentialdifferenzen änderen sich entsprechend der Wuchsstoffzugabe" and

on p. 765: „Wachstumsvariationen durch Zugabe von Wuchsstoff rufen ebenfalls eine Aenderung der Potentialdifferenzen hervor. Die stärker wachsende Zone erweisst sich als electropositiv. Angelegte Potentialdifferenzen bedingen Wachstumsänderungen am Hypocotyl von *Helianthus*. Liegt der Pluspol an der Zuwachszone, erfolgt eine kurze Steigerung der Zuwachsgeschwindigkeit, liegt der Minuspol an ihr, vermindert sich die Zuwachsgeschwindigkeit".

A high potential is attended with an intensive respiration. Though RAMSHORN does not state it distinctly, he does apparently not believe that the P.D. is a primary effect, but might be caused by a changed permeability. Subsequently the migration of ions would be facilitated and P.D. changes could be brought about.

KOCH (1934) let a current pass (tension: 4,5V) through auxin agar and stated that after short time the auxin had gathered at the positive pole. According KOCH in the hypocotyl of *Helianthus* by a P. D. of 4,5 V the auxin was shifted to the positive pole. In this way he succeeded in preventing photo- and geotropic reactions of hypocotyls by an electric current (a tension of 1,5—2 V was sufficient). Is this cataphoresis or electro-osmosis? The latter possibility was excluded by KOCH, p. 218: „Dass der Wuchsstoff durch einen elektro-osmotisch bewirkten Wasserström zum positiven Pol befordert wird, ist ausgeschlossen. In Agar wenigstens, der selbst negativ geladen ist, müsste die Elektro-osmose des Wassers zum negativen Pol hin stattfinden".

POHL (1936) later carried out a nice experiment. Two glass tubes filled with oat meal and water and fitted with platinum electrodes, were connected in such a way that an electric current could pass. After applying a P.D. of 15 volts for 24 hours, the two halves were separated and the oat meal extracted apart. It now appeared that all auxin present in the meal had assembled in the anode half and in this way even a greater output was obtained than with the normal extraction method, without a preceding electric current (see table 1).

TABLE 1.

	curvature in degrees of the test plants	
concentration	1	1 : 1
normal extract	11,4	1,2
anode extract	32,2	12,6
cathode extract	0	0

The behaviour of auxin, resp. indole-3-acetic acid in agar gels against a P.D., however, seems to be different from that in living

tissues. CLARK (1937 a, b; 1938) in a large number of experiments stated, that the longitudinal transport of indole-3-acetic acid in the plant cannot be affected by a P.D. applied from outside. According to him there is no relation between direction of transport and potential gradient in the plant. The same was found for auxin by KÖGL & HAAGEN SMIT & VAN HULSSEN (1936), who did not succeed in influencing the transport in the plant by means of a P. D. By a P.D., however, the dislocation of the growth substance in agar-agar is influenced. The increased sensitivity against auxin (KÖGL 1933) cannot be ascribed to an increased transport within the plant, but only to an accumulation of the growth substance near the wound surface of the coleoptile. Since indole-3-acetic acid and auxin very easily migrate in an agar gel under the influence of a potential difference and this does not happen in the plant, experiments on models have no physiological interest.

Speaking generally, it seems that one should be very cautious in interpreting results obtained on bio-potentials. RAMSHORN (1937) found the highest p o s i t i v e potentials in the zones of the fastest growth; according to him the tip is often positive against the base. CLARK (1935, 1937 a and b, 1938) regularly states the reverse. In a number of thorough experiments the tip was negative against the base. Even in cylinders of coleoptiles the top end proved to be negative with regard to the base.

There are also a few data on the influence of a P.D. on growth. CHOLODNY & SANKEWITSCH (1937) measured the growth of intact coleoptiles of *Avena* when a current of 10^{-7}—10^{-6} Amp. passed from the base to the tip. This current induced a short growth acceleration, usually followed by a growth inhibition. A current from the tip to the base caused a growth inhibition, that still lasted for a long time after switching off the current.

As for the effect of light on bio-potentials, I may refer to GLASS (1933). He stated that apical and basal leaves of *Elodea* respond to illumination of the apex by a strong increase of the potential (+ 100 mV) of the apex with respect to that of the base. The decrease in the magnitudes of the E.M.F.'s along the leaf from apex to base was uniform. In the roots of onions after illumination no rise of the P.D. was found. GLASS supposed that the P.D. effect is not a direct photo-electrical effect, but that some action of the chloroplast is the primary effect. He thus advocates the much referred opinion that to get a photo-effect on the P.D. the cell should contain chlorophyll. It seems, however, probable that this effect is not confined to chlorophyll only; perhaps other pigments, f.i. carotinoids, too may account for it, for etiolated plants can it show too.

Finally we owe some data on the effect of illumination on the P.D. in the *Avena*-coleoptile to CLARK (1935). After an all-round exposure to 600.000 M.C.S. changes in the P.D. were found, which ran about parallel to those of the growth. The fluctuations in the growth rate, however, were about half an hour ahead to those in the P.D. Retardations of the growth corresponded with positivation, accelerations with negativation of the tip.

It seemed worth while to investigate the P.D. between L- and D-side after an unilateral illumination of my subject, the *Avena* coleoptile.

By the courteous help of Dr. J. B. THOMAS I was able to obtain some measurements of the potential differences in the *Avena*-coleoptile after illumination. I thank him sincerely for his help and for the use of the equipment (THOMAS 1939). Table 2 and fig. 1 represent the data of one of some similar experiments on the P.D. after an exposure to 500 M.C.S. (390 erg/cm²) with unfiltered mercury light. These experiments had to be stopped when I was mobilized and had to join the army.

TABLE 2 (see fig. 1)

After illumination with 500 M.C.S. (unfiltered mercury light) in the *Avena* coleoptile a P.D. arises; L. becoming positive against D. Readings each two minutes, P.D. in millivolts. (Experiment on 17-5-'39)

time	P.D.	time	P.D.	time	P.D.
0 min.	— 0,32		+27,36		+21,62
	+ 0,81		+26,49	90 min.	+20,57
	+ 0.20		+22.50		+20,60
	— 0,94	50 min.	+21,11		+20,05
	+ 0,07		+21,47		+21,61
10 min.	+ 1,18		+18,99		+22,49
	— 1,32		+18,95	100 min.	+23,27
	+ 0,25		+17,74		+22,14
	— 1,47	60 min.	+23,88		+21,93
	— 5,20		+21,28		+20,66
exposure →			+22,12		+20,79
20 min.	— 7,65		+22,64	110 min.	+21,74
	— 9,50		+23,66		+21,28
	— 1,42	70 min.	+23,79		+21,48
	+ 2,73		+23,60		+22,00
	+10,14		+24,60		+21,31
30 min.	+ 7,32		+25,62	120 min.	+21,39
	+18,97		+27,09		+22,37
	+23,16	80 min.	+26,69		+21,67
	+26,50		+27,98		+21,99
	+23,49		+21,37		+21,73
40 min.	+25,09		+20,84	130 min.	+23,58
	+25,58				+24,61

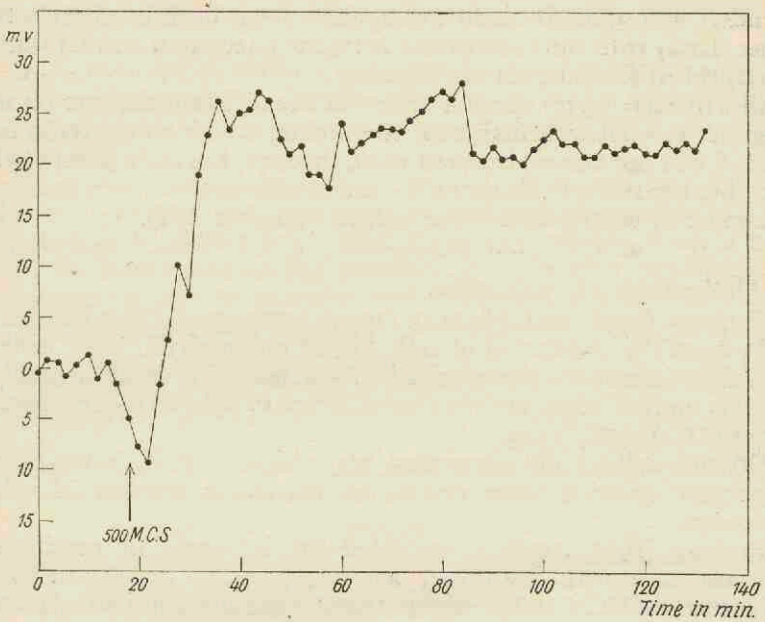

Fig. 1. After illumination with 500 M.C.S. (390 ergs/cm^2), white light, in the *Avena* coleoptile a P.D. arises. O = relative potential of the D- against the L-side; L becomes positive against D. Readings each 2 minutes.

The plants were cultivated in an usual air-conditioned dark room in the usual glassholders. The seedlings had the normal age of 90 hours. For the experiment they were placed with their glassholders in a small moist chamber with one glass side for the illumination. The opposite wall was of paraffin through which the electrodes were introduced. The glassholder was fixed in a paraffin block of a special shape which contained a vial of water, allowing the water-uptake by the roots during the experiment. The electrodes were long flexible gelatine-threads sticked on the epidermis without wounding it. This kind of electrodes allows the coleoptile to curve after the exposure. They were melted on the L- and D-side just below the tip. A third electrode was fixed at the basal region perpendiculary to the other two. This one was the zero; the measurements of L and D thus were done against the basal electrode, by substraction the difference between L and D could be found.

It appeared that L- and D-side had the same P.D. with respect to the base. Shortly after the exposure, however, the L-side became positive and maintained this level during about 100 minutes. Here the experiments were stopped. The coleoptiles in these experiments showed only a slight phototropic response; probably the gelatine electrodes still were too stiff to allow a normal curvature.

WALLER (1900), BOSE (1907) and BRAUNER (1927) did similar experiments but obtained opposite results. They probably used

an other light quantity than was applied in my experiments. On the other hand, with the controverse between RAMSHORN and CLARK in mind, this discrepancy is not amazing.

In THOMAS' (1939) thesis a review of the different theories on the origin of bio-potentials is given. Not feeling myself an expert in this field, I will not choose between these theories, but only point to the fact that the rising P.D. at the L- and D-side matches the transversal transport as supported by me lateron (Chapter VII).

e. *Photo-effects on respiration.*

GESSNER (1938) and FÖCKLER (1939) investigated the influence of light upon the respiration of cells free of chlorophyll. They believe that light stimulates the respiration. On the other hand a number of other authors could not state an influence of light on the respiration (see f.i. GAFFRON, 1939).

On this subject only few data are available; it is, however, an important question since respiration masters a number of other processes.

BONNER (1933, 1936) investigated the influence of respiratory poisons on growth. He found that transport and activity of auxin is blocked by HCN and phenylurethane. Pure auxin has no influence on respiration or on the respiratory quotient. With increasing age the growth rate decreases much more than the respiration. Because HCN and phenylurethane inhibits the respiration as well as the growth at the same rate, and since in the absence of oxygen no growth takes place, he concluded that the respiration was the essential condition for growth.

§ 3. Photo-Inactivation of growth substance.

SKOOG (1934) reported that in plants, treated with X-rays, the auxin content strongly decreased as compared to that of non-treated controls. The modified appearance of X-radiated plants was ascribed to an oxydative inactivation of the auxin. In 1935 the same inactivation could be stated with white light, when the plants previously had been stained with eosin. The *Avena*-coleoptiles grew normally in the dark after treatment with an 1 in 10^5 solution of eosin, but they had lost their phototropism.

The oxydative nature of the inactivation was derived from the fact, that also in vitro „auxin" proved to be inactivated by white light in presence of eosin. When, however, a water solution of growth substance was irradiated in an atmosphere of nitrogen, there was no inactivation, indicating that the reaction was an oxydation (p. 256).

His figures (summarized on p. 238, table 5) on this inactivation are convincing.

It is, however, doubtful which growth substance has been used by SKOOG. He speaks of "auxin obtained in highly active purified preparations from *Rhizopus suinus* and from urin" and of indole-3-acetic acid.

In order to study the eventual part of pH in this inactivation, I did some experiments with eosin myself with solutions of auxin-a, auxin-b and indole-3-acetic acid. Curiously enough, I never could find an inactivation in the presence of eosin after irradiating the solutions for 15—30 minutes with unfiltered mercury light (3300 ergs/cm²/sec) nor with blue light (λ = 4360 Å, 70 ergs/cm²/sec.).

The solutions were brought on pH = 4 by means of a 5 times diluted Mc. ILVAINE's standard buffer solution. Each solution was tested on 12 *Avena* test plants. For each test fresh solutions were prepared and irradiated. Eosin was added in a concentration 1 in 10^5. The auxin-b was obtained by extracting rice-bran. As a control blanc buffer solution-agar was tested too. The results are summarized in table 3.

TABLE 3.

	no growth substance				hetero-auxin 1 in 10^7				auxin-b of the same activity			
	dark		illum.		dark		illum.		dark		illum.	
	no eosin	with eosin	no eosin	with eosin	no eosin	with eosin	no eosin	with eosin	no eosin	with eosin	no eosin	with eosin
date												
13-2-'41	0	0	0	0	15,0	7,6	11,5	2,7				
14-2-'41	0	0	0	0	18,0	8,7	18,5	8,4				
17-2-'41					18,2	14,7	19,6	14,3	14,5	10,2	14,0	12,2
									18,7	13,8	17,6	14,7
18-2-'41	0	0	0	0	15,0	13,5	13,2	11,5	13,0	11,2	15,9	9,0
19-2-'41									13,3	9,0	12,7	7,7
Mean	0	0	0	0	16,6	14,1	16,4	12,9	12,9	9,2	12,9	9,2

Curvatures in degrees of the test plants. pH = 4, eosin concentration 1 in 10^5. Illumination during 15—30 minutes with unfiltered mercury light (3300 ergs/cm²/sec.). The activity of the auxin-b solution was the same as that of the solution containing hetero-auxin. See text.

The blanc buffer-agar did never give curvatures. On the other hand eosin strongly reduced the curvature, in the control dark solution as well as in the irradiated ones. There is, however, no question of any photo-inactivation of auxin-b or of indole-3-acetic acid in the presence of eosin. To avoid the

inhibition of the curvature by eosin, its concentration was reduced in the next set of experiments.

Accordingly the prescriptions by KÖGL, HAAGEN SMIT & ERXLEBEN (1933) auxin-a was prepared from urin and purified up to the extraction with petrol-ether. The auxin-a was taken up in a buffer solution of the desired pH and eosin (in controls water) was added. The eosin concentration finally was $\frac{1}{2}$ in 10^8; these solutions still were distinctly coloured. As a control physiologically aequivalent solutions of indole-3-acetic acid were prepared in a buffer solution of pH=4, with and without eosin. The concentration of the growth substances in these solutions was aequivalent to indole-3-acetic acid 1 in 10^4. In order to abolish the strong effect of pH of the agar blocks on the curvatures (see Chapter III § 5 b) all solutions were „neutralized" by diluting them 1000 times with a buffer solution of pH = 4 (that is about the optimal pH for the test) before bringing them in agar for the test. The illuminated preparations were irradiated with unfiltered mercury light (3300 ergs/cm²/sec) for half an hour.

The mean curvatures, obtained with the indole-3-acetic acid preparations, tested on 29 plants were:

not illuminated, without eosin	12,4°
not illuminated, with eosin	10,5°
illuminated, with eosin	13,0°

Table 4 gives the results obtained with auxin-a.

TABLE 4.

	pH = 4				pH = 6				pH = 8			
	dark		illum.		dark		illum.		dark		illum.	
	no eosin	with eosin	no eosin	with eosin	no eosin	with eosin	no eosin	with eosin	no eosin	with eosin	no eosin	with eosin
date												
27-2-'41	26,0	26,0	25,5	25,2	25,0	23,0	25,7	26,1	26,1	28,5	27,0	28,6
28-2-'41	8,4	17,0	4,7	16,6	8,0	14,0	7,0	14,0	13,6	12,0	5.0	2,2
1-3-'41	9,5	10,7	11,0	11,0	11,0	7,0	6,5	9,5	9,0	10,0	9,2	20,0
6-3-'41	20,0	17,0	22,0	11,7	18,0	13,0	16,0	15,0	9,0	9,0	5,5	15,0
Mean	16,0	17,7	16,6	16,1	15,5	14,2	13,8	16,1	14,4	14,9	11,7	16,4

Curvatures in degrees of the test plants. Auxin-a in solutions of pH = 4, = 6 and = 8 during the illumination brought to pH = 4 for the test. Eosin concentration during the illumination $\frac{1}{2}$ in 10^5, for the test brought to $\frac{1}{2}$ in 10^8. Illumination during 15—30 minutes with unfiltered mercury light (3300 ergs/cm²/sec.).

The inhibiting effect of eosin on the curvature has disappeared in this lower concentration. No indication of any photo-inactivation in the presence of eosin can be stated. On the contrary at pH = 6 and = 8 the curvatures are slightly higher after illumination with eosin than without. It might be that a slight inactivation occurred in the latter preparations.

From all this we may conclude that at none of the investigated pH eosin can act as a sensibilizer for the photo-inactivation of the growth substance as reported by SKOOG.

On the other hand I briefly surveyed in Chapter I the more recent work on the photo-inactivation of the auxin-a-lactone and its possible physiological interest. It is known from the work of C. KONINGSBERGER (1936) and SCHURINGA (1941) that in solutions auxin-a is in equilibrium with its lactone. At a low pH (about 4,5) a considerable amount (up to 60 %) of the auxin-a is present as auxin-a-lactone.

Apart from KONINGSBERGER & VERKAAIK (1938), whose work has already been discussed, also LARSEN (1939) gave evidence, that in the plant auxin-a is in equilibrium with its lactone. From etiolated pea seedlings he isolated two growth substances, an acid one and a neutral one ("Skototenin"). The latter amounts to about 30—50 % of the total amount of growth substance and would be inactivated by illumination. LARSEN himself mentioned the possibility that his "Skototenin" could be identical with the auxin-a-lactone.

