

# OSCILLATIONS IN THE REDISTRIBUTION OF THE GROWTH SUBSTANCE NAPHTHYLACETIC ACID AFTER PHOTOTROPIC INDUCTION\*

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## SUMMARY

The lateral distribution of the radioactivity from NAA<sup>14</sup>C in decapitated maize coleoptiles, after phototropic induction was studied. After a short unilateral irradiation (one second) with first positive radiant density (100lux) a transversal transport of the radioactivity away from the light sets in, followed by an oscillating redistribution.

## 1. INTRODUCTION

Both geotropic and phototropic stimulation lead to a lateral movement of auxin. This was proposed by CHOLODNY (1927) and first demonstrated by WENT (1928), and is known nowadays as the lateral-distribution-theory of Cholodny-Went.

The asymmetric distribution of the auxin after phototropic and geotropic induction was soon confirmed by other investigators; see WENT & THIMANN (1937).

Also BRIGGS *c.s.* (1957), and GILLESPIE & BRIGGS (1961) could show that phototropic and geotropic stimulation lead to an asymmetric transport of endogenous auxin to receivers placed at the base of the excised organs.

Gordon and his co-workers, however, suggested that phototropism following unilateral irradiation with light energy causing the first positive curvature and the geocurvatures of horizontally placed coleoptiles, might be the consequence of lateral inequalities in the ability of the tissues to transport auxin basipetally. See SHEN-MILLER & GORDON (1966) and NAQVI & GORDON (1966, 1967).

GOLDSMITH (1968) concludes, however, that "Even though the response of coleoptiles to stimulation is complex, present evidence still favors the conclusion that lateral movement of auxin is the basis of the asymmetric auxin distribution"

It must be noted that the lateral distribution theory of Cholodny-Went refers only to the first positive phototropic curvature, and the geocurvature as the reaction to gravity.

It should be noted that most investigators sampled their material for one observation only, after a certain arbitrarily chosen period of time. The redistribution of the auxin, after the stimulation preceding and causing autotropism or rectipetality, could possibly be the cause of the above-mentioned discrepancies in experimental results and in opinions.

BRAUNER & HAGER (1958) studied the geotropical perception on *Helianthus*

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seedlings. After stimulation in the horizontal position the seedlings were placed in a vertical position. After having attained their maximum curvature a counter-reaction sets in which results in an overshoot of the vertical position causing an oscillatory movement until equilibrium is attained. Brauner & Hager suggest that the counter-reaction is geotropically induced.

KARVÉ & SAWANT (1966) who could confirm Brauner & Hager's experiments, speak of a geotropically induced feedback oscillation. The fact that Brauner & Hager did not see this oscillating movement when the seedlings were turned in a horizontal position on a clinostat is no proof of its being geotropically induced. It could well be that the oscillatory movement must be ascribed to an endogenous autotropism. HERTEL & FLORY (1968) who studied the secretion of labelled IAA from corn coleoptile sections in receiver blocks after short intervals found indeed a longitudinal oscillating transport.

The experiments reported in this paper were performed in order to analyse the transversal distribution of the growth substance NAA in corn coleoptiles after phototropical induction.

## 2. MATERIAL AND METHODS

### 2.1 Material

Seeds of *Zea mais* (var. goudster hybr.) were, after having been soaked overnight in aerated tap water, laid out on moist filterpaper in diffuse daylight, for a period of 8 hours at room temp.  $\pm 20^\circ\text{C}$ , and subsequently planted in plastic pots in moist vermiculite. The pots were kept in an experimental dark room with a constant temperature of  $21^\circ\text{C}$  and a relative humidity of 90%, where the seeds grew in darkness for three days.

The coleoptiles were 3 cm long when used for the experiments which were performed in the same darkroom.

### 2.2 Radioactive compounds

The auxin  $\alpha$ -naphthylacetic acid- $1\text{-}^{14}\text{C}$  (NAA $^{14}\text{C}$ ) was obtained from The Radiochemical Centre, Amersham, England. The growth substance was applied in donor blocks of 3% agar with a volume of  $10\ \mu\text{l}$ . The concentration of the growth substance in the donor blocks was  $10^{-5}\ \text{M}$ . The receptor blocks were made of 3% agar only.

