Botany. — *Photo-inactivation of auxin in the coleoptile of Avena and its bearing on phototropism.* (Preliminary note). By W. F. F. OPPENOORTH. (Communicated by Prof. J. C. SCHOUTE.)

(Communicated at the meeting of October 28, 1939.)

Early in 1939 KONINGSBERGER and VERKAAIK reported some experiments in which the phototropic behaviour of deseeded and decapitated coleoptiles of *Avena* was investigated. By deseeding the coleoptiles were practically deprived of their own auxin and therefore the phototropic response could be due to the growth substances artificially applied in agar blocks to the coleoptiles in the usual way. These did not show any phototropic curvature with indole-3-acetic acid. With auxin-*a*, however, either extracted from coleoptile tips or pure chemical preparations from urin (obtained from KÖGL's laboratory), distinct phototropic responses appeared in continuous illumination with 100 M.C.

In vitro auxin-*a* in solutions is in equilibrium with its lactone. This auxin-*a*-lactone is readily inactivated by ultra-violet radiation and turns into the inactive lumi-auxin-*a*-lactone.

The authors concluded that also in the coleoptile the auxin-*a* is in equilibrium with its lactone. Within the plant a photochemical inactivation was postulated by visible light, carotinoids probably acting as "sensibilizers". Since this inactivation must be stronger at the light side of decapitated coleoptiles than at the shade side, the phototropic base response of such coleoptiles was ascribed to the photo-inactivation of the auxin-*a*-lactone fraction.

The eventual share of this process in the phenomena of phototropism of intact coleoptiles, however, was left undetermined. It seemed worth while to investigate phototropism once more from this photochemical angle. In the older investigations (WENT, 1928, VAN OVERBEEK, 1933 and many others) the auxin content had been determined by means of the diffusion method. With this method the total amount of auxin, delivered to an agar slice, e.g. by illuminated coleoptile tips, during a certain, relatively long time interval is estimated. It therefore easily may happen that, with this method, consistent but temporary differences in auxin content of light- and shade sides of coleoptile tips cannot be detected. Although WENT (1928), applying the diffusion method for the first time, reported a partly inactivation of growth substance of about 15 per cent, similar results were not reproduced by later authors. Consequently it is generally assumed that, according to the theory of WENT–CHOLODNY (see e.g. WENT and THIMANN, 1937, p. 166 a. f.), phototropism is due to an unequal "redistribution" of auxin in the illuminated organ.

The problem of photo-inactivation can only be solved by determining at

definite instants the auxin content of the coleoptile at the light- and at the shade side, that is: by means of the extraction method. This work proved to meet with a number of unexpected difficulties and, at present, can by no means be considered to be finished. Since the author, however, has been mobilized and his work therefore had to interrupted for an undefinite time, it seems appropriate preliminarily to publish the results obtained till thus far.

Material and methods.

All experiments were done with coleoptiles of *Avena*. The plants used for phototropic reactions, as well as those from which the tips were used for auxin extraction were always 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 95 %, temperature 22°5 C.), where the experiments were also taken. Eventual illuminations took place in an adjacent room, equally conditioned, but with blackened walls and ceiling.

As a light source for illuminations a high pressure mercury bulb from PHILIPS, Eindhoven, of the commercial "Philora" type was used, which — after a few minutes of preheating — gives light of a fairly constant intensity and rich in blue-violet (and also ultra-violet) radiation. This bulb had been mounted in a light-proof brass tube; the desired light quantity was administered by means of a photographic shutter. Usually 500 M.C.S. was applied; a light quantity of this order of magnitude induces about the maximum "first positive" phototropic curvature and therefore seemed appropriate to start this kind of investigations with. By placing the racks with plants almost parallel to the axis of the light beam, the light passed through the coleoptiles in the direction of the longest 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 light- and shade side had to be determined separately, it was necessary to split the isolated tips longitudinally before putting them into ether. This proved to be a very 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 a ebonite mould. This mould consists of two halves; 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 halves of the tips thus represent the light side (*L* in the tables and graph), the other those of the shade side (*D*). The sets of 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. 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 equivalents of indole-3-acetic acid.

The procedure of splitting and extracting illuminated coleoptile tips was not applied, until its reliability had been checked in a large 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 useful to discuss this essential part of the technique a little more in detail.

The extraction method.

