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ON THE INDOLEACETIC ACID CONVERTING ENZYME OF PEA ROOTS AND ITS RELATION TO GEOTROPISM, STRAIGHT GROWTH AND CELL WALL PROPERTIES

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CHAPTER 1

GENERAL INTRODUCTION

If the tip of a growing main root is placed at an angle with gravitation, then the rate of the elongation at the upper side becomes greater than that at the lower side and the tip curves in the direction of this force. This phenomenon is generally known as the positive geotropic reaction (or curvature) of the root. Although it has been described repeatedly, the knowledge of it is still superficial. Information on the detailed course of the reaction and of its physiological background is scanty.

In former experiments the difference of the growth rate at the upper and the lower side of horizontally placed root tips, by which the geotropic curvature is brought about, was not measured as a rule before the curvature was already in an advanced state. It was found then that the upper side of horizontally placed root tips had elongated slightly more than vertical roots in the same medium and time, whereas the elongation of the lower side had been considerably less (e.g. SACHS, 1874).

Until now the almost generally accepted interpretation of this behaviour of the roots is the CHOLODNY–WENT theory (CHOLODNY, 1924; WENT, 1926). According to this theory the growth regulating substance, presumably indoleacetic acid (IAA), would migrate laterally and accumulate at the lower side in geotropically exposed root tips. Since it is postulated that in root tips IAA is present in a supra-optimal concentration, the rate of elongation at this side would decrease and so a downward curvature of the tip would result.

If, however, the elongation at the upper and at the lower side of curving root tips is measured after short time intervals (AUDUS and BROWNBRIDGE, 1957) the course of the geotropic reaction can not be simply explained by the CHOLODNY–WENT theory. Moreover, experiments in which IAA was added made the authors conclude, that it is doubtful whether IAA is the substance governing the positive geotropic curvature of roots. BENNET–CLARK *et al.* (1959) also obtained results which do not match the CHOLODNY–WENT theory.

In the present investigation the question of the applicability of this theory has been approached in the following way.

In pea roots (and in other parts of numerous plants) an enzyme is present, generally indicated as "indoleacetic acid oxidase", which is able to convert indoleacetic acid into an inactive compound.

This enzyme was studied in vitro (cf. TANG and BONNER, 1947, 1948; WAGENKNECHT and BURRIS, 1950; GOLDACRE *et al.*, 1953; GALSTON *et al.*, 1953; RAY, 1958) and probably is active in vivo as well (GALSTON *et al.*, 1953; SIEGEL and GALSTON, 1953; GALSTON and DALBERG, 1954).

If this enzyme actually controls the concentration of IAA in roots, and if IAA is the substance which accumulates at the lower side of a horizontally placed root tip, it is likely that such an accumulation

can only occur, when the activity (or the concentration) of the enzyme is altered.

For that reason the enzyme activity i.e. the rate of conversion of IAA has been studied in the presence of an inhibiting substance i.e. caffeic acid (CA) (cf. RABIN and KLEIN, 1957; GORTNER and KENT, 1958) and of two promoting substances, namely 2,4-dichlorophenol (DCP) (GOLDACRE *et al.*, 1953) and p-cumaric acid (p-cum-A) (GORTNER and KENT, 1958; ELEMA, 1960), *in vitro* as well as *in vivo*.

Subsequently, the effect of DCP, p-cumaric acid and caffeic acid has been studied on the geotropic reaction and on the straight growth of the roots.

The geotropic reaction was followed by measuring the angle of curvature and the growth rates at the upper and at the lower side of the root tips.

It was hoped that the data obtained in this way would elucidate the question whether a correlation exists between the activity of the IAA converting system—affecting the IAA concentration—and the geotropic reaction.

Presumably growth phenomena are closely related with changes of the properties of the protoplasm and of the cell walls. Earlier only few experiments were made on changes of the suction force and of the osmotic value of cells of geotropically curved root tips (URSPRUNG and BLUM, 1924; OVERBECK, 1926) and on changes of the elasticity of the cell wall (HORREUS DE HAAS, 1929). Moreover these observations were made after rather long periods of geotropic exposure of the roots. It seemed therefore worth-while to investigate these items more closely. The changes of the magnitudes mentioned have been determined after short periods (less than 30 minutes, when the geotropic curvature of the roots becomes visible) and after longer periods of geotropic exposure. In connection with the other investigations, the influence of DCP, CA and IAA has been studied on the osmotic magnitudes and on the cell wall properties.

CHAPTER 2

IN VITRO EXPERIMENTS ON THE CONVERSION OF INDOLEACETIC ACID BY ENZYME PREPARATIONS OF PEA ROOTS

2.1. INTRODUCTION

It is generally acknowledged that indoleacetic acid (IAA) disappears from a solution through the action of enzyme preparations made from growing plants or plant parts. The enzyme, which is responsible for this phenomenon, is generally denoted in the relevant literature as indoleacetic acid oxidase, since the conversion of IAA is accompanied by the absorption of oxygen. In several cases it is known that the enzyme in question is a peroxidase. This, e.g., was stated by GALSTON *et al.* (1953), KENTEN (1955), PILET and GALSTON (1955),

RAY and THIMANN (1956) and RAY (1960). In other cases (FÅHRAEUS, 1961) it was shown that a phenol-oxidase is involved.

The term "indoleacetic acid oxidase" therefore is a collective noun, used in all cases in which the active enzyme in a crude enzyme preparation is unknown, the cases where a peroxidase is involved included.

The active component in enzyme preparations from pea roots that converts indoleacetic acid is unknown. It was tried to identify the character of this agent and to certify whether it is a peroxidase or a phenol-oxidase, or whether both enzymes are involved.

GOLDACRE *et al.* (1953) and many authors afterwards used 2,4-dichlorophenol (DCP) to increase the activity of their enzyme preparations, which of their own showed little or no activity. Therefore the effect of DCP on the disappearance of IAA from enzyme preparations of pea roots was studied as well. Moreover p-cumaric acid was tested since several authors consider it as a natural co-factor for the enzyme that converts IAA. GORTNER and KENT (1958) found it to be the case in pineapple and this may hold true for pea roots as well (cf. ELEMA, 1960; SONDHEIMER and GRIFFIN, 1960).

Several times diphenols have been mentioned that inhibit the IAA converting enzyme. One of the most active compounds among these inhibitors is caffeic acid (RABIN and KLEIN, 1957); for that reason the effect of this substance has also been tested.

2.2. MATERIAL AND METHODS

Pea roots, *Pisum sativum* var. "Vlijmse Gele Krombek", were grown in moist sand for 48 hours after the seeds had been soaked in aerated tap water for 20 hours. Then the tips, 5 mm long, were cut off and collected in an ice-cold phosphate-citrate buffer solution, pH 5.0. Subsequently they were ground in a mortar with glowed quartz sand and the pulp centrifuged at 600 g for 10 minutes. In order to remove all larger particles and also the mitochondria and to restrict the number of enzymes as far as possible, the supernatant was centrifuged for a second time at 30 000 g. After the first centrifugation the (turbid) supernatant (I) had the same activity as the pulp itself, whereas that of the resuspended precipitate was negligible. After decanting the supernatant (I) it was centrifuged at 30 000 g for 30 minutes after the addition of sucrose to a final concentration of 0.5 molar. The latter measure was intended to prevent the deterioration of the mitochondria during centrifugation. The precipitate (II), obtained in this way, proved to be almost inactive, whereas the specific activity of the (clear) supernatant (II) equalled that of supernatant (I) or even surpassed it.

The clear supernatant (II) was used as enzyme preparation; it is free from mitochondria, as could be shown by means of the method of SLATER and BONNER (1952). It does not contain any succinic acid dehydrogenase, which is known to be localized in the mitochondria. All activity of succinic acid dehydrogenase proved to be present in the precipitate (II).

The reaction mixture in which the disappearance of IAA (the oxidase activity) was followed contained 1 ml of the enzyme preparation (corresponding to about 6 mg dry weight), IAA in a final concentration of 50 μg/ml and a phosphate-citrate buffer of pH 5.0. The latter was added to a total volume of 10 ml.

The degradation of IAA from this reaction mixture was followed by means of Salkowski reagent (15 ml $FeCl_3$ 0.5 mol. 300 ml H_2SO_4 s.w. 1.84 and 500 ml aq. dest.). This gives a pink-red colour with IAA; the transmission of the light was measured 45 minutes after the addition of the reagent by means of a Zeiss spectrophotometer at 535 mμ. Within certain limits (50–56 μg/ml) the transmission is proportional to the concentration of IAA. The longer the enzym preparation acts upon the IAA the weaker the colour after addition of the Salkowski reagent, until the reaction finally becomes negative. The colour reaction is specific for IAA; other indole derivatives, such as indolepropionic acid and indolebutyric acid, yield a yellow colour with the reagent and indolealdehyde and tryptophane do not give any colour at all with Salkowski reagent.

The peroxidase activity of the enzyme preparation was checked by means of the conversion of pyrogallol. The reaction mixture then contained 1 ml pyrogallol 0.1 mol, 1 ml of the enzyme preparation, 1 ml H_2O_2 0.3 %, buffer solution pH 5.0 with which the final volume was brought to 10 ml.

The intensity of the yellow-brown colour of the purpurogallin formed was estimated 5 minutes after the addition of the enzyme preparation by means of the spectrophotometer at 400 mμ.

When the effect of a certain compound was tested on the activity of the enzyme preparation, 0.1 ml was added in a concentration 100 times higher than the final concentration, desired in the reaction mixture.

The cultivation of the roots and all manipulations during the experiments were carried out in an air conditioned room at 23° C and ca. 80 % relative humidity, in which only orange light (Schott filter OG–2) was used when needed. During the incubation the reaction mixtures were carefully shaken. The amount of IAA converted is indicated in the tables and figures as μg/ml.

2.3. The effect of 2,4-dichlorophenol on the activity of dialyzed and non-dialyzed enzyme preparations

In the investigation of the peroxidase and oxidase activity of the enzyme preparation 2,4-dichlorophenol (DCP) has been applied not only to increase the degradation of IAA but also to compare the effect of DCP on the activity of both enzyme systems themselves. The pH of the reaction mixtures was kept on 5.0 since in the presence of DCP the IAA converting power of the enzyme preparation is highest at pH 5.0. Without DCP the IAA disappears at the highest rate at pH 6.0 to 6.5 but this rate is still considerable at pH 5.0. The peroxidase activity is highest at pH 4.5 to 5.0. In order to

determine which enzyme is most active in the presence of DCP pH 5.0 has been chosen for these experiments.

As is apparent from column 1 and 2 in Table 1, the rate of conversion is notably increased by DCP in a concentration of 10^{-5} g/ml. The conversion of pyrogallol, however, is not affected by DCP. Also in the absence of DCP the enzyme preparation converts IAA (column 1). This activity, however, completely disappears after dialysis of the enzyme preparation (during 20 hours at 2° C in aq. dest.). The addition of DCP to the dialyzed preparation causes again the degradation of IAA in the reaction mixture at a high rate (column 3 and 4). It is generally believed that the activity of the enzyme preparation largely depends on the presence of a natural cofactor, which is washed away by dialysis. DCP would be a suitable substitute for the natural cofactor. As appears from a comparison of the figures of column 3 and 4 the conversion of IAA is dependent on such a cofactor, but the conversion of pyrogallol is not; dialysis has no effect upon the latter.

TABLE 1

The activity of peroxidase and the rate of the degradation of IAA by dialyzed and non-dialyzed enzyme preparations, with and without DCP 10^{-5} g/ml in the reaction mixture. The peroxidase activity is expressed in μg/ml purpurogallin produced in 5 minutes; the degradation of IAA in μg/ml IAA in 30 minutes.

	Non-dialyzed		Dialyzed	
	without DCP	with DCP	without DCP	with DCP
μg/ml purpurogallin produced	92	89	95	90
μg/ml IAA degraded	11.5	40.4	0.0	44.6

2.4. THE INHIBITION OF THE PEROXIDASE ACTIVITY AND OF THE OXIDASE ACTIVITY BY CYANIDE

It is known that cyanides inhibit all enzymes containing a heavy metal at their active site, as is the case with peroxidase which contains Fe^{++}. In the relevant literature it is mentioned that the activity of preparations of IAA oxidase can be completely checked by cyanide in a sufficiently high concentration (TANG and BONNER, 1948; WAGENKNECHT and BURRIS, 1950).

The conversion of IAA by an enzyme preparation of pea roots is almost entirely inhibited by a KCN concentration of 6.6×10^{-5} g/ml and higher. There remains, however, some degradation of IAA, whereas the oxidation of pyrogallol is completely checked (Table 2, column 3). Moreover the oxidation of pyrogallol is almost completely inhibited by a concentration of 2.2×10^{-5} g/ml, whereas the degradation of IAA is decreased only 50 % (Table 2, column 4).

Apparently the peroxidase of the enzyme preparation is more sensitive to KCN than the oxidase to which the IAA converting capacity is ascribed (cf. STUTZ, 1957).

TABLE 2

The inhibition of the peroxidase activity (μg/ml purpurogallin produced in 5 minutes) and of the degradation of IAA (μg/ml IAA converted in 30 minutes) by KCN. DCP 10^{-5} g/ml was added to increase the rate of degradation of IAA; no DCP was added to the control.

	Control	DCP 10^{-5} g/ml	DCP 10^{-5} g/ml + KCN 6.6×10^{-5} g/ml	DCP 10^{-5} g/ml + KCN 2.2×10^{-5} g/ml
μg/ml purpurogallin produced . .	94.0	92.6	0.0	1.4
μg/ml IAA degraded .	16.0	45.7	2.0	24.2

2.5. THE CONVERSION OF IAA UNDER ANAEROBIC CONDITIONS

It appears from the preceding sections that DCP only increases the rate of conversion of IAA and does not affect the oxidation of pyrogallol. The conversion of IAA is accompanied by the uptake of oxygen. Under anaerobic conditions no conversion of IAA occurs (TANG and BONNER, 1948; WAGENKNECHT and BURRIS, 1950; WAYGOOD *et al.*, 1956). DCP therefore should not have an effect on the degradation of IAA under anaerobic conditions (Table 3).

In these experiments IAA with or without additional compounds was pipetted in the main vial of a Warburg-manometer and the enzyme preparation in the side arm. After the replacing of the air in the manometer by nitrogen, the enzyme preparation was transferred into the main vial.

TABLE 3

The degradation of IAA under aerobic and anaerobic conditions and the effect of several compounds on the rate of it. The figures indicate μg/ml IAA degraded after 60 minutes.

Addition	Aerobic	Anaerobic
none (control)	20.7	0.0
DCP 10^{-5} g/ml	42.4 *)	0.0
KCN 6.6×10^{-5} g/ml	0.0	0.0
H_2O_2 0.03 %	19.5	16.6 **)

*) Conversion relatively small as compared with the control. After about 30 minutes in the presence of DCP the course of the degradation of IAA cannot be followed further colorimetrically in contrast to the control.

**) Oxygen is produced, possibly by the presence of katalase in the enzyme preparation.

Apparently no IAA is converted in the absence of oxygen. Also the acceleration of this conversion by DCP needs oxygen.

Hydrogen peroxide has no effect on the conversion of IAA under aerobic conditions; the colour reaction with Salkowski reagent is not influenced by it in the concentrations applied (cf. PLATT and THIMANN, 1956; SIEGEL and WEINTRAUB, 1952). The results reported in the last three sections can be summarized as follows:

1. The enzyme preparations of pea roots have the capacity to convert pyrogallol in the presence of H_2O_2. This reaction is not affected by DCP or by dialyzing the enzyme preparation. It is completely inhibited by 2.2×10^{-5} g/ml KCN.
2. They degrade IAA. This process needs oxygen and its rate is strongly increased by DCP. By dialysis a substance is removed from the enzyme preparation which is indispensable for the conversion of IAA. Addition of DCP restores the capacity to convert IAA. H_2O_2 has no effect on the degradation of IAA. KCN in a concentration of 2.2×10^{-5} g/ml inhibits this process about 50 %.