Besides the photo-inactivation of the auxin-a-lactone fraction, there are still two other ways in which the auxin-a and the auxin-b can be inactivated: the so called auto-inactivation and the oxydative inactivation. The auto-inactivation only has been studied in vitro (KÖGL, C. KONINGSBERGER & ERXLEBEN, 1936) and then occurs slowly in the lapse of several weeks. It is unknown, whether this type of inactivation has any physiological interest. To an oxydative inactivation many difficulties of the extraction method are ascribed, although the chemical details of this process are still unknown. VAN OVERBEEK (1935) has given some evidence that such an inactivation may have a physiological meaning. Also VAN RAALTE (1937) found a relation between the auxin content of root tips and the state of the redox system. The possibility may not be excluded that the redox system of living tissues is affected by illumination. A shift of the redox potential towards a higher rH in that case might cause an oxydative inactivation of auxin too. This could happen in the presence of certain dyes, discussed in § 5.

Finally I must mention that in the literature several indications are given of changes of the plasticity of the cell wall after illumination with various wave lengths, the shorter ones of which being claimed to be the most active in this regard. For the moment, however, it cannot be discriminated whether these changes are caused directly by changes of the physico-chemical properties of the cell wall, or indirectly via a photo-inactivation of auxin-a-lactone or by both. Such changes probably would result in a changed reactivity of the cell wall on illumination. Since in my own experiments (Chapter IV

§ 2) nothing could be stated on such changes in reactivity in the range of the first positive and the first negative curvature, this question will not be discussed further. It remains possible, however, that such phenomena are implicite with the second positive curvature, which has not been studied by me.

§ 4. The spectral distribution of the light sensitivity.

In most of the older experiments on phototropism white light has been used. Since long it is known that positive and negative curvatures alternate. This so called phototonus has been most thoroughly studied by ARISZ (1914), who found, that with ever increasing light quantities the first positive curvature is replaced by the first negative one and this on its turn by the second positive curvature. DU BUY (1933) claims that there is also a range of light quantities, where second negative and third positive curvatures would occur. The second positive curvature of ARISZ would be identical with the third one of DU BUY. These results have all been obtained with white light. Since it is possible that the action of the different spectral regions in phototropism would be different, it seemed worth while to study these phenomena with monochromatic light.

With monochromatic light only threshold values (of the first positive curvature) have been investigated. BLAAUW (1909) has given the first curves of the distribution of the light sensitivity in the spectrum, in which the light quantities have been accounted for. He found a maximum of this sensitivity at $\lambda = 4670$ Å for *Avena* and of $\lambda = 4950$ Å for *Phycomyces*. This sensitivity slowly decreases towards the shorter wave lengths and much more steeply towards the longer wave lengths. KONINGSBERGER (1922) studied the light-growthreactions of *Avena* in monochromatic light and found the same distribution of the activity of spectral light on the growth, thus greatly endorsing BLAAUW's theory on phototropism. Later investigators, a.o. DU BUY (1933) repeated BLAAUW's experiments. DU BUY critisizes the purity of the monochromatic light of other investigators, but he found the maximum very close to that of BLAAUW; for *Avena* this was at $\lambda = 4600$ Å. According to DU BUY the sensitivity very steeply drops towards the longer wave lengths. With $\lambda = 5460$ Å he still could get phototropic curvatures, but with $\lambda = 5780$ Å this proved to be impossible.

As stated above, thus far only threshold values have been studied. Only in DU BUY's experiments there is an indication that $\lambda = 4360$ Å can induce negative curvatures. With other wave lengths, however, negative responses have not yet been obtained. This possibly might be due to the low intensity of the monochromatic light used, since

negative responses only occur, if the light quantity used is administered within a certain time. It is therefore still to discriminate whether negative curvatures can be induced by wave lengths other than $\lambda = 4360$ Å.

§ 5. Pigments as sensibilizers in phototropism.

BÜNNING (1937) and WALD & DU BUY (1936) already suggested that carotinoids play a part in phototropism. BÜNNING compared the spectral distribution of the phototropic sensitivity of *Phycomyces* to the absorption spectrum of the carotinoids extracted from the sporangiophores. From the striking parallelism between these two BÜNNING concluded upon an important part of carotinoids in the perception of light. He and WALD & DU BUY showed the presence of carotene and carotinoids in the etiolated *Avena* coleoptiles and discussed the resemblance of the absorption spectrum of these pigments and the spectral sensitivity of *Avena*. The later investigations referred to in Chapter I made it probable that carotinoids act indeed as sensibilizers of the photo-inactivation of auxin-a-lactone. Especially SCHURINGA's work (1941) made this probability almost to a certainty.

Since in my own experiments only tips of *Avena*-coleoptiles of 3 mm were extracted, I tried to ascertain the statement by WALD & DU BUY that carotinoids are also present in these tips. After the extraction of auxin with ether, in which perhaps the major part of the carotinoids will have been dissolved too, the tips were gathered in acetone and kept in the refrigerator until several thousands of tips were gathered. The yellow solution then was decanted and centrifuged. By the kindness of the staff [1]) of the Biophysical Research of the Rockefeller Foundation, Utrecht, the absorption spectrum of this solution was measured (fig. 2).

The strong absorption in the ultra-violet being due to other substances (probably proteïns), the maximum at $\lambda = 4300$ Å still indicates the presence of carotinoids in the tips extracted with ether.

It certainly would be worth while to check whether carotinoids are also present in phototropic roots and absent in non-phototropic organs.

Several authors succeeded in sensibilizing non-phototropic roots by treating them with dye solutions, so that they curved phototropically after this treatment. METZNER (1923) for instance found that roots, grown in a solution containing fluorescein, were sensitive to light and showed phototropism. The same was stated for wheat roots by BLUM & SCOTT (1933) in solutions of erythrosin 1 in 10^7. They concluded (p. 535) "the wave length of the light producing the phototropic bending corresponds to the absorption spectrum of the dye

[1] To the members of which I tender my best thanks.

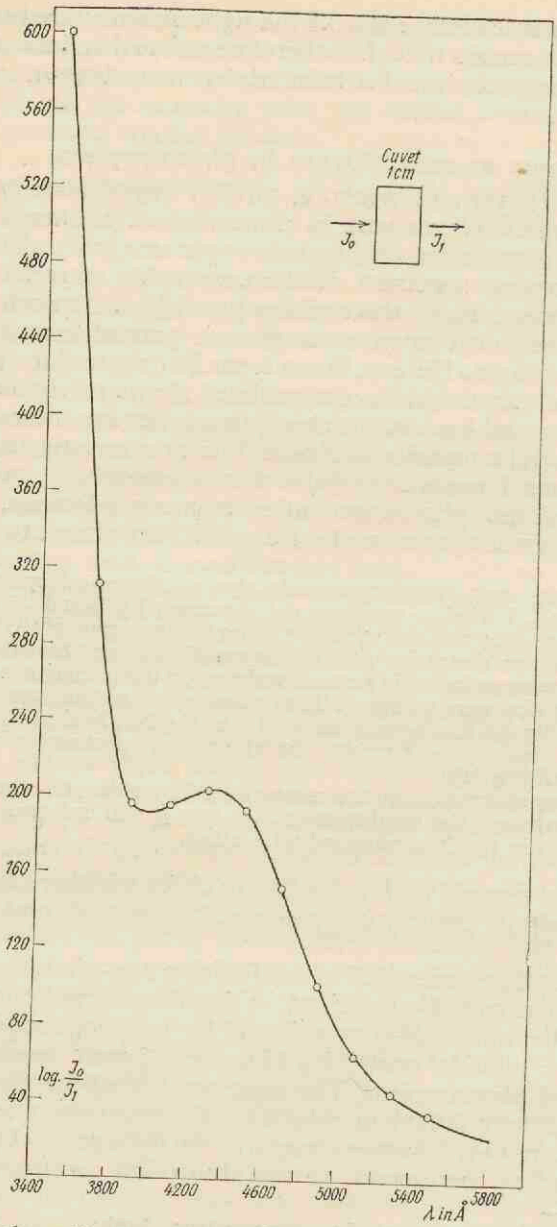

Fig. 2. The absorption spectrum of an acetone extract of 3 mm tips of *Avena* coleoptiles.

indicating that the dye acts as a photosensibilizer". Some scientists reported a detrimental effect of the dyes applied and did not get the same results. They observed an abnormal growth and a strongly decreased auxin content (BOAS & MERCKENSCHLAGER 1925, BOAS 1933, SCHWEIGHART 1935). It is likely that in these cases the dyes were applied in too high concentrations. In fact it would not be strange if further investigations would prove that phototropism of roots is attended with the presence of suitable photosensibilizing pigments.

In the above discussion special attention was paid to carotinoids. It remains possible, however, that also other pigments are involved in phototropism, not only in the photo-inactivation of the auxin-a-lactone fraction, but also in other processes. It will appear later that in phototropism also an increase of the auxin content plays a part. The nature of this increase still is unknown, but it seems likely that this process too is sensibilized by pigments, either by carotinoids or by other light absorbing substances.

Finally pigments possibly could play an indirect part in phototropism. At the end of § 3 I discussed the possibility of an oxydative inactivation of auxin by shifts of the redox system. Positivation of the redox potential (a raise of the rH) most probably would lead towards a decrease of the auxin content. Also in this process pigments might act as sensibilizers. In this connection I refer the conception of LAZAR (1935) and of BECK (1937) that carotinoids might act as oxygen vectors and to the statement by SYRE (1938) that erythrosin makes the redox system shift towards a higher rH. Such photochemical properties of pigments and dyes possibly largely depend on illumination and on light absorption. The present knowledge on bio-sensibilizers in physiological processes mastered by light still being very scanty, the urgency of further investigations in this field may be stressed.

CHAPTER III

MATERIAL AND METHODS.

§ 1. The plants used.

In all experiments coleoptiles of *Avena* were used. The plants used for phototropic reactions, as well as those from which the tips were taken for auxin extraction, always were of the same age as the test plants in the auxin test, that is 90 hours. They were grown in the

usual type of air conditioned dark room (relative humidity 96 %, temperature 23° C.) as described by NUERNBERGK & DU BUY (1930); where the experiments were taken too. Eventual illuminations took place in an adjacent room, equally conditioned, but with blackened walls and ceiling. By placing the racks almost parallel to the axis of the light beam, the light passed through the coleoptiles in the direction of the longer diameter of their elliptic cross section. Either immediately after the illumination, or after certain time intervals, the tips of the coleoptiles had to be removed for the extraction of auxin. Since the auxin content of the light- and shade-side had to be determined separately, the isolated tips had to be longitudinally split before being put into ether. This proved to be a delicate work, which demanded special precautions.

After cutting the about 10 mm long tips in the usual way with the decapitation scissors, the tips were only loosened a little, painstakingly taking care not to turn them along their longitudinal axis. As soon as a dozen of coleoptiles (one rack) was treated in this way, the tips were carefully removed from the primary leaf by means of tweezers and placed in an ebonite mould. This mould consists of two halfs; in each half twelve small furrows make a dozen of holes, when the halves are clasped together. In each hole fits exactly a coleoptile tip of 3 mm length. As soon as a dozen of tips is put into the holes in the correct orientation, they are cut to the same length of 3 mm with one stroke of a razor blade alongside the mould. Then the blade is pulled through the split between the two halves of the mould, so that the coleoptiles are split longitudinally as precisely as possible, in the plane of the short axis of their cross section. One set of the halves of the tips thus represent the light side (L in the tables and the graphs), the other those of the shade-side (D). The sets of the halves are picked up with tweezers and put into ether.

In order to control the reactivity of the test plants, with each test parallels ran with two to four different concentrations of indole-3-acetic acid [1]). By doing so, one can rule out the daily fluctuations in the reactivity of the test plants and the amounts of auxin in the coleoptile extracts can be expressed in aequivalents of indole-3-acetic acid.

The procedure of splitting and extracting illuminated tips was not applied until its reliability had been checked in a larger number of blank experiments with not-illuminated, "dark" coleoptile tips. Since it proved to be very difficult to obtain constant and reproducible results with the ether extraction of coleoptile tips, it seems

[1] The indole-3-acetic acid was obtained from FRAENKEL & LANDAU, Berlin.

useful to discuss this essential part of the technique a little more in detail.

§ 2. **The light used.**

For the experiments as a light source a high pressure mercury bulb from PHILIPS, Eindhoven, of the commercial "Philora" type (H.P. 300) was used, which — after a few minutes of preheating — gives a light of a fairly constant intensity and rich in blue, violet and ultra-violet. This bulb had been mounted vertically in a light-proof copper tube with a hole on one side; the desired light quantity was administrated by means of a photographic shutter.

At first unfiltered white light was applied (Chapter II). Lateron monochromatic light was used (Chapter III and IV).

To obtain monochromatic light of an intensity as high as possible a filter device was arranged. The main apparatus of the set is the "Dispersionsfilter" according to CHRISTIANSEN & WEIGERT, supplied by C. ZEISS, Jena. The transmitted wave length depends on the temperature of the filter, therefore it is placed in a thermostat, the temperature of which may vary 0,1° C. at most.

Fig. 3 shows the arrangement. L.S. is the H.P. 300 mounted in the copper tube. Its light, collected by lense L 1 (diameter 4 cm, focus 6 cm), has to pass diaphragm D 1. Lense L 2 (diameter 10 cm, focus 36 cm) makes a slightly diverging beam to obtain a correct dispersion by the filter (diameter 9,4 cm, thickness 5 cm). Lense L 3 (identical with L 2) collects the rays again, diaphragm D 2 (identical with D 1), connected with a photographic shutter, stands in its focus. Two large wooden compartments cut off all of the diffuse light. Behind D 2 a liquid filter (L.F.) is placed. By means of lense L 3 (diameter 7 cm, focus 36 cm) a parallel light beam is obtained. This had the advantage that, if wanted, several racks with plants could be placed behind each other, meanwhile taking care that the successive coleoptiles were not shaded by each other. In the parallel beam the distance between plants and light source does not affect the light intensity.

The lenses L 2, L 3 and L 4 are biconvex; the ratio of the radii of the two sides being 9 : 1. The most convex sides of L 2 and L 3 face each other.

Light, obtained in this way, proved to be fairly monochromatic [1]). Controlling it with a spectrograph only a weak trace of the neighbouring wave-lengths could be detected. The emission spectrum of

[1] In the arrangement and calibration of this equipment I had the unvaluable assistance of Dr. E. KATZ. I feel much indebted towards him for his courteous help and precious advices.

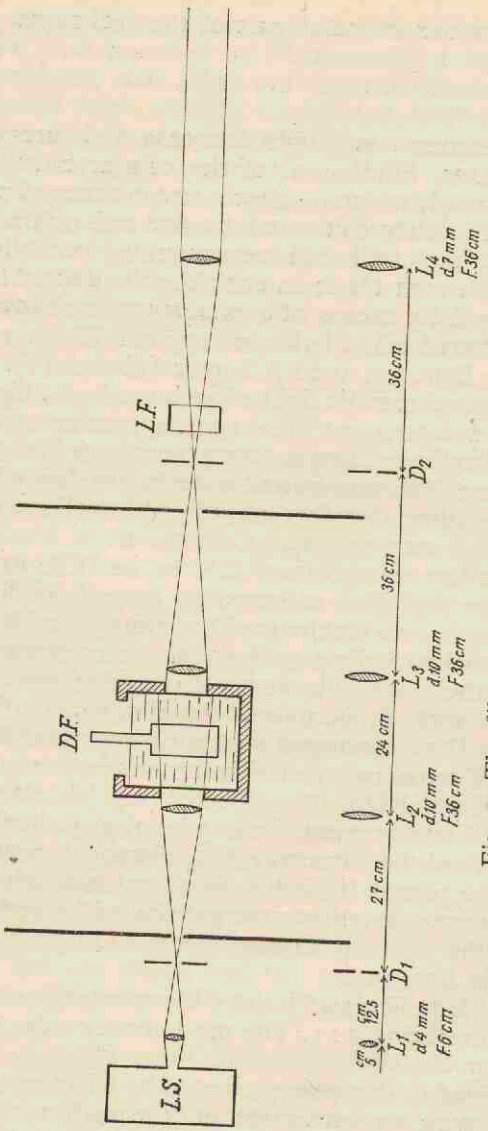

Fig. 3. The filter arrangement. Explanation see text.

the mercury bulb, being composed of lines or narrow bands, is especially adapted to this purpose.

With this equipment all wave-lengths could be obtained, if a

suitable light source is used. Intending to study the influence of $\lambda = 4360$ Å and of $\lambda = 5460$ Å, the mercury bulb was chosen because of its great intensity in these wave lengths.

In the first experiments with $\lambda = 4360$ Å it turned out that no negative response could be obtained. The intensity then was 22 erg/cm²/sec. Increasing the intensity up to 198 erg/cm²/sec. by changing the diaphragms of 1 mm by such of 10 mm and L 3 by a lense of shorter focus, negative reponses were obtained. These changes hardly did affect the purity of the light. Still in this case special filters were added to the equipment. To obtain:

$\lambda = 4360$ Å, a solution of Malachite green as a liquid filter was used; temperature of the thermostat was 46,3° C., and
$\lambda = 5460$ Å, an alcoholic solution of Victoria blue B; temperature of the thermostat 25,6° C.

The thickness of the filter was 5 cm. The filter solutions were prepared according to data from "The International Critical Tables" VII (1930).

The H.P. 300 was fed by the A.C. net of the town, the tension of which is not constant. In measuring the radiation intensity, it appeared that the fluctuations of the tension were rather small, and did not affect radiation for more than 5 %. This is, however, enough to affect the results if the exposure times are short, for instance 1/10 sec. For each extraction about 9 dozens of entire tips or 18 dozens of halves were needed. By exposing each dozen of plants apart the effect of these fluctuations greatly will neutralize each other. Further each point in the graph is the mean value of 8 or more extractions. Finally, the variability of the auxin content as found with the extraction method (see dark controls) is so great, that slight differences in the amounts of applied light energy cannot be of great importance. Therefore no further attemps were made to reduce this source of error.

§ 3. **The determination of the light intensity.**

The light intensity was measured with a thermopile after MOLL, constructed by KIPP & ZOON, Delft, or with a barrier-layer cell from TUNGSRAM.

