GROWTH-SUBSTANCE RELATIONS IN THE AVENA COLEOPTILE, STUDIED BY MEANS OF THE GEOTROPIC RESPONSE

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CHAPTER I

INTRODUCTION

Since the investigations of KNIGHT (1806) it is known that the curvature exhibited by orthotropic plant organs (roots, shoots, coleoptiles), when they are placed horizontally, is a response to the change with regard to the direction of gravity. This curvature is the result of a difference between the growth rate in the upper and that in the lower side of the organ.

The geotropic reaction of the *Avena* coleoptile has been studied extensively. It is a negatively geotropic organ, which means that it

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curves upwards when it is placed horizontally. The earlier work with this organ mainly concerned the influence of the angle of the coleoptile to the direction of gravity and the influence of external factors, e.g. the estimation of g.t.sin α (g = gravitational force), that is, the minimum time (t) a coleoptile must be kept at a given angle (α) to the vertical in order to obtain a perceptible curvature (RUTTEN-PEKELHARING, 1910); the influence of various temperatures on the presentation time (RUTGERS, 1912) and the geotropic curvature in the absence of free oxygen (VAN AMEYDEN, 1917). Geotropism had mainly been considered from the mechanical point of view, e.g. the theory of HABERLANDT (1900) and NĚMEC (1900) on the statolithic function of starch grains.

After the discovery by DARWIN (1880) that the perception of gravity is localized in the root tip, and the conclusions of ROTHERT (1894) that the same must be true for the coleoptile, and after the remarkable results of BOYSEN-JENSEN (1913), PAÁL (1914, 1919), SÖDING (1923) and WENT (1927, 1928), a new facet of the geotropic reaction was revealed. Particularly by the work of the last-mentioned author it became clear that the tip of the coleoptile is the production centre of a growth-regulating substance and that this growth substance plays a role in the development of the geotropic curvature, which indeed is a manifestation of differential growth.

That growth substance is a necessary factor was clearly shown by the work of WENT (1927). He demonstrated that if gelatine blocks containing growth substance, obtained by diffusion out of *Avena* coleoptile-tips, were placed on decapitated coleoptiles, a geotropic curvature resulted when the coleoptiles were placed horizontally, whereas, when blocks of plain gelatine were placed on the coleoptiles, no curvature occurred. This author, therefore, used a hydro-gel containing growth substance obtained from plant material to induce a geotropic curvature in decapitated coleoptiles. NUERNBERGK (1933) used auxin derived from urine, and found that if it is dissolved in an agar block and in this way supplied to a decapitated coleoptile, the coleoptile curves geotropically. His work therefore indicates that a growth substance, which had not yet been isolated from plant material, can also induce a geotropic curvature. BURKHOLDER (1941) was the first to study the effect of synthetic growth substances (e.g. α-naphthalene-acetic acid, indole-3-propionic acid, indole-3-butyric acid and indole-3-acetic acid) on the geotropic curvature of intact *Avena* seedlings and excised coleoptiles, the growth substances being either dissolved in water or mixed with agar. His conclusion that the growth substances reduce the curvature of the coleoptiles, however, has to be discounted in the light of later work (cf. ANKER 1954) which shows that the concentrations which he used were physiologically either too high or too low. ANKER (1954, 1956) made quantitative studies of the growth-substance requirements of excised, decapitated coleoptiles during the geotropic curvature; in his experiments the growth substances were dissolved in water and the coleoptiles submerged in the solutions. With three different growth substances (indole-3-acetic acid, indole-3-aceto nitrile and α-naphthalene-acetic acid) he demonstrated that the geotropic curvature is maximal in solutions of which the concentrations are sub-optimal for coleoptile growth, and that the geotropic curvature fails to appear in concentrations which are optimal and supra-optimal for coleoptile growth. An important feature of Anker's experiments is that they indicate the possibility of conducting quantitative experiments on geotropism with excised, non-decapitated coleoptiles in water, and with excised, decapitated coleoptiles in solutions of various growth substances.

To explain the differential growth of the upper and lower side in geotropic curvatures, CHOLODNY (1927) and WENT (1927) independently came to the same conclusions. The Cholodny-Went theory of tropisms was formulated in this way: "Growth curvatures, whether induced by internal or external factors, are due to an unequal distribution of auxin between the two sides of the curving organ. In the tropisms induced by light and gravity the unequal distribution is brought about by a transverse polarization of the cells, which results in a lateral transport of the auxin." (WENT and THIMANN, 1937). According to the theory, the auxin concentration on one side of a horizontally placed organ should be higher than on the other side. This view has been corroborated by the following investigations: DOLK (1930, 1936) on the coleoptiles of *Avena* and *Zea mays*; NAVEZ and ROBINSON (1933) on the coleoptile of *Avena*; DIJKMAN (1934) on the hypocotyl of *Lupinus* and VAN DER LAAN (1934) on the epicotyl of *Vicia faba*. Their results all show that the amount of growth substances diffusing from the lower half of the horizontally placed organ is higher than the amount diffusing from the upper half, but the question as to how these differences arise, is still unanswered.