Enzyme preparations of pea roots therefore show peroxidase activity as well as oxidase activity. The degradation of IAA must be ascribed to the oxidase component.

2.6. THE PRESENCE OF A PHENOL-OXIDASE IN THE ENZYME PREPARATIONS

From the relation between DCP (or another monophenol GOLDACRE *et al.*, 1953) and the conversion of IAA the question arose whether the enzyme preparation has the capacity to oxidize phenols, i.e. whether it contains a phenol-oxidase. For the investigation of this question use has been made of the fact that the oxidation products of many phenols, the quinones, readily form complexes with amino acids, which have a red colour.

The test was as follows: 0.5 ml of the enzyme preparation was added to a solution containing 0.5 ml catechol (10 %) and 0.5 ml proline (2 %). After 10 to 20 minutes at 25 to 30° C a bright red colour could be observed, which has been accepted as a proof for the presence of a phenol-oxidase (cf. the method of DRAWERT and GEBBING, 1963).

This observation and the results mentioned in the preceding sections make it likely, that the degradation of IAA is due to the action of a phenol-oxidase.

2.7. THE EFFECT OF 2,4-DICHLOROPHENOL AND P-CUMARIC ACID ON THE DEGRADATION OF INDOLEACETIC ACID IN VITRO

If DCP in a concentration higher than 10^{-8} g/ml is added to a reaction mixture containing IAA, enzyme preparation and buffer solution, the rate of conversion of IAA is increased. This effect is greatest with a DCP concentration in the reaction mixture of

10^{-5} g/ml (Fig. 1). The enzyme preparation can also convert IAA without the addition of DCP, but this capacity is lost by dialysis (section 2.3, Table 1). It is restored and even increased, however, after addition of DCP, which is considered as an adequate substitute of the naturally occurring cofactor. The latter could be p-cumaric acid (see section 2.1). Indeed p-cumaric acid proved to accelerate the conversion of IAA with a maximum effect in a concentration of 10^{-6} g/ml in the reaction mixture (Fig. 1). The activity of a dialyzed enzyme preparation is also restored by the addition of p-cumaric acid.

The maximum acceleration of the conversion of IAA that can be obtained is higher with DCP than with p-cumaric acid (Figs. 1 and 2).

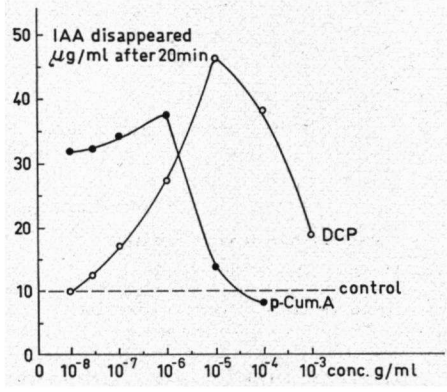

Fig. 1. The effect of the concentration of DCP and p-cumaric acid on the disappearance of IAA.

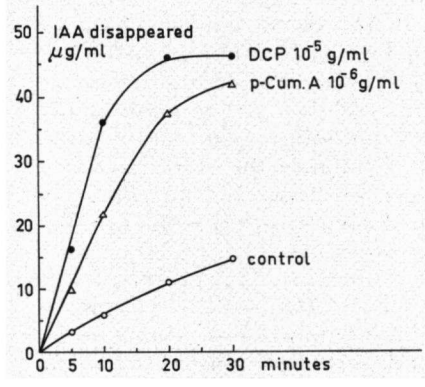

Fig. 2. Progress curves for the action of the IAA converting enzyme with DCP and p-cumaric acid and without addition.

The data thusfar obtained lead to the supposition that the added phenols act as a substrate of the phenol-oxidase present in the enzyme preparations. This has been tested by the addition of DCP and of p-cumaric acid to the dialyzed enzyme preparation at different times

before the addition of IAA. It showed that the rate of IAA conversion was higher after a pre-incubation of the phenols mentioned than when the phenols were added simultaneously with the IAA to the enzyme preparation. The highest rate of degradation of IAA was obtained at a pre-incubation time of 60 minutes with 10^{-5} g/ml DCP or 10^{-6} g/ml p-cumaric acid preceding the addition of IAA. With longer pre-incubation times the enhancing effect decreased again (Fig. 3).

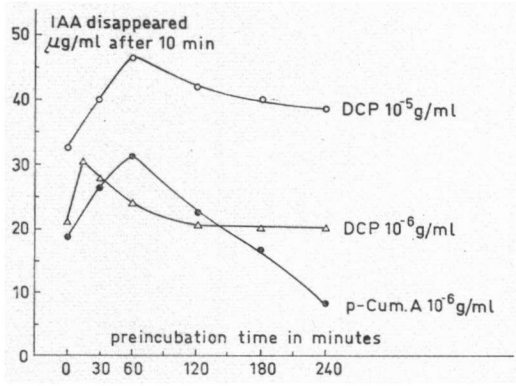

Fig. 3. The effect of various periods of pre-incubation with DCP and p-cumaric acid on the subsequent disappearance of IAA.

With DCP in a concentration of 10^{-6} g/ml the highest rate of conversion of IAA was reached already after a pre-incubation time of 15 minutes. The addition of p-cumaric acid 10^{-6} g/ml more than 2 to 3 hours prior to the addition of IAA resulted in a slower conversion of IAA than if p-cumaric acid had been added simultaneously with IAA. In Fig. 3 the amounts of IAA converted are shown after an incubation during 10 minutes. The course of the graphs after incubation times of 5 to 20 minutes is in principle similar.

Possibly DCP and p-cumaric acid are transformed by the enzyme into compounds that enhance the conversion of IAA in the reaction mixture. After a certain lapse of time the concentration of these compounds seems to decrease and consequently the rate of degradation of IAA becomes slower.

Quite different results after a pre-incubation with DCP were described by FURUYA and GALSTON (1961). If DCP had been added to a dialyzed enzyme preparation of plumules of etiolated pea shoots or of peas irradiated with red light, this had no effect on the conversion of IAA added afterwards. Pre-incubation with DCP in a non-dialyzed enzyme preparation of the same origin caused a decrease of the rate of the conversion of IAA, which became greater as the pre-incubation time had been longer. The inhibiting effect of DCP could be annulled by the addition of Mn^{++}. Therefore DCP and Mn^{++} ions had an antagonistic effect in this case.

From investigations of WAGENKNECHT and BURRIS (1950), WAYGOOD

et al. (1956), HILLMAN and GALSTON (1956) and STUTZ (1957) and from own experiments (Fig. 4, method as described in section 2.2)

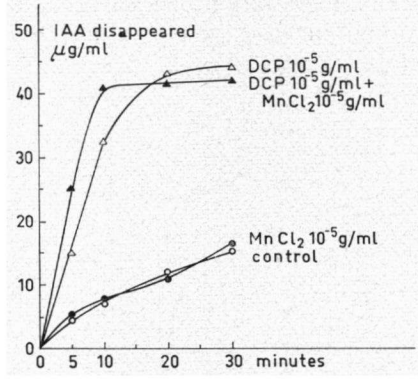

Fig. 4. The effect of Mn^{++} on the disappearance of IAA with and without DCP in the reaction mixture.

it appears that the combined effect of DCP and Mn^{++} ions is larger than that of each substance apart. Manganese ions increase in our and in other experiments (KENTEN, 1955; HILLMAN and GALSTON, 1956) the rate of degradation of IAA only if DCP or a natural cofactor is present in the enzyme preparations.

2.8. THE EFFECT OF CAFFEIC ACID ON THE DEGRADATION OF IAA IN VITRO

The conversion of IAA by enzyme preparations of pea roots is inhibited after the addition of caffeic acid in not too low a concentration. This was also reported for a number of enzyme preparations of different origin (RABIN and KLEIN, 1957; STUTZ, 1957; GORTNER and KENT, 1958; VARGA and KÖVES, 1962). The disappearance of IAA from the enzyme preparation is even entirely stopped when the concentration of caffeic acid in the reaction mixture amounts to 10^{-6} g/ml or higher, whereas it has no effect in concentrations of 10^{-8} g/ml or less (Fig. 5).

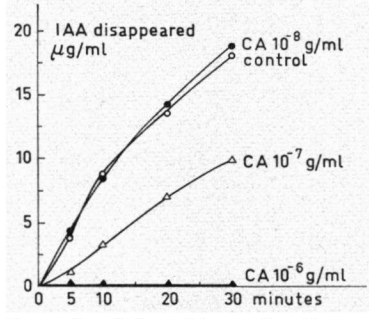

Fig. 5. The effect of caffeic acid on the disappearance of IAA.

If caffeic acid in a concentration that stops the degradation of IAA (10^{-6} g/ml) and DCP in a concentration (10^{-5} g/ml) that produces a maximum rate of the degradation of IAA are added simultaneously to an IAA containing enzyme preparation, DCP has no notable effect (Fig. 6).

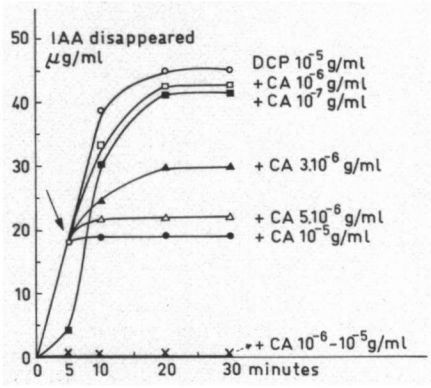

Fig. 6. The effects of various concentrations of caffeic acid added simultaneously with, or 5 minutes after, DCP to the reaction mixture.

If DCP 10^{-5} g/ml and caffeic acid 10^{-7} g/ml are added simultaneously the disappearance of IAA is retarded in the beginning, but after some time it reaches the same rate as when DCP alone would have been present (Fig. 6). The reaction overcomes the inhibiting effect of caffeic acid.

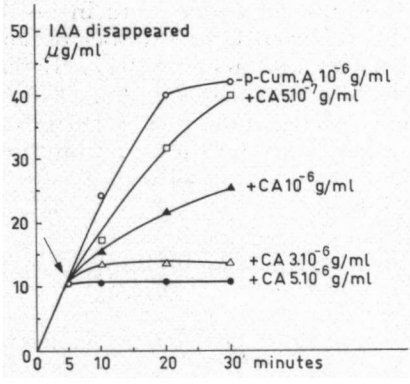

Fig. 7. The effects of various concentrations of caffeic acid added to the reaction mixture 5 minutes after p-cumaric acid.

The enhanced degradation of IAA, once started by DCP or p-cumaric acid, can be retarded or checked by caffeic acid. The measure in which this occurs depends on the concentration of the added caffeic acid (Figs. 6 and 7). The addition took place 5 minutes

after the reaction had started. In this case the inhibiting effect of caffeic acid was smaller than if caffeic acid in the same concentration had been added simultaneously with DCP (Fig. 6). It may be concluded that the inhibition of the conversion of IAA by caffeic acid is hampered notwithstanding DCP or p-cumaric acid are already in contact with the enzyme.

It appears that the rate of degradation of IAA depends on the ratio of the concentrations of caffeic acid and DCP or p-cumaric acid in the reaction mixture. This suggests a competition between caffeic acid and DCP or p-cumaric acid for some active site of an enzyme. RABIN and KLEIN (1957) reported a competitive inhibition of IAA oxidase by chlorogenic acid, of which caffeic acid is the active component.

2.9. DISCUSSION

In enzyme preparations of tips of pea roots probably a phenol-oxidase is present. The results of the experiments reported in this chapter yield a number of arguments which indicate that this enzyme occupies a key position in the degradation of IAA.

1. The degradation of IAA, which does not occur in dialyzed enzyme preparations, is resumed after the addition of DCP or p-cumaric acid. Apparently this conversion depends on the presence of some monohydroxyphenol.
2. These monophenols are transformed probably by the enzyme preparation into compounds that accelerate the conversion of IAA.
3. Dihydroxyphenols, like caffeic acid, inhibit the conversion of IAA, probably because they compete for the active sites of the enzyme with monophenols, of which the reaction products are indispensable for the degradation of IAA.
4. No IAA is degraded under anaerobic conditions.

The compounds produced by the phenol-oxidase are unknown, but possibly the monophenols are transformed into dihydroxyphenols, resp. quinones. The latter substances may act as hydrogen acceptors and be reduced again to dihydroxyphenols. According to KENTEN and MANN (1950) and KENTEN (1955) quinones can be reduced by Mn^{++} ions, which would accelerate the conversion of IAA. In the case of the enzyme in preparations of pea roots Mn^{++} accelerates the IAA degradation in the presence of a monophenol.

Therefore, in enzyme preparations of pea roots the existence of an oxido-reduction cycle in which Mn^{++} ions and phenols are involved, as supposed by KENTEN and MANN and KENTEN and endorsed by GOLDACRE (1961) for other enzyme preparations, must not be excluded. Besides, a disappearance of IAA as stated by LEOPOLD and PLUMMER (1961), where the oxidation products of diphenols (the quinones) appeared to form "complexes" with IAA, must be kept in mind.

The peroxidase activity of the enzyme preparations should be distinguished from the activity of the phenol-oxidase. This does, however, not include that these activities should be ascribed to

different enzymes. With enzyme preparations of *Lupinus*, which show much resemblance with those of pea roots, STUTZ (1957) did not succeed in separating the peroxidase and oxidase activity by means of electrophoresis. Both activities may be localized in the same enzyme (cf. also RAY, 1960).

Whatever the case may be, the degradation of IAA by enzyme preparations is connected with the transformation of monophenols. The products of this reaction probably cause the conversion of IAA.

In the next chapter will be reported whether the system found *in vitro* is also active *in vivo*.

CHAPTER 3

THE ACTION OF THE INDOLEACETIC ACID CONVERTING SYSTEM OF PEA ROOTS IN VIVO

3.1. INTRODUCTION

In the preceding chapter the system has been described through which IAA is converted by an enzyme preparation of root tips of peas. If this system has a physiological meaning it should have the same capacity in intact root tissue.

In several cases the conversion of IAA in vivo has been demonstrated. GOLDACRE *et al.* (1953) and GALSTON and DALBERG (1954) determined the decrease of the IAA concentration in solutions in which sections of pea shoots had been put. If these sections first had been in a DCP solution (GOLDACRE *et al.*) the rate of the decrease of the IAA concentration was accelerated. SIEGEL and GALSTON (1953) estimated the uptake and the binding of IAA by tips of pea roots. From the difference between the quantities of IAA present in the medium and present in the root tips as "free" and "bound" IAA they calculated the amount of converted auxin. In these cases the degradation of IAA has been determined indirectly.

In the following experiments a direct method has been applied. The disappearance of IAA from tips of pea roots has been followed and the effect on this process of the phenols DCP, p-cumaric acid and caffeic acid has been studied. In this way it was certified whether the system through which IAA disappears from *in vitro* enzyme preparations also operates *in vivo*.

3.2. THE CONVERSION OF IAA IN ROOT TIPS OF PEAS

The natural IAA concentration in root tissue is too low to be determined by means of Salkowski reagent. The latter does not yield a red colour with intact root tips nor with a crude pulp of them. Therefore IAA must be taken up by the root tips in a fairly high concentration before the degradation of it can be studied.

The experiments ran as follows: root tips (50 tips, 5 mm long) were transferred into an IAA solution (5 ml; 10^{-4} g/ml) and remained in it for different times. Then they were rinsed in aq. dest. and

ground in a mortar in 1 ml ice-cold aq. dest. Subsequently 1 ml trichloro-acetic acid (5 %) was added in order to precipitate the proteins. After centrifuging to eliminate the larger particles the supernatant was decanted and Salkowski reagent was added to the supernatant and to the precipitate (cf. SIEGEL and GALSTON, 1953).

In another series of experiments the root tips remained for one hour in the IAA solution and were then transferred into aq. dest. in which they were kept during different times before they were treated in the way described above. Table 4 gives the results.