### 2.3 Transport tests

The coleoptiles were decapitated by cutting  $\pm 1,5\ \text{mm}$  of the top, after which they were freed from the primary leaves. From the decapitated coleoptiles a 13 mm long section, was cut. These sections were placed with their basal ends on the edge of a razor-blade which was mounted in a perspex holder, great care being taken that the base was cut in two equal halves to a depth of about 2 mm. Receiver blocks were placed on this holder on either side of the razor-blade and the coleoptiles were positioned in such a way that the cut surfaces of the split basal end of the coleoptiles made good contact with the receiver blocks.

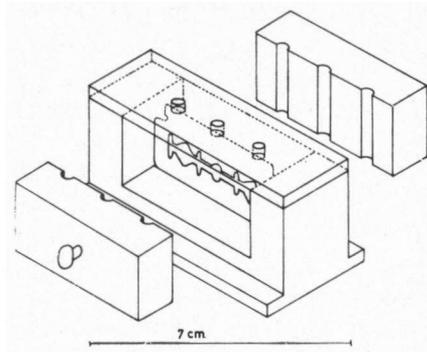


Fig. 1. Explanation see text.

In most experiments the distribution of the  $^{14}\text{C}$  between the light and shadow sides was estimated after cutting the coleoptiles lengthwise into two halves.

For this purpose a razor-blade holder was made as shown in *fig. 1*.

In the top plate of this perspex holder holes were bored into which the basal ends of the coleoptile cuttings fitted exactly. The edge of the razor-blade was fixed just below and precisely on the central line of the holes. The coleoptile sections were placed in a vertical position in these holes on the razor blade. The donor blocks were placed on the apical ends of the coleoptile sections. Five minutes after adjustment of the donor blocks the coleoptiles were illuminated from one side by a light flash of one second's duration and an intensity of 100 Lux., ordinary incandescent light being used.

Observations were made on the influence of this illumination on the lateral transport of the growth substance after intervals of 30 minutes.

This was done by taking away the donor blocks and pressing gently on the top end of the coleoptile cuttings, which were held in a vertical position by the grooves in the perspex holder, and resting on the razor blade, were cut into two halves.

Donor- and receiver blocks as well as both halves of the coleoptile samples were stored separately in vials in 2 ml 50% ethanol.

After grinding of the tissue the scintillation liquid was added to the vial and the samples assayed for radioactivity in a liquid scintillation counter (Nuclear Chicago Mark I).

The composition of the scintillation fluid was the same as described by Veen (1967).

Quenching was corrected by the use of an external standard.

### 3. RESULTS

**3.1 Transport of NAA- $^{14}\text{C}$  in maize coleoptiles after unilateral irradiation.** Coleoptiles on receiver blocks for extended periods  
The aim of the investigation was to study the lateral transport of a growth sub-

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stance in a corn coleoptile following unilateral irradiation with a light quantity causing the first positive phototropic reaction.

The growth substance naphthylacetic acid (NAA) was chosen as being similar to and as effective in its growth activity as IAA (HERTEL & FLORY 1968).

For experimental purposes it is preferable to IAA due to its stability. Its movement in tissue is shown to be polar and active (VEEN 1967).

NAA-<sup>14</sup>C was applied in donor blocks of agar gel. The donor blocks were placed on the apical surface of the decapitated maize coleoptile. In this experiment six coleoptile sections, 13 mm long, were used.

These pieces were carefully positioned on the edge of a razor blade, with their split base on two receiver blocks which were placed on either side of the razor blade. The coleoptile sections were then irradiated with 100 Lux light intensity for one second and kept in darkness until used for sampling.

Receiver blocks were sampled for analysis after extended periods of time.

The results of this experiment are shown in *fig. 2*.

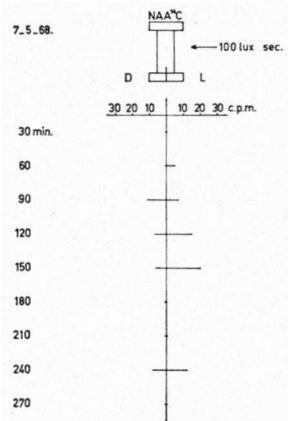


Fig. 2. Time-course of movement of <sup>14</sup>C in 13 mm sections from light and shadow halves of corn coleoptiles. Radioactivity counted in receiver blocks, background (19 cpm) subtracted.

In the top part of *fig. 2* the set-up of the experiment is shown, the coleoptile with the donor block, the two receiver blocks on either side of the razor blade and the irradiation from one side.