BRAUNER (1926, 1927, 1928) showed that in horizontally placed tissue, living or dead, as well as in a suitable model (two electrolyte solutions separated by parchment paper) a transverse electrical polarity is established. The upper side of a horizontally placed coleoptile becomes electrically negative with respect to the lower side. Brauner called this phenomenon "geo-electric effect". Further investigations on the difference in potential between the upper and lower side of horizontally placed plant organs were carried out by BRAUNER and AMLONG (1933), CLARK (1937) and SCHRANK (1944, 1945*a*, 1945*b*). In all cases the same transverse electrical polarity could be demonstrated. CHOLODNY (1926, 1927) had already suggested that the transverse movement of growth substances would be the result of an e.m.f., and WENT (1932) formulated "Eine botanische Polaritätstheorie" (a theory of polarity in plants) after investigating the penetration of basic and acidic dyes into stem sections of *Impatiens*. According to this theory the lateral movement of growth substances would be due to a cataphoretic transport: the negatively charged anion of the growth-substance molecule would be transported to the positively charged lower side of the horizontally placed organ, resulting in an increase in the growth substance concentration in the lower side. As yet, no direct evidence has been brought forward to prove that growth substances are displaced inside the plant by electric potentials, and the question still remains whether the potential differences shown to exist in a horizontally placed plant organ are the primary effect, and, if so, whether they are adequate to displace growth substances and thus produce a geotropic curvature. In this connection SCHRANK (1945*a*) stated that "the fact that the transverse electrical polarity is established prior to the unequal distribution (of growth substances) does not necessarily mean that it is the orienting force or polarity which is essential for lateral hormone transport. It means only that the transverse electrical polarity fulfills the prime requirement of being established in the correct sequence."

Although the insight into the problem of "geotropism-growth substance" is still far from complete, the expectation seems justified that by more detailed studies of the geotropic reaction at precise dosages of growth substance, a better understanding of the relation between reacting organ and growth substance will be obtained.

From what already has been stated it can be concluded with certainty that the relation between growth substance and tissue in the upper side of a horizontally placed organ is different from that in the lower side, because—as in the case of the coleoptile of *Avena*—the lower side grows more rapidly than the upper side. This does not necessarily have to imply that more growth substance is available at the lower side. The results of Dolk and others indeed seem to indicate this possibility, but the validity of their conclusions is limited by the possibility, that in the case of equal distribution of growth substance between the upper and lower half of the coleoptile, growth substance diffuses more easily from the lower half of the coleoptile, in other words, is more mobile than the growth substance in the upper half. As professor Koningsberger, in whose laboratory this investigation was carried out, remarked during preliminary discussions concerning this subject, it cannot be excluded that a physiological difference is induced between the upper and lower side when the organ is placed horizontally, so that in this position their relation to growth substance would be different. It is not unconceivable that a difference in sensitivity to growth substance could arise, or that the reactivity to equally distributed growth substance would be increased or decreased. Fig. 1 gives a clear picture of these two factors and it also indicates the possibility of discriminating between them. The relationship is different: an increase in the sensitivity is accompanied by a lower optimal concentration, a higher reactivity yields a stronger reaction at the same optimum concentration. In practice, however, an overlapping of the two curves will often frustrate the attempt to discriminate between the two theoretical possibilities.

It is also possible that, due to a shift in the potential, more growth substance would migrate from the lipophylic phase of the protoplasm to the hydrophylic phase, so that, as in the case of the experiments of Dolk, more growth substance would be obtained in the hydrophylic block of agar at the lower side.

The method of ANKER (1954, 1956), which consists of studying growth and geotropism with coleoptiles separated from the seed and submerged in solutions, has the advantage that growth substance can be supplied at will from an external medium, within a wide range of conditions and positions of the experimental objects, so that *quantitative* studies can be carried out. The principle of this technique was therefore adopted.

The present investigation was primarily undertaken to gain more insight into the geotropic reactions of the *Avena* coleoptile, but in a

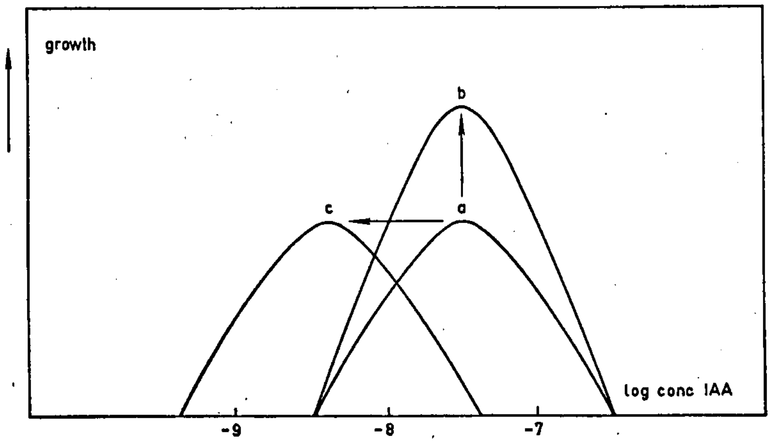

Fig. 1. Arbitrary optimum curves, showing the changes in reactivity and sensitivity of a tissue to growth substance. a → b: increased reactivity; a → c: increased sensitivity.

certain sense the geotropic reaction was also used as an aid to study diverse growth-substance effects on the coleoptile tissue. The most interesting questions investigated were: 1). How does a coleoptile previously kept horizontally in water react if it is subsequently kept vertically in growth substance? Does some "preparatory reaction" ("Stimmungsänderung") take place which later on can be released ("ausgelöst") by growth substance, or, in other words, does a geo-induction occur in the absence of growth substance? 2). Is a back curvature after a counter-induction in the other direction possible by means of growth substance already present in the coleoptile, or is it impossible that growth-substance molecules which have already partaken in a growth-reaction, are mobilized in order to promote growth at another site in the tissue, in this case at the other side of the coleoptile? 3). Must an induced curvature first be completed before the curvature in the opposite direction, caused by rotation through 180° (the upper side becoming the lower side) begins to develop or does the latter start immediately, and 4). What is the relation between the rate at which the original curvature proceeds and that of the back curvature?