It appears that the IAA taken up by the root tips disappears; the detectable amount of auxin, as well in the supernatant as in the precipitate, decreases with time even when the root tips remain in the IAA solution.

TABLE 4

The conversion of IAA in root tips, (I) continuously kept in an IAA solution of 10^{-4} g/ml and (II) after pretreatment with IAA (1 hour) kept in aq. dest.

Hours in		µg/ml IAA in supernatant		Colour of the precipitate after addition of Salkowski	
IAA	aq. dest.				
I	II	I	II	I	II
1	—	20	—	strongly red	—
2	1	8	5	faintly red *)	faintly red
—	2	—	0	—	very faintly red
4	3	7	0	faintly red	very faintly red

*) The indication "faintly red" resp. "very faintly red" means that these colours do not differ notably when compared.

3.3. THE EFFECT OF DCP AND P-CUMARIC ACID ON THE DISAPPEARANCE OF IAA FROM ROOT TIPS

It was tested whether the connection between the disappearance of IAA and the presence of DCP and p-cumaric acid, as reported in Chapter 2, also exists in root tips. 250 root tips (5 mm long) were placed for 1 hour in an IAA-solution (5 ml; 10^{-4} g/ml) and then divided in 5 samples of 50 tips each. One sample was immediately desintegrated and treated as described in section 3.2. The second was transferred into aq. dest. and kept in it for 1 hour. The other samples were put in a DCP solution of 10^{-5} g/ml for resp. 15, 30 and 60 minutes. After these times the root tips were ground and treated as described in section 3.2.

In another experiment the root tips, after the pretreatment with IAA, were put in a solution of p-cumaric acid 10^{-6} g/ml. The results are given in Table 5.

It appears that the rate of disappearance of IAA, taken up by the root tips, is increased by a treatment with DCP or p-cumaric

acid. Apparently the degradation of IAA in root tips is controlled by the same system as found in *in vitro* experiments.

TABLE 5

The effect of DCP and p-cumaric acid on the disappearance of IAA in tips of pea roots

Treatment after 1 hr pre-treatment with IAA 10^{-4} g/ml	µg/ml IAA in supernatant	Colour of precipitate after addition of Salkowski
immediately ground	15	strongly red
60 min. in aq. dest.	5	moderately red
15 min. in DCP 10^{-5} g/ml	0	I) faintly red
30 min. in DCP 10^{-5} g/ml	0	II) fainter red than I
60 min. in DCP 10^{-5} g/ml	0	III) fainter red than II
60 min. in p-cumaric acid 10^{-6} g/ml.	0	same colour as II

3.4. THE UPTAKE OF DCP BY AND ITS CONVERSION IN TIPS OF PEA ROOTS

From the effect of DCP on the disappearance of IAA in root tips follows, that DCP is taken up by these tips. The rate of this uptake was studied as follows. 80 seedlings were placed for one hour with their roots in DCP solutions. Then the roots were rinsed with aq. dest. and the tips, 5 mm long, were cut. From these tips enzyme preparations were made (2 ml in buffer pH 5.0) as described in section 2.2. One ml of the enzyme preparation was added to an IAA solution of 5×10^{-5} g/ml (total volume 10 ml) and then the disappearance of IAA was followed by means of Salkowski reagent (cf. section 2.2). The results are presented in Fig. 8. Pretreatment with DCP *in vivo* gives an increase of the rate of disappearance of IAA from the enzyme preparations *in vitro*. DCP concentrations lower than 10^{-7} g/ml, however, have no effect. A comparison of the rate of disappearance of IAA in enzyme preparations of root tips treated with DCP (Fig. 8) with that of enzyme preparations to which DCP

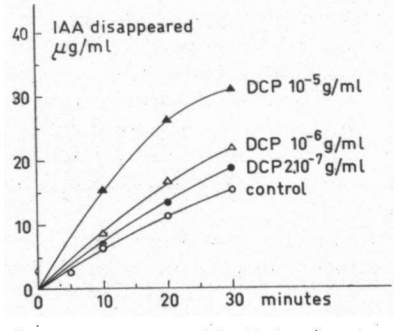

Fig. 8. The effect of pretreatment with DCP *in vivo* on the conversion of IAA *in vitro*.

was added *in vitro* (Fig. 1) shows that in the first case a treatment of the roots with 10^{-5} g/ml DCP gives a conversion of 31 μg/ml IAA after 30 minutes (Fig. 8) which equals the amount found for 10^{-6} g/ml DCP when added *in vitro* (Fig. 1).

One ml enzyme preparation from 40 treated root tips, diluted 10 times in the reaction mixture (10 ml) therefore is equivalent with 10^{-6} g/ml DCP. The 40 root tips thus contained 10 μg DCP, i.e. 0.25 μg per tip.

The volume of a tip is about 4 mm^3. The DCP concentration within a root tip therefore is about 62 μg/ml, whereas that of the surrounding solution was 10 μg/ml. Apparently there is a notable accumulation of DCP in the root tips.

In these experiments the roots were pretreated with DCP during one hour. It was found that the effect on the rate of degradation of IAA is the same for a pretreatment with DCP (10^{-5} g/ml) during ½, 1, 2, 3 and 5 hours. Evidently the concentration of DCP (or its reaction products) taken up by the roots remains high for a long time.

From the following experiments it is clear, that DCP is converted in the root tissue. Root tips (each lot 100, 5 mm long) were placed for resp. 0, 1, 2 and 4 hours in 5 ml DCP solution 5×10^{-5} g/ml, thus containing 250 μg DCP each. They then were ground and treated as described in section 3.2. In the supernatant the amount of phenols, more properly that of reducing substances (except for the control mainly DCP) was determined by means of the reagent of FOLIN–CIOCALTEU. The commercial product (B.D.H.–England) was diluted with the same volume aq. dest. To 0.5 ml of the supernatant 0.1 ml of the diluted reagent and 1 ml 2 % Na_2CO_3, dissolved in 0.1 N NaOH, were added. In the presence of phenol after some time a bright blue colour appears, the intensity of which was measured spectrophotometrically at $\lambda = 750$ mμ.

In Table 6 the results are presented.

TABLE 6

The quantity of phenols (DCP) present in extracts from root tips kept for different times in a DCP solution of 5×10^{-5} g/ml. The figures indicate μg DCP in 0.5 ml of the extracts.

	Hours in DCP 5×10^{-5} g/ml			
	0	1	2	4
μg DCP in 0.5 ml extract	4	50	40	15

It is apparent that a substantial part of the DCP taken up by the root tips is converted into non-reducing compounds. The results may be slightly influenced by reducing substances already present (control: time 0) or newly produced, but the diminishing of the DCP content is clear. The colour of the reagent was not affected by the addition of trichloro-acetic acid or aq. dest.

The conversion of DCP in the root tips endorses the view (cf. 3.3) that the degradation of IAA in the presence of DCP *in vivo* is mediated by the same enzyme system as *in vitro*.

3.5. The inhibition of the conversion of IAA in root tips by caffeic acid

The influence of caffeic acid on the disappearance of IAA in root tips was investigated in a similar way as has been described for DCP and p-cumaric acid. Root tips (200, 5 mm long) were kept for one hour in 5 ml IAA solution 10^{-4} g/ml, then rinsed in aq. dest. and divided in 4 samples, one of which was kept for 1 hour in aq. dest., the others were put in solutions of caffeic acid of resp. 10^{-5}, 10^{-6} and 10^{-7} g/ml.

Subsequently the treated root tips were rinsed in aq. dest., ground and treated as described in section 3.2. The results are collected in Table 7 and show clearly that IAA disappears during a stay of the root tips in aq. dest. but not from root tips which were put in solutions of caffeic acid, even not in a concentration of 10^{-7} g/ml, which *in vitro* inhibits the conversion of IAA for 50 % only (2.8 and Fig. 5).

Table 7

The effect of caffeic acid on the disappearance of IAA in root tips of peas.

Treatment after 1 hr pre-treatment with IAA 10^{-4} g/ml	µg/ml IAA in supernatant	Colour of precipitate after addition of Salkowski
immediately ground	18	strongly red
1 hr in aq. dest..	5	moderately red
1 hr in caffeic acid 10^{-5} g/ml. . . .	18	strongly red
1 hr in caffeic acid 10^{-6} g/ml. . . .	16	strongly red
1 hr in caffeic acid 10^{-7} g/ml. . . .	18	strongly red

3.6. The uptake of caffeic acid by tips of pea roots

The fact that caffeic acid completely inhibits the degradation of IAA in root tips in concentrations of an order of magnitude lower than *in vitro* suggests, that this compound is accumulated in the root tips. This was tested in the following experiments.

Seedlings, 48 hours old, were placed with their roots in solutions of caffeic acid in concentrations of resp. 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9} g/ml for 1 hour. Then tips of 5 mm length were cut off and enzyme preparations were made (cf. section 2.2). The inhibiting power of these preparations on the degradation of IAA is represented in Fig. 9. It is apparent that caffeic acid in a concentration of 10^{-7} g/ml, if applied to the root tips, completely stops the conversion of IAA *in vitro* and that 10^{-8} g/ml caffeic acid even exerts a clear inhibition,

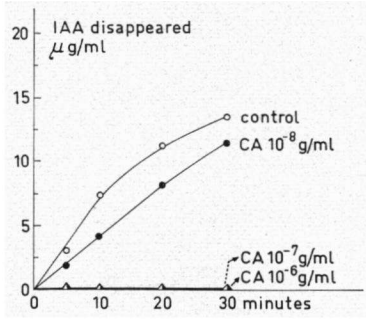

Fig. 9. The effect of pretreatment with caffeic acid *in vivo* on the conversion of IAA *in vitro*.

whereas this concentration when applied *in vitro* has no effect at all (cf. Fig. 5).

This proves that caffeic acid is accumulated in the root tips, or, as another possibility, that it is converted by the root tips after its uptake into a compound that has a greater inhibiting power than caffeic acid itself (not published data support this).

3.7. Changes in the IAA converting capacity of pea roots and other tissues effected by addition of IAA from outside

It was found that, when 48 hours old seedlings are placed with their roots during 3, 6, 12 or 24 hours in solutions of IAA 10^{-8} to 5×10^{-8} g/ml, the IAA converting power of enzyme preparations of these root tips is not altered. The same holds true for IAA concentrations of 10^{-5} and 10^{-6} g/ml, when applied during 3 and 6 hours.

This statement is different from that of Galston and Dalberg (1954) who found an influence of pretreatment of sections of pea epicotyls with IAA. If pretreated during 10 to 20 minutes with an IAA solution (optimum concentration 10^{-7} molar) sections cut from the young parts showed an increased power to convert IAA, which was also the case with crude homogenates of such sections.

Sections cut from older parts of the epicotyls did not show this effect of such a pretreatment. This is explained by the authors by assuming that older parts had already for some time a natural supply with IAA and therefore are adapted to eliminate IAA, whereas young parts need 10 to 20 minutes to acquire this capacity. They suppose that in the latter case IAA first has to induce the formation of an adaptive enzyme, the IAA oxidase. This, however, is not the only possible explanation of the experimental results. It is not excluded that either the content of substances that inhibit the conversion of IAA is higher in young parts than in older sections, or that of substances that increase the rate of that conversion higher in older than in young sections.

Audus and Bakhsh (1961) found, different from the reported own

results, that the IAA converting capacity in pea roots is increased by a pretreatment with IAA (or triiodobenzoic acid). They could, however, not find any induced synthesis of an enzyme.

It seems more probable that an increased capacity to convert IAA after pretreatment with IAA might be due either to the removal of an inhibitor from the enzyme, as can be deduced from experiments of PILET (1959), or to an increase of the concentration of natural cofactors. The latter has been demonstrated by GARAY (1962), who found in *Lupinus* that after a pretreatment with IAA the concentration of monophenols (that enhance the rate of conversion of IAA) had increased.

3.8. THE EFFECT OF GEOTROPIC INDUCTION ON THE IAA CONVERTING ACTIVITY OF PEA ROOT TIPS

From the preceding sections it is clear that the rate of degradation of IAA in root tips is dependent on the presence in the tissue of monophenols, enhancing this rate, and of diphenols, decreasing this rate. The possible physiological meaning of this IAA converting system was shown by the fact that it can be changed through the addition of substances from outside. This meaning, however, would be elucidated if it could be demonstrated that this system also alters when the rate of the growth of an organ changes, the latter fact being ascribed to a change in the auxin concentration in the growing tissue.

According to the CHOLODNY–WENT theory such a change would be responsible for geotropism. This theory postulates a lateral shift of the auxin transport in horizontal root tips so that auxin accumulates at the lower side. It is likely that such an accumulation can be maintained only if the activity of the IAA converting system is lowered at the lower side. Therefore it was investigated, whether the geotropic induction affects the IAA converting system.

One lot of 100 pea roots was placed horizontal and another lot remained in the vertical position. After 20 to 40 minutes tips of 5 mm length were cut off and enzyme preparations made in the usual way (see section 2.2). Then the IAA converting capacity of these preparations was tested.

The results of 6 experiments were averaged and plotted in Fig. 10. In all experiments the rate of degradation of IAA by the action of enzyme preparations of vertical root tips surpassed that of preparations of horizontal root tips. These data and those of the preceding sections together make it likely that in the horizontal position either the concentration of inhibiting substances increases or that of cofactors of the IAA converting system decreases.

If in the horizontal position IAA actually accumulates at the lower side of the root tips, the rate of the IAA conversion should be more decreased at that side than at the upper side.

This was investigated in a series of experiments, in which lots of 150 pea roots were placed either vertical or horizontal for 20 to

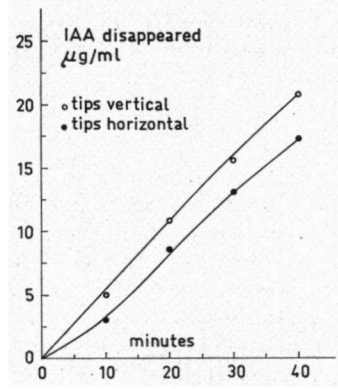

Fig. 10. The influence of geotropic induction on the activity of the IAA converting enzyme of pea root tips.

40 minutes. With a special apparatus the root tips then were cut off and simultaneously split into a lower and an upper half. The same equipment was used for the vertical roots which therefore were split also into longitudinal halves. All halves were kept apart and then collected to make enzyme preparations and to test these on their IAA converting capacity in the usual way. In Table 8 the averaged results of 6 experiments are presented. From these it appears that the rate of degradation of IAA by enzyme preparations of the lower as well as of the upper halves of the horizontal roots is less than that effected by preparations of the halves of the vertical roots. Further, as an average, the rate of IAA conversion in enzyme preparations of the lower halves is lower than that of preparations of the upper halves. In 2 of the 6 experiments, however, no differences were found. This probably has to be ascribed to the inevitably laborious method. During the cutting of the root tips and the collecting of the halves (1 to 2 minutes for 5 roots) differences might fade away by reactions at the large wound surfaces.

TABLE 8

The degradation of IAA by enzyme preparations of the upper and lower halves of tips of horizontally placed pea roots. As control similar preparations of halves of longitudinally split vertical roots were prepared. The figures indicate µg/ml IAA degraded.

Incubation time in minutes	Tips vertical		Tips horizontal	
	I	II	upper half	lower half
10	8.1	8.2	6.5	4.6
20	13.6	13.8	10.9	8.2
30	21.0	21.0	17.0	15.0
40	24.5	23.3	22.2	22.0

In conclusion it can be said that the activity of the IAA converting system is decreased in the horizontal root tips. This indicates that this system is of physiological interest.

3.9. Discussion

From the effect of DCP, p-cumaric acid and caffeic acid on the degradation of IAA in tips of pea roots can be concluded that the conversion of IAA in root tips is mediated by the same system as found for enzyme preparations *in vitro*. Moreover it was found that also DCP is converted in root tips, as is the case in enzyme preparations *in vitro*. It is therefore probable that in pea roots the concentration of IAA as well as that of mono- and diphenols is governed by the same enzyme. This, generally indicated as "IAA oxidase", probably is a phenol-oxidase (cf. Chapter 2).