In the beginning the prescription, given by VAN RAALTE (1937) was followed. Immediately before the extraction the ether was freed from peroxides by redistilling it over CaO and $FeSO_4$. 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 acidified distilled water to remove the acid. The ether fraction subsequently was evaporated to a volume of 0.5 cm^3 and brought in a small test tube with an agar slice and 0.1 cm^3 of a buffer solution of pH 4.5. The rest of the ether was evaporated by means of an air current on a water bath.

Several authors (THIMANN, 1934, VAN OVERBEEK, 1936, WENT and THIMANN, 1937) report an inactivation of auxin during the extraction by enzymatic processes; for that reason they are afraid of peroxides in the ether. SKOOG (1935) moreover found that inactivation readily occurs in vitro in the presence of eosin (1 in 10^5), the latter substance probably acting as photocatalyst.

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. 4 different extracts from 24 coleoptile tips, made on the same day, gave the results of table I.

The same variability was found when diluting the extract. 3 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

TABLE I.

	No. of test plants	Mean curvature in degrees
extract *a*	22	7
„ *b*	24	9.5
„ *c*	12	18.5
„ *d*	21	12
indole-3-acetic acid 2.5 in 10^8	28	3.5
idem 5 in 10^8	28	8
idem 1 in 10^7	21	16

represented in table II; each experiment was done on a different day and consists of three parallels (*a*, *b* and *c*).

TABLE II.

	Curvatures in degrees:								
	Expt. No. 89			90			91		
Concentration:	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*
5 : 1	8.5	2	3.5	6	3	8	3.5	4	—
1 : 1	2	0.5	0.5	2	1.5	3	0	0.5	—
1 : 5	1	0	0	1	1	1.5	0	0.5	—
indole-3-acetic acid 2.5 in 10^8	3			6			3.5		

It can easily be understood that also the results obtained with split coleoptiles 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 III gives the results:

TABLE III.

	Curvature in degrees:													
	Expt. No. 92		94		95		97		99		100		Mean	
Concentration:	*F*	*B*	*F*	*B*	*F*	*B*	*F*	*B*	*F*	*B*	*F*	*B*	*F*	*B*
5 : 1	5	5	2	7	22	16.5	4	3	2	3	1	4	6	6.4
1 : 1	0.5	4	0	2	12	6	0	0	1	3.5	1	1.5	2.4	2.8
1 : 5	1	0	0	0	3	0	0	0	0	0	0	0	0.6	0
indole-3-acetic 2.5 in 10^8	6		4		4.5		7		4		7		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 error had to be found in the operations preceding the final evaporation of the ether.

In many cases the amount of auxin, extracted from the coleoptiles, was extremely low too. Therefore a number of variations on the extraction method, described in literature, were tested. The sulphuric acid was replaced by hydrochloric and 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 and cold or hot water proved to be unsuitable media for extraction.

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 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 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 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.

Further the possibility of enzymatic oxidation was considered. To eliminate the responsible enzymes, the tips were dipped into boiling water or the action of enzymes was inhibited with H_2S. The yield of auxin, however, was not increased. Also extreme low temperatures did not help. The dry-ice extraction after DU BUY (1938) gave largely varying results too. 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.

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 cm³ of ether extract was brought in a 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 8 experiments gave the same results as with the buffer solution as intermedium, but at least it has the advantage that the material can be reduced to one third.

After all these modifications the variations in the individual experiments

were not yet eliminated, but in a series of experiments a fairly reliable mean value was obtained. In table IV 5 experiments are resumed, in each of which two parallel sets of 60 coleoptile tips were extracted and tested in the concentration 5 : 3.

TABLE IV.

Concentration 5 : 3	Curvature in degrees: Expt. No. 145	146	147	148	149	Mean
Parallel *a*	9	2	0.5	1	1.3	2.7
„ *b*	5.2	1.5	1.5	3.5	1.7	2.7
indole-3 acetic acid 5 in 10^8	5.5	6.5	6	5.5	4	5.5
idem 2.5 in 10^8	5.5	2.5	3	2	2	2

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 V 240 halves of coleoptiles (in the controls 120 entire tips) have been extracted and tested on 24 plants, the concentration thus being 5 : 1.

TABLE V.