The results of this investigation, although mainly obtained by means of a study of the geotropic reaction, moreover yielded a contribution to the knowledge with regard to the regeneration of a physiological tip, and to the uptake, the transport and the fixation of growth substance in the coleoptile of *Avena*.

CHAPTER II

MATERIAL AND METHODS

Unhusked seeds of *Avena sativa* (var. "Siegeshafer", 1954 harvest) were placed in water in a suction flask and evacuated on the water tap for approximately 10 minutes. After they had been washed a few times, they were left to soak in tap water for two hours. The soak water was discarded and the seeds were sown in moist, sterilized vermiculite in flats and put to germinate in a ventilated darkroom which was kept at a temperature of 23° C and at a relative humidity of about 90 %. When the seedlings were just appearing above the surface of the vermiculite, 72 hours after soaking, they were exposed to orange light for 24 hours in order to suppress mesocotyl growth. Throughout the investigation, which was carried out in the above-mentioned darkroom, the only light used was from incandescent lamps, filtered by Scott OG 2 filters, so that no phototropic responses could occur.

The geotropic reaction was studied with coleoptiles which had reached a height of 2–3 cm. Such coleoptiles are physiologically still young, and show very active growth. Only straight coleoptiles of the same length were used. The coleoptile was severed from the mesocotyl, and after the primary leaf had been extracted through the opening at the base of the coleoptile, it was placed in a VAN DER WEIJ (1932) microtome (guillotine). By means of this instrument, the hollow coleoptile cylinders were simultaneously cut to a uniform length of 16–17 mm, measured from the tip of the coleoptile. In the case of decapitation, however, this manipulation was preceded by the removal of exactly 1 mm of the tip of the coleoptile; the same instrument was used.

The coleoptiles were then mounted on an apparatus similar to that described by ANKER (1954). By means of such an apparatus 12 coleoptiles could simultaneously be brought from the vertical into the horizontal position and, after being brought back to the vertical position, they could automatically be turned 90° round the vertical axis, in order that shadowgraphs could be made of the curvatures. A few minor modifications, however, were made: 1) The pins onto which the coleoptiles were slipped, were slightly conical-shaped, so that coleoptiles of varying diameter could easily be mounted on the apparatus; 2) the base onto which the pins were fixed, was altered in such a way, that the coleoptiles could be rotated through 180°, so that the former upper side became the lower side. Two such apparatuses were used: one for the experimental series and the other for the control series. The two series of experiments were always carried out at the same time, because even in the course of a single day the results may vary considerably (cf. ANKER, 1954). When the coleoptiles were mounted on the apparatus, care was taken that they were placed in such a way that the curvatures would develop in the plane of the two vascular bundles. According to ANKER (1954), the geotropic curvatures in this plane are more uniform and more easily measurable than the curvatures in the plane of symmetry.

In the greater part of the experiments the geotropic curvature of decapitated coleoptiles took place in an aqueous solution of indole-3-acetic acid (IAA) containing 0.1 mg/l. This concentration was chosen because it is optimal for the geotropic curvature of decapitated coleoptiles (cf. ANKER, 1954). The curvature of non-decapitated coleoptiles took place in water, except where otherwise stated. The fluid (one liter), either a solution of IAA or water, was contained in a cuvette made from transparent perspex, which could be raised on a platform to such a height that the coleoptiles were 1.8 cm below the surface of the solution when they were placed horizontally. In those cases that the coleoptiles were kept vertically in the solution, the platform was raised to such a height that the tips of the coleoptiles were just below the surface.

Although no experiments were undertaken on the necessity of aerating the solutions, purified air was led through, with the additional benefit of keeping the solution in constant circulation.

All shadowgraphs were made on Gevaert Document Rapid photographic paper because of its sensitivity to the phototropically inactive light used in the darkroom. A series of shadowgraphs could thus be made of the same coleoptiles. Because coleoptiles are rarely perfectly straight, shadowgraphs were also made before each experiment, so that the actual curvatures could be measured. The photographic paper was held in a plate holder by means of a glass negative on which millimeter squares had been photographed. The fact that the coleoptiles were photographed together with the squares greatly facilitated the estimation of the curvatures. The plate holder was at a distance of 1 cm behind the coleoptiles and the lamp used for exposing was at a distance of 80 cm from the plate holder. The shadowgraphs thus obtained were of good contrast and the curvatures could be measured to a high degree of accuracy by means of a protractor.

In general, the variability in biological material is rather considerable. Therefore each experiment was repeated from three to six times and the mean values calculated. The variation is expressed as the standard error of the mean, calculated by means of the formula

$$SE_m = \pm \sqrt{\frac{\Sigma (v-m)^2}{n(n-1)}} \quad \text{where}$$

n = the number of coleoptiles

$\Sigma (v-m)^2$ = the sum of the squared deviations from the mean.