The results reported in this chapter affirm in principle those of Goldacre *et al.* (1953) and of Siegel and Galston (1953). They give moreover a more accurate presentation of the mechanism by which IAA is eliminated. Quite recently Zenk and Müller (1964) have demonstrated also in *Avena* coleoptiles an *in vivo* active IAA converting enzyme system.

The fact that the rate of conversion of IAA is affected, i.e. decreased, by a geotropic induction, indicates that the IAA converting system is of physiological interest. It further suggests that diphenols (caffeic acid) which inhibit the IAA conversion, and monophenols (p-cumaric acid, DCP) which enhance it, are involved in the changes of the growth rate resulting in a geotropic curvature.

So, an unequal distribution of auxin can probably be acquired by a different enzymactivity at the opposite sides of horizontal root tips, thus without lateral transport.

In the next chapter the results will be reported of experiments on the geotropism of pea roots and on the effect of DCP, p-cumaric acid and caffeic acid on the geotropic reaction.

Chapter 4

THE GEOTROPIC REACTION AND THE GROWTH OF PEA ROOTS IN DIFFERENT MEDIA

4.1. Introduction

It was intended to investigate whether a relation could be found between the geotropic reaction and the straight growth of pea roots and the IAA converting system. The latter therefore was influenced by the addition of DCP, p-cumaric acid and caffeic acid to the medium of the roots.

For a comparison of geotropic responses under different conditions information was needed on the course of the geotropic curvature under standard conditions. To this purpose observations of the

curving organs must be made after short intervals. In the majority of the older experiments on geotropism only the final curvature after a rather long time has been measured. Positive and negative phases during the geotropic reaction, as e.g. found by RUFELT (1957) and by AUDUS and BROWNBRIDGE (1957), can only be detected by repeated measurements after short intervals.

AUDUS and BROWNBRIDGE did not only follow the geotropic curvature but they also measured the growth rate at the upper- and lower side of the curving roots. By doing so they obtained information on the way in which a positive and a negative phase during the reaction originated. Since their results do not match the CHOLODNY–WENT theory and are important in connection with the following sections, they are briefly resumed. After a geotropic exposure in the horizontal position during 40 minutes the roots were rotated on the horizontal axis of a clinostat. The tips of the roots first showed a positive geotropic curvature due to a strong inhibition (33 %) of the growth rate at the lower side, which was accompanied by a slight increase (9 %) of the growth rate at the upper side. Subsequently the growth rate at the upper side strongly decreased (38 %) but that at the lower side decreased still further (78 % as compared to that of vertically growing controls). Then a negative phase of the curvature follows, about 2 hours after the start of the experiments, because the growth rate at the lower side increased considerably (to 90 % of that of the controls), whereas that at the upper side increased much slower.

The authors concluded that this behaviour is incompatible with the CHOLODNY–WENT conception. The same holds true for the results obtained when IAA had been applied to .he roots. They believe, that the transversally acting gravity does not induce a lateral distribution of growth substance in roots but leads to a de novo production of an inhibiting substance in the lower side.

This publication and the results reported in the preceding chapters were the starting point for the own experiments. In these, however, as in those of RUFELT (1957), the roots were not placed on a clinostat but they remained with their non-curving base in the horizontal position. The rate of the straight growth and the course of the geotropic reaction were followed in aq. dest. and in solutions of DCP, p-cumaric acid, caffeic acid and IAA.

4.2. MATERIAL AND METHODS

Seeds were soaked for about 20 hours in aerated tap water. In order to secure a straight growth of the roots each seed was placed on the top of a vertical hole pushed in moist coarse sand with a rod. After the roots had grown for 24 to 28 hours (about 1 cm long) the seeds were placed in plexiglass holders on a jar filled with aq. dest. in which the roots grew for another period of 20 to 24 hours until the straight roots were 3.5–4 cm long. The seedlings then were used for the experiments.

For these experiments small special frameworks were used as sketched in Fig. 11. The seeds were fixed on steel pins (1), mounted

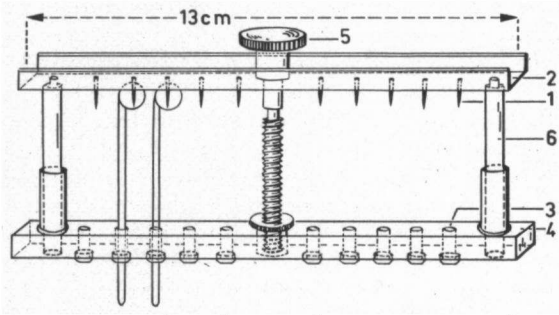

Fig. 11. Apparatus used at the experiments on geotropism and growth.

on a metal strip (2). A plexiglass strip (4), which can slide along two metal rods (6) and can be adjusted by means of a screw (5), is mounted on the metal strip (2). In the plexiglass strip 10 metal tubes (3) are mounted in holes drilled perpendicularly in the strip. Into these tubes the roots are introduced by turning up the strip by means of the screw (5). The root tips protrude from the tubes and the length of the freely protruding part can be chosen as desired by turning the screw (5); each frame thus can hold 10 roots.

For the study of geotropical reactions the frames are placed on one of their narrow sides in plexiglass cuvettes. For the measurement of the straight growth the frames were placed with their metal strip (2) on the top of such a cuvette. In these cuvettes the seedlings were immersed either in aq. dest. or in solutions of compounds, the influence of which had to be investigated.

During the experiments shadowgraphs of the roots were made with a beam of orange light (SCHOTT filter OG2) through the cuvette on strips of Gevaert Document Rapid paper or on Graphic Ortho film. In the latter case the shadowgraphs were projected at a 10-fold magnification, drawn and then measured. The ends of the tubes (3) give completely sharp zero marks on the pictures.

The variation is expressed as the standard error of the mean,

$$SE_m = \sqrt{\frac{\Sigma\,[(x - \bar{x})^2}{n(n-1)}}$$

where n = the number of roots and $\Sigma\ (x - \bar{x})^2$ = the squared deviations of the mean.

4.3. THE GEOTROPIC REACTION AND THE STRAIGHT GROWTH OF PEA ROOTS IN DISTILLED WATER

The growth at the lower and the upper side of horizontally placed roots and the straight growth of vertically growing roots (control) were measured from successive shadowgraphs made at ½ or 1 hour intervals. Some characteristic examples of the geotropic reaction (i.e.

the most frequently occurring types of reaction) are presented in Figs. 12 and 13. The upper part gives the geotropic curvatures in

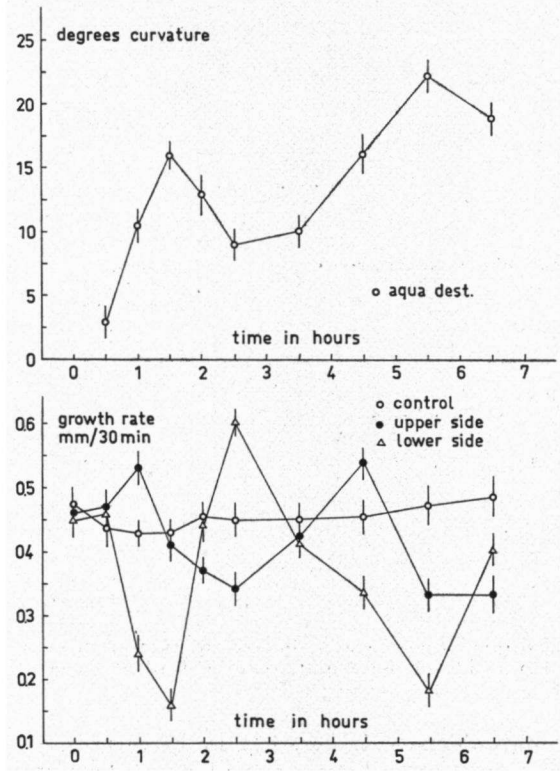

Fig. 12. The geotropic reactions and the growth rate at the upper and at the lower side of horizontal roots in distilled water.

degrees against time, the lower part the growth rates at the lower and upper sides and that of vertical roots. The absolute growth rates at the lower and upper sides show a considerable individual variance, especially during the first 1½ hours, whereas the rate of the geotropic curvature has only a slight variance. The following course shows alternating phases of positive and negative reactions. The first positive phase lasts for ca. 1½ hours and then a negative phase starts. The time after which the latter changes into the second positive phase varies considerably i.e. between 2½ and 3½ hours after the beginning of the experiment.

In the horizontal position the growth rates at the upper and lower side differ insignificantly during the first ½ hour and the curvature consequently is slight. Then a phase follows during which the difference between the growth rates becomes very large, either because that at the upper side is slightly increased and that at the

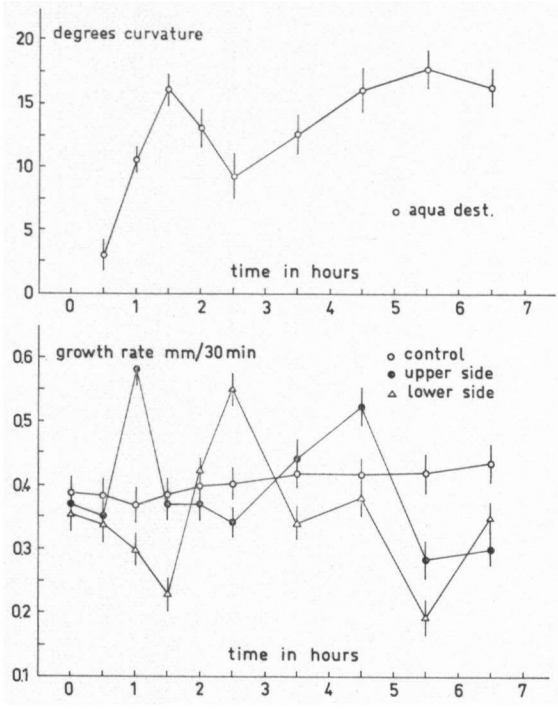

Fig. 13. The geotropic reactions and the growth rate at the upper and at the lower side of horizontal roots in distilled water.

lower side strongly decreased (Fig. 12), or because that at the upper side is strongly increased and that at the lower side slightly decreased (Fig. 13). Subsequently the growth rate at the upper side slows down to that of the vertical controls whereas meanwhile that at the lower side is retarded further. This period is called the first positive phase. Between ½ to 1½ hours after the start of the experiment the upper side grows faster than the lower side, so that a positive geotropic curvature develops.

About 1½ hours after placing the roots horizontal the much retarded growth rate at the lower side suddenly increases steeply and surpasses that at the upper side and that of the controls. During this phase the already started decrease of the growth rate at the upper side gradually continues. Since the increase of the growth rate at the lower side occurs in the same cells that were retarded during the first positive phase, the positive curvature decreases, as a rule from 5° to 10° but sometimes the roots become entirely straight. This phase is indicated as the first negative one.

During the 6 to 7 hours lasting experiment still a second positive phase occurs, caused by a similar alternation of the growth rates at the upper and lower side, followed by a second negative phase.

As already mentioned there is a considerable variation in time at which these phases start (cf. Figs. 12 and 13). The rate of curving during the second positive phase is always slower than that during the first positive phase. Both second phases, however, do not differ in principle from the first ones.

In Fig. 14 the alternation of positive and negative phases are

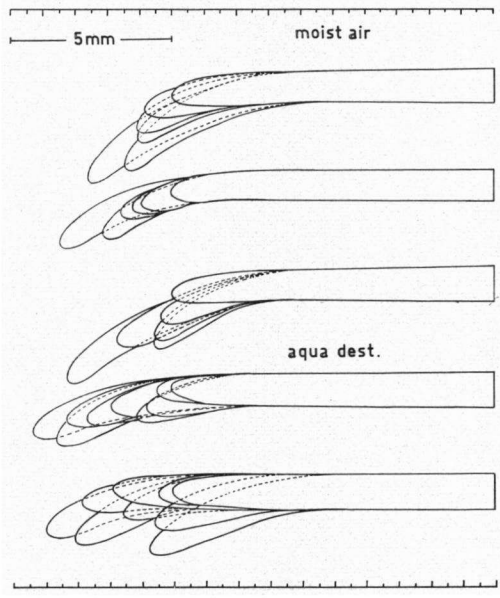

Fig. 14. The main types of geotropic reactions of horizontal roots in moist air and in distilled water.

shown for two roots growing in aq. dest. (roots 4 and 5), as drawn from successive shadowgraphs. Between the start of the first and the second positive phase the root has elongated 2 to 3 mm. A great part of the cells which have been involved in both the first positive and negative phase are now shifted too far from the tip to participate in the second phases. The cells at the extreme tip, however, are in the same situation as the older ones were at the beginning of the experiment.

At the end of the experiment the total elongation of the upper side as well as that of the lower side of horizontal roots is less than that of vertically growing controls.

In principle the described changes of the growth rate at the upper and lower side during the first positive and negative phases of the geotropic reaction match the observations of AUDUS and BROWNBRIDGE (1957), whereas the graphs of the course of the geotropic curvature show much resemblance with the results obtained by RUFELT (1957).

4.4. The geotropic reaction and the straight growth of pea roots in moist air

Most former investigations on geotropism of roots have been made with roots growing in moist air. In order to check whether these results are comparable with those obtained with roots growing in water, a number of experiments were made with roots in air at saturated humidity. The applied method was the same as described in section 4.2. Fig. 15 shows the results of these experiments. It is

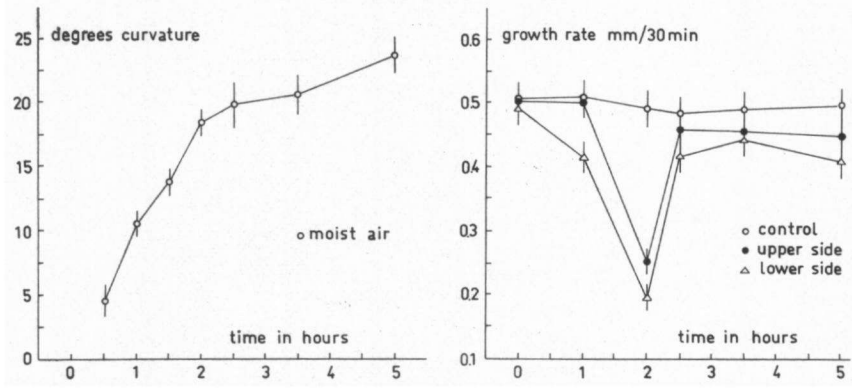

Fig. 15. The geotropic reaction and the growth rate at the upper and at the lower side of horizontal roots in moist air.

apparent that the first negative phase, which is so pronounced in water, is only very weak in air and occurs much later (between 2½ and 3½ hours). In Fig. 14 typical specimen of the successive shapes of the geotropic curvature (roots 1, 2 and 3) are given.

During the entire duration of the experiments (5 hours) the growth rate of the lower side remained less than that of the upper side. At both sides the growth is strongly inhibited during the phase in which the rate of increase of the curvature is greatest.

The pattern of the geotropic reaction therefore proves to be much simpler in moist air than in water. The course of the curvature as described matches that found e.g. by Brumfield (1955), Ching *et al.* (1956) and Larsen (1957). The strong decrease of the growth rate at both the upper and the lower side resembles the overall growth inhibition observed by Bennet-Clark *et al.* (1959) at geotropically curving roots.

As for the explanation of the different behaviour of horizontal roots in moist air and in water the investigations of Rufelt (1957b) may be of interest. From these it is apparent that the magnitude of the negative reaction of the roots largely depends on the oxygen tension in the medium and that it is suppressed by increasing the oxygen tension. This may account for the difference between roots growing in moist air and in water (see also Lundquist and Rufelt, 1961).