Along the straight line the radioactivity in the receiver blocks is demonstrated, in counts per minute, that of the non-illuminated "dark" side "D" being shown on the left part of the line and that of the illuminated "light" side "L" on the right.

From the data shown in *fig. 2* there is an indication that after the first 90 minutes the greater part of the <sup>14</sup>C is found in the receiver blocks on the "dark" side but that gradually, after 150 minutes, more <sup>14</sup>C is found in the receiver blocks on the "light" side.

This suggests a transversal redistribution of the radioactivity after unilateral irradiation.

### 3.2. The proportional distribution of $\text{NAA}^{14}\text{C}$ in the coleoptile after phototropic stimulation

The data from the first experiment indicate that 90 minutes after phototropic stimulation more growth substance is found in the dark side of the coleoptile than in the irradiated side but that at a later period, 120 minutes after irradiation, this is reversed. In order to study the transversal distribution of labelled NAA in phototropically stimulated coleoptiles some experiments were done with the same set-up as that of the first experiment but instead of collecting the  $^{14}\text{C}$  in receiver blocks the coleoptiles were cut lengthwise after extended periods and used for analyses. The sum of the radioactivity found in both halves at a certain time after irradiation was taken as 100 per cent and the relative amounts of the radioactivity in the receiver blocks were expressed as percentages of this total amount. In *fig. 3* the percentages found in both halves after increasing time periods are plotted on the horizontal lines.

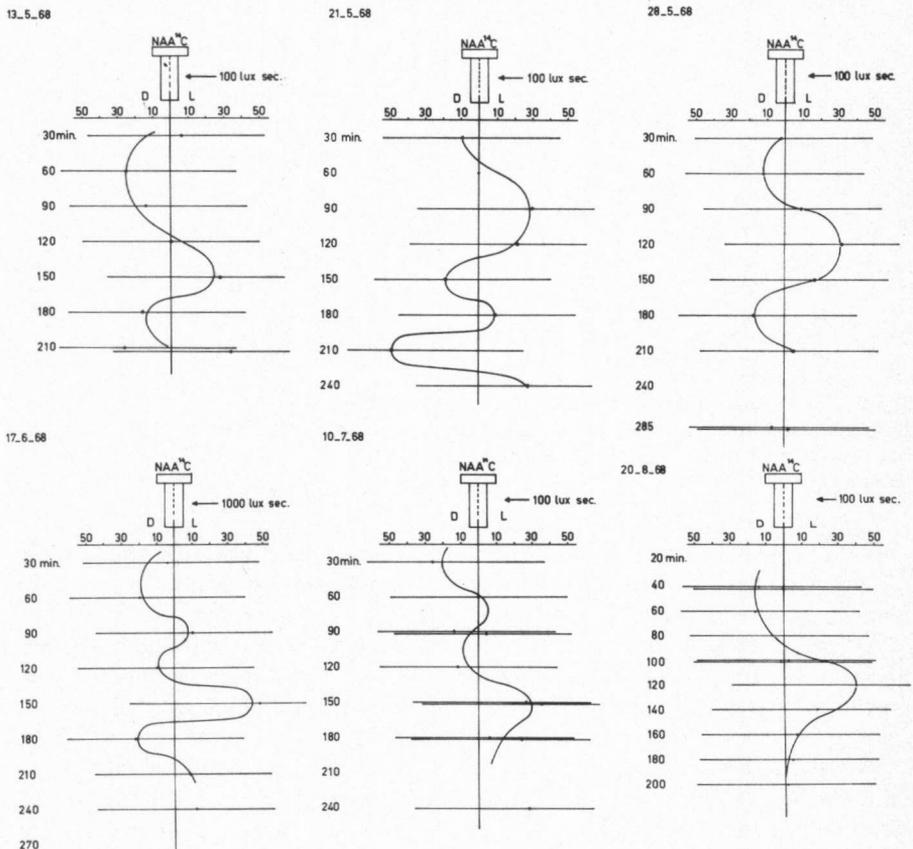


Fig. 3. Time-course of the distribution of  $^{14}\text{C}$  in corn coleoptiles after unilateral irradiation. Sum of radioactivity in both halves D and L taken as 100 per cent. The curve shows the difference of the percentages.

When it is assumed that the relative growth of both sides of the coleoptile is governed by the difference in the amounts of the growth substance present in both halves, this difference is more important than the actual amounts.

The curve shows this proportionate difference of both sides after the various periods.