Concentration 5 : 1	Curvature in degrees: Exp. No. 151		152		153		154		Mean	
	F	*B*	*F*	*B*	*F*	*B*	*F*	*B*	*F*	*B*
split tips	4.5	3 4	2.3	4.0	2.5	2.5	2.4	1.6	2.9	2.9
control: entire tips	6.0		2.3		1.5		2.3		3.0	
indole-3 acetic acid 2.5 in 10^8	3.7		1.0		2.5		2.1		2.3	
idem 5 in 10^8	8.0		2.2		4.3		4.2		4.7	
idem 1 in 10^7	8.0		12.1		—		—		10.0	

The means of 4 replications in table V 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.

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 cm³ ether in Erlenmeyer flasks of 25 cm³, cooled

in a beaker with shredded ice. For each extract, as a rule, 9 dozens of tips or 18 dozens of half tips were used; the volume of ether thus was 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 botles with ether were placed in a light tight box and transported into the refrigerator, where they remained for at least 5 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. As a matter of precaution all manipulations for the extraction and evaporation of ether were done in a dark room in the same orange light as used in the air conditioned dark rooms.

Possible factors in phototropism.

Since it was intended to study the eventual part of photo-inactivation of auxin in phototropism, it had to be determined first whether other factors have a share in this phenomenon and, if so, to what extent.

According to the WENT–CHOLODNY theory it could be expected that the so called redistribution, that is a lateral shifting of the auxin towards the shade side, would prove the most important factor. The most direct evidence for such 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 and XIII can also be concluded that at the light side a part of the growth substance has been inactivated (or consumed, VAN OVERBEEK), but in his table XIV this inactivation (or consumption) is only very weak.

WENT (1928) too has already given data in experiments with *Avena*-coleoptiles, in which the auxin from light- and shade side has been trapped separately, by placing the coleoptiles over a razor blade. WENT stated that the amount of growth substance obtained from the shade side of illuminated coleoptiles is higher than that of halves of dark controls. In his table XXII he only gives relative figures; with 1000 M.C.S. this ratio was 57 : 50. It is questionable, however, whether this small difference is real. A decrease of the total amount of growth substance by the illumination of 16 % in WENT's experiments can be ascribed to photo-inactivation. From his tables XX and XXI some inactivation can be concluded too. For a transversal transport WENT's table XXIII is the most conclusive one; it is reprinted in our table VI, with the relative figures in brackets.

The difference in the second interval apparently must be ascribed to "redistribution". This is supported by our own experiments done by means of the diffusion method, but in a distinctly different way. Immediately

TABLE VI. (WENT's table XXIII.)

Illuminated with 100 M.C.S. diffusion method; tips on agar during:	Curvature in degrees:		
	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)

after the illumination with 500 M.C.S. the coleoptile tips were split and the halves placed on agar slices, those of the light- and of the shade side apart. Table VII gives the means of 5 experiments; sets of 36 half tips, 3 mm long, were placed on one agar slice each. After one and two hours the same half tips were transferred on to a new agar slice.

TABLE VII.

Illuminated with 500 M.C.S. diffusion method; half tips on agar during:	Curvature in degrees (auxin in per cent of dark controls, 1st hour)			
	Dark Controls (split tips)	*L*	*D*	Total *)
1st hour	3.9 (100)	1.4 (36)	2.3 (59)	3.7 (48)
2nd hour	2.8 (70)	1.8 (46)	1.9 (49)	3.7 (48)
3rd hour	2.2 (56)	1.2 (30)	1.4 (36)	2.6 (33)

*) Twice as many split tips as dark controls.

A redistribution in our experiments was prevented by splitting the coleoptiles, which fact explains the difference between WENT's figures and ours in the later periods.

Also an experiment, in which the coleoptile tips were cut and split partly immediately after the 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 VIII gives the means of two experiments.

TABLE VIII.

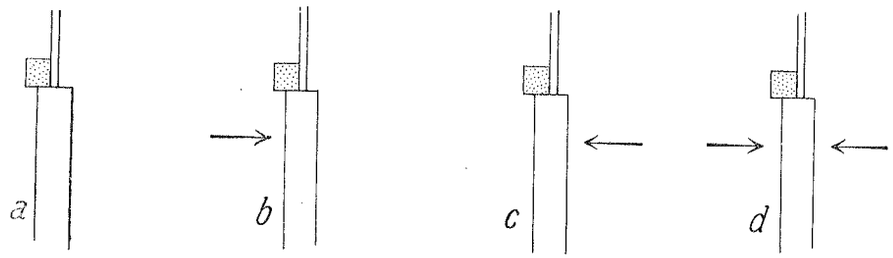
Illuminated with 500 M.C.S. Split tips on test plants:	Curvature in degrees (dark control (split tips) 5°5) In brackets per cent auxin of dark control (≙ 100)		
	L	*D*	Mean
immediately after illumination	5 (91)	5 (91)	5 (91)
one hour ,, ,,	3.1 (56)	7.2 (131)	5.2 (94)
two hours ,, ,,	3.2 (58)	6.1 (111)	4.7 (84)