Notwithstanding the more complicated geotropic reaction of roots in water than in air, the first medium was chosen since the compounds, which influence had to be investigated, could be applied best to the roots in solutions. This possibly could have the advantage that the successive phases of the geotropic reaction would appear to react differently on the applied substances. This would be the more important because the data presented in the last sections can by no means be interpreted in terms of the CHOLODNY–WENT theory.

4.5. THE EFFECT OF DCP ON THE GEOTROPIC REACTION AND ON THE STRAIGHT GROWTH

As Fig. 16 shows, the development of the geotropic curvature in

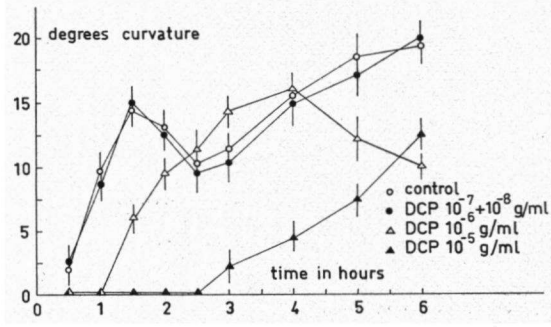

Fig. 16. The geotropic reactions in solutions with various concentrations of DCP.

solutions of DCP in concentrations of 10^{-6} g/ml and higher is different from that in aq. dest. In a concentration of 10^{-6} g/ml DCP the first positive phase of the curvature is strongly retarded and in a concentration of 10^{-5} g/ml the curvature starts after 2½ hours and even at the end of the experiment (after 6 hours) the first positive phase had not yet finished.

The growth rate of the upper and lower side of horizontal roots was measured in DCP 10^{-5} g/ml. As Fig. 17 shows the growth is retarded strongly and equally at both sides. After 1½ hours the growth rate increases again but not before 2½ hours after the start of the experiment a difference between the growth rate of the upper and lower side becomes apparent and it remains about constant during the further course of the experiment. As could be expected as long as the growth retardation at the upper and lower side is equal (2½ hours) no geotropic curvature develops. The moment at which a difference of the growth rate at the upper and lower side starts depends on the DCP concentration.

As we have seen DCP enhances the degradation of IAA in roots (section 3.3) and it is converted itself in the roots (section 3.4) as well. If IAA would be unequally distributed by a geotropic exposure, DCP must have the capacity to decrease the IAA concentration in

the root to such an extent, that no significant difference of the IAA concentration at the upper and lower side can be obtained.

If DCP is converted into a compound that can not influence the IAA concentration, then after a certain lapse of time the latter maybe increases again and the capacity of the root to curve geotropically will be restored.

This explanation is endorsed by a comparison of the growth rate of the upper and lower sides of horizontal roots (Fig. 17) with that of vertical roots in a solution of DCP which contains 10^{-5} g/ml (Fig. 18). In a concentration of 10^{-7} g/ml DCP, where the geotropic

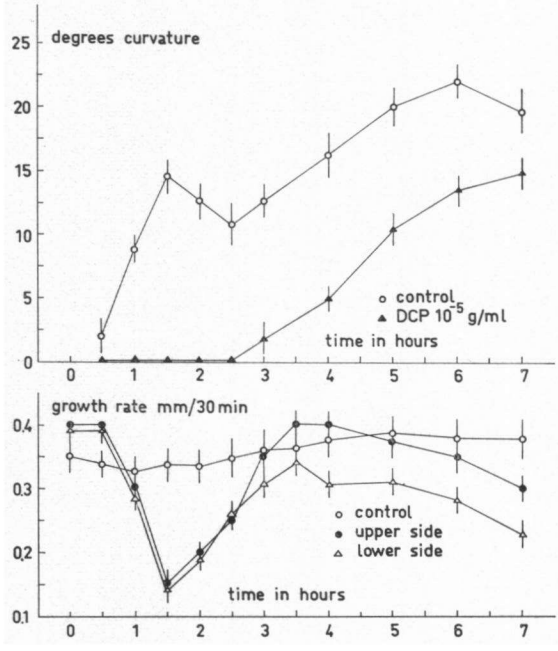

Fig. 17. The effect of DCP 10^{-5} g/ml on the geotropic reaction and on the growth rates at the upper and at the lower side of horizontal roots.

reaction is not markedly affected, the growth rate of vertical roots soon shows a quick increase, followed by a decrease and after about 3 hours the final growth rate becomes steadily higher than that of the controls. This particular case would fit in the assumption that in the roots the natural IAA concentration is supra-optimal. The results obtained with DCP suggest that IAA plays a part in the growth and consequently in the geotropism of roots (cf. AUDUS and SHIPTON, 1952).

4.6. THE EFFECT OF P-CUMARIC ACID ON THE GEOTROPIC REACTION

The course of the geotropic curvature in different concentrations of p-cumaric acid is presented in Fig. 19. In a concentration of

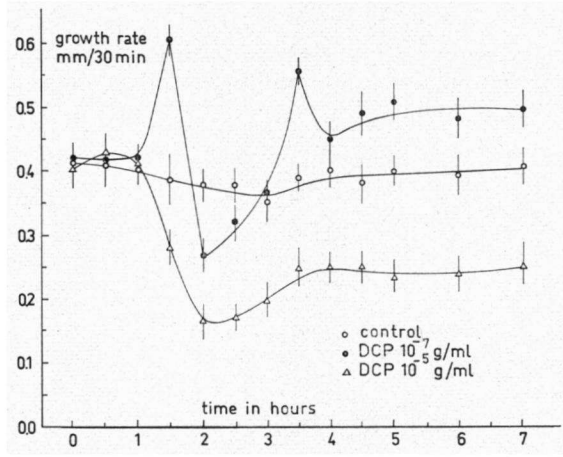

Fig. 18. The growth rate of roots vertical in solutions of DCP (added after one hour).

10^{-9} g/ml the second positive phase of the reaction is more pronounced than that of the controls. In 10^{-8} g/ml also the first positive phase is enhanced, whereas in 10^{-7} g/ml the course is the same (not drawn in Fig. 19) as in water.

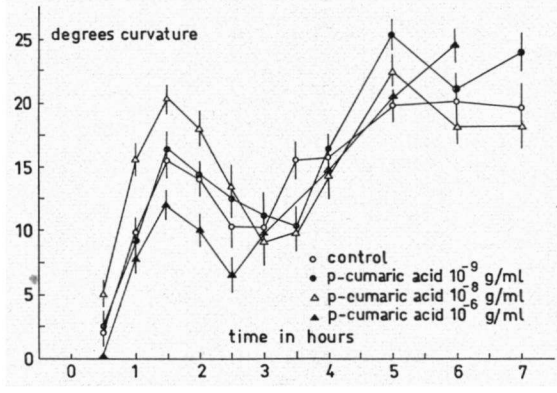

Fig. 19. The effects of various concentrations of p-cumaric acid on the geotropic reaction.

In 10^{-6} g/ml p-cumaric acid, however, the first positive phase is reduced and retarded but the second positive phase remains enhanced. The reduction of the first positive phase can be explained by a reduction of the IAA concentration in the roots by p-cumaric acid (cf. the effect of 10^{-6} g/ml DCP, Fig. 16). Apparently the enhancement of the second positive phase is due to a prolongation of the reaction, i.e. by a longer lasting difference of the growth rates at the upper and lower side, which in p-cumaric acid is more pronounced than

in low concentrations of DCP (Fig. 16). p-Cumaric acid may be converted (cf. Chapter 2, Fig. 3) into a compound which effect on the geotropic reaction does not match the effect of p-cumaric acid on the IAA degradation.

4.7. The effect of caffeic acid on the geotropic reaction and on the straight growth

The course of the geotropic curvature in different concentrations of caffeic acid is presented in Fig. 20. The reaction is strongly

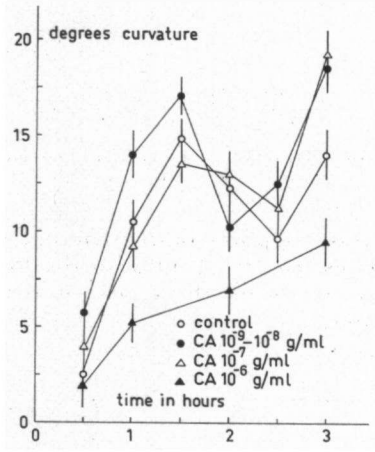

Fig. 20. The effects of various concentrations of caffeic acid on the geotropic reaction.

inhibited by 10^{-6} g/ml caffeic acid. In the concentration 10^{-7} g/ml the first and the second positive phase of the geotropic curvature are accelerated initially. In 10^{-8} and 10^{-9} g/ml (the figures have been averaged in the graph) this acceleration is more pronounced and the peaks of both phases are higher. After 30 minutes the angle of the curvature in 10^{-7} to 10^{-9} g/ml caffeic acid is larger than that of the controls. This indicates that in these cases the difference of the growth rate at the upper and at the lower side must be larger than that of roots in water. The lower part of Fig. 21 shows how this difference is realized. The growth rate at both sides is increased, that at the upper side, however, significantly more than that at the lower side. This difference is maintained during the second half hour, whereas the growth rate itself becomes slower than that of the vertical control roots. Both the first and the second positive phases of the geotropic curvature start earlier in a solution of caffeic acid than in water, whereas the second positive phase reaches an earlier and higher peak in 10^{-8} g/ml caffeic acid than in water. The course of the positive and negative phases, however, are similar to those in water (cf. Figs. 12 and 13).

It may be concluded that caffeic acid in low concentrations accelerates the positive phases of the geotropic reaction. This may be due to a slight inhibition of the IAA (and monophenol) converting system. In that case one could imagine that the accumulation of substances, involved in the geotropic reaction, at the lower side—i.e. IAA or some monophenol—is facilitated.

The growth rate of horizontal roots in a caffeic acid solution of 10^{-8} g/ml is generally lower than that of roots growing vertically in water (Fig. 21). The same holds true for roots growing vertically in 10^{-8} g/ml caffeic acid (Fig. 22). This may be explained also by

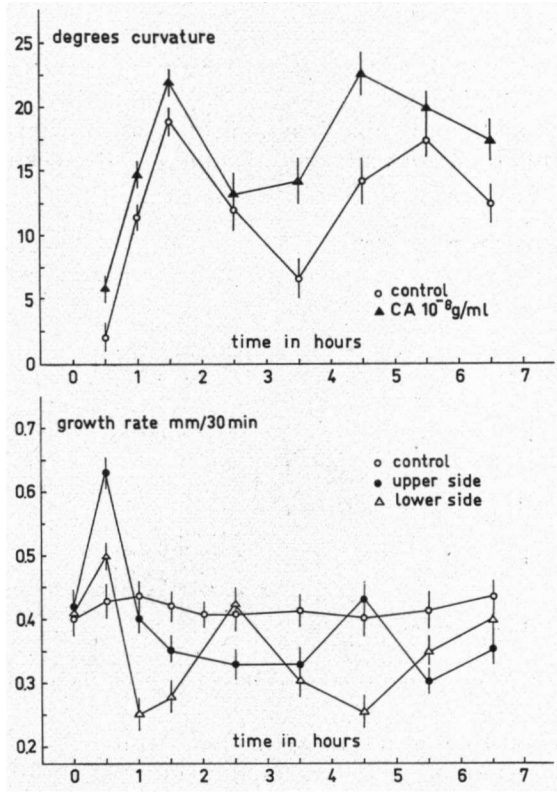

Fig. 21. The effect of caffeic acid (10^{-8} to 10^{-9} g/ml) on the geotropic reaction and on the growth rate at the upper and at the lower side of horizontal roots.

an increase of the concentration of IAA or of that of some monophenol to a supra-optimal level.

As in 10^{-7} g/ml DCP (Fig. 18) also in 10^{-8} g/ml caffeic acid short initial periods of an increased growth rate occur; obviously such periods are not a specific effect of caffeic acid or DCP.

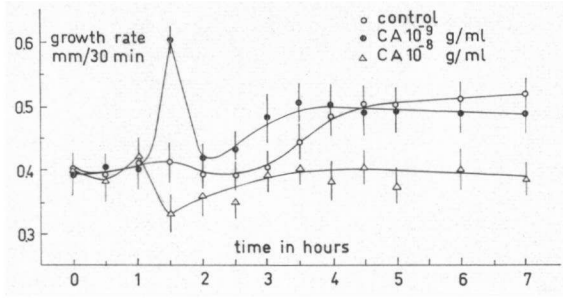

Fig. 22. The growth rate of vertical roots in solutions of caffeic acid (added after one hour).

4.8. The effect of IAA on the geotropic reaction and on the straight growth

The course of the geotropic reactions of roots growing in solutions of IAA of different concentrations is presented in Fig. 23. In a

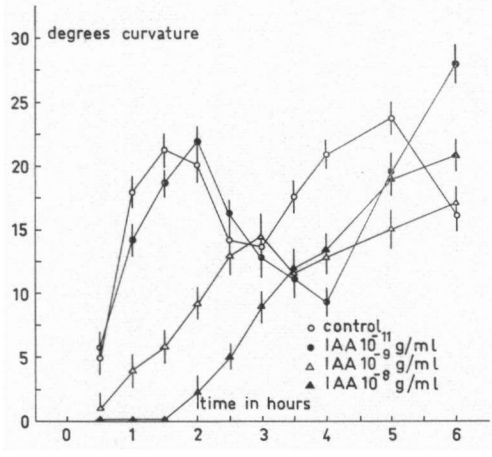

Fig. 23. The effects of various concentrations of IAA on the geotropic reaction.

concentration of 10^{-11} g/ml IAA the first positive phase is slightly retarded and it lasts longer than in the controls. In 10^{-9} g/ml this effect is much more pronounced and in 10^{-8} g/ml there is a lag time preceding a still much more retarded first positive phase than in 10^{-9} g/ml IAA. With increasing concentrations of IAA the rate at which the first positive phase develops, decreases and the duration of this phase increases. The first negative phase, being more pronounced and lasting longer in 10^{-11} g/ml IAA than in water, flattens out in 10^{-9} g/ml IAA and it does not appear within the duration of the experiment in 10^{-8} g/ml IAA.

Fig. 24 shows how the first positive phase of the geotropic reaction

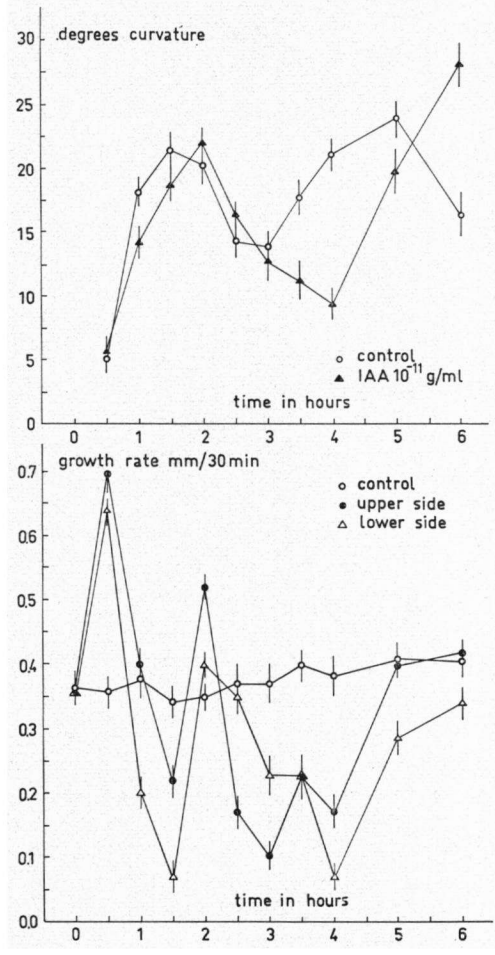

Fig. 24. The effect of IAA 10^{-11} g/ml on the geotropic reaction and on the growth rate at the upper and at the lower side of horizontal roots.

in 10^{-11} g/ml IAA is realized by alternating but synchronous phases of increased and decreased growth rates at the upper and lower side. The first negative phase is caused by a stronger decrease of the growth rate at the upper side than at the lower side and not by an increase of the growth rate of the lower side as found in water (Figs. 12 and 13) and in caffeic acid (Fig. 21). It is striking that the alternating changes in the growth rate at both sides, though different in magnitude, are all in the same direction. In 10^{-8} g/ml IAA (Fig. 25) the growth after a short lasting acceleration comes to a complete standstill for at least half an hour. Then it is resumed but the rate remains lower than that of the vertical controls in water. The growth rate at the upper side is higher than that at the lower

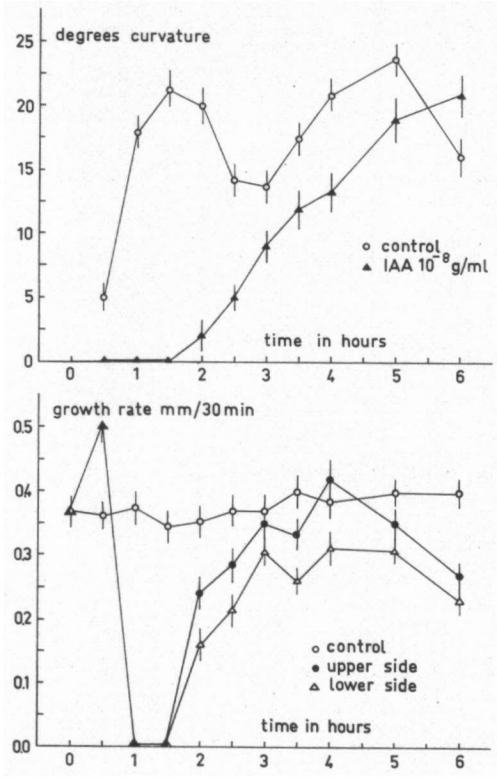

Fig. 25. The effect of IAA 10^{-8} g/ml on the geotropic reaction and on the growth rate at the upper and at the lower side of horizontal roots.

side and here also the growth curves of both sides run parallel.