*Fig. 3* shows six experiments. In most experiments the greater part of the  $^{14}\text{C}$  is found in the non-irradiated side of the coleoptile shortly after irradiation. From the non-irradiated "dark" side the radioactivity shifts to the irradiated side and then back again to the "dark" side. In other words after a short phototropical stimulation the growth substance is redistributed over both sides of the coleoptile through a transversal oscillating transport process.

It must be kept in mind that the same phenomenon can be explained by a different uptake of the growth substance from the donor at the "light" and "shadow" side. Therefore, experiments were carried out with donor blocks applied at different positions.

### 3.3 The influence of asymmetrically applied growth substance and its redistribution after unilateral irradiation

In these experiments the donor blocks with labeled NAA were applied either on the apical surface of the irradiated "L" side or on the apical surface of the non-irradiated "D" side of the coleoptile.

After application of the donor blocks the coleoptiles were irradiated as described earlier.

After irradiation the coleoptiles were left in darkness for prolonged periods before they were sampled for analysis. For this purpose they were cut lengthwise.

In *fig. 4* the set-up of the experiment and the  $^{14}\text{C}$  distribution in the dark "D" side and in the irradiated "L" side is plotted.

*Fig. 4* shows two experiments of both possibilities, two with the donor blocks on the irradiated side and two with the donor blocks on the non-irradiated "dark" side.

It can be seen in *fig. 4* that with the donor blocks on the irradiated side, most of the  $^{14}\text{C}$  is found in the irradiated side throughout the whole experiment but that the radioactivity is also found in the non-irradiated half of the coleoptile.

In the experiment of 7/6/'68 two peaks can be distinguished, one after 120 minutes and the second after 240 minutes.

In the experiment of 24/6/'68 we find one peak after 60 minutes and the second peak after 150 minutes.

In these cases the movement of the radioactivity is away from the light.

The two lower graphs in *fig. 4* show two experiments in which the donor blocks were placed on the non-irradiated side. In these cases the lateral movement of the growth substance occurred towards the light. The data of these four experiments suggest that the lateral movement of the growth substance is enhanced in the direction away from the light and depressed in the direction towards the light. This is in agreement with the results of DELA FUENTE & LEOPOLD (1968).