The thermopile was chosen because of its non-selectivity in the different regions of the spectrum, but is not very sensitive. The photo-electric cell on the other hand is much more sensitive, but it has a selective spectral sensitivity. So the photoelectric cell had to be calibrated with the thermopile.

In the beginning in the filter arrangement diaphragms of 1 mm were used. The obtained monochromatic green $\lambda = 5460$ Å and blue $\lambda = 4360$ Å was too weak to measure it with the thermopile, therefore the photo-electric cell was used. It was necessary to re-calibrate the photo-electric cell after some time.

Lateron, when using diaphragms of 10 mm, the intensity allowed the use of the thermopile.

The electric currents, delivered by these instruments were estimated with a bifilar-galvanometer from C. ZEISS, Jena [1]). Its highest sensitivity amounts to $7{,}5 \times 10^{-9}$ Amp/scale unit. This means that with the photo-electric cell one scale unit corresponds to 0,216 erg/cm²/sec. at $\lambda = 4360$ Å and with the thermopile to 33 erg/cm²/sec. at the same wave-length. This accuracy matches with my purpose.

§ 4. The auxin extraction method.

In the beginning the prescription for auxin extraction, given by VAN RAALTE (1937) was followed. As a matter of precaution all manipulations for the extracting and evaporation of the ether were done in a dark room in the same orange light as used in the air conditioned rooms. Immediately before the extraction the ether was freed from peroxides by redistilling it over CaO and $FeSO_4$ (ether 400 cm³, $FeSO_4$ 10 gr, CaO 1 gr, H_2O 40 cm³).

The coleoptile tips were thoroughly ground with washed quartz sand under ether and a few drops of a 0,1 n H_2SO_4 solution. Then the ether was decanted and the residue washed twice more with ether. After this, the extract was shaken with slightly accidified distilled water to remove the acid. The ether fraction subsequently was evaporated to a volume of 0,5 cm³ and brought into a small test tube with 0,1 cm³ of a buffer solution of pH=4,5 and an agar slice. The rest of the ether was evaporated on a water bath by means of an air current.

Several authors (THIMANN 1934, VAN OVERBEEK 1936, WENT & THIMANN 1937) report an inactivation of auxin during the extraction by enzymatic processes; for that reason they are also afraid of peroxides in the ether.

Following the prescription mentioned above, it was first tried to find out how many tips, or halves of tips were needed to obtain measurable curvatures in the test. The results proved to be very uncertain and variable. Four different extracts from 24 coleoptile tips, made on the same day, gave the results of table 5.

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[1] I have to thank Dr. E. NUERNBERGK for leaving this instrument at my disposal and Mr. J. G. HAGEDOORN for calibrating of the thermopile.

TABLE 5.

Experiment on 22-5-'38	number of test plants	mean curvature in degrees
extract a	22	7,0
extract b	24	9,5
extract c	12	18,5
extract d	21	12,0
indole-3-acetic acid		
2,5 in 10^8	28	3,5
5 in 10^8	28	8,0
1 in 10^7	21	16

Sometimes the same variability was found when diluting the extract. Three different extracts of 84 coleoptile tips were tested in 3 different concentrations. Concentration 1 : 1 means that one agar slice, divided into 12 blocks and tested on 12 plants, contains the extract of 12 tips; concentration 5 : 1 contains 5 times as much, that is the extract of 60 tips etc. The results are represented in table 6; each experiment is done on a different day and consists of three parallels (a, b and c).

TABLE 6.

Curvatures in degrees of the test plants.

Exp. on	10-6-'38			15-6-'38			16-6-'38			mean
	a	b	c	a	b	c	a	b	c	
Concentration of the extract										
5 : 1	8,5	2,0	3,5	6,0	3,0	8,0	3,5	4,0	—	4,8
1 : 1	2,0	0,5	0,5	2,0	1,5	3,0	0	0,5	—	1,3
1 : 5	1,0	0	0	1,0	1,0	1,5	0	0,5	—	0,6
indole-3-acetic acid 2,5 in 10^8		3,0			6,0			3,5		4,1

In the mean values, however, there is a certain proportionality. It can easily be understood that the results obtained with split coleoptiles also were uncertain.

Since the halves of the not illuminated tips ought to have equal auxin contents, we will indicate them arbitrarily as F(ront) and B(ack). In each experiment 28 dozens of tips were extracted; table 7 gives the results.

TABLE 7.

Curvatures in degrees of the test plants.

Exp. on	17-6-'38		20-6-'38		21-6-'38		23-6-'38		27-6-'38		28-6-'38		mean	
	F	B	F	B	F	B	F	B	F	B	F			B
Concentration														
5 : 1	5,0	5,0	2,0	7,0	22,0	16,5	4,0	3,0	2,0	3,0	1,0	4,0	6,0	6,4
1 : 1	0,5	4,0	0	2,0	12,0	6,0	0	0	1,0	3,5	1,0	1,5	2,4	2,8
1 : 5	1,0	0	0	0	3,0	0	0	0	0	0	0	0	0,6	0
indole-3-acetic acid 2,5 in 10^8	6,0		4,0		4,5		7,0		4,0		7,0		5,5	

Since in spite of all fluctuations in the individual experiments, the means match reasonably well and in most cases the different concentrations show a fair proportionality, the source of the error had to be found in the operations preceding the final evaporation of the ether. It turned out, that the principal source of the variability lies in grinding the tips; here the most serious destruction of auxin was found. The auxin in the ether solution was relatively stable.

In many cases the amount of auxin, extracted from the coleoptiles, was extremely low too. Therefore a number of modifications of the extraction method, described in literature, were tested. The sulphuric acid was replaced by hydrochloric and by acetic acid; both gave worse results. Also the use of chloroform instead of ether, as practised by THIMANN (1934) and BOYSEN JENSEN (1936), with hydrochloric and acetic acid failed to improve the yield.

Alcohol 96 % and cold or hot water proved to be unsuitable media for extraction. The destruction of the auxin, caused by enzymatic processes, perhaps could be prevented by boiling the living tips; therefore hot water was used. (Table 8A).

Since the water, with which the sulphuric acid was washed away, contained some ether, which was therefore lost together with eventual auxin dissolved in it, it was tried to wash with a saturated solution of ammonium sulphate, but this meant no improvement either.

When VAN OVERBEEK (1938) reported that the acid could be omitted in the extraction of *Avena*, I had arrived at the same conclusion myself. Also the grinding of the tissue proved to be superfluous with coleoptile tips (table 8B).

With regard to indications in literature, it was tested whether auxin is inactivated by oxidation during the evaporation of the ether. To that purpose the evaporation was done on a hot waterbath by

TABLE 8.

A. Curvatures in degrees of the test plants.

Extraction with:	Relative concentration of the extract:	
	10	1
ether	17	8,0
alcohol	7,0	3,0
water	5,0	2,0

Comparison of the extraction with ether, alcohol and hot water (100° C.).

B. Curvatures in degrees of the test plants.

Experiment No.		Relative concentration of the extract:		
		50	10	1
109	with sand	9,5	6,0	0
	without sand	12,0	5,0	0
110	with sand	2,0	1,0	0
	without sand	4,0	1,0	0

Comparison of the extraction with and without grinding with sand.

means of a nitrogen current. The yield of auxin was only a little increased but it is a great advantage, that the agar slice with the auxin preparation can be kept much longer in a nitrogen atmosphere than in the air. The activity is preserved for at least 48 hours, which has a special advantage, when the test plants have been spoiled for some reason.

The possibility of enzymatic oxidation was considered too. To eliminate the eventually responsible enzymes, the tips were dipped into boiling water or the action of enzymes was checked with H_2S. The yield of auxin, however, did not increase. Extreme low temperature did not help either. The dry-ice extraction after DU BUY (1938) too gave largely varying results. Since, however, the evaporation of the ether is much less at a lower temperature, and also to inhibit eventual enzymatic effects, the ether further was cooled with ice during the treatment of the coleoptile tips in the dark room and the extracts were kept in the refrigerator.

Finally it was tried to omit the buffer solution and to soak the agar slice directly with the extract during the evaporation of the ether. To that purpose the 0,5 cm^3 of ether extract was brought in the small test tube with an agar slice only. It was hoped that, during

the evaporation of the ether with nitrogen, the auxin would enter the agar. This method in eight experiments gave the same results as with the buffer solution as intermedium, but it has at least the advantage that the material can be reduced to one third (table 9).

After all these modifications the variations in the individual experiments were not yet eliminated, but a fairly reliable mean value was obtained. In table 10 five experiments are resumed, in each of which two parallel sets of 60 coleoptile tips were extracted and tested in the concentration 5 : 3.

With this method also a number of blank experiments with split tips was taken. The tips were not illuminated and therefore the two sets of F- and B-halves should give the same results. In each experiment of table II 240 halves of tips (in the controls 120 entire tips) have been extracted and tested on 24 plants, the concentration thus being 5 : 1.

The means of four replications in table 11 fairly match each other. Since it proved to be impossible to get reliable figures from one experiment (mean of 24 test plants), it was decided to use statistical values and to take into account only the means of at least four replications of each experiment. This means a lot of more work, but it was absolutely necessary, since the kind of processes, which destroy the auxin or are responsible for the fluctuations, could not be detected.

The procedure of the extraction practised for the experiments, described in this paper, briefly can be resumed as follows.

The halves of the coleoptile tips, obtained in the way described above, were picked up with tweezers and put into 20 cm^3 ether in Erlemeyer flasks of 25 cm^3, cooled in a beaker with shredded ice. For each extract normally nine dozens of entire tips or 18 dozens of half tips were used; the volume of ether thus being relatively very large. Immediately before using it the ether was freed from peroxides by redistillation. As soon as the operations in the air conditioned dark room were finished, the bottles with ether were placed in a light-tight box and transported into the refrigerator, where they remained for at least five hours. Then the ether was decanted and evaporated to a volume of 0,5 cm^3. This volume was brought together with an agar slice into a small type of test tube. Then the rest of the ether was evaporated on a hot water-bath by means of a current of nitrogen. The tightly closed tubes with the agar slice in an atmosphere of nitrogen were left for one night in the refrigerator and the preparations were tested the next day.

TABLE 9.

Comparison between agar slices soaked in buffer solution (a and b) and not soaked (c). Concentration 5 : 3. Curvatures in degrees of the test plants.

	Experiment on									
	14-10-'38	27-10-'38	28-10-'38	1-11-38'	2-11-'38	3-11-'38	4-11-'38	7-11-'38	8-11-'38	9-11-'38	mean
a	5,5	4,5	5,0	4,0	6,0	5,5	5,5	3,0	9,0	3,0	5±0,4
b	4,0	4,0	5,0	6,5	11,5	7,0	7,0	3,0	11,0	3,0	6±1,0
c	—	—	4,0	13,5	4,0	10,0	6,0	3,0	4,0	2,0	6±1,4
indole-3-acetic acid 2,5 in 10^8	5,0	4,0	4,5	7,5	3,0	3,0	4,0	1,0	5,0	3,0	4±0,5

TABLE 10.

Curvatures in degrees of the test plants. The agar blocks were not soaked.

Concentration 5 : 3	Exp. on					
	14-11-'38	15-11-'38	16-11-'38	17-11-'38	18-11-'38	mean
parallel a	9,0	2,0	0,5	1,0	1,3	2,7±1,6
idem b	5,2	1,5	1,5	3,5	1,7	2,7±0,7
indole-3-acetic acid: 5 in 10^8	5,5	6,5	6,0	5,5	4,0	5,5±0,13
2,5 in 10^8	5,5	2,5	3,0	2,0	2,0	2,4±0,16

Two parallel sets of 60 coleoptile tips (not illuminated) were extracted and tested on 36 plants, the concentration thus being 5 : 3. This was repeated 5 times. The variation of the individual experiments were not yet eliminated, but a fairly reliable mean value was obtained.

TABLE 11.

Blank experiments with split tips. Curvatures in degrees of the test plants.

Concentration 5 : 1	Exp. on									
	22-11-'38		23-11-'38		24-11-'38		25-11-'38		mean	
split tips	F	B	F	B	F	B	F	B	F	B
	4,5	3,4	2,3	4,0	2,5	2,5	2,4	1,6	2,9±0,5	2,9±0,5
control entire tips	6,0		2,3		1,5		2,3		3,0±1,0	
indole-3-acetic acid 2,5 in 10^8	3,7		1,0		2,5		2,1		2,3±0,6	
5 in 10^8	8,0		2,2		4,3		4,2		4,7±1,2	
1 in 10^7	8,0		12,1		—		—		10,0	

Recently several times (FRÖSCHEL 1940, LINSER 1940, RUGE 1939, VOSS 1939) the presence of growth inhibiting substances in plant extracts has been mentioned. With my own extracts never positive curvatures were obtained in the *Avena* test. Since no indication of the interaction of such growth inhibitors was found in my experiments, they are left out of consideration.

§ 5. The analysis of agar blocks containing auxin.

a) *The test.*

For the estimation of the auxin content of the agar blocks the *Avena*-test of WENT (1928) in its later modification (VAN DER WEY 1931) was used. For the test the pure line of oats "Segrehafer" was used from Svalöv, kindly supplied by Prof. A. ÅKERMAN, director of the Experiment Station of the "Svensk Utsädes Förening". The coleoptiles were decapitated twice, with an interval of $1\frac{1}{2}$ hours. Immediately after the second decapitation the agar blocks were placed upon the stumps. These agar blocks were obtained by deviding up an agar slice of $8 \times 6 \times 0{,}9$ mm into 12 equal parts. In this paper an agar *slice* means a sheet 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.

Shadowgraphs of the test plants were taken 2 hours 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 average of 10—36 plants, the mean error being calculated from the formula:

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

From the first experiments it appeared that for each extraction a large number of tips was needed to get measurable curvatures in the test. Therefore a more sensitive test method would be desirable. The deseeded test according to SKOOG (1937) did not give reliable results. The sensitivity one time was much higher, another time the same as that of the standard test of WENT. Therefore the deseeded test was not used.

FUNKE (1939) propagated a new test. The coleoptiles were cut off near above the mesocotyl and then prepared in the usual way. This method did not give an important increase in the sensitivity. Therefore at last the old standard test was maintained.

Failing to find a better test method, I tried to increase the auxin content of the tips according to GORTER & FUNKE (1937). They found that *Raphanus* hypocotyls contained more auxin in a dry atmosphere (50 %) than when cultivated at a higher air humidity (100 %). Some experiments in this direction were taken. One part of the plants was cultivated as normally in the air-conditioned dark room but at an air humidity of 50 %, the other part in another room at an air-humidity of 96 %.

It appeared that there is some variation in auxin content, but the mean of these experiments almost yields the same result. The air humidity therefore practically has no influence on the auxin content of the tip of the *Avena* coleoptile.

b) *The pH of the agar blocks.*

It is a well known fact that the presence of electrolytes affects the auxin curvature. KÖGL & HAAGEN SMIT (1931) already used a solution of 160 mg/l KCl + 0,2 cm^3/l of glacial acetic acid to dilute their hetero-auxin standards. I found that the pH of the agar blocks too is very important. As already is shown in table 3 and 4, referring to experiments on inactivation, and in table 12, the concentration of indole-3-acetic acid 1 in 10^7 at pH = 4 induces a curvature of about 13°, while the same concentration at pH = 8 did not yield a curvature at all.

TABLE 12.

Influence of pH of the agar blocks in the test.
Curvatures in degrees of the test plants.

pH = 4				pH = 6				pH = 8			
H.A. in 10^8				H.A. in 10^8				H.A. in 10^8			
0	10	5	2,5	0	10	5	2,5	0	10	5	2,5
0	14,4	12,1		0	11,2	4,0		0	3,3	1,5	
0	12,0	7,0		0	6,3	0		0	0	0	
	17,0	7,0	4,3		9,2	0,5	0		1,1	0	0
mean:											
0	14,5	8,7	4,3	0	8,9	1,5	0	0	1,5	0	0

H.A. = hetero-auxin = indole-3-acetic acid.

Therefore the most suitable pH of the agar blocks was tested. These experiments were carried out with indole-3-acetic acid and with auxin-a, extracted from oats. Plotting the auxin curvature

against pH an optimum curve is obtained with a peak at pH = 4. So the agar slices, used in the test, were soaked in a buffer of pH= 4 during one night. The next day they were brought in the test tubes as described above.

CHAPTER IV

PRELIMINARY EXPERIMENTS.

§ 1. The first orientation.

Since I intended to study the eventual part of photo-inactivation of auxin in phototropism, it had first to be determined which other factors participate in this phenomenon and to what extent.

In view of the CHOLODNY-WENT theory the so called redistribution, that is a lateral shifting of the auxin towards the shade side, should be expected to be the most important factor. The evidence, given by WENT (1928) and VAN OVERBEEK (1933) for this redistribution, will be discussed first. It may be mentioned ahead, that in many cases it will be very difficult or even impossible to discriminate between a lateral transport of auxin and an increase of the synthesis of auxin at the shade side of the coleoptile. The latter possibility will be discussed later, but should be kept in mind and critically judged whenever accounting for the results of experiments on redistribution of auxin.

WENT first exposed his plants vertically from the top side and found an inactivation as read from his tables XX and XXI, reprinted in my table 13. He studied also the delivery of auxin after unilateral illumination in experiments, where the auxin from the light- and the shade side was trapped separately, by placing tips over a razor blade after exposure to light. He states that the amount of growth substance obtained from the shade side of illuminated coleoptiles is higher than it would be of halves of the dark controls. In his table XXII (my table 14) he only gives relative figures, but an inactivation of 16 % is detectable. In his table XXIII (my table 15) he does not give dark controls, but it is the most conclusive one in favour of a transversal transport, especially the figures of the second interval. I decided to repeat experiments of this type.

The most direct evidence, however, for a transversal transport has been given by VAN OVERBEEK (1933) in experiments with sections of hypocotyls of *Raphanus*, supplied with agar blocks containing growth substance and unilaterally illuminated. From his tables XII, XIII and XIV (my tables 16, 17 and 18) it can also be concluded that in

TABLE 13 (WENT's tables XX and XXI, 1928).

Delivery of auxin after illumination from the top side with 1000 M.C.S. (diffusion method).

	XX	XXI	1000 M.C.S. ↓
not illuminated (control)	100	100	
illuminated, first halve hour	72	82	
illuminated, second ,, ,,	88	92	
illuminated, third ,, ,,	87	94	

TABLE 14 (WENT's table XXII, 1928).