10^{-11} g/ml IAA is already sufficient to retard and to prolong the first positive phase of the geotropic reaction. This indicates that the endogenous auxin concentration in the roots must be very low. In higher IAA concentrations (10^{-9} and 10^{-8} g/ml) the geotropic reaction is delayed, probably until sufficient IAA has been converted in the root tip (Fig. 23 and lower part of Fig. 25) to make a reaction possible. If the first negative phase of the geotropic reaction, as it occurs in water (Figs. 12 and 13), is caused normally by a decrease of the IAA concentration because conversion takes place at the lower side to such a low concentration that the growth rate is enhanced, then it is likely that IAA in the medium, especially in higher concentrations, retards or even prevents this negative phase.

Apart from a short lasting acceleration of the growth rate at the start of the experiment, in both concentrations 10^{-11} and 10^{-9} g/ml IAA the growth rate of horizontal roots is lower than that of roots growing vertically in water. This is different from the growth of vertical roots in solutions of IAA 10^{-11} and 10^{-9} g/ml (Fig. 26).

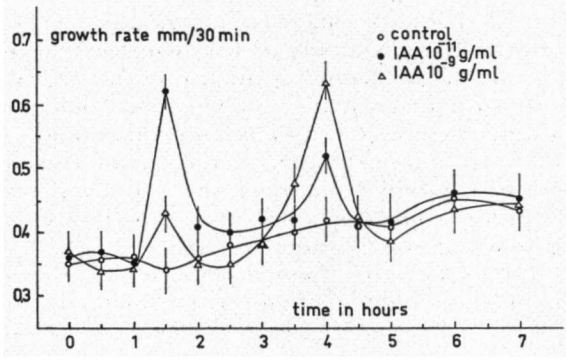

Fig. 26. The growth rate of vertical roots in solutions of IAA (added after one hour).

In both concentrations an increase of the growth rate occurs in the first hour, which is more pronounced in 10^{-11} g/ml than in 10^{-9} g/ml IAA. A similar acceleration of the growth has been stated in 10^{-7} g/ml DCP and in 10^{-8} g/ml caffeic acid. After three hours in IAA a second peak appears in the growth curve, but now that in 10^{-9} g/ml IAA is higher than that in 10^{-11} g/ml.

The first peaks seems to be a non-specific effect, for they are similar to those in DCP and caffeic acid (Figs. 18 and 22). The second peaks can possibly be explained by the assumption that after some time the level of 10^{-11} g/ml IAA has been so much reduced that the second peak is low (cf. the similar peak in the growth curve in 10^{-7} g/ml DCP in Fig. 18), whereas by the conversion of IAA the concentration of 10^{-9} g/ml IAA has been reduced to a promoting one.

4.9. Discussion

During the positive and negative phases of the geotropic reaction of roots placed horizontally in water, considerable differences in the growth rate at the upper and lower side occur (cf. Audus and Brownbridge, 1957). Moreover a strong inhibition of the growth at both sides in horizontally growing roots in moist air has been found (cf. Bennet-Clark *et al.*, 1959). These phenomena are not well compatible with the Cholodny–Went theory. If IAA were the substance that is unequally distributed, like in coleoptiles 30 percent at the upper side and 70 percent at the lower side, then the observed behaviour of the roots can not be explained. Moreover the straight growth of vertical roots in IAA solutions (Fig. 26) does not indicate that the natural IAA concentration in the root is supra-optimal, which is a postulate of the Cholodny–Went theory in its original form.

It could, however, be possible that both positive phases of the geotropic reaction are due to an unequal distribution of IAA. In that case the effect of 10^{-5} g/ml DCP on the geotropic reaction (Fig. 16) and on the straight growth (Fig. 18) could be explained

by a reduction of the IAA concentration in the root so that it is too low to be distributed unequally to a sufficient extent in horizontal roots and to maintain the normal growth rate in vertical roots. The inhibition of the straight growth by caffeic acid could be due to an inhibition of the IAA converting system and consequently an increase of the IAA concentration in the root. For the same reason the earlier and prolonged first positive phase of the geotropic reaction in a caffeic acid solution could be ascribed to an earlier starting and longer lasting unequal distribution of IAA.

The addition of IAA retards the first positive phase of the geotropic reaction. This indicates that it hampers the unequal distribution of some substance, which is not necessarily IAA itself, but it can be the case.

The effect of p-cumaric acid during the first positive phase has much in common with that of caffeic acid. This suggests that p-cumaric acid is converted in the root into a compound that has the same effect as caffeic acid (cf. Fig. 3, Chapter 2).

Nothing is known about the way in which the negative phase of the geotropic reaction is realized. For a comparison of the course of the latter in different media some characteristic specimina of subsequent stages are presented in Fig. 27. In the negative phase

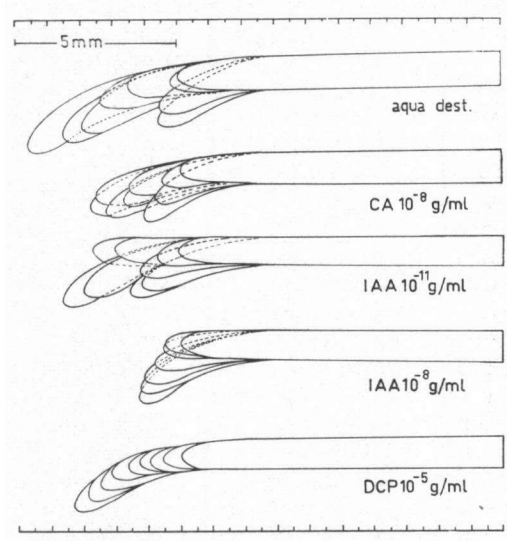

Fig. 27. Characteristics of the geotropic reactions in solutions of various compounds. The time intervals between the successive stages of the geotropic reactions correspond with the respective graphs.

the growth rate of cells of which the growth was strongly reduced, is suddenly strongly increased for some time. This suggests that a substance which inhibits the growth during the first positive phase

at the lower side is eliminated, thus allowing a short lasting but considerable increase of the growth rate.

It may be interesting to state that the negative phase is only weak or absent in moist air (high oxygen tension), in a high DCP concentration and in high IAA concentrations. These are all conditions which enhance the activity of IAA-oxidase.

In water (low oxygen tension) and in caffeic acid the negative phase is more pronounced. In both cases the activity of the IAA converting system may be considered to be low.

Finally it may be mentioned that there is no direct relation between the absolute growth rate of horizontal roots and the magnitude of the geotropic curvature during the first positive phase. This gets clear from a comparison of the growth rate of horizontal roots in moist air, in DCP and in 10^{-8} g/ml IAA. Apparently DCP prevents the origination of a difference in growth rate between the upper and lower side of the horizontal root. DCP therefore may be called a selective inhibitor of geotropism (cf. AUDUS and BROWNBRIDGE, 1957b).

CHAPTER 5

THE EFFECT OF DCP, CAFFEIC ACID AND IAA WHEN APPLIED AT DIFFERENT STAGES OF THE GEOTROPIC REACTION

5.1. INTRODUCTION

In the preceding chapter the subsequent stages have been reported which develop during the geotropic reaction. A first positive and a first negative phase could be distinguished, often followed by a second positive and a second negative phase, during the course of the experiment. It was proved that the first negative phase can be suppressed more easily by substances added to the medium than the first positive phase, whereas the start and the duration of the latter is influenced by such substances.

It was thought worth-while to investigate whether it was possible to get more information on the nature of the subsequent phases by adding different compounds to the medium at different stages of the geotropic reaction. The experiments were as follows. A series of frames with roots were placed with the roots horizontal in water. At different moments one or more frames were transferred into a solution of the substance of which the effect had to be investigated. The registering of the course of the geotropic reaction was continued in these solutions. The controls remained horizontal in water.

5.2. THE EFFECT OF DCP SUPPLIED AT DIFFERENT STAGES OF THE GEOTROPIC REACTION

Since 10^{-5} g/ml DCP has a more marked effect than lower concentrations this concentration was chosen. The results are presented in Fig. 28; *a* gives the course of the geotropic curvature of the controls

in aq. dest. and *b* that of roots that were in a DCP solution during the whole experiment (cf. Fig. 16). When the roots are transferred into the DCP solution after one hour, that is when the first positive phase is in its full development (Fig. 28c), DCP has no significant

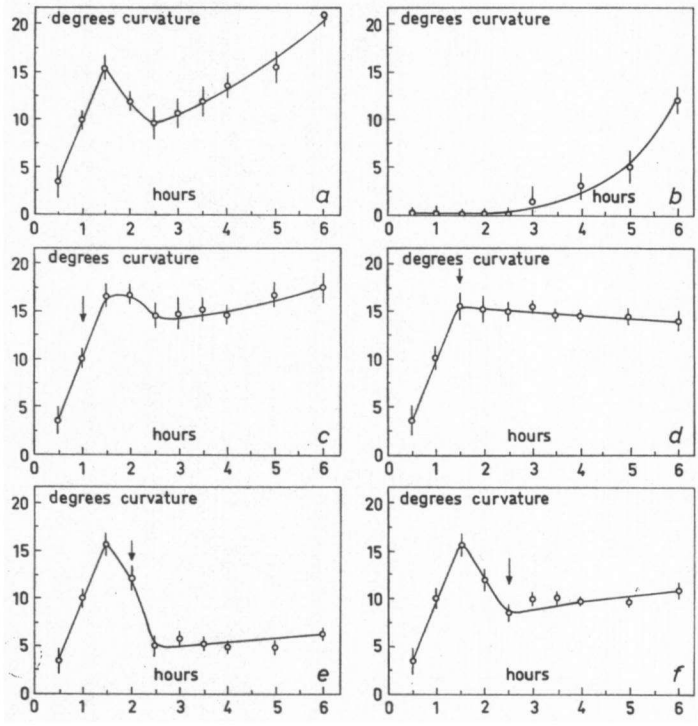

Fig. 28. The effects of addition of DCP (10^{-5} g/ml) at different stages during the course of the geotropic reaction.

effect on the magnitude of the angle of curvature at the end of this phase though there is a tendency to increase it.

The start of the first negative phase is delayed and the phase itself and the second positive phase are flattened. When the roots are brought into the DCP solution when the first positive phase has just finished (after 1½ hour, Fig. 28d) the following phases are completely suppressed and the angle of the geotropic curvature remains constant during the next hours of the experiment. After a transfer into the DCP solution during the first negative phase (after 2 hours, Fig. 28e) this reaction is prolonged. Consequently the angle of the curvature becomes smaller than that of the controls. The second positive phase is fully suppressed. After a transfer into the DCP solution at the end of the first negative phase (after 2½ hours, Fig. 28f) the second positive phase is almost completely suppressed.

The influence of DCP on an existing difference in growth rate between the upper and lower side is only weak but it tends to prolong this difference. It further prevents the origination of any new difference in growth rate. Since the effects of DCP on the positive phases and on the negative phase are similar, it seems justified to conclude that the subsequent phases are governed by the same physico-chemical principle.

5.3. The effect of caffeic acid supplied at different stages of the geotropic reaction

Since concentrations of 10^{-7} g/ml caffeic acid and lower have no distinct effect on the geotropic reaction in progress, this compound has been supplied in these experiments in a concentration of 10^{-6} g/ml. In Fig. 29a the course of the geotropic reaction in aq. dest.

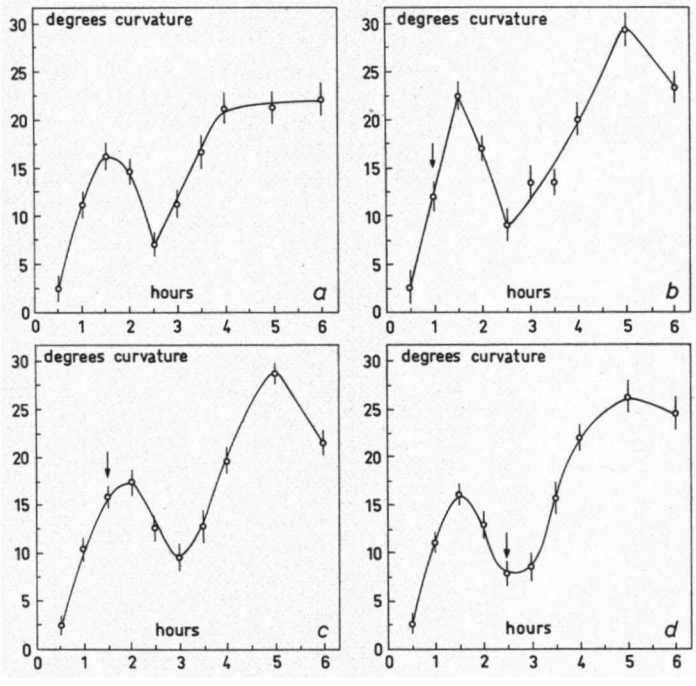

Fig. 29. The effects of addition of caffeic acid (10^{-6} g/ml) at different stages during the course of the geotropic reaction.

is presented. Fig. 29b gives this course when the roots are transferred into the caffeic acid solution during the first positive phase (after 1 hour). The latter then continues at the same rate it had at the moment of transfer, whereas this rate decreases when the roots are kept in water. The angle of curvature at the end of the first positive phase therefore is larger in caffeic acid than in water during the

same period. The first negative and the second positive phase are more pronounced too in this case. When the roots are transferred into the caffeic acid solution at the end of the first positive phase (after 1½ hour, Fig. 29c), then the first negative phase is delayed and the second positive phase enhanced. The latter also occurs when the transfer takes place at the end of the first negative phase (after 2½ hours, Fig. 29d).

In general it can be stated that caffeic acid prolongs a once started phase of the geotropic reaction and that it enhances the following phases. This means that an already existing as well as a newly originating difference in growth rate between the upper and the lower side is prolonged by caffeic acid.