Although it is not possible in the last experiments to discriminate between transversal transport and changed synthesis of auxin in the

coleoptile tip, it seems well established that redistribution actually plays a certain part in the phototropic response on illumination with 500 M.C.S.

Other possible factors 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 our experiments, the decapitated test plants were illuminated with 500 M.C.S. before the application of the auxin agar, as indicated in the head of table IX.

TABLE IX.



a. dark control	*b*, *c* and *d* illuminated before the test with 500 M.C.S. in the direction of arrows		
curvature in degrees:			
8.1	7.0	7.5	6.9

From these figures we may conclude that after an illumination of 500 M.C.S. no essential change in the reactivity of the tissue could be demonstrated.

The only factor therefore, that can be expected to play a part in our experiments with auxin extraction is, next to an eventual photo-inactivation, the shifting of the auxin eventually coherent with a change in its synthesis.

The auxin content of coleoptile tips after illumination.

In this section the results of 39 experiments will be reported. Each experiment is either an extraction immediately after the 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 coleoptiles and putting the halves into ether took about 5 minutes. In this way on 9 different moments after illumination estimations of the auxin content at the previous light- (L) and shade side (D) were made. As a control and comparison in each experiment a blank set ran with plants that were not illuminated; the curvature obtained with the extract of their split tips is used as a standard ($=$ 50; entire tips $=$ 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 were treated with indole-3-acetic acid in different concentrations and, finally, one set was used 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. 1. These plants