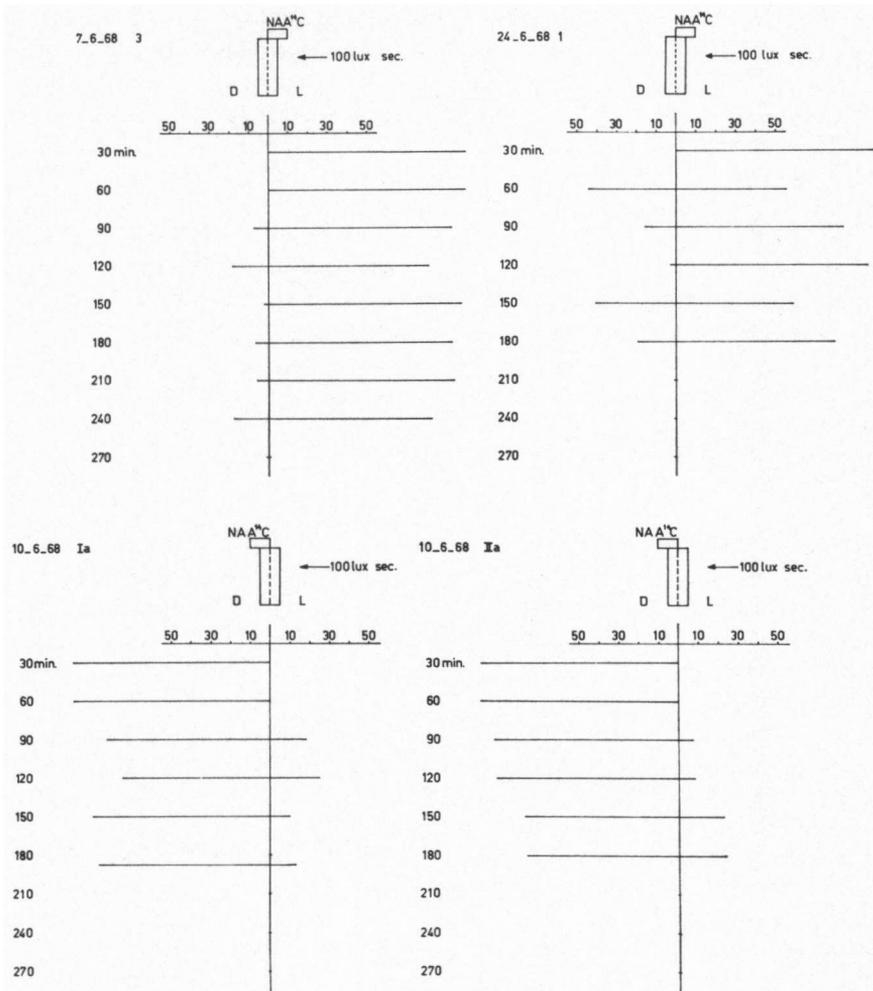


Fig. 4. Time-course of the distribution of  $^{14}\text{C}$  absorbed from  $\text{NAA}^{14}\text{C}$  in donor blocks, asymmetrically applied, after unilateral irradiation. Sum of radioactivity in both halves (D and L) taken as 100 per cent.

Moreover the data confirm the previous experiments, *i.e.* the oscillation of the auxin transport.

**3.4 The distribution of NAA in the non-irradiated coleoptile**  
 The experiments described above all suggest an oscillating redistribution of the growth substance after phototropic stimulation. The question can be posed as to what extent the lateral distribution of the growth substance was influenced by cutting the coleoptiles lengthwise.

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In order to test this, several control experiments were made. In these experiments the set-up was the same as in all the others except that the coleoptiles were not irradiated.

After decapitation and removal of the primary leaves the coleoptiles were put in the holder on the edge of the razor-blade and a donor block with radioactive NAA was applied on the apical surface. The explants were left in darkness until they were sampled for analysis.

For this purpose they were cut lengthwise and analysed in the usual way.

The amount of radioactivity, was plotted as percentages of the total amount in both halves every 30 minutes over a prolonged period; in the same way as was done in the previous experiments.

The results of these experiments are shown in *fig. 5*.

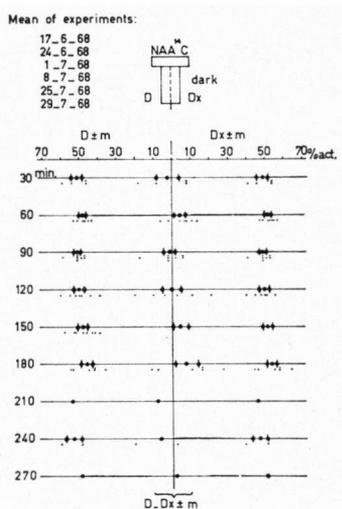


Fig. 5. Time-course of the distribution of  $^{14}\text{C}$ , absorbed from NAA  $^{14}\text{C}$ , in both halves (D and Dx) of non-irradiated corn coleoptiles.

In *fig. 5* the percentages of NAA in the dark halves D and Dx are plotted left and right of the axis.

The differences of these percentages are indicated by the dots along the axis. The mean values of the percentages after each interval are also indicated.

It can be seen that the amount of radioactivity in both halves is  $\pm 50\%$ .

The mean values of the differences of the percentages are irregularly scattered, left and right of the axis.

The oscillating alternation of maxima and minima in the lateral distribution of the growth substance, as seen after irradiation, was not found.

This leads to the conclusion that the transverse oscillating distribution of the radioactivity is due to the irradiation.

### 3.5 One irradiated coleoptile on receiver blocks which were renewed every 30 minutes

In a previous experiment, described under 3.1 a number of coleoptiles were used, the receiver blocks of which were sampled every 30 minutes.

In the experiments described in this section, only one coleoptile was used for each experiment and this was transferred onto new receiver blocks every 30 minutes.

The amount of radioactivity found in the receiver blocks shows how much of the  $^{14}\text{C}$  of the growth substance  $\text{NAA}^{14}\text{C}$  was secreted after, each 30 minutes period from the start of the experiment (the irradiation) and on which side of the coleoptile, dark or irradiated it was to be found.

Altogether 4 experiments were carried out.

The results are shown in *fig. 6*.