5.4. The effect of IAA supplied at different stages of the geotropic reaction

In preliminary experiments it was found that the IAA concentration has to be fairly high in order to affect the course of a once started phase of the geotropic reaction. Therefore a concentration of 3×10^{-8} g/ml IAA was chosen. Fig. 30a presents the course of the geotropic reaction of the controls in water. When the roots are transferred into the IAA solution during the first positive phase (after 1 hour, Fig. 30b) the rate of curving is retarded and this phase

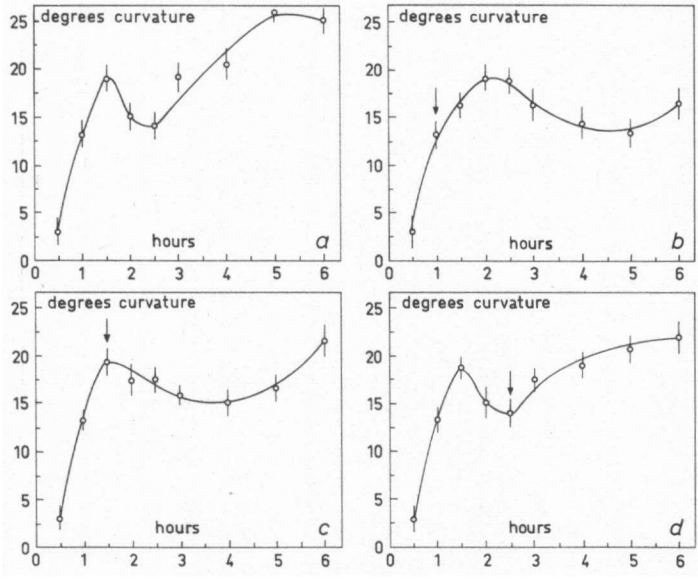

Fig. 30. The effects of addition of IAA (3×10^{-8} g/ml) at different stages during the course of the geotropic reaction.

is prolonged as well as the following phases. When the transfer into IAA takes place at the end of the first positive phase (after 1½ hour,

Fig. 30c), and at the end of the first negative phase (after 2½ hours, Fig. 30d) the next phase starts at the same moment as in the controls but the rate of reaction is strongly retarded.

IAA does not only retard and prolong a phase that has already started but its influence on the following phases is the same. A lower rate of the development of the geotropic reaction means a smaller difference between the growth rate at the upper and lower side. It may be that the addition of IAA affects the slope of the transversal gradient of the unequally distributed substance. It has, however, no influence on the origination of the unequal distribution itself (Fig. 30c and d).

5.5. Discussion

From the results reported in the preceding sections the following conclusions can be drawn:

1. DCP prevents the origination of a difference in growth rate between the upper and the lower side of the horizontal root tip.
2. Caffeic acid enhances and prolongs this difference in growth rate.
3. IAA decreases the difference in growth rate but does not affect the moment at which such a difference starts.
4. The subsequent positive and negative phases of the geotropic reaction are governed by the same physicochemical principle.

From Chapters 2 and 3 follows that in case 1 much IAA is converted in the root and in case 2 there is no conversion.

If IAA is the substance that is unequally distributed during the geotropic reaction, these results suggest that this distribution can be regulated by the IAA converting system. The effect of IAA could then be explained by admitting that added IAA reduces the slope of the transversal gradient of the internal IAA concentration and consequently the difference in growth rate between the upper and the lower side.

One can argue against an unequal distribution of IAA by stressing the fact that DCP has only little effect on a difference in the growth rate at the upper and at the lower side already existing at the moment at which DCP is added. Although also in this case DCP will promote the conversion of IAA, this does, however, not necessarily mean that the difference between the growth rates at the upper and at the lower side changes. The latter does not depend on the absolute IAA concentration in the root (cf. Chapter 4) but on the difference in concentration at the upper and at the lower side.

Conclusion 4 evokes the problem how a negative phase of the geotropic reaction can be explained by an unequal distribution of IAA. One then has to assume that IAA is accumulated at the lower side of the root and converted to such an extent, that at a certain moment its concentration causes an increase in the growth rate (Fig. 26).

The fact that the rate at which the curvature develops during

the first positive phase decreases in control roots, pleads in favour of such a conversion of IAA. However, some other factor responsible for the negative phases must be involved, since in a solution of caffeic acid, which inhibits the conversion of IAA, these phases nevertheless do occur.

CHAPTER 6

THE EFFECT OF DCP, CAFFEIC ACID AND IAA ON THE SUCTION FORCE AND ON THE PROPERTIES OF THE CELL WALL OF GEOTROPICALLY REACTING TISSUES OF ROOT TIPS OF PEAS

6.1. Introduction

The geotropic reactions of root tips are performed by often quickly changing growth rates at the upper and the lower side (Chapter 4). Cell elongation is effected by the uptake of water. Changes in the growth rate therefore must be accompanied by changes in the water uptake. The latter changes may be caused by changes of the permeability of the protoplasmic membranes, of the suction force (S), of the osmotic value (O) of the cell sap, and of the wall pressure (W). The latter depends on the ratio between the plastic and elastic properties of the cell wall.

Experiments were made to investigate the effect of geotropic exposure on the mentioned properties with the exception of the permeability. Since the geotropic reaction is influenced by DCP, caffeic acid and IAA, also the effect of these compounds on these properties has been studied.

6.2. Methods

The changes of S, O and W in tissues from the growing zone of roots were estimated according to the principle of the method of Ursprung and Blum (1924). The value of S and O can be calculated from the changes in length of the tissue in solutions of different osmotic values. In their formula $S = O - W$, S is the suction force, measured when the tissue is in equilibrium with the surrounding medium. The osmotic value of the tissue O at incipient plasmolysis can be determined cryoscopically or plasmometrically. The wall pressure W is the difference between O and S. When O is determined plasmometrically the original length of the root tissue and that at incipient plasmolysis is measured. The original O of the tissue can be found by $\pi_1 V_1 = \pi_2 V_2$. This equation, however, is only applicable if πV is actually constant.

This has been checked by determining the product πV before and after plasmolysis. The volume V of 4 mm cylinders of root tissue was assumed approximately proportional with their length L, which therefore was used as measure for V. In this case O has been measured

cryoscopically of press juice from a large number of root cylinders. In turgescent cylinders O was found to be 0.13 mol. and in plasmolyzed cylinders 0.20 mol. The product πV in the first case $0.13 \times 84.5 = 10.9$ and in the latter $0.20 \times 60.3 = 12.0$. This issue shows an acceptable difference.

For the experiments the roots (grown as described in 4.2) were removed from the seed and with a special microtome the tip of 2 mm was cut off. The next section of 4 mm, comprising the major part of the growing zone, was used. In a number of experiments the cylinders of horizontal roots were longitudinally split in an upper and a lower half. When transferred into water these halves strongly curved. For that reason they were photographed and the changes in their length were measured later on at a 10-fold magnified projection.

Mannitol in 0.0 to 1.0 mol. solutions was used for the estimation of S and O. The cylinders floated in the solutions which were stirred by aeration. The length of the cylinders was measured by a microscope with an eye-piece micrometer (one scale unit = 57 μ), immediately after cutting and then after they had been for one hour in a mannitol solution.

The elasticity of the cell walls of the tissue was determined by measuring the length of the cylinders after they had been for one hour in aq. dest. (L_t) and then after they had been for one hour in a mannitol solution (L_p). The difference $L_t - L_p$ is indicated as elastic extension, that is $dL = L_t - L_p$. The relative elastic extensibility of the cell walls can be expressed as

$$E = \frac{L_t - L_p}{L_p} \times 100\ (\%).$$

The plasticity of the cell walls of the cylinders is expressed as the difference between the length of the plasmolyzed cylinders L_p after a given treatment and their length when they are plasmolyzed immediately after cutting (L_{p0}), thus $dP = L_p - L_{p0}$. The relative value of the plastic extensibility then is

$$P = \frac{L_p - L_{p0}}{L_p} \times 100\ (\%).$$

In the tables and figures the changes in length are presented in scale units (of 57 μ) of the eye-piece micrometer.

6.3 The suction force and the osmotic value of cylinders from the growing zone of vertical roots

If cylinders are transferred into aq. dest. immediately after cutting, their length rapidly increases. Notwithstanding the fact that the roots had grown for 24 hours in aq. dest. they are not fully turgescent. The suction force and the osmotic value were estimated by measuring the length of the cylinders in mannitol solutions in a range of concen-

trations. The values found were plotted in a graph of which Fig. 31 gives an example. The point of intersection with the abscissa indicates

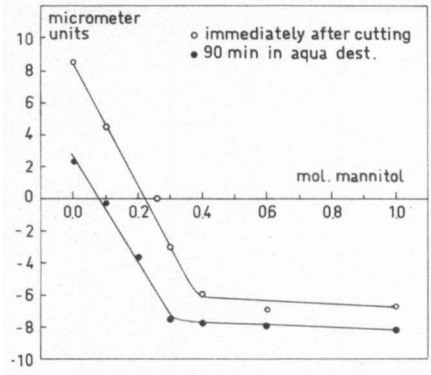

Fig. 31. The effect of immersion in distilled water of cylinders from the growing zone of vertical pea roots on the suction force and on the osmotic value.

the value of the suction force and that where the curve becomes asymptotic that of the osmotic value at incipient plasmolysis. Suction force and osmotic value of cylinders decrease during their stay in water.

Roots grown in moist sand are less turgescent than those grown in aq. dest. The wall pressure in the first was much less than in the latter (Table 9).

TABLE 9

The values of S, O and W in atm and the extension in length of cylinders from the growing zone of vertically growing roots, measured immediately after cutting and after a stay in aq. dest. The values are the mean of 5 experiments (150 cylinders). The standard error of the mean does not exceed $\pm$ 0.33.

Hours in aq. dest.		S	O	W	Extension in length
1)	0	5.8	9.8	4.0	8.3
	0 *)	7.1	9.7	2.6	14.4
2)	1.5	1.9	6.8	4.9	2.1
	4	1.2	5.8	4.6	1.5

*) Cylinders from roots grown in moist sand.
1) and 2) Experiments presented in Fig. 31.

6.4. THE EFFECT OF GEOTROPIC EXPOSURE ON THE SUCTION FORCE AND OSMOTIC VALUE

The roots were placed horizontal for 20 to 30 minutes. A geotropic curvature then is still absent or just incipient. Then cylinders were cut and transported into mannitol solutions of different concentrations. The estimated values are presented in Table 10.

TABLE 10

The effect of a geotropic exposure during 20 to 30 minutes on *S*, *O* and *W* in atm and the extension in length of cylinders from the growing zone of roots. The values are the mean of 5 experiments (150 cylinders). The standard error of the mean does not exceed ± 0.28.

Roots	*S*	*O*	*W*	Extension in length
Vertical	6.0	9.8	3.8	9.5
Horizontal	4.5	8.7	4.2	7.5

It is clear that the suction force of cylinders from geotropically exposed (horizontal) roots is lower than that of cylinders from vertical roots. This lower *S* has to be due to a lower *O* and to a slightly higher *W* (Table 10).

The results of the experiments in which the cylinders were longitudinally split and *S*, *O*, *W* and the extension in length of the upper and lower halves was determined separately, proved to be hardly quantitatively reproducible. The extension in length of both halves was less than that of the intact controls. As a rule this extension, i.e. the water uptake was less in the lower than in the upper half.

6.5. THE SUCTION FORCE, THE OSMOTIC VALUE AND THE EXTENSION IN LENGTH OF CYLINDERS OF ROOTS THAT HAVE BEEN PLACED HORIZONTAL IN SOLUTIONS OF DCP, CAFFEIC ACID AND IAA

Geotropic exposure proves to provoke changes in *S*, *O* and *W* that preceed the geotropic reaction. Since DCP, caffeic acid and IAA affect the geotropic reaction it has been investigated whether these substances have an effect on *S*, *O* and *W* in horizontal roots. The results are presented in Table 11.

TABLE 11

The values of *S*, *O* and *W* in atm and the extension in length of cylinders from the growing zone of roots which have been geotropically exposed during 20 to 30 minutes in solutions of DCP, caffeic acid and IAA. The values are the mean of 5 experiments (150 cylinders). The standard error of the mean does not exceed ± 0.25.

Treatment	*S*	*O*	*W*	Extension in length
vertical in water	6.4	10.0	3.6	8.9
horizontal in water	5.0	8.8	3.8	6.5
horizontal in DCP 10^{-7} g/ml. . .	6.3	8.8	2.5	7.2
horizontal in DCP 10^{-5} g/ml. . .	4.4	8.4	4.0	5.2
horizontal in CA 10^{-8} g/ml . . .	5.6	8.6	3.0	6.7
horizontal in CA 10^{-6} g/ml . . .	5.5	8.7	3.2	7.0
horizontal in IAA 10^{-11} g/ml . .	5.2	8.3	3.1	6.8
horizontal in IAA 10^{-8} g/ml . . .	4.4	8.6	4.1	5.9

In the first place it is evident that the osmotic value in the cylinders is not affected by any of the applied substances. In a solution of 10^{-7} g/ml DCP the suction force (S) is not lowered because in this medium the wall pressure (W) is reduced. In 10^{-5} g/ml DCP and in 10^{-8} g/ml IAA, S is lower than in horizontal root cylinders in water because W is higher. The other series of Table 11 do not show consistent differences from the horizontal controls in water.

As far as differences are found in S and W they presumably are due to changes of the properties of the cell walls.

6.6. The effect of geotropic exposure on the elastic and plastic extensibility of the cell walls

The roots were placed horizontal for 20 to 30 minutes and then cylinders were cut and their elastic and plastic extensibility determined as described in section 6.2. Table 12 gives the results:

Table 12

The elasticity and the plasticity of cylinders from vertical and horizontal roots. The values are the mean of 6 experiments ($\pm$ 250 cylinders). The standard error of the mean does not exceed $\pm$ 0.30.

Roots	dL	E (%)	dP	P (%)
Vertical (control)	10.8	15.4	6.8	11.8
Horizontal	9.1	13.2	5.8	9.4

After an exposure in the horizontal position, so short that a geotropic curvature is still absent or just incipient, the elastic as well as the plastic extensibility of the growing tissue prove to be decreased.

Efforts made to determine these changes separately of the upper and lower halves of split cylinders failed here too (see 6.4) because quantitatively the results were not reproducible, especially those bearing on the plasticity. It proved, however, that the elastic extensibility of the upper halves always surpassed that of the lower ones.

6.7. The effect of DCP, caffeic acid and IAA on the elastic and plastic extensibility of the cell walls of root cylinders

The roots were placed for 20 to 30 minutes either vertical or horizontal in solutions of DCP, caffeic acid or IAA; then the roots were sectioned and the cylinders treated as mentioned in the former section. Of each compound two concentrations were chosen; a high one in which the geotropic reaction is inhibited and another in which this reaction is only weakly inhibited or even promoted (cf. Chapter 4). Table 13 presents the results.

TABLE 13

The effect of DCP, caffeic acid and IAA on the elastic and plastic extensibility of the cell walls of cylinders of vertically and horizontally placed roots. The standard error of the mean does not exceed ± 0.35.

Treatment	E (%)	P (%)
aqua dest. vertical	15.8	12.2
aqua dest. horizontal	13.3	10.0
DCP 10^{-5} g/ml vertical	13.4	9.7
DCP 10^{-5} g/ml horizontal	14.0	7.6
DCP 10^{-7} g/ml vertical	14.4	10.8
DCP 10^{-7} g/ml horizontal	14.5	9.7
CA 10^{-5} g/ml vertical	14.0	10.0
CA 10^{-5} g/ml horizontal	12.9	9.4
CA 10^{-9} g/ml vertical	14.8	12.2
CA 10^{-9} g/ml horizontal	13.0	9.7
IAA 10^{-8} g/ml vertical	11.8	10.7
IAA 10^{-8} g/ml horizontal	12.7	10.5
IAA 10^{-11} g/ml vertical	13.1	12.0
IAA 10^{-11} g/ml horizontal	13.7	10.4

The elastic extensibility of cylinders cut from vertical roots is in all solutions lower than in water. The measure of the decrease of E depends on the applied compound and on its concentration. In cylinders of roots that stayed horizontal in caffeic acid or in IAA, E had the same value as those of horizontal roots in water. In DCP solutions in both concentrations E is increased as compared with the horizontal controls.