CHAPTER III

TIME RELATION BETWEEN UPTAKE OF GROWTH SUBSTANCE AND GEOTROPIC CURVATURE

A. THE UPTAKE OF GROWTH SUBSTANCE IN THE HORIZONTAL POSITION

When coleoptiles, that have been separated from the seed and decapitated, are kept horizontally in water, there is at first either no curvature at all or a very slight one only, the magnitude of the curvature depending on the small amount of growth substance present in the coleoptiles after decapitation. However, 120 to 150 minutes after decapitation, the regeneration of the physiological tip is completed and then the rate of curving begins to increase. Soon afterwards it reaches a constant value. This is shown in Fig. 2. In solutions of growth substances of appropriate concentration, on the other hand, decapitated coleoptiles show a well-marked curvature after 75 minutes which may practically equal that of non-decapitated coleoptiles in water. An experimental period of this short duration has the advantage that the experiment is completed before an appreciable regeneration of the physiological tip, which otherwise might obscure the results, has been effected. It is therefore clear that the geotropic curvature is dependent

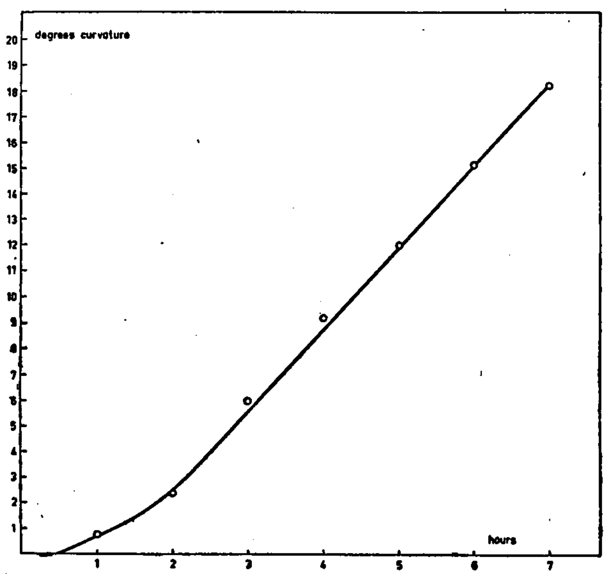

Fig. 2. Curvature of decapitated coleoptiles kept in a horizontal position in water for 7 hours.

upon the presence of growth substance. The increase of the curvature depends on the amount of growth substance available, and its rate of development may therefore be used as a measure of the uptake of the growth substance and its utilization, a utilization, of course, depending upon the uptake of the growth substance.

When supplying growth substance from an external medium to decapitated coleoptiles, one meets the question whether it is possible for the coleoptiles to give a full geotropic response if they are provided with growth substance for only part of the time that they are exposed to a unilateral action of gravity. In other words, can a flood (store) of growth substance be utilized in a subsequent geotropic reaction, or do the uptake of growth substance and the curvature only proceed simultaneously?

This question was investigated in the following series of experiments. (a) During an experimental period of 75 minutes, decapitated coleoptiles were kept horizontally. During this period they were kept for either 0, 15, 30, 45 or 60 minutes in a solution of growth substance (0.1 mg IAA/l) and for the rest of the time in water. Decapitated coleoptiles that were kept for 75 minutes in the growth substance solution served as controls. The coleoptiles used in the experimental series were kept in the growth-substance solution (i) from the start of the experiment, (ii) during the latter part of the experiment or (iii) in such a way that they were brought once more in water for the last 15 minutes of the experiment, as described below and shown in Fig. 3 in which the results obtained are shown.

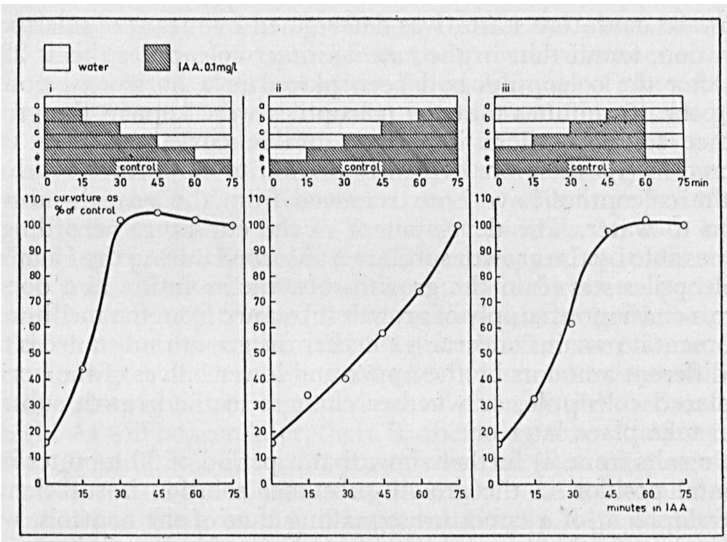

Fig. 3. Curvature of decapitated coleoptiles kept during part of the 75 minutes period of geotropic exposure in a growth-substance solution (0.1 mg IAA/l). Magnitude of the curvature expressed as a percentage of the curvature of the controls which were kept in the growth-substance solution for the entire experimental period. (Average curvature of the controls: 10° = 100 %).

All coleoptiles kept horizontally for 75 minutes:

(i) (a) 75 minutes in water
(b) 15 minutes in growth-substance solution, 60 minutes in water.
(c) 30 ,, ,, ,, ,, ,, 45 ,, ,, ,,
(d) 45 ,, ,, ,, ,, ,, 30 ,, ,, ,,
(e) 60 ,, ,, ,, ,, ,, 15 ,, ,, ,,
(f) 75 ,, ,, ,, ,, ,, (controls).

(ii) (a) 75 minutes in water
(b) 60 minutes in water, 15 minutes in growth-substance solution.
(c) 45 ,, ,, ,, 30 ,, ,, ,, ,, ,,
(d) 30 ,, ,, ,, 45 ,, ,, ,, ,, ,,
(e) 15 ,, ,, ,, 60 ,, ,, ,, ,, ,,
(f) 75 minutes in growth-substance solution (controls).