Delivery of auxin after unilateral illumination with 1000 M.C.S. (diffusion method).

not illum. control		100	1000 M.C.S. → 
illum. light side	27 (32 %)	84	
illum. shade side	57 (68 %)		

TABLE 15 (WENT's table XXIII, 1928).

Delivery of auxin after unilateral illumination with 100 M.C.S. (diffusion method).

tips on agar during:	Curvatures in degrees of the test plants			100 M.C.S. → 
	L	D	Total	
first 75 minutes	6,8 ± 0,4(42)	9,6 ± 0,8 (58)	16,4 (100)	
next 75 minutes	1,8 ± 0,8(12)	15,0 ± 1,0 (88)	16,8 (100)	

TABLE 16. (VAN OVERBEEK's table XII, 1933, modified)

L → 	illuminated one-sidedly		dark controls	
	light side	shade side	front	back
	1,0 ± 1 0,8 ± 1	4,3 ± 1,5 5,8 ± 1	5,1 ± 0,8	5,0 ± 1,5
mean	0,9	5,0	5,0	5,0
total transported	5,9		10	
difference in light and dark	4,1			

TABLE 17 (VAN OVERBEEK's table XIII, 1933, modified).
Curvatures in degrees of the test plants.

L →	illuminated		not illuminated	
	A	B	A	B
	53 (37 %)	88 (63 %)	20 (50 %)	19,8 (50 %)
mean per experiment	6,5	11,0	10	10
total transported	17,5		20	
difference in light and dark	2,5			

TABLE 18 (VAN OVERBEEK's table XIV, 1933, modified).
Curvatures in degrees of the test plants.

L →	illuminated		not illuminated	
	A	B	A	B
	31,7 (30 %)	75,8 (70 %)	67 (63 %)	40 (37 %)
mean per experiment	3,5	8,4	8,4	5,0
total transported	11,9		13,4	
difference in light and dark	1,5			

the light a part of the growth substance has been inactivated. In his table XII, where no auxin was supplied but the auxin from the cotyledon-petioles was collected, this inactivation is very pronounced. At that time a photo-inactivation of auxin in vitro had not yet been shown and so its possibility too was mistaken (DU BUY 1934).

Since in WENT's table XXIII no dark control is recorded as a standard, I have repeated experiments on this subject, but in a distinctly different way. Immediately after illumination with 500 M.C.S., the coleoptile tips were cut off and split; the halves, those of the light- and of the shade side apart, were placed on an agar slice.

Table 19 gives the means of 5 experiments; sets of 36 half tips, 3 mm long, were placed on one agar slice each. One and two hours after illumination the same sets of half tips were transferred to a new agar slice.

A redistribution in my experiments was prevented by splitting the tips directly after exposure, which fact explains the difference between WENT's figures and mine for the later periods. In my

TABLE 19.

Delivery of auxin after illumination with 500 M.C.S. (diffusion method). Immediately after exposure the tips are split and put on agar. Curvatures in degrees of the test plants.

half tips on agar during:	dark controls (split tips)	illuminated L	illuminated D	Total[1]	
1st hour	3,9(100%)	1,4 (36 %)	2,3 (59 %)	3,7 (42 %)	500 M.C.S. →
2nd hour	2,8(100%)	1,8 (64 %)	1,9 (68 %)	3,7 (66 %)	
3rd hour	2,2(100%)	1,2 (55 %)	1,4 (64 %)	2,6 (59 %)	

Mean of 5 experiments.

TABLE 20.

Illuminated tips were split and put directly on the test plants. Curvatures in degrees; those of the dark controls being 5,5°. Mean of three experiments.

	L	D	Total
immediately after illumination	5,0 (91%)	5,0 (91%)	10,0 (91%)
one hour after illumination	3,1 (56%)	7,2 (131%)	10,3 (94%)
two hours after illumination	3,2 (58%)	6,1 (111%)	9,3 (84%)

experiment a long lasting decrease of the auxin delivery both by the light- and by the shade halves is visible. Another experiment, however, in which the coleoptile tips were cut and split partly immediately after illumination and partly resp. one and two hours afterwards, pleads in favour of a transversal transport. In this experiment the split tips were directly placed unilaterally on the decapitated test plants (no diffusion into agar, table 20).

Although it is not possible in the last experiment to discriminate between transversal transport and changed synthesis of auxin in the split coleoptile tip, it seems well established that redistribution actually plays a certain part in the phototropic response after illumination with 500 M.C.S.

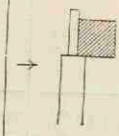
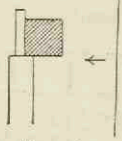
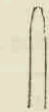
WILDEN (1939) yet applied another method. She placed the entire illuminated tip on the test plants and found 17 : 83 as the comparative concentrations of auxin of the light- and the shade side after 130—140 minutes (first phototropic curvature). Also in these experiments there were no dark controls as a standard.

Now it has been pointed out that no definite conclusion can be drawn from experiments by means of the diffusion method, via agar

1) Twice as many split tips as dark controls.

TABLE 21.

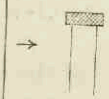
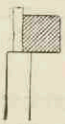
Illumination of the test plants with 500 M.C.S. just before putting on the auxin agar.
Curvatures in degrees of the test plants.

			
dark control	illuminated in the direction of the arrows		phototropic curvature
8,1°	7,0°	7,5°	6,9°	+ 11,0°

Mean of 5 experiments.

TABLE 22.

Illumination of the test plants with 500—960.000 M.C.S. just before putting on the auxin agar. The arrows indicate the direction of the light beam.
Curvatures in degrees of the test plants.

				
phototropic curvature	auxin-agar illuminated	blanc-agar illuminated control	auxin-agar dark control	normal test control
+ 9,4°	+ 0,7°	+ 0,5°	0°	8,8°

blocks or directly. The extraction method in this case should be applied.

Other possible factors, involved in phototropism, are a change of the reactivity of the illuminated tissue or a change in the rate of transport of auxin. In order to investigate whether these factors, which can hardly be separated from each other, did play a part in my experiments, the decapitated test plants were illuminated with 500 M.C.S. *before* the application of the auxin agar, in the way indicated in the head of table 21 by means of arrows. This experiment further was modified as indicated in table 22. When the light energy was raised from 500 M.C.S. to 960.000 M.C.S. no curvature could be obtained (table 22), neither with auxin agar nor with blank agar (as a control). From these data we may conclude, that after an

illumination with 500 M.C.S. no essential change of the reactivity of the tissue could be detected. DU BUY (1933) reported the same results.

The only factor therefore, that can be expected to play a part in my experiments by means of the auxin extraction method is, besides an eventual photo-inactivation, the lateral shifting of the auxin eventually attended with a change in its synthesis.

§ 2. The auxin cont ent of coleoptile tips after illumination with 500 M.C.S. (390 ergs/cm^2), unfiltered mercury light.

In this paragraph the auxin content of the tips of *Avena* coleoptiles after radiation with white mercury light will be reported. Since about the maximum of the first curvature is obtained with a light quantity of about 500 M.C.S., this amount of light energy has been applied for the first orientation. In all experiments, reported in this paragraph, the plants were irradiated with 500 M.C.S. (= 390 ergs/cm^2 for the mercury bulb). Each experiment is an extraction either immediately after illumination, or after 15 or 30 minutes, 1, 2, 3, 4, 5 or 6 hours after the illumination. It could not be avoided that decapitation, splitting of the coleoptile tips and putting the halves into the ether took about 5 minutes. In this way at 9 different moments after illumination determinations of the auxin content of the previous light- (L) and shade-side (D) were made. As a control and comparison in each experiment an equal number of plants, but not illuminated, were extracted; the curvature obtained with the extract of their split tips is used as a standard (100 %); in each experiment the values of the halves of the illuminated tips are calculated as per cent of those of the "dark" split tips. Further in each experiment control sets of test plants were treated with indole-3-acetic acid in different concentrations and finally at least one set of coleoptiles was used in each experiment to control the phototropic curvature. Several of the latter sets have been recorded photographically and the average course of the phototropic curvature is represented in fig. 4. These plants were not rotating on a clinostat; especially in the later hours the phototropic response will have been decreased by geotropic interaction; according to ARISZ (1915), on the clinostat the phototropic curvature would have increased for many hours. For each experiment (on one day) 50 racks with 12 coleoptiles each were needed. It appeared impossible to clinostat such a large number of water-grown plants, therefore never a clinostat was used.

The results of the extraction experiments have been summarized in table 23 and fig. 5.

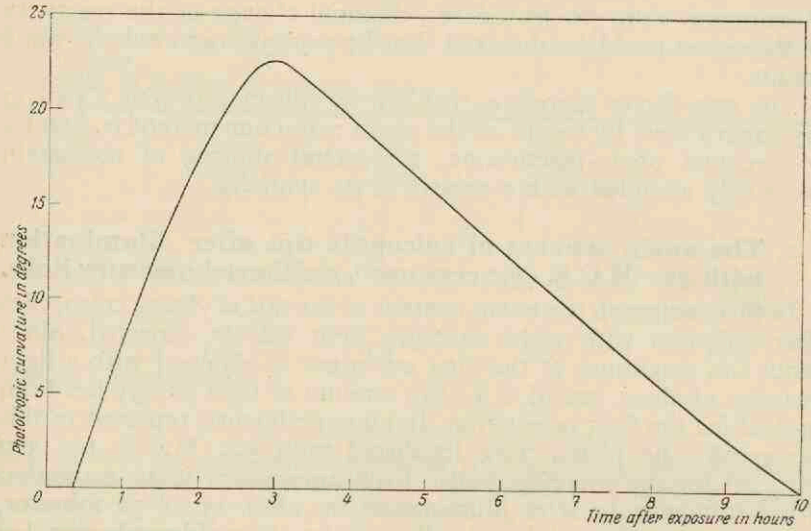


Fig. 4. The average course of the phototropic curvature after illumination with 500 M.C.S. (390 ergs/cm²), unfiltered mercury light.

TABLE 23.

Auxin content after:	Mean curvature in the test in degrees			Photo-tropic curv.	Relative auxin concentration		
	C	L	D		C	L	D
5 minutes	10,0	6,3	6,8	20	100	63	68
¼ hour	5,7	2,9	4,1	20	100	51	72
½ hour	5,5	2,5	6,1	20	100	45	111
1 hour	10,0	3,2	10,1	20	100	32	101
2 hours	10,0	3,7	11,3	22	100	37	113
3 hours	4,0	3,0	6,0	20	100	75	150
4 hours	5,3	5,9	4,9	18	100	111	87
5 hours	7,0	5,7	7,2	20	100	80	103
6 hours	6,0	5,0	5,4	20	100	83	90

The auxin content of illuminated coleoptile tips (3 mm) at different times after exposure to 500 M.C.S. white light (390 ergs/cm²), exposure time = 1 second.
The values are means of at least 4 replications.
As a control each day the phototropic curvature was determined too (2 hours after exposure).

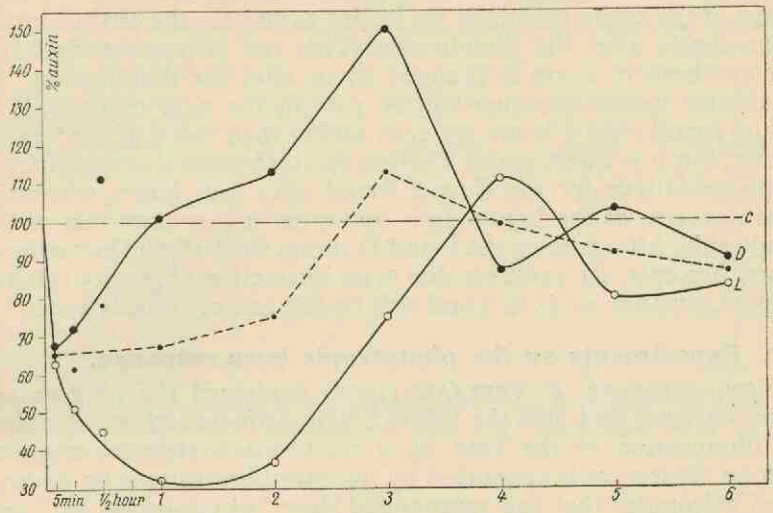


Fig. 5. The course of the auxin content of L- and D-side of the tips after an illumination with 500 M.C.S. (390 ergs/cm²), unfiltered mercury light.

The most striking result is that, immediately after the illumination (that is after about 5 minutes), the auxin content proves to be decreased for about 30 %, as well at the light as at the shade side. There is no doubt that this decrease must be due to a photo-inactivation of auxin, that is of auxin-a-lactone. It is evident, however, that this inactivation, occurring equally strong *at both sides* of the coleoptile tip with a light quantity of 500 M.C.S. cannot be related with any phototropic response, since it cannot induce a different growth rate at both sides. On the other hand this sudden decrease of the auxin content may be responsible for the so called "long" light-growth-reaction after all-round illumination. According to the ideas of WENT (1928), it seems demonstrated once more that phototropism is not merely depending on a light-growthreaction. CHOLODNY (1931) too has endorsed this idea.

A difference of the auxin content of the light- and shade side sets in later and reaches its maximum not until one or two hours have passed after the illumination. At that time the auxin content at the shade side begins to surpass that of the "dark" controls.

The long lasting decrease of the auxin content of the light side, coinciding with a gradual increase at the shade side, speaks in favour of a lateral transport. After two hours, however, the total amount of

auxin in the entire coleoptile tip begins to surpass the auxin content immediately after the illumination. This can only mean that also the synthesis of auxin is changed by or after the illumination. To this point special attention will be paid in the next chapters. The values found after 3 hours are even higher than those of the "dark" plants, but it is questionable whether this difference is consistent; the same holds true for the figures found after four hours, where the auxin content of the light-side is somewhat higher than that of the shade-side. After 3 hours the L and D curves show slight fluctuations; these, however, are probable due to an interaction of gravity (phototropic curvature $= \pm 22°$) and will be left out of consideration.

§ 3. Experiments on the phototropic base response.

KONINGSBERGER & VERKAAIK (1938) explained the phototropic base response and also the "short" light-growthreactions, induced by illumination of the base by a photo-inactivation of auxin-a-lactone. This view is supported by my own observations on decapitated coleoptiles that had regenerated their "physiological tip" and were illuminated with about 500 M.C.S. In these coleoptiles the phototropic curvatures were only weak and never did surpass 5° (see table 24, fig. 6).

According to KOCH (1934) a lateral transport seems hardly possible in the hollow base of the cylindrical coleoptile and chiefly must be governed in the extreme solid tip. This too pleads in favour of the conclusion of KONINGSBERGER & VERKAAIK. In this regard one may speak of a special phototropic function of the tip of *Avena* (see PAÁL, 1919; and BOYSEN JENSEN & NIELS NIELSEN, 1925).

I found another strong indication in this direction. It is known that when the primary leaf has peared through the coleoptile no longer a phototropic response can be obtained. By means of the extraction method however, it appeared to me that in the first time the auxin content of the tip has not yet decreased. We may therefore conclude that no phototropic curvature occurs while no lateral shifting of the auxin is possible. So the tip is not only the auxin producer.

KONINGSBERGER & VERKAAIK (1938 p. 12) concluded: "The base response in decapitated coleoptile tips is to be ascribed only to the partial photo-inactivation of the auxin-a; no evidence of a "redistribution" (lateral transport) of the growth substance in the base of the coleoptile, as postulated by the CHOLODNY-WENT theory, could be obtained".

At first sight the reader will feel an apparent controversy between my experiments on the auxin content of illuminated tips and those

TABLE 24.

Experiment on:	17-3-'39		18-3-'39	
Time between decapitation and illumination	number of test plants	curvature in degrees	number of test plants	curvature in degrees
2 hours	27	2,4	24	0,6
3 hours	28	3,4	23	1,9
4 hours	31	4,3	33	4,5
5 hours			34	4,0
6 hours			33	4,3
Control, not decapitated, normal phototropic curvature			21	15,0

The phototropic curvature of decapitated coleoptiles illuminated at different times after decapitation. Illumination with a 97 Watt lamp on 2 meters during 20 seconds.

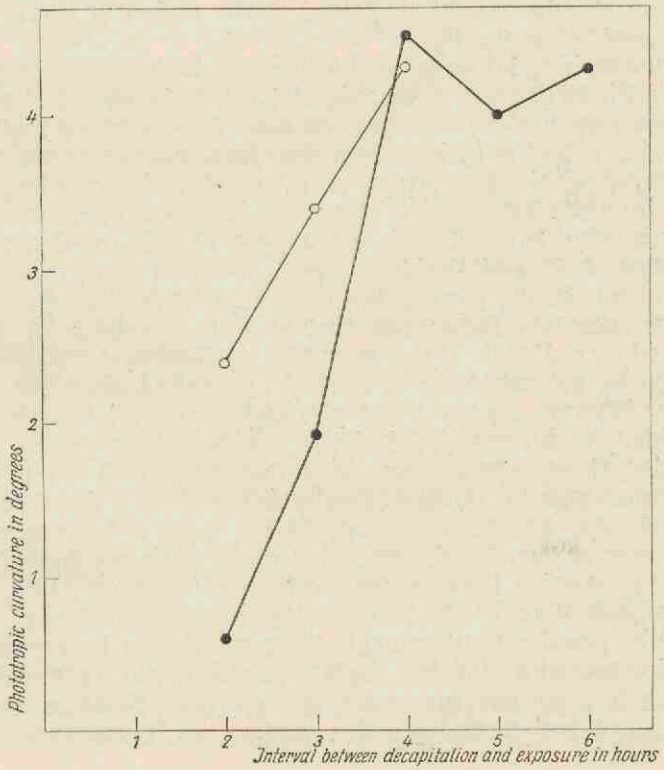


Fig. 6. The phototropic curvature of decapitated coleoptiles in relation to different regeneration times after an illumination with 500 M.C.S.

of KONINGSBERGER & VERKAAIK. In fact, however, there is no discrepancy, but our investigations are complementary to each other.

KONINGSBERGER & VERKAAIK studied the phototropic base response, caused by a long lasting illumination of 100 M.C.S., that is an amount of light energy far within the range of the second positive phototropic curvature, when the entire coleoptiles are illuminated.