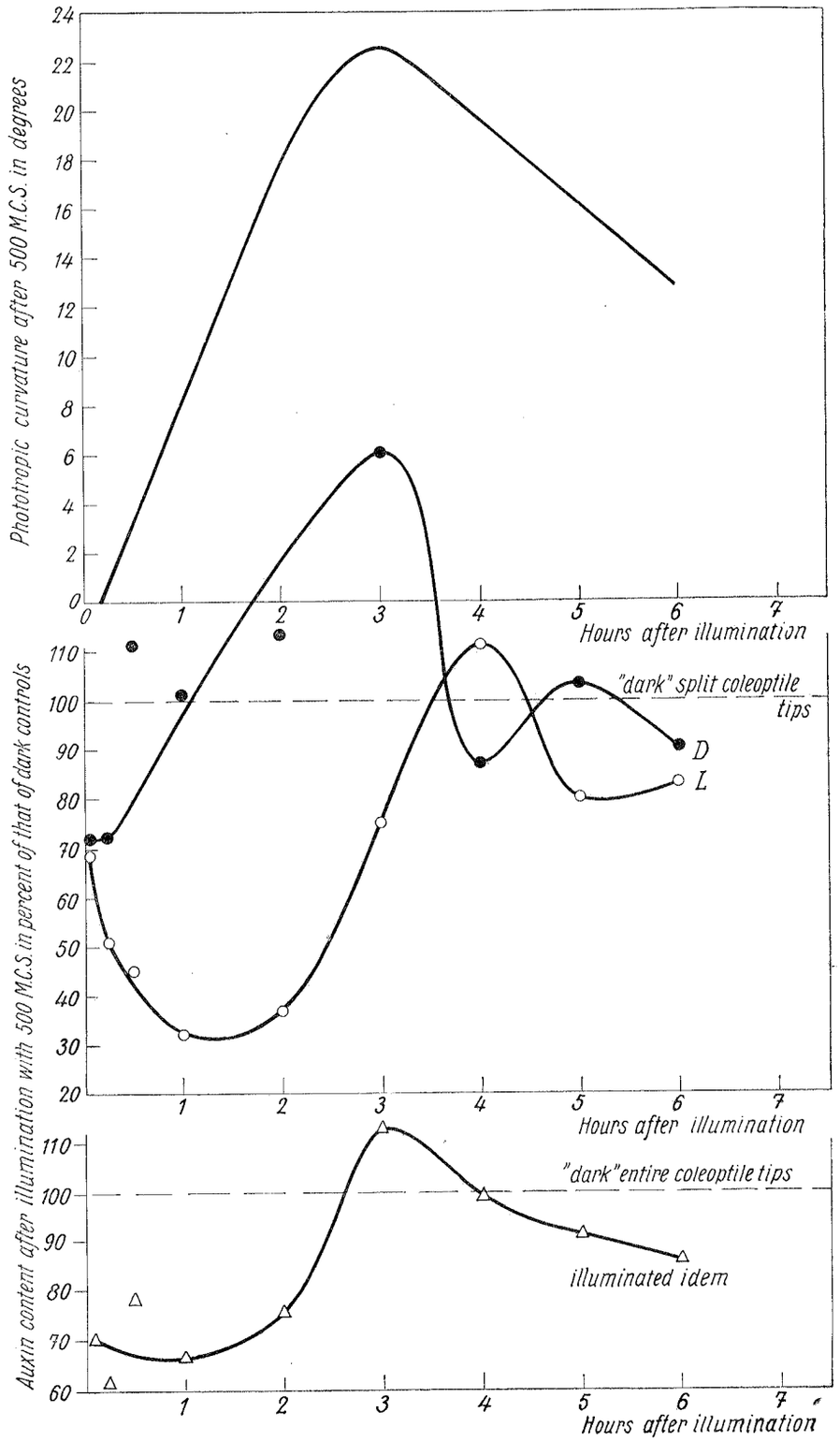


Fig. 1.

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. The results of the extraction experiments have been summarized in table X and in fig. 1.

TABLE X.

Time after illumination with 500 M.C.S.	Auxin content in per cent of split „dark" tips *)			Auxin content in per cent of the illuminated tips	
	L	*D*	Sum	*L*	*D*
immediately (± 5 minutes)	34	36	70	49	51
15 minutes	26	36	62	41	59
30 minutes	23	55	78	29	71
1 hour	16	51	67	24	76
2 hours	18	57	75	25	75
3 hours	37	75	112	33	67
4 hours	55	44	99	56	44
5 hours	40	51	91	44	56
6 hours	42	45	87	48	52

*) The split "dark" coleoptiles being = 50.

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 *at both sides* of the coleoptile tip at a light quantity of 500 M.C.S. *equally strong*, cannot be related with any phototropic response, since it cannot induce a differentiated growth rate. On the other hand, this sudden decrease of the auxin content may be responsible for the so called "slow" photo-growth-reaction. According to the ideas of WENT (1928), it seems demonstrated once more that the photo-growthreaction is not directly linked to phototropism.

A difference in the auxin content of light- and shade side sets in later and reaches its maximum not until 1 to 2 hours have passed after the illumination. At that time the auxin content of the shade side begins to surpass that of the "dark" controls. It seems completely impossible readily to explain the phototropical response by a photo-inactivation of auxin alone, but still much more experimental work will be wanted duly to interpret the reported data. The long lasting decrease of the auxin content at 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. The values found after 3 hours are even higher than those of "dark" plants, but it is questionable whether this difference is consistent; the same holds true for the figures found after 4 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, probably too weak to be real.

It would perhaps be possible, but certainly premature to try to estimate the phototropic response in terms of the auxin content of the light- and shade side. To this purpose it seems indispensable to repeat the same experiments with a variety of light quantities. Perhaps a mathematical treatment of the different curves would then also enable to disentangle the coinciding effects of transversal transport and change in synthesis of auxin.

Discussion of the results.

All illuminations reported in this paper have been done with a light quantity of 500 M.C.S., giving about the maximal "first positive" phototropic response. It was ascertained that with this light quantity the ability to react of the base and the transport of auxin are not measurably affected. On the other hand strong evidence was obtained again that „redistribution" of auxin, probably combined with a change in the synthesis of auxin plays an important part in phototropism.

The method of auxin extraction taught that immediately after the illumination with 500 M.C.S. the auxin has been inactivated for about 30 %, both at the light- and at the shade side of the coleoptile tip. This phenomenon can be explained as a photo-inactivation of the auxin-*a*-lactone fraction, according to the equilibrium, stated in solutions by KÖGL, C. KONINGSBERGER and ERXLEBEN (1936):

auxin-*a* ⟷ auxin-*a*-lactone (both active)
↓ light!
lumi-auxin-*a*-lactone (inactive)

and supposed within the plant by KÖGL (1936).