(iii) (a) 75 minutes in water
(b) 45 minutes in water, 15 minutes in growth-substance soln., 15 min. water
(c) 30 „ „ „ 30 „ „ „ „ „ 15 „ „
(d) 15 „ „ „ 45 „ „ „ „ „ 15 „ „
(e) 0 „ „ „ 60 „ „ „ „ „ 15 „ „
(f) 75 minutes in growth-substance solution (controls)

From the results obtained in series (i) it can be seen that the amount of growth substance taken up during 15 minutes (one-fifth of the experimental period) is already sufficient to ensure a curvature of about 44 % of the control. In subsequent experiments (e.g. Fig. 4, p. 13) it was found that curvatures which could be measured by the method employed, only became apparent 20 to 30 minutes after the coleoptiles had been placed horizontally. Schrank (1945*a*), by whom the moment the upward curvature starts, was determined by means of microscopic observation, found that in the case of intact coleoptiles this is 22 minutes after the coleoptiles had been placed in a horizontal position. During the 15 minutes that the coleoptiles were kept in the growth-substance solution, there is no measurable curvature. The values registered in (i)*b* therefore evidently pertain to curvatures developing after the coleoptiles have been removed from the growth-substance solution to water. The development of the curvature accordingly is made possible by the growth substance absorbed during the 15 minutes the coleoptiles stayed in the growth-substance solution and does not require a continuous supply of growth substance from the medium. For the moment it remains uncertain whether the growth substance is taken up in different amounts by the upper and lower halves of the horizontally placed coleoptile, or whether changes in the growth substance pattern take place later on.

The results from (i) further show that a period of 30 minutes in the horizontal position in the growth-substance solution is sufficient for the development of a curvature equalling that of the controls, which had been kept in the growth-substance solution for the whole period of 75 minutes. There is no indication that the rate of the development of the curvature is more rapid in the beginning, i.e. during the latter part of the first 30 minutes, than afterwards. It will be shown later that the rate of increase of the curvature remains constant over a period of several hours. One may safely assume that the rate of development of the curvature is the same in both experimental and control coleoptiles throughout the experimental period. This was confirmed by the experiments described in a following section (p. 13). If the geotropic curvature is dependent upon the immediate availability of growth substance, which is likely since the geotropic curvature is a growth phenomenon, one must assume that by an excessive growth-substance uptake during the period of 30 minutes, the further development of the curvature is ensured for another 45 minutes. Even if some of the growth substance should be lost by diffusion into the surrounding water, a sufficient amount, at any rate, remains available to ensure the further development of the geotropic curvature during the period the experiment lasts.

The results of series (ii) and (iii) agree very well with each other: in both sets of experiments there is an increase in the magnitude of the curvature with an increase in the time the coleoptiles are kept in the growth-substance solution. During the time that the coleoptiles are kept in water the curvature remains negligible; in fact, the development of the curvature only starts after the coleoptiles have been placed in the growth-substance solution. In the following instances the period elapsing between the moment of introducing the growth substance and the end of the experiment will be designated as "conditioning time". In experiment *c* of series (ii) and in experiment *b* of series (iii) the conditioning time is 30 minutes and the curvature is 36 % and 35 % respectively of that of the controls. With a conditioning time of 45 minutes (experiment *d* of series (ii) and experiment *c* of series (iii)), the curvatures are 56 % and 62 % respectively, of the controls, which is to be regarded as a sufficient agreement. In all cases observed it is seen that a transfer of the coleoptiles from the growth-substance solution to water (series (iii)), does not affect the magnitude of the subsequent curvature, at least if the experiment is continued for a limited period only. The results of experiment *e* of series (ii), however, do not agree with those of experiment *d* of series (iii). In experiment *e* of series (ii) the curvature is 74 % of that of the controls while in experiment *d* of series (iii) the curvature equals that of the controls. In both cases the conditioning time is the same, viz. 60 minutes, and as it was shown in the two pairs of experiments described previously that a period of 15 minutes in water following the immersion in the growth-substance solution does not affect the final magnitude of the curvature, the difference in degree of curvature cannot be understood unless one assumes that the values obtained in experiment *d* of series (iii) are incidentally too high. As will be seen later, there is, after a lag time ("latency time") of 20 to 30 minutes a linear proportionality between the magnitude of the curvature and the conditioning time when the coleoptiles are kept horizontally in the growth-substance solution (p. 14). A conditioning time of 60 minutes therefore should yield a smaller curvature than one of 75 minutes.

(b) The fact has already been mentioned that during an immersion of only 15 minutes in the growth-substance solution enough growth substance is taken up to ensure at the end of the 75 minutes a curvature far in excess of that shown by decapitated coleoptiles kept horizontally in *water* for 75 minutes.

An exact estimation of the rate of growth-substance uptake from the observed curvatures, however, is impossible since the uptake proceeds more rapidly than the utilization. If this leads to an accumulation of growth substance in the coleoptile, so that the inner concentration becomes higher than that of the solution, it becomes possible that part of it is lost again by diffusion. If the coleoptiles are transferred to water, as was done in the present experiments, even without accumulation some auxin may be lost in this way. The probability of growth-substance loss is very small when the submersion time is short, since the length of the section is relatively great and a diffusion through the cuticle is not to be expected at the concentration used.