I examined the auxin content of the coleoptile tips at different intervals after illumination with a light quantity causing about the maximum of the first positive phototropic curvature.

In both cases a certain photo-inactivation of auxin has been found, which can be responsible for the light-growthreactions. In the experiments of KONINGSBERGER & VERKAAIK auxin *present in the base* was inactivated, which may correspond with the "short" light-growthreaction of the base. In my case 30 % of *the auxin in the tip* was inactivated, which certainly also would have happened after an all-round illumination. This fact probably may be responsible for the "long" light-growthreaction, since it must take time for the auxin in the tip to be transported to the region of actual growth (5—7 mm below the tip).

Phototropism, however, in both cases should be interpreted in quite a different way. According to KONINGSBERGER & VERKAAIK, the base response can be explained entirely by a photo-inactivation of auxin. It had to be investigated whether the same holds true for the second positive phototropic curvature of the intact coleoptile too.

As for the first positive curvature (my own experiments) this explanation is impossible since with 500 M.C.S. the degree of inactivation at L- and D-side is equal. We have seen, that the first experiments strongly are in favour of a redistribution of auxin. But further there are indications that also the synthesis of auxin is changed by radiation. If this would be confirmed, it certainly would have to be accounted for in the light-growthreactions too. In this respect WENTS's fig. 3, 4 and 5 (1925) are very interesting. After illumination with 500 M.C.S. first a decrease of the growth rate was recorded, there upon an increase so that the growth rate grew even larger than that in the dark, finally followed by a decrease till the original value had been reached. This is quite in keeping with the auxin content curve of the entire coleoptile tip in my fig. 5 (p. 331).

The interaction between "redistribution" and a possible change in synthesis of auxin still is obscuring our problem. I hoped that it would be possible to disentangle these two factors by repeating the experiments with different light quantities, not only with such causing first positive curvatures, but also with those in the region of negative and second positive curvatures. It was thought to be

possible that light quantities, smaller or greater than 500 M.C.S., would have a different effect on the photo-inactivation and would also affect the redistribution and an eventual auxin synthesis in a different way.

CHAPTER V

EXPERIMENTS WITH MONOCHROMATIC LIGHT.

§ 1. Introduction.

Many indications are found in literature that the different wave lengths have a different effect in phototropism and on the growth processes. BLAAUW (1909) a.o. have analyzed the long known fact (MÜLLER, 1872) that the phototropic sensitivity is not equal for the different wave lengths. KONINGSBERGER (1922) showed that the wave lengths $< \lambda = 4800$ Å cause a long lasting reaction with a small energy quantity (1—5 ergs/cm^2), wave lengths $> \lambda = 4800$ Å cause short lasting reactions, for which much larger light quantities are needed. It seems possible that the difference in activity of the different wave lengths is not only quantitative but also qualitative. With white light in that case phototropism and light-growthreactions would be complicated by the quality of the light and the simplest reactions would occur on monochromatic illumination. For that reason I used monochromatic light, since this seemed to offer a possibility to discriminate between photo-inactivation, transversal transport and changed synthesis of auxin.

The monochromatic light, used in these experiments was obtained as described in Chapter III § 3.

The auxin from the illuminated coleoptile tips was extracted by means of the described method and estimated by means of the *Avena* test as in the preceding experiments. Each figure in the tables represents the mean curvature of 12—24 testplants on the same day. From these values the means of parallel series (different days) were calculated and the auxin content of the exposed tips was expressed in percent of the dark values. They were plotted in a curve, auxin percent against the time elapsed after exposure to light. Since each experiment takes a lot of time a limited number of curves could be obtained.

I tested the following wave lengths and light quantities:

1st. $\lambda = 4360$ Å; 330 ergs/cm^2; this quantity induced about a maximal first positive curvature of — in my case — 15°.

The light quantity, used in the experiments with white light (fig. 8) induces a maximal response too though the absolute light quantities slightly differ. The curves of the auxin content proved to differ much too.

2nd. $\lambda =$ 4360 Å; 3.000 ergs/cm²; this quantity induces a first negative curvature. When the intensity per time unit is large enough, negative curvatures with monochromatic light were obtained.

3rd. $\lambda =$ 5460 Å; 26.400 ergs/cm²; this quantity was the smallest one which with this wave length induced a positive curvature of 15°.

It was planned to compare the fate of auxin after an exposure with the light quantities of $\lambda =$ 4360 Å and $\lambda =$ 5460 Å which induced equal curvatures. To obtain this quantity of energy with $\lambda =$ 5460 Å an exposure of 20 minutes proved to be necessary. It is possible that this long lasting exposure complicates the result. With the highest intensity used a negative response could not be obtained with $\lambda =$ 5460 Å.

§ 2. **$\lambda =$ 4360 Å; E = 330 ergs/cm². Exposure time = 15 sec., phototropic curvature = + 15°.** (table 25, fig. 7).

This energy quantity induces the maximal response in the region of the first positive phototropic curvature. In this case one may expect a higher auxin content in the relatively fastergrowing D-side. This is shown indeed by the curves.

As after the exposure to white light (fig. 5, p. 331) here too the auxin content in the L- as well as in the D-side decreases with 30—40 %. The auxin content of the L-side continues to decrease during the first hour after the exposure, then it increases slowly again. In the D-side the auxin content starts to increase during the first hour, then it gradually falls off until 3 hours after the exposure the contents of L- and D-side are about equal, namely 75 % of that of non-radiated controls.

After the inactivation the auxin content of the L- side only slowly increases; after 3 hours this increase is not more than ± 10 % (of the content of the controls).

So the bending, induced by 330 ergs/cm², $\lambda =$ 4360 Å, probably must chiefly result from a transversal transport of auxin from the L- to the D-side in the (solid) coleoptile tip. The increase of the auxin content, which must be due to some change in the rate of auxin synthesis, is only slight, as the auxin content of the entire

exposed tip indicates (broken line in the figure; these values are obtained by halving the sum of L- and D-values).

The decrease of the auxin content of the total coleoptile, undoubtedly due to a photo-inactivation, can entirely account for the long lasting light-growthreaction (retardation) as registered by KONINGSBERGER (1922) for the same spectral region (4200—4400 Å and 4400—4600 Å) for much smaller light quantities (2 ergs/cm²).

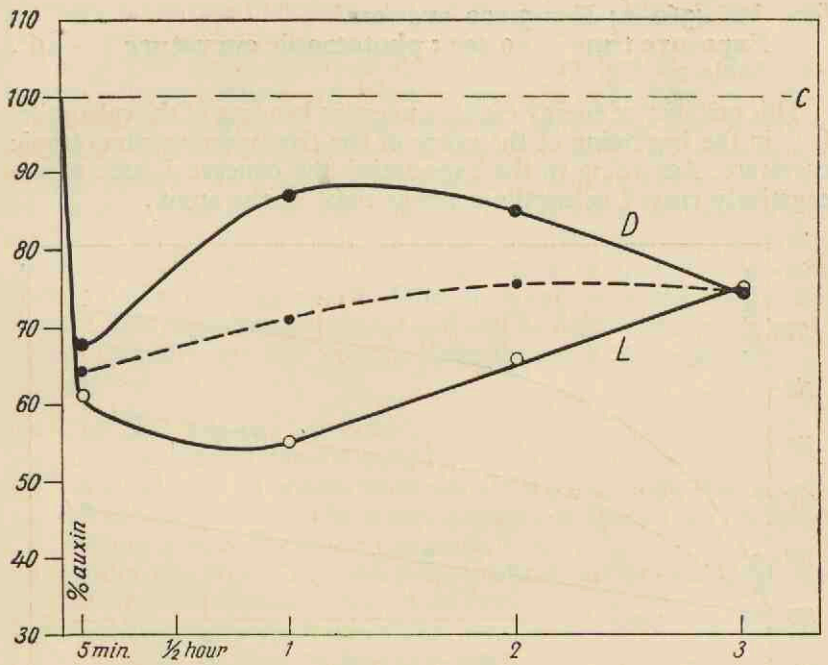


Fig. 7. The course of the auxin content of L- and D-side of the tips after an illumination with 330 ergs/cm², λ = 4360 A.

A comparison of fig. 5, obtained with an exposure to white light and fig. 7 teaches that the synthesis of auxin is not or almost not increased until 2 hours after the exposure. Then in the white light curve a consistent increase is shown, but equal in both sides of the coleoptile. We did not find this large increase in fig. 7, here at most a slight increase is visible. Three hours after exposure the auxin content of the controls is not yet attained.

Characteristic for the energy quantity 330 ergs/cm², λ = 4360 Å is:

1st. a maximal first positive curvature,
2nd. inactivation of auxin (30—40 %) both in L- and D-side,
3rd .this inactivation can account for the long lasting growth retardation after all-round illumination (KONINGSBERGER 1922),
4th. a probable transversal transport during the first hour after the exposure,
5th. a slightly increased auxin synthesis lateron.

§ 3. **λ = 4360 Å; E = 3.000 ergs/cm². Exposure time = 10 sec.; phototropic curvature = — 6°.** (table 26, fig. 8).

This quantity of energy causes a negative bending of the coleoptile. It is in the beginning of the range of the first negative phototropic curvature. According to the expectation the concave L-side of the negatively curved coleoptile contains most of the auxin.

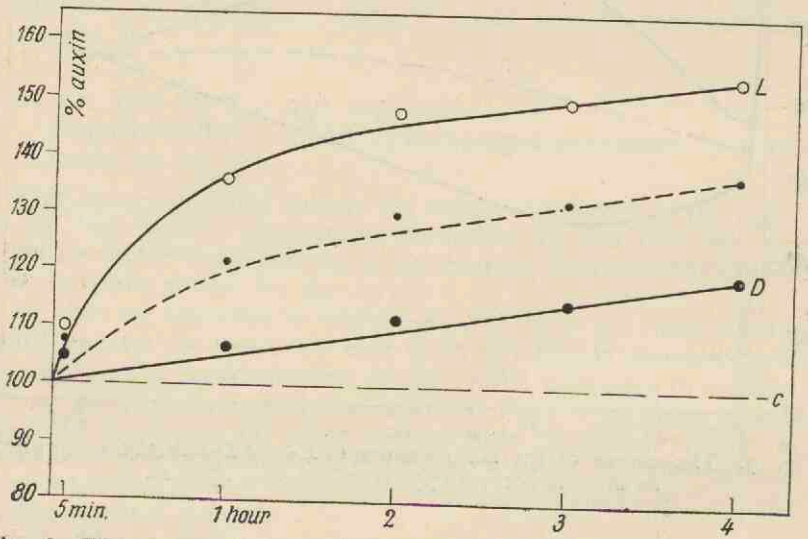


Fig. 8. The course of the auxin content of L- and D-side of the tip after an illumination with 3.000 ergs/cm², λ = 4360 A.

In fig. 8 it is shown that no inactivation could be stated. On the contrary, in fact the determinations immediately (5 min.) after exposure turned out on L = 110 %, D = 105 % of the controls. The increase of these values, being small and perhaps not real, is neglected when drawing the curves. The increase of the auxin

content in the D-side shows a linear course, in contrast to that of the L-side, where the increase is the largest during the first hour after the exposure, then it follows a same linear course as in the D-side.

The course of the mean auxin content of the entire t/p becomes linear after the first hour. Up to the end of the fourth hour the increase proceeds steadily and is still important. Experiments during a longer time fail (owing to gravity), so we cannot say whether and when the auxin content of the tip grows normal again. The after effect of 3.000 ergs therefore is much greater than that of 330 ergs (of the same wave length).

The degree of photo-inactivation of the auxin-a $\rightleftarrows$ auxin-a-lactone system certainly will depend upon the light quantity, but it is still unknown how. It seems, however, impossible from a photochemical point of view, that it would not occur within a certain range of light energy. We therefore must assume, that also here a part of the auxin is inactivated, but that this inactivation immediately is overbalanced by an increase of the auxin content. This increase cannot be but a synthesis either a production or an activation or a liberation of auxin. The nature of this phenomenon is still completely obscure. It is, however, consistent and will be discussed later (p. 347).

Characteristic for the energy quantity of 3.000 ergs/cm² $\lambda = 4360$ Å is:

1st. the first negative curvature,
2nd. no inactivation can be stated,
3rd. in stead of it an almost immediately starting, long lasting and steady increase of the auxin content is found, for at least during 4 hours after the exposure.
4th. under the prevailing conditions nothing can be concluded on an eventual lateral transport of auxin.

§ 4. **$\lambda = 5460$ Å; E = 26.400 ergs/cm². Exposure time 20 min.; phototropic curvature = + 15°.** (table 27, fig. 9).

This light quantity administered in 20 minutes induces a strong positive curvature. In contrast to the previous experiments no short exposure times could be applied to obtain the large light quantity; the time of 20 minutes approaches more or less that of continuous illumination.

The reaction time of the phototropic curvature and the time of cutting the tips is counted from *the end* of the exposure time and therefore the abscissa of fig. 9 actually should be shifted for ± 20 minutes to the left for a comparison with the other figures.

Firstly fig. 9 shows that no inactivation can be stated. An increase of the auxin content is found, it is strongest in the D-side and most prominent during the first hour after exposure. The maximum is attained after about two hours, then a rather steep decrease sets in after about 3 hours and after about 4 hours the dark value is attained again. Longer lasting experiments being impracticable, we cannot say whether the dark value further is maintained or not.

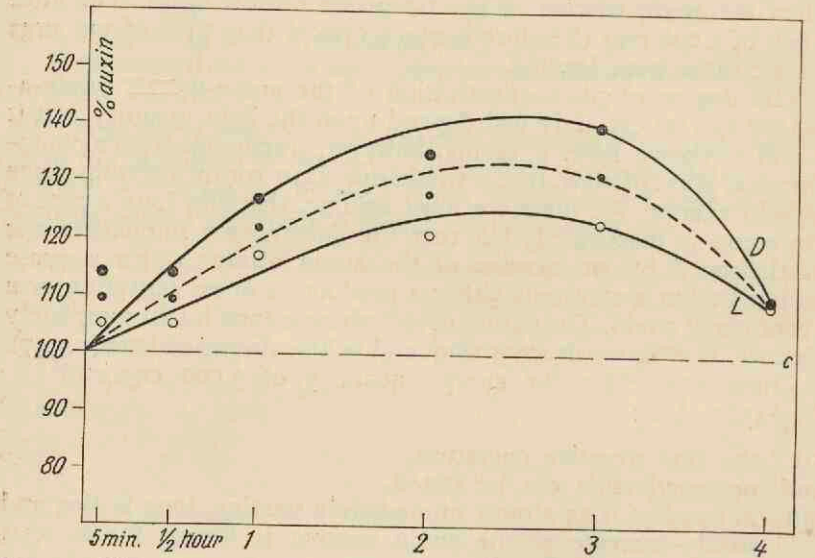


Fig. 9. The course of the auxin content of L- and D-side of the tip after an illumination with 26.400 ergs/cm², λ = 5460 Å.

A typical difference with the preceding experiments is, that the divergency between the curves of the L- and D-side lasts for such a long time. In the other cases after 1—2 hours the curves either converged or ran parallel to each other. Here the increase of the auxin content of the D-side remains for about three hours ahead as compared to that of the L-side.

Characteristic for the light quantity of 26.400 ergs/cm², λ=5460 Å is:

1st. a positive curvature,
2nd. no photo-inactivation of auxin can be stated,
3rd. nothing can be concluded on a transversal transport,
4th. an increase of the auxin content of L- and D-side during about 2—3 hours, followed by a rather steep decrease to the original

level. This increase is and remains stronger in the D-side than in the L-side.

§ 5. Discussion of the factors determining the auxin-content.

At the end of the preceding chapter we expressed the hope that we would be enabled to discriminate between the three phenomena which come into play in the phototropic response by using monochromatic light. These three phenomena were: a) photo-inactivation of auxin, b) a transversal transport of auxin and c) a changed synthesis of auxin.

Illumination with 330 ergs/cm², $\lambda = 4360$ Å, causes a photo-inactivation of auxin of 30—40 % at both sides and probably also a transversal transport. The auxin content later only slightly increases; this must be due to an auxin synthesis, increased in some way. When increasing the light quantity up to 3.000 ergs/cm², $\lambda = 4360$ Å, however, no photo-inactivation of auxin can be found, but only a distinct increase in the auxin content in both sides, in the L-side, however, to a much larger extent. No conclusions can be drawn on a lateral transport of auxin.

With illumination with 26.400 ergs/cm², $\lambda = 5460$ Å, no photo-inactivation of auxin could be detected either. Also here a pronounced, long lasting increase of auxin content was found, but that in the D-side is more pronounced. A conclusion on lateral transport of auxin cannot be drawn.

a) *The photo-inactivation of auxin.*

From these series it appears that photo-inactivation of auxin cannot always be stated. In the next chapter we will investigate by which light quantities photo-inactivation of auxin can be shown and by which not. There this process will be treated more in detail.

b) *The transversal transport of auxin.*

One only can conclude upon a lateral transport of auxin when a *de*crease of the auxin content of one side runs parallel with an *in*crease of that in the other side. Such a parallelism only was found for the auxin curves of L- and D-side in the experiments with 500 M.C.S. (white light) and with 330 ergs/cm², $\lambda = 4360$ Å. In these cases it seems justified to argue in favour of a lateral transport. Also here, however, such a transport is attended with, or followed by a change in the auxin content, which only can be due to a change in "synthesis" of auxin (in the broadest sense). It is, therefore, a question of personal feeling whether one wants to explain the opposite

parallel changes of the auxin content of L- and D-sides by a lateral transport or by a different change of the auxin synthesis at both sides. The same holds true for the other curves with 3.000 ergs/cm², $\lambda = 4360$ Å (negative curvature) and with 26.400 ergs/cm², $\lambda = 5460$ Å, (positive curvature). One may argue the differences between the L- and D-side by a lateral transport attended with a homogenously increased auxin synthesis, or by an increased auxin synthesis different in both sides of the coleoptile. This question will be discussed more thoroughly at the end of this paper.

c) *The changed synthesis of auxin.*

In all four series of experiments an increase of the auxin content could be shown, which must be due to some increased synthesis of auxin.