The plastic extensibility P of cylinders of vertical roots is lower in the solutions than of the controls in water, the lower concentrations of caffeic acid and IAA excepted. Where P is lowered it has about the same value as that of cylinders of horizontal roots in water.

P of the cylinders cut from horizontal roots that were in the solutions is about equal to that of the horizontal controls, with the exception of 10^{-5} g/ml DCP where P has an extra low value.

The conclusion is that the elastic and plastic extensibility of vertical roots is lowered in solutions of DCP, caffeic acid and IAA. In horizontal roots E and P have about the same value in the solutions as in water. Only in DCP, E is increased. This means that E and P in horizontal roots are not affected by compounds that do affect the geotropic reaction, either by inhibiting it (10^{-5} g/ml caffeic acid; 10^{-8} g/ml IAA) or not affecting it (10^{-7} g/ml DCP, 10^{-11} g/ml IAA) or increasing it (10^{-9} g/ml caffeic acid). There is therefore no correlation between E and P and the incipient geotropic reaction.

6.8. CHANGES OF THE ELASTIC AND PLASTIC EXTENSIBILITY OF CELL WALLS AND OF WATER UPTAKE DURING THE COURSE OF THE GEOTROPIC REACTION

The negative result reported in the former section urged to investigate whether also in the course of the geotropic reaction no

correlation could be found between the effect of DCP, caffeic acid and IAA on the geotropic reaction and on changes of E and P. A number of frames with 10 roots each were prepared and placed in aq. dest. or in solutions of the compounds mentioned.

In each series after 30, 60 etc. minutes one frame was taken out of the fluid, the roots sectioned and the cylinders tested on their extension in length (water uptake) and on their elastic and plastic extensibility. One frame was used to follow the course of the geotropic reaction by making shadowgraphs. The results are presented in Fig. 32; the elastic and plastic extensibility are expressed as dL

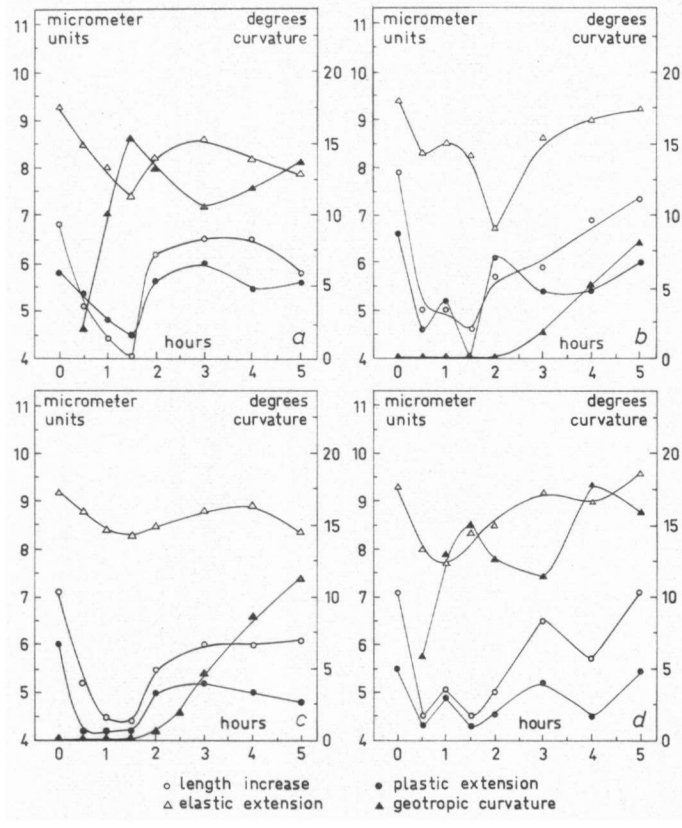

Fig. 32. The elasticity, the plasticity and the water uptake of cylinders from the growing zone of horizontal pea roots at different moments during the course of the geotropic reaction.

and dP respectively. All graphs show that dL, dP and the water uptake decrease after the roots have been placed horizontal. The values reach a minimum after 1½ hour and then gradually increase.

This course is accompanied by an increase of the angle of curvature in water (Fig. 32a) and in 10^{-8} g/ml caffeic acid (Fig. 32d) which reaches a peak after 1½ hour and then decreases. The direction of the changes of dL, dP and the water uptake therefore is the reverse of that of the geotropic reaction and this holds true for the further duration of the experiment. It is, however, obvious that in 10^{-5} g/ml DCP (Fig. 32b) and in 10^{-8} g/ml IAA (Fig. 32c) similar changes of dL, dP and water uptake occur that are not accompanied by a noticeable geotropic reaction. In these cases there is no correlation between the cell wall properties and the geotropic reaction. From the latter it is apparent that more is needed than a change of the elastic and plastic extensibility of the cell walls and of the water uptake.

6.9. Discussion.

When roots are placed horizontal changes occur in physiological factors which are closely related with the growth. The suction force and so the water uptake are decreased by a geotropic exposure because the osmotic value decreases and the wall pressure increases by a lowering of the elastic extensibility of the cell walls. At least in short lasting experiments these changes are greater at the lower than at the upper side of the root. In principle these results match those of Ursprung and Blum (1924) and of Overbeck (1926) as far as the elastic extensibility is concerned. They did not state a change of the osmotic value. The experimental technique and the moment at which the determinations were made by these authors are, however, different from the present investigation. A different result was reported by Horreus de Haas (1929) who found an enhanced elastic extensibility at the upper as well as at the lower side of geotropically exposed roots, that at the upper side being larger than that at the lower side.

It may be of interest to note that in geotropically negative organs, e.g. hypocotyls of *Helianthus annuus* Brauner and Brauner (1961, 1962) found an increase of the suction force and of the elastic extensibility at the lower side whereas in the present investigation in positive geotropic organs, i.e. pea roots, a lowering of these qualities was found.

From the results of short as well as of long lasting experiments it proves that changes of the suction force and of the elastic and plastic extensibility of the cell walls occur almost independently of the presence or absence of DCP and IAA in the medium. In water and in caffeic acid (Fig. 32a and d) these changes seem to be correlated with the geotropic reaction, but in DCP and IAA they apparently are uncoupled.

A geotropic reaction therefore is not merely mediated by changes of osmotic magnitudes and properties of the cell walls in the growing tissue (cf. Chapters 4 and 5).

CHAPTER 7

GENERAL DISCUSSION

Phenols can be converted by enzyme preparations of pea roots *in vitro* and in root tissue *in vivo*. The active component may be a phenol-oxidase, though this needs further investigation. If besides such compounds (of which 2,4-dichlorophenol, p-cumaric acid and caffeic acid have been investigated) also indoleacetic acid is present, the latter disappears from solutions as well as from root tissue. This disappearance is linked with the conversion of p-cumaric acid or DCP and presumably of other monophenols that were not yet tested. The conversion of these monophenols, however, is not dependent on the presence of IAA. Quite recently ENGELSMA (1964) demonstrated a linkage between the conversion of p-cumaric acid and that of IAA by means of a peroxidase from horse-radish. In this case, however, p-cumaric acid is not converted in the absence of IAA. The enzyme responsible for the degradation of IAA in pea roots therefore is different from the peroxidase from horse-radish and from peroxidase in pea roots as has been expounded in Chapter 2.

The degradation of IAA is inhibited by caffeic acid and presumably by related diphenols in general. The rate of degradation of IAA depends on the ratio between the concentration of mono- and that of diphenols (mono- and dihydroxycinnamic acids). From investigations of GOLDACRE *et al.* (1953), GALSTON and DALBERG (1954) and SIEGEL and GALSTON (1953) it could be concluded that IAA applied from outside is converted in a living tissue. For pea roots it now has been shown in a more direct way that added IAA disappears from the tissue and that the phenols that were investigated influence this process.

The activity of the IAA converting system is lowered by a geotropic exposure of the root. This statement seems a convincing argument for the physiological importance of the IAA converting system. As the results reported in Chapter 2 show, the decrease of the activity of this system in horizontal pea roots may be due either to an increase of the concentration of an inhibitor (caffeic acid) or to a decrease of that of a promoting substance (DCP or p-cumaric acid). In this connection it may be interesting to mention the observation of CZAPEK (1897, 1902) and of METZNER (1936) that in geotropically exposed root tips of *Vicia faba* an increase of the content of a diphenol; dioxyphenylalanine occurs. It would be worth-while to re-investigate the meaning of this early statement. Probably mono- and diphenols therefore play an important part in the geotropic reaction. This part could either be a direct or an indirect one, i.e. by regulating the IAA concentration in the reacting tissue. For the latter possibility arguments were found in a study of the effect of DCP, p-cumaric acid and caffeic acid on the geotropic reaction and on the straight growth (Chapters 4 and 5).

As for the straight growth, it should be mentioned that NITSCH

and NITSCH (1962) found a correlation between the effect of several phenols thereupon and on the activity of the IAA converting system whereas VARGA and KÖVES (1962) and STENLID (1963) did not find a direct relation.

No arguments were found to conclude that the supposed unequally distributed substance cannot be IAA. AUDUS and LAHIRI (1961) found that after a geotropic exposure the content of a compound that could not be identified, quickly increased in root tips of *Vicia faba*. According to these authors, however, it is not IAA. The demonstration of IAA in extracts from pea roots has ever met difficulties (cf. GORTER, 1932; AUDUS and GUNNING, 1958). This possibly has to be ascribed to the action of the IAA converting system during the extraction. Negative results might be due to the applied methods and do not prove that IAA does not occur in pea roots.

The conventional interpretation of geotropism in roots according to the CHOLODNY–WENT theory is far too simple. Especially the alternating changes of the growth rate at the upper and at the lower side of horizontally growing roots do not fit in the frame of this theory. In principle the objections mentioned in this paper are similar to those raised by AUDUS and BROWNBRIDGE (1957). These authors believe that the strong decrease of the growth rate at the lower side is caused by the production of some growth inhibitor.

This inhibitor, however, could equally well be IAA, since a strong increase of the IAA concentration also strongly inhibits the growth of roots.

The CHOLODNY–WENT theory postulates that the natural IAA concentration in the root tip is supra-optimal. The experiments on which this conception is founded—decapitation of the roots, the application of IAA from outside—offer by no means a convincing proof.

LARSEN (1956) correctly pointed out that in all former experiments the effect of IAA has been determined a very long time (17 hours or even more) after its application. Since the IAA taken up by the roots is converted in the root, it is doubtful whether after such a long time actually the effect of IAA is measured.

From experiments in which the rate of elongation was measured at short intervals (Chapter 4), it appeared that the growth rate in IAA solutions of a concentration of 10^{-11} g/ml and 10^{-9} g/ml IAA is enhanced and not lowered. This is incompatible with the conception that the IAA concentration in roots is supra-optimal. Moreover it is useless to connect the effect of added IAA on the growth measured after 17 hours, with the geotropic reaction, which already gets apparent after 30 minutes (cf. RUFELT, 1961).

Finally it may be remarked, that if IAA is actually unequally distributed in the horizontal root tip, this must not necessarily be due to some lateral transport. A change of the activity of the IAA converting system—i.e. a change in the ratio mono/diphenols—at the upper—and at the lower side can account for a difference in the IAA concentration as well.

The changes of the properties of the cell walls, as reported in Chapter 6, caused by a geotropic exposure, curiously enough, prove to be almost independent of the presence of DCP, caffeic acid and IAA, substances which all exert a notable influence on the geotropic reaction. This does not necessarily mean that these changes have no connection with the geotropic reaction: the regulation of these changes, however, lays beyond the scope of the IAA converting system.

The course of the positive phase of the geotropic reaction of roots can tentatively be explained by assuming that:

1. the activity of the IAA converting system is lowered in the horizontal position (either by an increase of the concentration of an inhibitor of this system (diphenol) or by the decrease of that of a promoting substance (monophenol). This decrease in activity is stronger at the lower than at the upper side of the root tip.
2. this decreased activity can result in a local accumulation of IAA and so in an unequal distribution of IAA.
3. this unequal distribution—according to the classic theory—causes a difference in growth rate between the upper- and the lower side and as a consequence a geotropic curvature gets apparent. Simultaneously changes in *S*, *O* and *W* occur which are controlled otherwise.
4. the rate of curving during the positive phase of the reaction decreases with time because the activity of the IAA converting system is gradually restored.

During the geotropic reaction a negative phase can occur which is dependent on the medium of the roots. The way in which this phase is realized can as yet not be elucidated.

SUMMARY

The character of the enzymic conversion of IAA in 48 hours old roots of *Pisum sativum* var. 'Vlijmse Gele Krombek' has been investigated in enzyme preparations as well as in the root tip tissue.

From experiments with enzyme preparations it appeared that IAA is not converted by a peroxidase but by an oxidase. This oxidase possibly is a phenol-oxidase.

The latter has the capacity to convert DCP and p-cumaric acid in vitro. This conversion enhances the degradation of IAA in the solution. IAA is not converted if this solution does not contain a naturally occurring or added monophenol. The conversion of IAA therefore is linked with that of a monophenol as a co-factor.

Caffeic acid (a diphenolic compound) inhibits the degradation of IAA in a solution.

Also in the tissue of the root tips, the conversion of IAA is enhanced by the addition of DCP or of p-cumaric acid and inhibited or even checked by addition of caffeic acid. DCP itself is converted in the root. Apparently the same IAA converting system is active as well in the living tissue as in enzyme preparations *in vitro*.

No influence of externally supplied IAA could be found on the rate of the IAA conversion in the root tips.

In geotropically exposed, i.e. horizontally placed, roots the activity of the IAA converting system is lowered. This points towards a physiological meaning of this system.

The effect of DCP, p-cumaric acid and caffeic acid has been investigated in relation to their effect upon the IAA converting system on the geotropic reaction and on the straight growth. In these experiments the roots had to grow in water or in solution of the mentioned compounds.

Therefore the geotropic reaction, i.e. the course of the curvature and the growth rate at the upper and at the lower side of the horizontal root, was followed in different media: moist air, in water and in solutions of the mentioned compounds.

The geotropic reaction shows alternating positive and negative phases, which are more pronounced in submersed roots than in roots growing in moist air.

The effect of DCP, p-cumaric acid and caffeic acid on the geotropic reaction and on the straight growth suggest that IAA actually is the regulating factor of both processes in roots.

No indication has been found that IAA is present in the root in a supra-optimal concentration. The observed phenomena are therefore incompatible with the CHOLODNY–WENT theory in its classic form.

If DCP is added at different stages of the geotropic reaction, it suppresses the next positive or negative phase of this reaction.

Caffeic acid on the other hand promotes the origination of a difference in growth rate between the upper and the lower side of the horizontal root and prolongs the duration of such a difference.

IAA added to the medium decreases the difference in growth rate between the upper and the lower side.

The results endorse the conception that IAA regulates the geotropic reaction and that the IAA concentration in its turn is governed by the activity of the IAA converting system.

If IAA is actually unequally distributed in the horizontal root, this unequal distribution has not necessarily to be ascribed to some lateral transport, but more likely to a different activity of the IAA converting system at the upper and at the lower side of the root tip.

Finally the changes of several cell properties—suction force, osmotic value, wall pressure, plastic and elastic extensibility of the cell walls—have been determined in geotropically exposed roots. The influence of DCP, caffeic acid and IAA on these properties has been studied.

The suction force, the osmotic value and the elastic and plastic extensibility are lowered after placing the roots horizontal (measured after 20 to 30 minutes).

Although these magnitudes are changed by the addition of DCP, caffeic acid and IAA in vertically growing roots, similar changes were not observed in horizontal roots, whereas these substances do affect the geotropic reaction.

In experiments of long duration the elastic and plastic extensibility of the cell walls and the water uptake show changes in water and in solutions of caffeic acid, the direction of which is opposite to that of the geotropic reaction. In solutions of DCP and IAA, however, no correlation has been found between these changes and the geotropic reaction.

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