To investigate the influence of an immersion in a growth-substance solution, shorter than 15 minutes, decapitated coleoptiles were kept in a growth-substance solution (0.1 mg IAA/l) for 5 and for 10 minutes and then in water for 70 and 65 minutes respectively. In these series of experiments, the controls were decapitated coleoptiles kept horizontally in *water* for 75 minutes, in contrast to the previous series of experiments, where coleoptiles kept horizontally in *growth substance* for 75 minutes served as the controls.

The results are presented in Tables *Ia* and *Ib* respectively.

TABLE Ia

Curvature of decapitated coleoptiles kept horizontally, first in a growth-substance solution (0.1 mg IAA/l) for 5 minutes and then for a further 70 minutes in water. Controls: kept horizontally in water for 75 minutes.

Curvature in degrees	
5 minutes in the growth-substance solution, 70 minutes in water	75 minutes in water (controls)
4.6	3.0
4.1	4.1
4.2	2.8
4.7	4.0
4.8	2.8
Av. 4.5 ± 0.14	3.3 ± 0.29

TABLE Ib

Curvature of decapitated coleoptiles kept horizontally, first in a growth-substance solution (0.1 mg IAA/l) for 10 minutes and than for a further 65 minutes in water. Controls: kept horizontally in water for 75 minutes.

Curvature in degrees	
10 minutes in the growth-substance solution, 65 minutes in water	75 minutes in water (controls)
4.4	2.3
5.0	2.6
5.4	3.3
4.6	2.3
Av. 4.8 ± 0.22	2.6 ± 0.24

From the results it can be seen that when the coleoptiles are kept for only 5 minutes in a growth-substance solution, the curvature is 1.4 times as high as the curvature of the coleoptiles kept in water all the time. When kept for 10 minutes in the growth-substance solution, the curvature is 1.8 times as high. It is clear that the growth substance taken up during these 5 or 10 minutes is subsequently used for the development of the geotropic curvature which, however, starts only some 20 minutes later. This means that at least part of the IAA is retained during this interval and that it remains available for the development of the geotropic curvature.

The curvature of these coleoptiles was much smaller than that of coleoptiles which are kept in a growth-substance solution for 75 minutes, which is usually 10° or more. The amount of growth substance taken up in such short periods is therefore insufficient to ensure the development of a curvature of maximum magnitude.

(c) In the experiments described under (a) it was found that a period of 30 minutes in a horizontal position in a solution of growth substance is sufficient to ensure the further development of the geotropic curvature in water for at least 45 minutes and at the same rate as in the control coleoptiles which remained in the growth-substance solution. It was now investigated how long the effect which an immersion during 30 minutes in a growth-substance solution exercises on the further development of the curvature in water, really lasts. The experimental procedure is given below and the results are shown in Fig. 4.

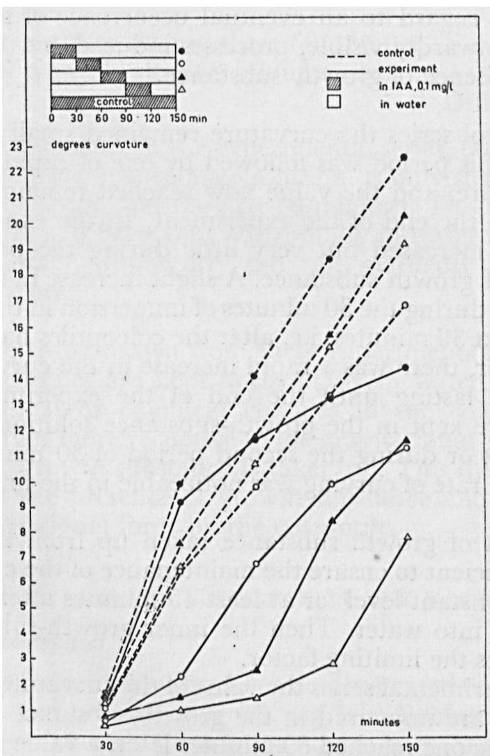

Fig. 4. Curvature of decapitated coleoptiles kept horizontally in water before and after a period of 30 minutes in a growth-substance solution (0.1 mg IAA/l). Controls: decapitated coleoptiles kept horizontally in the growth-substance solution for the whole of the experimental period.

Decapitated coleoptiles were kept horizontally for 150 minutes: in total they were kept in water for 120 minutes and for the remaining 30 minutes in a growth-substance solution (0.1 mg IAA/l). The distribution of the periods in the growth-substance solution varied in the different series of experiments. The controls were kept horizontally in a solution of growth substance for the entire experimental period of 150 minutes. The time-table for the different series is as follows:

(a) 30 minutes in the growth-substance solution, 120 minutes in water.
(b) 30 minutes in water, 30 minutes in g.s., 90 minutes in water.
(c) 60 „ „ „ 30 „ „ „ 60 „ „ „
(d) 90 „ „ „ 30 „ „ „ 30 „ „ „
(e) 150 minutes in the growth-substance solution (controls).

This procedure also enabled one to study the possible influence of a period passed horizontally in water, preceding that in which the growth substance was added, on the rate of curving. In this way data could be obtained with regard to an eventual occurrence of introductory, although not outwardly visible, processes induced by the gravitational force in the absence of growth substance.

In the control series the curvature remained small during the first 30 minutes. This period was followed by one of rapid increase in the rate of curvature, and the value now reached remained more or less constant up to the end of the experiment. In the experimental series the curvature increased but very little during the period preceding the addition of growth substance. A slight increase in the curving rate was noticeable during the 30 minutes of immersion in the IAA solution. During the next 30 minutes, i.e. after the coleoptiles had been brought back into water, there was a rapid increase in the curvature, followed by a decrease lasting until the end of the experiment. When the coleoptiles were kept in the growth-substance solution at the start of the experiment or during the second period of 30 minutes, a marked decrease in the rate of curving was noticeable in the final period of the experiment.