In the experiments with small light quantities i.c. 500 M.C.S. (white light) and 330 ergs/cm², $\lambda = 4360$ Å, this increased synthesis was only slight and preceded by a marked photo-inactivation of auxin. With 330 ergs/cm², $\lambda = 4360$ Å, the inactivated amount of auxin was 30—40 % and thus the main auxin content of the tip decreased to $\pm$ 65 % of the dark value. Three hours later it had slowly increased up to $\pm$ 75 %. About the same was found for 500 M.C.S. (white light). During the second hour the increase amounts only to $\pm$ 15 %, the auxin level rising from $\pm$ 60 % to $\pm$ 75 % of the dark value. Then, however, a markedly quick increase occurs so that one hour later, 3 hours after the exposure, the auxin level of the dark controls has been surpassed. This last feature fails in the curve of 330 ergs/cm², $\lambda = 4360$ Å. A longer continuation of these experiments is impossible since geotropism interacts with the phototropism and obscures the results. The later fluctuations found with 500 M.C.S. therefore will not be valued.

Far more prominent is the increased auxin content in the experiments with 3.000 ergs/cm², $\lambda = 4360$ Å, and with 26.400 ergs/cm², $\lambda = 5460$ Å. With 3.000 ergs/cm², $\lambda = 4360$ Å (negative curvature) the increase immediately appears and it lasts for 4 hours at least. It is most prominent in the L-side. Due to the long exposure time things are a little different in the experiments with 26.400 ergs/cm², $\lambda = 5460$ Å. Here after about half an hour (since the radiation began) an increased auxin content is found, being strongest at the D-side. It lasts not so long; after 4 hours the surplus production practically has disappeared from the tip.

We thus have to conclude that light induces either immediately or later an increase of the auxin synthesis in the coleoptile tip. The nature of this synthesis still being completely obscure, we cannot

discriminate between a real synthesis or a photo-activation or a liberation of auxin from a bound state. Also this question, which for the present is a completely unknown item of our problem will be discussed at the end of this paper.

CHAPTER VI

THE PHOTOTROPIC CURVATURE AS A FUNCTION OF THE LIGHT ENERGY (λ = 4360 Å)

§ 1. Introduction.

In the preceding chapter it turned out, that photo-inactivation of auxin cannot always be stated. It had to be investigated how this phenomenon is related with the amount of light energy. For it cannot be understood why by a certain amount of light energy auxin is inactivated, and why by increasing this energy no inactivation is detectable.

In the beginning, working with monochromatic blue light, λ = 4360 Å, no negative phototropic curvature could be obtained. Fig. 10 shows the curve (broken line) when the phototropic reaction is plotted against the log. of the light energy. The light intensity was 22 ergs/cm²/sec. The reason why no negative response could be obtained was the rather low intensity. This turned out when, in order to get more suitable exposure times, the intensity was raised up to 198 ergs/cm²/sec., by making the light beam narrower and by opening the diaphragms. Now negative curvatures could be obtained indeed. So it seems that the curvature (see the graph) obtained with unfiltered white light presumably does not differ from that induced by monochromatic blue λ = 4360 Å. Only if the light intensity is sufficiently high — i.c. the time of exposure short enough (cf. ARISZ 1914) — negative curvatures will appear. When using an intensity, i.c. an energy-quantity per time unit — not large enough to produce a negative curvature — the positive curvature does not show the rapid decrease with increasing light quantity. There is only a slow decrease which is converted into an increase again without entering the domain of negative curvatures or even reaching zero. So the curve does show a flat sinking slope, but a slight one only. It should be realised that in these cases the exposure-times amounted to 20—60 minutes and therefore were very long. For the present

I will confine myself to the short-exposure effect, since with the long one complicating time effects may be involved. Therefore I have studied the short-exposure effect only.

§ 2. **Experiments with different light quantities, $\lambda = 4360$ Å** (table 28, fig. 10).

The next series of experiments was carried out with monochromatic blue light $\lambda = 4360$ Å. with varying light quantities. The auxin extraction took place immediately after illumination. In Chapter V I accounted for the course of the auxin content during several hours after an illumination with 330 ergs/cm² and with 3.000 ergs/cm² of the same wave length. The former quantity causes an inactivation of the auxin for about 30—40 %; with the latter light quantity no inactivation could be stated. It was strange not to find any inactivation by increasing the light energy. So it seemed worth while to test other light quantities to see whether inactivation takes place or not. With monochromatic blue light the smallest energy used was 35 ergs/cm²; in this case no inactivation was detectable. Exposure to 150 ergs/cm² had an equal negative result. So the phototropic curvature in this region cannot be induced by a partly photoinactivation of auxin, but must be due to either a transversal transport or an altered auxin synthesis in the tip or to both. The applied amounts of light energy fall within the range, where the phototropic curvature is proportional with log. light energy. When the light energy surmounts about 330 ergs/cm² this proportionality disappears and here the first positive phototropic curvature reaches its maximum. In this region the photo-inactivation of auxin occurs, but this cannot have a share in the curving process, since it is practically equal in both sides. On the other hand the auxin content immediately after the application of 150 ergs/cm², $\lambda = 4360$ Å, namely L = 85%, D = 115 % as compared to the dark controls may be explained in favour of a lateral transport of auxin. One should remind, however, that it is hard to understand, how such a quick lateral move of auxin can be realized. It is true, that VAN DER WEY (1932) estimated the rate of the longitudinal auxin transport on 2 mm in 10—12 minutes. There is, however, no indication that a lateral transport is based on the same mechanism (see CLARK 1938).

When the light quantity is increased further the phototropic response decreases. In this range of energy no inactivation is detectable either. I used 700, 1000 and 1400 ergs/cm², $\lambda = 4360$ Å. So it turned out that only in a narrow region at about 330 ergs/cm², that is in the region of the maximum of the first positive phototropic curvature, inactivation can be stated. Moreover this inactivation

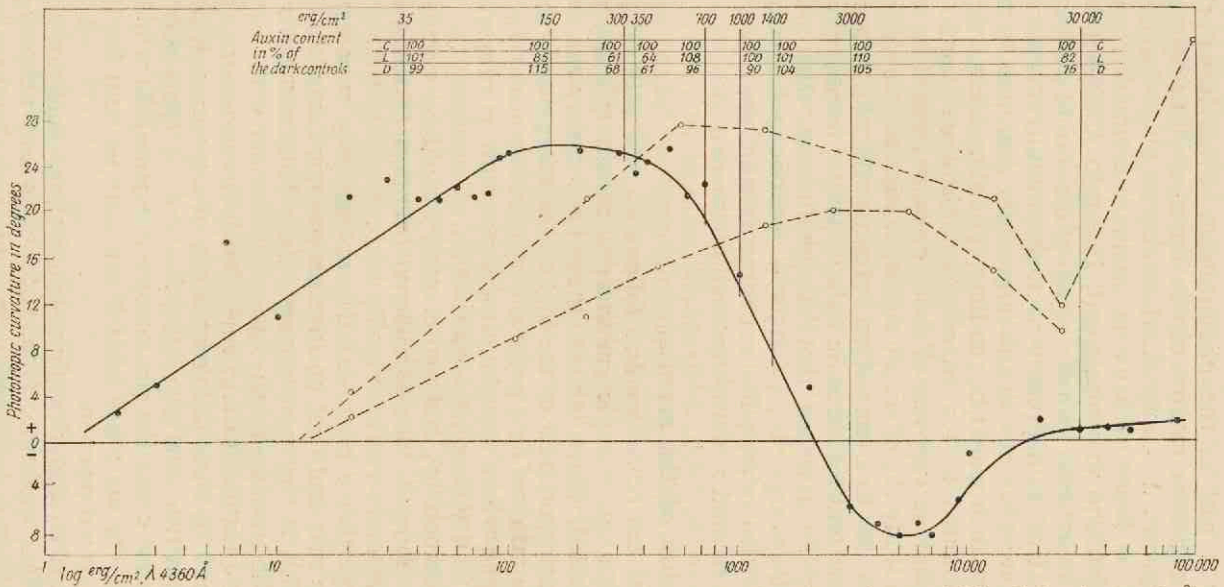


Fig. 10. The relation of phototropic curvature and irradiated energy for monochromatic blue light, $\lambda = 4360$ Å. The full line represents the average curvature induced by an intensity of 198 ergs/cm²/sec.; broken lines: two experiments with an intensity of 22 ergs/cm²/sec.

cannot play a part in the phototropism of the *Avena* coleoptile.

The first negative curvature can be obtained with a light quantity of 3.000 ergs/cm², $\lambda = 4360$ Å. Here also no photo-inactivation of auxin could be found. Finally an auxin determination was made after an exposure to 30.000 ergs/cm², $\lambda = 4360$ Å. The phototropic curvature is about zero, here the region of the second positive curvature begins. Here again a photo-inactivation of auxin is obvious. No experiments were taken with still larger light quantities because the exposure time then would be too long. The time needed for 30.000 ergs/cm² amounted to 100 seconds for this wave length. To get 100.000 ergs/cm² an exposure of about 5 minutes would be required. Intending to study the effect after short exposures, to avoid eventual complications due to a time effect, experiments with larger energy quantities were omitted, because light of greater intensity could not be obtained. To ascertain that the photo-inactivation of auxin, obtained with 330 ergs/cm², $\lambda = 4360$ Å, was not an illusive incident, at the end of this series a few experiments with 350 ergs/cm² were taken. They showed a photo-inactivation of auxin too.

§ 3. Discusion of the results.

The results can be resumed as follows.

To induce a phototropic curvature a certain quantity of light energy is needed. When increasing the light energy the curvature proves to be proportional to the log. of the energy until the curve flattens and a maximal first positive curvature is reached (fig. 10).

In the region where the phototropic curvature is proportional to log. light quantity no photo-inactivation of auxin by the applied doses of radiation (35 and 150 ergs/cm²) could be stated. Phototropism here must be due to a lateral transport of auxin or by a change in the auxin synthesis or by both.

It is strange that a photo-inactivation was only apparent in the region of the maximal first positive phototropic curvature (330,350 ergs/cm²). This is the more strange, since KONINGSBERGER (1922) recorded light-growthreactions after illumination with very much smaller light quantities (2 ergs/cm²). But it is consistent and it was regularly found in three different series of experiments, one with white light and two with blue light, $\lambda = 4360$ Å. This inactivation, however, cannot cause the phototropic curvature, since it is equal at both sides. On the other hand it can fully account for the long lasting light-growthreaction.

Still stranger is, that this photo-inactivation could not be found with larger light quantities (700, 1000, 1400 and 3.000 ergs/cm²). As we pointed out on page 339 it seems impossible that the inactivation

would not occur in this range of light energy, that cannot be attained without passing the critical range of inactivating light quantities. The most probable explanation is offered by the assumption that also the auxin synthesis is changed by these amounts of light energy so quickly that the inactivation is immediately overbalanced.

The inactivation found with still higher amounts of light energy (30.000 ergs/cm^2) then only can mean that there also the lactone fraction of the newly produced auxin-a is inactivated. That would mean, that the equilibrium auxin-a $\rightleftarrows$ auxin-a-lactone establishes itself within 100 seconds (exposure time). On its turn this suggestion offers a possibility to account for the time-effect (ARISZ 1914), but we will discuss this question later.

Meanwhile the data collected on the photo-inactivation are completely enigmatic. I therefore tried to find an explanation in the following direction. SCHURINGA (1941) found that the equilibrium auxin-a: auxin-a-lactone is catalyzed by H-ions and that the photo-inactivation auxin-a-lactone $\rightleftarrows$ lumi-auxin-a-lactone by ultra-violet radiation is readily achieved in an ethanolic solution at pH = 4. It would be possible that the internal pH of the coleoptile tip shifts towards a lower value under the influence of the light. So I tried to alter this internal pH artificially. VAN SANTEN (1940) showed that the internal pH of coleoptile sections equals that of the medium when they have been soaked for 12—24 hours in buffer solutions. In my experiments the entire plants were soaked for 12 hours in buffer solutions of different pH. They then were exposed to light in the air and placed back again in the solution. As controls plants were used that had been soaked in tap water. I first compared such water soaked coleoptiles to normal "air-grown" coleoptiles. The phototropic curvature was smaller in the "tap water coleoptiles". The curvatures shown by coleoptiles from the buffer solutions pH = 4, = 6 and = 8 (see table 29) were different indeed. The growth rate, however, proved to be different too; the plants in pH = 8 grew more slowly than the others. The differences in phototropism therefore may be considered primarily due to differences in growth rate. These experiments therefore are not conclusive. Though they fail to elucidate the curious relation between light energy and photo-inactivation of auxin, it still remains possible that the internal pH is affected by light in such a way that the energy / inactivation relation depends on it. I only did not succeed in giving experimental support to this possibility.

Another possibility is that this relation depends on specific properties of the photo-sensibilizer. According to several authors and more especially SCHURINGA (1941) carotinoids are the physiological sensi-

TABLE 29.

	energy in ergs/cm² (λ = 4360 Å)		
	32	70	254
in pH 4		14,7	
in pH 6		13,5	
in pH 8		7,0	
in tap water	10,5	12,0	20,0
in air	14,7	21,0	25,0

A comparison of the phototropic curvature (in degrees) of plants soaked for 12 hours before exposure in tap water, buffer solutions of pH=4,=6 and=8 and of normal „air-grown" plants.

bilizers in the photo-inactivation of auxin-a-lactone. The possibility that the action of the sensibilizer could be affected by light-absorption in the medium, had not to be excluded. SKOOG (1935) reported an auxin inactivation by light in the presence of eosin. Since this dye is water soluble and carotinoids not, I treated auxin solutions with eosin and then irradiated them. I could, however, not confirm the results of SKOOG. Neither by white light, nor by monochromatic blue light, tested at different pH's an inactivation of auxin could be stated (see Chapter II § 3).

CHAPTER VII

GENERAL DISCUSSION AND SUMMARY.

§ 1. The types of phototropic reactions; Phototonus.

From the preceding experimental data can be concluded upon a threefold part played by auxin in *phototropism:*

1st. a photo-inactivation of the auxin-a-lactone fraction,
2nd. a change of the synthesis of auxin, induced by light,
3rd. a lateral transport of auxin.

The last item only has a certain degree of probability. The present technique does not enable to discriminate with certainty between a change of the auxin synthesis and a lateral transport. Only in those cases, where the total auxin content of the illuminated tips is 100 % of-or less than-that of the dark controls and where a decrease of the auxin content of one side is attended with a simultaneous parallel increase of that of the other side, a lateral transport seems evident. When, however, the total amount of auxin in the tip increases and a change in the auxin synthesis is apparent, one cannot conclude

whether the differences between L- and D-side are due to a lateral transport or to a different synthesis or to both. For the *light-growth-reactions* the lateral transport can be left out of consideration.

Before considering these three processes from a theoretical point of view, I will survey the different types of phototropic responses studied with monochromatic light. With $\lambda = 4360$ Å I succeeded in inducing the first positive, the first negative and the threshold of the second positive curvature. I stated a photo-inactivation of the extractable auxin for resp. 30 — 40 % and 20 — 25 % in the regions of the maximum of the first positive reaction and in that of the threshold value of the second positive reaction. Although this inactivation, being about equal at both L- and D-side, cannot in itself explain the phototropic response, it certainly complicates the phototropic phenomena, known as phototonus, in this spectral region.

This follows from the fact that I could not obtain other than positive reactions with $\lambda = 5460$ Å; all light quantities of this wave length yielded positive curvatures. I only studied the course of the auxin content after a radiation with 26.400 ergs/cm² of $\lambda = 5460$ Å. No auxin inactivation could be stated, but the auxin synthesis proved to be increased, the auxin content of the D-side being more increased than that of the L-side. The first extraction being started 25 minutes after the beginning of the illumination (which lasted 20 minutes), the possibility remains, that a photo-inactivation escaped from our observation and had been balanced by an increased synthesis. This, however, seems not probable. Although with this wave length I only studied the effect of one light quantity and I did not investigate the curvatures due to the less frangible wave lengths of the spectrum, it seems justified to conclude that phototropism, as far as it occurs in this spectral region, can only be positive and chiefly is due to an increased auxin synthesis; in the tip this increase being stronger in the D-side than in the L-side.

The only data available on light-growthreactions in this part of the spectrum (KONINGSBERGER 1922) show short lasting and very weak growth retardations with 6 ergs/cm², $\lambda = 5300$—5700 Å and 8 ergs/cm², $\lambda = 5700$—6200 Å; with 60 ergs/cm², $\lambda = 6200$—7000 Å a much stronger, short lasting growth retardation was found. These reactions, which are typical base responses, can be left out of consideration, since my experiments exclusively deal with changes of the auxin content in the tip. Nowhere, however, KONINGSBERGER has reported an acceleration of the growth rate, as could be expected from my own data. Perhaps his observation time (2 hours) has been too short to this purpose.

Since the increasing auxin content found by me must be due to

an increased auxin synthesis, which in its turn is caused by the radiation, it seems impossible to explain the different auxin contents of L- and D-side by a higher auxin synthesis in the D-side, that received less light energy. Most probably this difference must be ascribed to a lateral transport.