V. J. KONINGSBERGER and VERKAAIK (1938) gave evidence that this equilibrium actually occurs within the coleoptile and suggested that carotinoids possibly would act as sensibilizers and cause the inactivation by visible wave lengths.

Until recently in vitro this inactivation had only been observed by ultraviolet radiation. In this regard it is very interesting, that, shortly after the publication by KONINGSBERGER and VERKAAIK, Prof. KÖGL verbally communicated they had succeeded in his laboratory to show that auxin-*a*-lactone is also inactivated by the longer wave lengths of the visible light

in the presence of carotene. The question of the photo-inactivation of auxin-*a*-lactone since has been studied again in KÖGL's laboratory by G. J. SCHURINGA, who also had to stop his research as a result of the mobilization. We have, however, been kindly authorized to report that SCHURINGA found that the spectral region of inactivation is shifted by carotene for about 1000 Å.U. towards the visible light. This means that the maximum of inactivation in the presence of carotene exactly coincides with the spectral region, which has the maximum effect in phototropism (4670 Å.U., BLAAUW, 1909; 4800—4360 Å.U. DU BUY, 1933), and in photo-growthreactions (V. J. KONINGSBERGER, 1922) and with the maximum absorption band of carotene extracted from *Avena* coleoptiles (BÜNNING, 1937).

The inactivation within the coleoptile, caused by unilateral illumination with 500 M.C.S., being as strong at the shade side as at the light side, this factor, curiously enough, cannot be responsible for the phototropic curvature, since it cannot induce a different growth of both sides. On the other hand the "slow" photo-growthreactions, caused by illumination of the coleoptile tip, can readily find their explanation in this inactivation. After WENT's work (1928), this is the strongest indication that photo-growthreaction and phototropism are not directly related.

As KONINGSBERGER and VERKAAIK (1938) pointed out, the phototropic base response and also the "short" photo-growthreaction, induced by illumination of the base, both can find their explanation in the photo-inactivation of auxin-*a*-lactone. This view is supported by my own observations on decapitated coleoptiles that had regenerated their "physiological" tip. In these coleoptiles the phototropic curvatures were only weak and never did surpass 5°. A lateral transport seems impossible in the hollow base of the cylindrical coleoptile and to be governed in the extreme, solid tip. In this regard, one may speak of a special phototropic function of the tip in *Avena*.

Since the auxin content of the tip after about two hours after the illumination begins to surpass that immediately after the illumination, also the synthesis of auxin must have been changed by or after the irradiation.

The interaction between "redistribution" and change in synthesis still is unknown. It seems possible to disentangle these two factors by repeating the reported experiments with different light quantities. It was planned to do so, not only for light quantities causing "first positive" curvatures, but also for those of the region of the negative and "second positive" curvatures. It is possible that light quantities, smaller or greater than 500 M.C.S., have a different inactivating effect and also affect the redistribution differently. The authors hopes soon to be able again to continue his research along this program. He feels much indebted towards the director of this institute, Prof. Dr. V. J. KONINGSBERGER, for his interest and especially for his help in the redaction of this preliminary report.

Summary.

1. A method is described to determine the auxin content of the light- and shade side of illuminated coleoptiles separately by means of ether extraction. The extraction method is amply discussed. The results of this method are rather unstable; only statistical values can be used.

2. Immediately after an illumination of 500 M.C.S. about 30 % of the auxin in the coleoptile tip proves to have disappeared, as well from the light- as from the shade side. It is assumed that the lactone fraction of the active compounds: (auxin-*a* + auxin-*a*-lactone) is inactivated by the radiation, carotinoids acting as sensibilizers.

3. This inactivation, being equal at both sides, cannot explain the phototropic curvature; along different ways strong evidence is given that phototropism is chiefly due to a redistribution of auxin, according to the WENT–CHOLODNY theory. The extreme, solid coleoptile tip is responsible for this redistribution.

4. Moreover, the synthesis of auxin probably is changed by or after the illumination.

5. The photo-growthreactions, both of the "slow" and of the "short" type, caused by an all-sided illumination can find their explanation in the stated inactivation of auxin. The same holds true for the weak phototropic base response of the coleoptile.

6. Photo-growthreactions and phototropism therefore are not directly related.

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September 1939.

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