The amount of growth substance taken up from the solution apparently is sufficient to ensure the maintenance of the curving rate at a more or less constant level for at least 45 minutes after the transfer of the coleoptiles into water. Then the inner growth-substance concentration becomes the limiting factor.

If in the experimental series the value of the curvature at the moment the coleoptiles are immersed in the growth-substance solution is subtracted from the one reached 60 minutes later, a value is obtained that is nearly the same as that found in the control series 60 minutes after the beginning of the experiment. The method of calculation is shown below:

(a) IAA added at time zero:

Curvature at 0 minutes . . . . :	0.0°
„ after 60 minutes . . . . :	9.2°
„ of controls after 60 minutes . . . . :	9.9°
Difference:	0.7°

(b) IAA added after 30 minutes:

Curvature at that moment . . . . :	0.6°
,, 60 minutes later . . . . :	6.8°
Increase of curvature during these 60 minutes . . . . . :	6.2°
Curvature of controls after 60 minutes . . . . :	6.5°
Difference:	0.3°

(c) IAA added after 60 minutes:

Curvature at that moment . . . . :	1.6°
,, 60 minutes later . . . . :	8.5°
Increase of curvature during these 60 minutes . . . . . :	6.9°
Curvature of controls after 60 minutes . . . . :	7.6°
Difference:	0.7°

(d) IAA added after 90 minutes:

Curvature at that moment . . . . :	1.6°
,, 60 minutes later. . . . . :	7.8°
Increase of curvature during these 60 minutes . . . . . :	6.2°
Curvature of controls after 60 minutes . . . . :	6.7°
Difference:	0.5°

In all cases the curvature in the experimental series, 60 minutes after submersion in the growth-substance solution, is only slightly less than that of the control series after the first 60 minutes. The length of time that the coleoptiles had been kept horizontally in water before growth substance was added, apparently did not markedly influence the subsequent curvature.

The conclusion to be drawn from these experiments is that during the time the coleoptiles are kept horizontally in water, there are no preparatory physical or chemical changes in the coleoptile which would make it either more receptive to the gravitational force, or more reactive once the growth substance is taken up. This indicates a close relation between the presence of growth substance and the "perception" of the gravitational force by the coleoptile.

B. The uptake of growth substance in the vertical position

(*1*) *Decapitated coleoptiles*

In the previous section (A) it has been demonstrated that decapitated coleoptiles kept horizontally in a 0.1 mg/l IAA-solution for only a short time, take up enough growth substance to ensure a prolonged development of the geotropic curvature when transferred to water.

It was now investigated what geotropic curvatures decapitated coleoptiles exhibit in water, when the growth substance was taken up in a vertical position.

(a) Decapitated coleoptiles were kept vertically in a solution containing 0.1 mg IAA/l for periods ranging from 5 to 90 minutes and then horizontally in water for a further 75 minutes The experiments therefore were completed before an appreciable regeneration of the physiological tip could have been effected. It is known from the work of GORTER (1927) and confirmed by the present results (p. 40) that no regeneration of the physiological tip occurs when the decapitated coleoptiles are supplied with IAA. According to TSI-TUNG LI (1930) the process is completed 120 to 150 minutes after 1 mm of the tip of a coleoptile still attached to the seed, is removed.

In the control series decapitated coleoptiles were kept vertically in water for the same length of time as the coleoptiles of the experimental series were kept vertically in the growth-substance solution, and then for 75 minutes horizontally in the growth-substance solution. The curvatures were measured after the coleoptiles had been in the horizontal position for 75 minutes; the results are shown in Table II.

TABLE II

Curvature of decapitated coleoptiles, first kept vertically in a growth-substance solution (0.1 mg IAA/l) for various lengths of time and then horizontally in water for 75 minutes. Controls: decapitated coleoptiles first kept vertically in water for the same period as the experimental coleoptiles were kept vertically in the growth-substance solution, and then horizontally in a growth-substance solution (0.1 mg IAA/l) for 75 minutes. Curvatures in experimental and control series measured after the same length of time.

Minutes in vertical position	Curvature in degrees		Curvature expt. as % of the controls
	Experimental coleoptiles	Control coleoptiles	
0	1.5 ± 0.19	8.7 ± 0.84	17
5	5.1 ± 1.27	12.0 ± 1.76	43
10	5.1 ± 0.82	13.6 ± 0.72	38
20	5.9 ± 0.44	13.8 ± 0.44	43
25	3.6 ± 0.07	10.9 ± 0.20	33
30	3.9 ± 0.22	14.8 ± 0.33	26
40	5.3 ± 0.75	13.9 ± 1.30	38
60	5.3 ± 0.54	14.2 ± 1.82	37
90	6.7 ± 0.83	15.6 ± 0.52	43

From the results presented in Table II it is clear that there is not much difference in the curvature of the coleoptiles, whether they were kept in the growth-substance solution for only 5 minutes or for 90 minutes. Although there is some variation in the absolute value of the curvatures, they all fluctuate between 26 % and 43 % of the value reached by the curvature of the controls. In all instances the curvature is larger than that of coleoptiles which were placed horizontally in water but were not previously kept vertically in a growth-substance solution, for in that case the curvature measured only 1.5° ± 0.19.