The uniform phototropic behaviour of *Avena* against light of $\lambda = 5460$ Å suggests that the complicated phenomena of phototonus must be due to the action of the short wave lengths (i.c. $\lambda = 4360$ Å), which consist of photo-inactivation and increased auxin synthesis. These two factors, eventually combined with a lateral transport, compete and interact with each other and the result, observed in the phototropic response, is called "phototonus".

a. *First positive curvature.*

The strangest result of my experiments with light quantities $\lambda = 4360$ Å in the range of the first positive curvature is that a photo-inactivation of auxin only could be stated in the region of the maximal curvature (330—350 ergs/cm^2). It is possible that some inactivation occurred indeed with smaller amounts of light energy, but that they were only too slight to be detected with our method. On the other hand SCHURINGA (1941) found a practically complete inactivation of auxin-a-lactone by ultra-violet light $\lambda = 3340$ Å, with 11,4 ergs/cm^2, and, in the presence of β-carotene, by violet $\lambda = 4360$ Å with 6,9 ergs/cm^2. It is not clear why such an inactivation could not be found under physiological conditions. As I pointed out on p. 346 also KONINGSBERGER (1922) found consistent light-growth-reactions after illumination with 2 ergs/cm^2, $\lambda = 4200$—4400 Å and $\lambda = 4400$—6400 Å, which indicates that a photo-inactivation must have occurred. Perhaps another statement by SCHURINGA (1941) can account for my curious negative result. Estimating the quantum output of the photo-chemical inactivation of auxin-a-lactone, this author reasoned that one light quantum $\nu = 9.10^{14}$ ($\lambda = 3340$ Å) inactivates at least more than 3.10^6 molecules of auxin-a-lactone. He suggests that each auto-inactivation of auxin originates by "germs" of lumi-auxin-a-lactone and that these germs catalyze or sensibilize the further inactivation. When applying this idea upon our case one can admit that a very slight inactivation initiated by radiation, later-on automatically could increase in the dark. This hypothesis deals with a kind of "photo-mechanical induction" advocated in plant physiology as early as 1878 by JULIUS WIESNER. Since my own auxin determinations with low light quantities were done only immediately (i.c. $\pm$ 5 minutes) after the illumination, it must be left to further investigations to check this possibility. In favour of it pleads the fact

that after all-round illumination with light quantities within the range of the first positive curvature always long lasting light-growth-reactions have been found as retardations of the growth rate (KONINGSBERGER, 1922; VAN DILLEWIJN, 1927). Further the auxin content of the L-side, in the region of the maximum of the first positive curvature, continues to decrease for about one hour after the illumination.

On the other hand the almost parallel course of the curves of the auxin content of the L- and D-side strongly suggest a lateral transport.

The main auxin content of the entire tip, however, immediately after the illumination decreased to 65 %, soon begins slightly to increase which points in the direction of a slight change of the auxin synthesis by the radiation. One can also explain the differences of the L- and D-side by different changes of the auxin synthesis. Perhaps this question would have found a more conclusive answer if only the extreme solid tips had been extracted (200—300 μ). From a technical point of view this would have been too difficult, so that I always extracted tips of 3 mm.

b. *First negative curvature.*

The increase of the auxin synthesis, only slight in the range of the first positive curvature, entirely prevails in the region of the first negative reaction. As we discussed on p. 339 it cannot be understood that with these amounts of light energy no photo-inactivation would occur, so that, for the moment, the only possible explanation is, that a decrease of the auxin content by photo-inactivation is overbalanced by an immediate and strong increase of the auxin synthesis. The possible nature of this increase will be discussed later in § 5. We here will mention that negative curvatures only can be obtained — as already stated by ARISZ (1914) — when the amount of light energy required is administered within a certain time. This time effect most probably must be due to the light-*intensity*, the latter determining the slope of the curve of the change of the auxin synthesis. It is in agreement with this hypothesis that the auxin content of the L-side shows a steeper increase than that of the D-side. On the other hand this difference also can be explained by a lateral transport, in this case from the D- towards the L-side. When comparing the curves of the auxin contents with the light-growthreactions as studied by VAN DILLEWIJN (1927) we have to choose those experiments in which only the extreme tip ($\frac{1}{2}$ mm) had been illuminated. They show either a growth acceleration or practically no change. Since VAN DILLEWIJN applied white light, the amounts of light energy

cannot be compared. In the experiments, in which the entire coleoptile was illuminated, the light-growthreactions are complicated by strong base responses, which obscure the comparison.

We still have to mention that negative curvatures could not regulary be obtained with 3.000 ergs/cm^2, $\lambda = 4360$ Å. On several days the plants don't curve at all, they are indifferent. This variability, perhaps due to the same factors as that in the *Avena*-test, can be understood from the complicated nature of the processes, described above, involved in the negative phototropic response.

c. *Second positive curvature.*

At about the threshold value of amounts of light energy, inducing a second positive curvature, I found a distinct inactivation of auxin again. Although I did not further study the course of the auxin content in the range of the second positive curvature, it is possible to predict the factors on which the auxin content of the tip must depend: an increase of the auxin synthesis depending upon the light intensity and a photo-inactivation of the auxin-a-lactone already present, and of that newly delivered by the increased synthesis. If the increase of the auxin synthesis is so steep (high light intensity) that the establishment of the equilibrium auxin-a $\rightleftharpoons$ auxin-a-lactone cannot keep pace with it, negative curvatures will result. If the light intensity is lower, the increase of the auxin synthesis will be too slow to overrun the photo-inactivation of the auxin-a-lactone fraction. In this case only second positive curvatures will result. This seems the most probable explanation of the time effect as stated by ARISZ (1914), many other authors and myself. It remains possible that also a lateral transport is involved in the distribution of the auxin in the second positive curvature.

Further, in all cases, also a photo-inactivation of auxin present in the sub-apical regions of the coleoptile occurs (KONINGSBERGER & VERKAAIK, 1938).

The latter phenomenon accounts for the short lasting base response of the light-growthreactions, which always is a retardation of the growth rate. The later course of the light-growthreaction depends on the interaction of the increased synthesis in the tip and the photo-inactivation of auxin as discussed above.

In all cases, where the establishment of the equilibrium auxin-a $\rightleftharpoons$ auxin-a-lactone in newly synthezised auxin can keep pace with the rate of photo-inactivation, second positive curvatures will occur. This always will be the case with continuous illuminations. The reasoning, given here, matches the statement by KONINGS-

BERGER (1922), that in continuous light the "sensitivity" of the light-growthreaction for a higher light intensity is fully preserved. This can be explained by the fact that to each light intensity a specific increase in the auxin synthesis belongs and consequently an increased amount of auxin-a-lactone, liable to photo-inactivation.

Further the conclusion of HAIG (1935) that in *Avena* "there should be two photo-receptor systems, which have separate loci" matches my view that photo-inactivation of auxin in the entire coleoptile interacts with changes of the auxin synthesis in the tip, perhaps attended with a lateral transport in the tip too.

§ 2. The photo-inactivation of auxin-a-lactone.

The photo-inactivation of a part of the auxin, as stated in the more basal regions of the coleoptile by KONINGSBERGER & VERKAAIK (1938), certainly also occurs in the tip of the coleoptiles. The course of this inactivation in its dependence on the light energy still is enigmatic. The inactivation only could be stated in the region of the maximum of the first positive curvature [light energy = 500 M.C.S. (390 ergs/cm^2), white light; or 330—350 ergs/cm^2, λ = 4360 Å] and at the threshold of the second positive curvature (light energy = 30.000 ergs/cm^2, λ = 4360 Å). On p. 347 I tried to account for the fact that no photo-inactivation could be stated for amounts of light energy below 330 ergs/cm^2, λ = 4360 Å, by referring to the data collected by SCHURINGA (1941) on the photo-inactivation of auxin-a-lactone in vitro and the "photo-mechanical induction" assumed by him. As most probable explanation for the fact that no photo-inactivation was found in the region of the decreasing first positive and in that of the negative curvatures I assumed that the inactivation is masked and overbalanced by a steeply increasing auxin synthesis.

Since in vitro pure solutions of auxin-a-lactone are only inactivated by ultra-violet radiation, in the coleoptile the inactivation by visible light must be sensibilized. According to BÜNNING's (1937) and DU BUY & OLSON's (1938) statement that carotinoids play a part in phototropism, KONINGSBERGER & VERKAAIK (1938) suggested that these substances would act as sensibilizers in the photo-inactivation of auxin in the coleoptile. This view is strongly supported by the work of SCHURINGA (1941). The rate of sensibilization being directly proportional to the light absorption i.c. the light gradient, there is — as far as the photo-inactivation is concerned — no reason to join ATKINS' view (1936): "a relationship is suggested between the phototropic curvature and the light gradient of the photosensitive area, rather than a direct photochemical effect of some growth regulator". Further ATKINS (1936) leaves open the possibility that also chloro-

phyll and anthocyanids could act as photo-sensibilizers. For the present there is no indication that this view holds true.

It remains possible, however, that in the increase of the auxin synthesis by light (§ 5) also photo-sensibilizers are involved, others than carotinoids. My results, inclusively those obtained with light of $\lambda = 5460$ Å, suggest that these sensibilizers absorb a broader region of the spectrum than carotinoids do.

§ 3. The increase of the auxin synthesis by light.

Earlier or later in all experiments with different light quantities an increase of the auxin content was apparent. After exposure to small light quantities (500 M.C.S., white light and 330 ergs/cm², $\lambda = 4360$ Å) the increase of the auxin content is only slow and slight but with larger light quantities this increase is the only prominent feature of the auxin curvature not only with short wave lengths, but also with $\lambda = 5460$ Å. This increase of the auxin content of the tip can only be identical with an increase of the auxin synthesis, if this term be used in its broadest sense. The nature of this synthesis still is completely unknown. We only tried to argue that the rate of the increase of the auxin synthesis depends on the light quantity administered per time unit, i.c. on the light intensity. This dependency seems logical for each kind of photo-catalyzed synthesis, either for an inversion of an inactive precursor into auxin, or for a liberation of free auxin from a bound state. The last mentioned possibility gets a special interest in connection with WENT's (1938) opinion on the occurrance of auxin in the tissue. He believes that auxin in the cell occurs in a "free" and in a "bound" state. The former is diffusable and can be transported, the latter is bound somewhere to the plasm and cannot diffuse nor be transported. With the diffusion method only the free moving auxin is determined, with the extraction method both. In the statement by STEWART & WENT (1940, p. 710): "light apparently does not decrease the amount of free moving auxin in the *Avena* coleoptiles, in contrast to its effect on bound auxin" should be read in photo-chemical terms: "auxin-a" for "free moving auxin" and „auxin-a-lactone" for "bound auxin". It is, however, hard to be understood how the polar acid itself could be free and would not be adsorbed to the protoplasm, while the neutral lactone would be. Moreover this does not agree with the fact that the auxin-a-lactone is transported in the plant: in the *Avena*-test it has the same activity as auxin-a. It seems much more likely that the free auxin consists of the auxin-a $\rightleftarrows$ auxin-a-lactone system and the bound auxin of some adsorbed and therefore inactive precursor, that is

liberated by light [1]). Instead of the quoted lines we would prefer to read: "light apparently can decrease the amount of free moving auxin in the tip of the *Avena* coleoptiles and liberate the bound auxin". Since the latter most probably is adsorbed to some boundary layer of the protoplasm this version of WENT's hypothesis would include a change in the permeability. This in its turn could account for changes in the normal basipetal transport, known as lateral transport.

§ 4. The lateral transport of auxin.

The course of the auxin content of L- and D-sides in all experiments can be explained by factors other than a lateral transport. There is not one experiment which gives evident proof of a lateral transport. As we stated earlier (p. 342) it is more or less a question of personal feeling, whether one accepts a lateral transport or not. Personally, however, I'm convinced that this lateral transport exists on the following reasons:

1st. the experiment by BOYSEN JENSEN, referred to on p. 295,
2nd. the distribution of auxin after exposure to light quantities inducing the maximum of the first positive phototropic curvature,
3rd. the fact that after exposure to 26.400 ergs/cm^2, $\lambda = 5460$ Å the D-side continuously has a higher auxin content than the L-side,
4th. the fact that all changes in the auxin content found in the L- and D-sides are too small entirely to account for the phototropic response. Presumably they coincide with changes in the physico-chemical properties of the protoplasm and/or of the cell wall, f.i. of the permeability of the protoplasm as explained in the end of the preceding paragraph,
5th. the different results of WENT's experiments (1928) on the auxin delivery by diffusion of coleoptile tips split only at their base and my experiments on the auxin diffusion from entirely split tips (p. 324). Also my experiments resumed in table 19 as compared to those of table 20 show consistent differences.
6th. the fact that geotropism entirely is ascribed to such a lateral transport.

Since the data on geotropism of the earlier authors (WENT, 1928; CHOLODNY, 1929, 1930; DOLK, 1930 and DIJKMAN, 1934) were all obtained by means of the diffusion method, I decided to check them by means of the extraction method. In two replications sets of coleoptiles were either decapitated and extracted or placed horizontal and then decapitated and extracted after 1,

[1] VOSS (1939) suggests that auxin, inactivated by the scutellum, can be stored in this inactive form in the cells of the seedlings.

2, 3, 4 and 5 hours. The results are given in table 30; they only confirm that no changes occur in the total auxin content, so that geotropism must be entirely due to a dislocation of auxin towards the lower side.

TABLE 30.

Experiment on:	3-10-'40	2-10-'40	Mean
Control	7,0	5,0	6,0
After 1 hour	6,4	5,4	5,9
After 2 hours	6,0	5,0	5,5
After 3 hours	5,0	4,9	5,0
After 4 hours	5,0	4,0	4,5
After 5 hours	4,6	4,8	4,7
Hetero-auxin			
1 in 10^7	5,5	8,5	7,0
$\frac{1}{2}$ in 10^7	4,3	4,0	4,2
$\frac{1}{4}$ in 10^7	2,3	1,5	1,9

Curvature in degrees of the test plants. The auxin content of the coleoptile tips at different times after they had been placed horizontal. Below: standard tests with indole-3-acetic acid.

Many authors believe that geotropism and phototropism are of a different nature. In my opinion they have at least one important factor in common: the lateral transport of auxin, for *Avena* in the extreme solid tip of the coleoptile.

Still less than proving the existence of a lateral transport in phototropism, I succeeded in elucidating the mechanism of such a change of the polarity. In the end of § 3 only a hypothetical possibility has been mentioned: the light would liberate an auxin precursor ("bound auxin") adsorbed to the boundery layer of the protoplasm in the tip cells of the coleoptile. Consequently the properties of that layer and also its permeability would change and this change would involve all still deficiently investigated phenomena referred to in Chapter II, such as changes in viscosity of the cell wall, of bio-potentials, of respiration, of the protoplasmic streaming and also of the turgor etc.

This merely hypothetical explanation can also account for long distance effects of illumination. PROBST (1927) for instance stated an increase of the growth rate of *Avena*-coleoptiles when the roots are illuminated. He ascribed this to an increased permeability of the root cells by which the water supply of the coleoptile would be promoted.

§ 5. Light-growthreactions and phototropism.

As far as the photo-inactivation of auxin is concerned, it has turned out with certainty that phototropism is not a special case of

a light-growthreaction after all-round illumination. In those cases were this photo-inactivation was stated, it was equal at the D- and the L-side and therefore cannot account for the phototropic response. On the other hand this inactivation fully can be responsible for the "tip-reaction" as far as it consists of a long lasting growth retardation. BLAAUW's theory therefore cannot be entirely valid.

If one attributes a part to lateral transport in phototropism, as I do, the discrepancy with BLAAUW's theory is largest in the range of the first positive curvature. In this connection it must be mentioned that the course and the shape of the first and the second positive curvature are different. In the first positive reaction the curvature starts in a narrow section just under the tip; it then gradually shifts towards the base, while the tip region straightens out again or, under the influence of gravity, curves in the opposite direction, the coleoptile becoming S-shaped. In the second positive curvature the entire coleoptile curves simultaneously. Furthermore it lasts much longer: the first positive curvature disappears after 2—3 hours, the second one, according to DU BUY (1933), lasts for 5 hours.

This feature of the second positive curvature agrees with an inactivation of the auxin-a-lactone fraction present all along the coleoptile. The short base response of the light-growthreaction will be due to this inactivation. With an unilateral illumination this inactivation in basal zones will — according to the strong light gradient in this region — be less at the D- than at the L-side, so that at least part of the second positive curvature can be explained in BLAAUW's terms. That presumably is not the case with the reactions induced by the tip, which, however, in this range of the light quantities have not yet been thoroughly studied.

§ 6. Summary.

By means of the extraction method data were collected on the course of the auxin content of the light- and shade-side of coleoptile tips of *Avena* after unilateral illumination. As light quantities were applied:

a) 500 M.C.S. (390 ergs/cm^2), white light and 330 ergs/cm^2, monochromatic blue light of $\lambda = 4360$ Å, both inducing about the maximum of the first positive curvature,
b) 3.000 ergs/cm^2, $\lambda = 4360$ Å inducing negative curvatures,
c) 26.400 ergs/cm^2, $\lambda = 5460$ Å inducing positive curvatures.

Further a number of preliminary experiments were carried out with other amounts of monochromatic light ($\lambda = 4360$ Å). The results can be resumed as follows:

1. The part of auxin in phototropism is threefold:
   a. a photo-inactivation of auxin-a-lactone,
   b. a change of the synthesis of auxin induced by the light,
   c. a lateral transport.
2. Although no proof could be given for a lateral transport, there is evidence that it actually plays an important part in phototropism.
3. With the longer wave lengths ($\lambda = 5460$ Å) only positive curvatures could be obtained; negative curvatures occurred after exposure to adequate amounts of light $\lambda = 4360$ Å of high intensity.
4. This difference is reduced to a different interaction of the three factors, mentioned under 1 (see p. 341).
5. Photo-inactivation of auxin was stated in the region of the maximum of the first positive curvature (light energy: 500 M.C.S (390 erg/cm^2), white light; 330 ergs/cm^2, $\lambda = 4360$ Å) and at the threshold of the second positive curvature (light energy: 30.000 ergs/cm^2, $\lambda = 4360$ Å).
6. This photo-inactivation can account for the long lasting growth-retardation of the so-called "tip" light-growthreaction, but not for the phototropic response since it is equal at the light- and the shade-side of the tip.
7. BLAAUW's theory, reducing phototropism to special cases of light-growthreactions, can therefore not be entirely valid, especially not for the first positive curvature.
8. Always, and especially after exposure to larger light quantities, the synthesis of auxin is increased. The unknown nature of this increased synthesis has been discussed (§ 3, p. 354) and the rate of the increase has been ascribed to the light quantum per time unit, i.c. the light intensity.
9. It was tried to explain phototonus in terms of an interaction between photo-inactivation and rate of the increase of auxin synthesis (§ 1, p. 350).
10. Theories are given to explain the different phototropic responses (§ 1, a, b, c, p. 350). Especially in the first positive reaction a prominent part is ascribed to the lateral transport of auxin. In the second positive reaction the photo-inactivation of auxin-a-lactone all alongside of the coleoptile greatly attributes to the curvature.

The investigations were carried out in the Botanical Laboratory of the State University, Utrecht. I owe much to Prof. Dr. V. J. KONINGSBERGER for his interest in my work and his valuable criticism.

TABLE 25.

The auxin content of illuminated tips after illumination with 330 ergs/cm², $\lambda = 4360$ Å at different times after exposure.