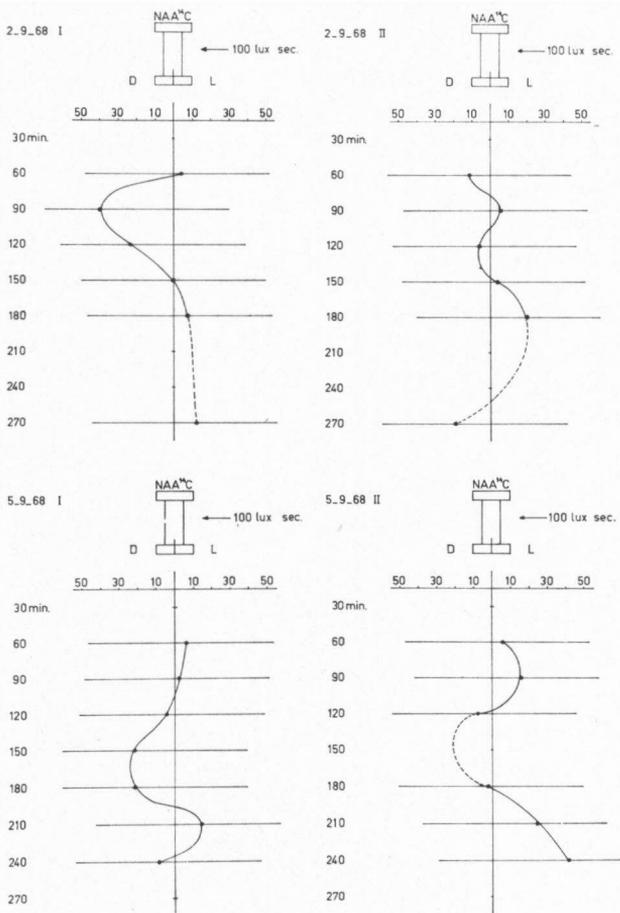


Fig. 6. Time-course of movement of  $^{14}\text{C}$  in unilateral irradiated corn coleoptile. Radioactivity counted in receiver blocks renewed every 30 minutes.

In *fig. 6* the amount of growth substance in the receiver blocks is plotted, as usual, as percentages of the total amount in both blocks after a time period of 30 minutes.

It can also be seen that without cutting the coleoptile the growth substance is transported into the receiver blocks by a transverse oscillating process in which maxima and minima alternate on both sides of the coleoptile.

#### 4. DISCUSSION

It was felt that in order to study the lateral distribution of the growth substance NAA after phototropic induction in maize coleoptiles, it was necessary to analyse the growth substance in the tissues during prolonged periods.

No attempt was undertaken to identify the radioactivity in receiver blocks or in the tissue. There is however evidence that only the unaltered auxin is transported through plant tissue into receiver blocks (VEEN 1966).

The nature of the radioactivity in the tissue is unknown, but it is highly probable that the auxin is at least partly immobilised (GOLDSMITH 1968).

The results obtained from the experiments described in paragraphs 3.1, 3.2, 3.3 and 3.5, allow the conclusion that after a short unilateral irradiation of the coleoptiles a transverse transport of the growth substance away from the light sets in, resulting in a temporary uneven distribution of the growth substance on both sides of the coleoptile. These findings are in agreement with the theory of Cholodny-Went. This asymmetric distribution of the growth substance is followed by a redistribution process.

The experiments with asymmetrically applied donor blocks, described in paragraph 3.3, show, that when the donor was placed on top of the irradiated side the radio activity appeared at certain times in the non-irradiated side of the coleoptile as well, in the form of "waves". When the donor blocks were placed on the non-irradiated side of the coleoptiles the radioactivity also appeared in the irradiated side, in this case against the light.

The experiments show that the redistribution of the growth substance after the light flash can not be explained as a simple diffusion process. The results of the experiments suggest that the irradiation induces a transverse oscillating redistribution of the growth substance.

It must be born in mind, that the basipetal transport of the growth substance in non-irradiated coleoptiles is polar and longitudinally oscillating as was found by HERTEL & FLORY (1968).

Although unilateral irradiation induces a transverse polarity which enhances the transport of the growth substance away from the light and depresses the movement towards the light (DELA FUENTE & LEOPOLD 1968) the normal basipetal transport remains.

This explains the oscillating distribution of the growth substance in both halves of the coleoptiles.

We do not know by what mechanism the asymmetric distribution of the growth substance is accomplished.

The result of the experiments suggest, however, that the unilateral light induction not only induces the transport of the growth substance away from the light but that at the same time a counter-reaction is induced. It is hypothesized that this counter-reaction, depending on the light intensity will give rise to negative phototropic curvatures, as was found earlier by ARISZ in 1915, and which functions as a regulation mechanism.

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