Exp. on:	Control not illum. C	Illuminated L	Illuminated D	Phototropic curvature	H.A. ... × 10^{-7} 1	H.A. ... × 10^{-7} ½	H.A. ... × 10^{-7} ¼
Immediately after exposure.							
16-9-'40	17.0	14.4	14.0	10.4	13.1	8.1	3.3
	9.2	4.8	7.0	21.3	8.0	3.1	0.6
24-9-'40	—	6.6	5.7				
		6.3	—				
mean:	13.1	8.0	8.9				
1 hour after exposure.							
17-9-'40	6.0	3.2	5.0	13.8	—	7.0	4.8
	7.5	3.5	6.7				
18-9-'40	5.4	4.5	5.3	11.3	11.6	5.4	0
	5.2	2.8	4.2				
	—	2.6	4.6				
mean:	6.0	3.3	5.2				
2 hours after exposure.							
19-9-'40	6.0	2.8	3.2	8.0	—	9.2	3.4
	4.4	4.7	4.5				
		—	4.0				
20-9-'40	6.0	4.0	4.3	10.0	—	—	—
	7.4	5.1	7.4				
		3.3	7.0				
mean:	6.0	4.0	5.1				
3 hours after exposure.							
25-9-'40	4.8	2.8	3.6	21.7	inactive		
	3.0	3.8	3.3				
26-9-'40	3.2	3.0	3.1		7.0	3.5	—
	4.6	4.3	2.8				
		2.8	2.5				
27-9-'40	9.0	5.4	6.6	15.0	inactive		
mean:	4.9	3.7	3.6	18.3			

Continuation Table 25.

Summary of the mean values.

Extraction after exposure:	In degrees			Phototropic curvature	In %		
	C	L	D		C	L	D
Immediately:	13.1	8.0	8.9	16.0	100	61	68
After 1 hour:	6.0	3.3	5.2	12.5	100	55	87
After 2 hours:	6.0	4.0	5.1	9.0	100	66	85
After 3 hours:	4.9	3.7	3.6	18.3	100	75	74

TABLE 26.

The auxin content of illuminated tips after illumination with 3.000 ergs/cm², $\lambda = 4360$ Å at different times after exposure.

Exp. on:	Control not illum. C	Illuminated L	Illuminated D	Phototropic curvature	H.A. ... × 10^{-7} 1	$\frac{1}{2}$	$\frac{1}{4}$
Immediately after exposure.							
24-10-'40	5.8	5.8	6.5	—11.0	7.3	3.6	1.5
	5.3	5.6	5.6				
25-10-'40	8.7	9.5	7.3	— 9.0	8.5	4.0	0
	8.6	7.0	5.7				
31-10-'40	4.3	7.0	7.0	— 1.6			
	7.6	7.0	8.0		8.9	4.5	1.0
1-11-'40	3.1	5.1	3.7				
	4.0	5.2	6.1				
mean:	5.9	6.5	6.2	— 7.2	8.2	4.0	1.2
1 hour after exposure.							
7-11-'40	4.4	8.3	9.3		8.8	4.6	3.0
	3.6	8.6	4.5				
8-11-'40	5.5	5.8	4.8	— 7.8	10.5	6.5	4.0
	4.8	8.0	4.9				
14-11-'40	4.3	8.6	6.5	— 4.0	25.5	17.5	11.2
	12.0	8.0	7.2				
mean:	5.8	7.9	6.2	— 6.0	9.6	5.5	3.5
2 hours after exposure.							
5-11-'40	—	3.3	3.6	—10.			
	3.8	—	—		8.3	3.9	2.1
6-11-'40	4.0	5.2	3.5	— 8.7			
	3.3	4.3	2.5				
18-11-'40	0.9	—	—	— 9.0	6.6	3.4	0.9
	0.8	1.8	1.5				
mean:	2.5	3.7	2.8	— 9.1	7.5	3.7	1.5

Continuation Table 26.

Exp. on:	Control not illum. C	Illuminated L	Illuminated D	Phototropic curvature	H.A. ... × 10^{-7} 1	$\frac{1}{2}$	$\frac{1}{4}$
3 hours after exposure.							
29-10-'40	6.5	7.4	7.9	— 4.0	7.2	5.1	0
	2.0	5.4	5.1				
30-10-'40	7.7	10.0	6.6	— 6.0	8.0	6.0	0
	2.2	3.0	2.3				
12-11-'40	1.0	7.6	6.5	-10.0	—	—	7.3
	1.2	3.3	2.5				
13-11-'40	9.9	8.4	4.8	— 5.0			
	4.5	10.3	6.7				
mean:	4.6	6.9	5.3	— 6.2	7.6	5.5	—
4 hours after exposure.							
21-10-'40	5.2	7.0	8.5	— 8.0	8.2	5.5	3.3
	4.5	6.0	7.6				
	2.0	4.0	2.1	— 1.3	0	0	0
22-10-'40	3.4	6.0	3.4				
	4.3	7.6	4.3	— 9.0	8.0	5.7	2.5
4-11-'40	3.7	5.3	2.0				
mean:	3.9	6.0	4.7	— 6.1	8.1	5.6	2.9

Summary of the mean values.

Extraction after exposure:	In degrees C	L	D	Phototropic curvature	In % C	L	D
Immediately:	5.9	6.5	6.2	— 7.2	100	110	105
After 1 hour:	5.8	7.9	6.2	— 6.0	100	136	107
After 2 hours:	2.5	3.7	2.8	— 9.1	100	148	112
After 3 hours:	4.6	6.9	5.3	— 6.2	100	150	115
After 4 hours:	3.9	6.0	4.7	— 6.1	100	154	120

TABLE 27.

The auxin content of illuminated tips after illumination with 26.400 ergs/cm², $\lambda = 5460$ Å at different times after exposure.

Exp. on:	Control not illum. C	Illuminated L	Illuminated D	Photo-tropic curva-ture	H.A. ... × 10^{-7} 1	$\frac{1}{2}$	$\frac{1}{4}$
			Immediately after exposure.				
15-2-'40	4.6	2.5	3.0	9.0	10.1	5.1	2.0
	4.5						
1-7-'40	5.7	6.3	5.9				
		8.4	9.8	9.4	12.4	7.2	5.4
2-7-'40	7.7	5.8	6.6				
		6.2	6.1	9.9	5.7	4.6	2.2
3-7-'40	8.4	11.3	12.2	12.2	8.7	7.1	4.9
12-8-'40	5.7	9.5	7.1	15.0	—	—	—
	8.0	3.3	8.0				
mean:	6.4	6.7	7.3	11.1	9.2	6.0	3.7
			30 minutes after exposure.				
20-6-'40	—	4.7	7.0	9.1	9.6	8.3	6.2
		4.7	7.8				
27-6-'40	5.8	3.0	2.5	10.3	11.4	5.2	3.1
		6.1	2.5				
28-6-'40	7.7	—	6.0	8.3	12.7	8.6	5.3
	5.2	10.3	—				
16-8-'40	10.5	9.8	10.1	23.3	10.0	5.0	2.6
	5.7	9.5	10.4				
19-8-'40	8.0	10.7	14.6	9.3	11.3	6.1	2.6
	5.2						
	9.7	10.0	12.3				
mean:	7.2	7.6	8.1	12.0	10.7	7.1	4.0
			1 hour after exposure.				
4-7-'40	7.2	10.8	8.4	13.9	10.5	3.8	0
5-7-'40	4.6	5.0	8.6	9.9	13.3	8.0	3.3
		6.4	9.0				
8-7-'40	6.2	8.3	9.7	10.4	12.2	8.6	4.8
		7.9	4.2				
13-8-'40	8.3	7.0	11.0	21.0	—	—	—
	5.7	7.2	6.0				
mean:	6.4	7.5	8.1	14.0	12.0	6.8	4.0

Continuation Table 27.

Exp. on:	Control not illum. C	Illuminated L	Illuminated D	Phototropic curvature	H.A. ... × 10^{-7} 1	H.A. ... × 10^{-7} $\frac{1}{2}$	H.A. ... × 10^{-7} $\frac{1}{4}$
		2 hours after exposure.					
30-7-'40	5.0	3.4	12.0	16.5	12.7	7.8	4.9
		8.8	6.6				
31-7-'40	3.3	5.9	4.2	20.0	8.4	4.7	1.8
		4.7	7.6				
1-8-'40	10.5	17.0	7.0	12.5	10.3	5.8	2.4
		8.0	13.3				
9-8-'40	6.6	6.4	8.0	—	—	—	—
	6.1	6.5	9.4				
mean:	6.3	7.6	8.5	16.3	10.5	6.1	3.0
		3 hours after exposure.					
2-8-'40	5.3	7.7	8.4	20.0	12.5	4.8	3.3
		9.7	10.7				
5-8-'40	6.3	8.2	9.2	21.0	—	—	—
		5.8	9.1				
6-8-'40	3.6	5.2	5.0	14.0	11.9	6.5	3.4
	4.8	6.6	7.7				
7-8-'40	10.0	10.0	11.2				
	5.8	6.0	6.1	15.0	—	—	—
mean:	6.0	7.4	8.4	17.5	8.6	5.0	2.9
		4 hours after exposure.					
8-8-'40	3.5	10.0	10.3	—	—	—	—
	8.5	7.3	4.3				
14-8-'40	5.0	7.6	7.8	17.3	—	—	—
	7.1	—	—				
15-8-'40	9.9	6.1	9.0	12.0	—	—	—
	6.6	6.0	7.3				
mean:	6.8	7.4	7.5	14.7	—	—	—

Summary of the mean values.

Extraction after exposure:	In degrees C	In degrees L	In degrees D	Phototropic curvature	In % C	In % L	In % D
Immediately:	6.4	6.7	7.3	11.0	100	105	114
After 30 min.:	7.2	7.6	8.1	12.0	100	105	114
After 1 hour:	6.4	7.5	8.1	14.0	100	117	127
After 2 hours:	6.3	7.6	8.5	16.3	100	121	135
After 3 hours:	6.0	7.4	8.4	17.5	100	123	140
After 4 hours:	6.8	7.4	7.5	14.7	100	109	110

TABLE 28.

The auxin content of illuminated tips immediately after exposure with different light quantities, $\lambda = 4360$ A.

Exp. on:	Control not illum. C	Illuminated L	Illuminated D	Photo-tropic curva-ture	H.A. . . . $\times 10^{-7}$ 1	$\frac{1}{2}$	$\frac{1}{4}$
			Illumination with 35 ergs/cm².				
4-3-'41	14.1	11.5	10.0	13.0	5.4	5.3	0
	8.0	4.0	5.8				
5-3-'41	5.2	13.5	7.0	19.0	6.5	3.7	0
	6.0	—	10.0				
7-3-'41	10.8	7.2	5.6	14.7	15.0	7.6	4.0
	9.0	—	9.5				
10-3-'40	14.0	11.0	22.0	15.0	18.6	13.2	
	22.0	20.0	18.0				
mean:	11.1	11.2	11.0	15.3	11.4	7.5	—
			Illumination with 150 ergs/cm².				
19-11-'40	2.5	1.2	3.8	+33.0	10.5	8.3	4.3
	2.1	0.7	3.8				
20-11-'40	5.7	3.1	4.5	+20.4	14.4	12.0	6.0
	2.6	2.0	2.6				
22-11-'40	5.0	5.1	5.4	+31.0	13.1	10.0	5.5
	3.2	5.6	4.3				
25-11-'40	6.7	5.9	8.0	+28.0	12.5	8.1	5.4
mean:	4.0	3.4	4.6	+28.0	12.6	9.6	5.3
			Illumination with 350 ergs/cm².				
28-3-'41	7.0	4.0	—	23.0	26.0	14.0	6.0
	7.0	2.3	2.3				
31-3-'41	5.7	5.2	5.4	18.0	18.6	12.0	3.5
	3.6	3.3	2.8				
mean:	5.8	3.7	3.5	20.5	22.3	13.0	4.7
			Illumination with 700 ergs/cm².				
20-3-'41	6.3	7.1	10.5	26.0	14.0	14.0	13.2
	9.0	6.0	10.4				
21-3-'41	—	8.0	4.8	29.5	17.4	8.0	5.0
	—	4.0	3.0				
24-3-'41	2.3	1.6	1.6	—	5.7	2.6	
	1.4	1.1	2.7				
25-3-'41	1.4	3.1	3.8	—	15.6	6.8	2.4
	4.4	10.9	3.6				
27-3-'41	13.1	6.7	1.6	24.5	27.3	16.6	
	2.7	7.0	7.0				
mean:	5.1	5.5	4.9	27.0	16.0	9.6	6.9

Continuation Table 28.

Exp. on:	Control not illum. C	Illuminated L	Illuminated D	Photo-tropic curva-ture	H.A. . . . × 10^{-7} 1	$\frac{1}{2}$	$\frac{1}{4}$
Illumination with 1.000 ergs/cm².							
17-3-'41	18.6	13.4	8.5	20.0		19.0	8.0
	7.5	10.8	8.5				
18-3-'41	29.4	25.5	27.4	17.4		10.0	4.2
	26.2	23.0	25.7				
19-3-'41	14.0	21.1	8.1	27.0	25.4	18.4	10.0
	6.3	13.6	13.0				
20-3-'41	12.0	8.4	—	17.6	22.0	14.8	9.3
	7.4	4.6	4.1				
mean:	15.2	15.1	13.6	21.0	23.7	15.5	7.9
Illumination with 1.400 ergs/cm².							
11-3-'41	9.0	9.0	12.0	15.0	16.0	8.6	
	8.0	4.4	12.0				
12-3-'41	6.0	5.0	4.0	12.5	10.0	4.0	
	6.4	6.2	6.2				
13-3-'41	15.0	18.5	13.5	17.0	17.0	6.0	
	10.0	12.0	10.0				
14-3-'41	10.5	9.0	8.0	19.0	9.7		
	6.7	9.0	9.5				
mean:	9.0	9.1	9.4	16.0	13.2	6.2	
Illumination with 30.000 ergs/cm².							
2-4-'41	25.7	22.0	13.0	—1.0	14.0	7.7	2.2
	6.6	20.0	19.0				
3-4-'41	20.7	10.7	—	—1.0	15.2	7.7	0
	15.0	12.3	19.3				
4-4-'41	15.0	8.6	5.8	+3.0	10.7	8.0	0
	8.0	—	11.0				
7-4-'41	13.1	12.2	9.7	+3.0	(7.0)	(20.0)	(11.8)
	20.6	11.5	10.7				
mean:	16.9	13.9	12.9	+1.0	13.3	7.8	0

Continuation Table 28.

Summary of the mean values.

Energy in ergs/cm² λ = 4360 Å	Curvature in degrees			Phototropic curvature	Curvature in % of the control		
	C	L	D		C	L	D
35	11.1	11.2	11.0	15.3	100	101	99
150	4.0	3.4	4.6	28.0	100	85	115
330	13.1	8.0	8.9	15.0	100	61	68
350	5.8	3.7	3.5	20.5	100	64	61
700	5.1	5.5	4.9	27.0	100	108	96
1.000	15.2	15.1	13.6	21.0	100	100	90
1.400	9.0	9.1	9.4	16.0	100	101	104
3.000	5.9	6.5	6.2	−6.0	100	110	105
30.000	16.9	13.9	12.9	+1.0	100	82	76

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STELLINGEN

I

Op physiologische gronden moet aangenomen worden, dat in de plant het evenwicht auxine-a = auxine-a-lacton zich binnen drie minuten instelt.

II

Het is zeer waarschijnlijk, dat het vermogen om phototropisch te reageeren berust op de aanwezigheid van carotinoïden.

III

Bij Chlorella pyrenoidosa gaat de basale ademhaling tijdens de oxydatieve verwerking van toegevoerde organische stoffen onveranderd door.

IV

Uit de proeven van MELCHERS moet de conclusie getrokken worden, dat voor het in bloei komen van planten de samenwerking van florigeen en vernaline noodig is.

G. MELCHERS, 1941, Biol. Zbl. 61.

V

De pollenanalytische methode mag, met eenige voorzichtigheid, ook op aeolische afzettingen toegepast worden.

VI

De bouw van de angiosperme embryozak, ook wanneer deze van het normale type afwijkt, kan verklaard worden door aan te nemen, dat in principe twee gereduceerde archegoniën aanwezig zijn en een vegetatief prothallium ontbreekt.

H. NILSSON, 1941, Botaniska Notiser.

VII

De tegengestelde reactie ten gevolge van exstirpatie van de pedaalganglia, wanneer de slakkenvoet (Helix pomatia) in „Muskel-" dan

W. F. F. OPPENOORTH Jr.

wel in „Zentraltonuskonstanz" verkeert, moet verklaard worden door de wisselwerking tusschen de periferie en de twee antagonistische tonuscentra in de pedaalganglia in verband met hun specifieke reactie op de grootte van den rekkingslast.

VIII

Met behulp van gistingsproeven kan worden bepaald of het aneurine-disulfide een physiologische beteekenis heeft.

O. Zima & K. Ritsert & Th. Moll, 1941, Hoppe-Seyler 267.

IX

Het heidepodsolprofiel is genetisch één geheel en is ontstaan door uitlooging der bovenliggende en inspoeling in de daaronder liggende lagen. Dit profiel kan ook in den huidigen tijd nog ontstaan.

X

Door uitwendige omstandigheden is de stofwisseling der planten zoo te beïnvloeden, dat zoowel de aantasting door als de verspreiding van parasitaire plantenziekten zeer bemoeilijkt wordt.

F. Alten & H. Orth, 1940, Phyt. Zts. 13,
J. M. Fife & V. L. Frampton, 1936, Jrn. of Agric. Res. 53.

XI

Actieve resistentie tegen virusziekten berust waarschijnlijk niet op een verandering van het virus zelf, maar op een verandering in de plant.

J. Oortwijn Botjes, 1940, Tijds. over Plantenziekten 46.

XII

Het uiteengaan der chromosomen bij de kerndeeling geschiedt door middel van trekdraden.

T. Shimamura, 1940, Cytologia 11.
W. J. Schmidt, 1939, Chromosoma 1.

XIII

Het chromosoom in het leptoteen-stadium der reductiedeeling is niet uit één enkelvoudige draad opgebouwd.

H. Matsuura & T. Haga, 1940, Cytologia 10.