Although the growth substance was provided in a concentration sub-optimal for growth (ANKER, 1956), the results indicate that the amount taken up in the vertical position is not entirely consumed in the ordinary growth process, but that part of it remains available for a subsequent geotropic reaction. Whether the coleoptiles remain from 5 to 90 minutes vertically in the growth-substance solution, this part proves to be independent of the time during which the growth substance is taken up. This would indicate that the uptake of growth substance proceeds more rapidly than its fixation to, or utilization by the protoplasm. Since both processes are proceeding at a constant rate, a steady state is soon reached, in which a certain amount of growth substance ("free auxin") remains available in the cells. In the previous section (p. 10) there were indications for the assumption that the growth substance, once it has been taken up by the coleoptile, is stored in such a way that it cannot be lost into the surrounding water. It therefore does not seem likely that the growth substance taken up in the vertical position is lost into the water when the coleoptiles are placed horizontally, and that the small size of the curvature would be due to this cause.

(b) From the preceding series of experiments it is clear that in any case part of the growth substance taken up in the vertical position can be used in the development of a geotropic curvature when the coleoptiles are placed horizontally in water.

Next, the possibility was investigated of a summation of the effects due to the amount of growth substance taken up first during a stay in the vertical position and then in a horizontal one in the same solution. In view of the results obtained by ANKER (1954, 1956), a summation would result in a small decrease in the magnitude of the curvature, since the concentration of 0.1 mg IAA/l in the medium was already nearly supra-optimal.

Decapitated coleoptiles were kept vertically in a growth-substance solution (0.1 mg IAA/l) for different lengths of time and then horizontally in the same solution for a further 75 minutes. In the control series the coleoptiles were kept vertically in water for the same length of time as the experimental ones were kept vertically in the growth-substance solution, and then they were kept horizontally in the growth-substance solution for 75 minutes. The results are given in Table III.

TABLE III

Curvature of decapitated coleoptiles first kept vertically in a growth-substance solution (0.1 mg IAA/l) for various lengths of time and then horizontally for a further 75 minutes, also in the growth-substance solution. Controls: decapitated coleoptiles first kept vertically in water for the same period as the experimental coleoptiles were kept vertically in the growth-substance solution, and then horizontally in the growth-substance solution for 75 minutes.

Minutes in vertical position	Curvature in degrees		Curvature expt. as % of controls
	Experimental coleoptiles	Control coleoptiles	
10	10.7 ± 0.91	11.2 ± 1.94	96
20	11.9 ± 0.84	12.7 ± 1.22	94
40	11.2 ± 1.12	11.7 ± 1.07	96
80	13.6 ± 1.58	12.4 ± 0.26	110

The curvature of the experimental series is slightly smaller than that of the control ones. If the differences between the curvature of the control and the experimental series were significant, this could easily be explained by the observations of Anker just-mentioned. These results agree well with the explanation (p. 10) that a constant ratio between the uptake and utilization of the growth substance is soon reached, so that a certain amount of free growth substance remains available in the cells.

(c) ANKER (1956) has shown that no geotropic curvatures occur in IAA solutions of concentrations that are optimal for straight growth (0.6 mg IAA/l) or higher. It seemed worthwhile to investigate the effect exercised by a high growth-substance concentration applied before the coleoptiles were placed horizontally, on the magnitude of the geotropic reaction, when the latter is carried out in a solution of optimal IAA concentration. Therefore experiments were carried out in which the coleoptiles were kept vertically for periods of 10, 20, 40, and 80 minutes in a solution containing 1.0 mg IAA/l and then horizontally for a further 75 minutes in a solution containing 0.1 mg IAA/l. In this case the controls were kept vertically in water for the same length of time as the coleoptiles of the experimental series were kept vertically in the growth-substance solution and then horizontally in a growth-substance solution (0.1 mg IAA/l) for 75 minutes. The results are shown in Table IV and in Fig. 5.

TABLE IV

Curvature of decapitated coleoptiles first kept vertically in a growth-substance solution (1.0 mg IAA/l) for various lengths of time and then horizontally in a growth-substance solution (0.1 mg IAA/l) for 75 minutes. Controls: decapitated coleoptiles kept vertically in water for the same period as the experimental coleoptiles were kept vertically in the growth-substance solution, and then horizontally in a growth-substance solution (0.1 mg IAA/l) for 75 minutes.

Minutes in vertical position	Curvature in degrees		Curvature in experimental series as % of those of controls
	Experimental coleoptiles	Control coleoptiles	
10	7.6 ± 0.51	10.6 ± 0.76	72
20	6.1 ± 0.31	9.9 ± 0.75	62
40	5.5 ± 0.40	10.3 ± 0.65	53
80	4.9 ± 0.45	12.7 ± 0.96	39

It is clear that a "flooding" of the coleoptile with growth substance, prior to a geotropic exposure, has a strongly decreasing effect on the magnitude of the curvature. Even such a short period as 10 minutes has a marked depressing effect on the ultimate magnitude of the curvature, again indicating that the uptake of growth substance by decapitated coleoptiles is a rapid process.

There is no reason to suppose that the growth-substance concentration was supra-optimal for straight growth. The geotropic curvature already decreases considerably at concentrations which are far below the saturation value for straight growth. The relation between the optimal concentrations for straight growth and for geotropism is dealt with by ANKER (1956). Maximal geotropic curvature occurred in that limited range of concentrations where small differences in the concentration cause large differences in straight growth. The interpretation of the present decrease in curvature is obvious on account of the results of ANKER (1956). Here also the concentration inside the coleoptiles was supra-optimal for the geotropic curvature and about optimal for straight growth.

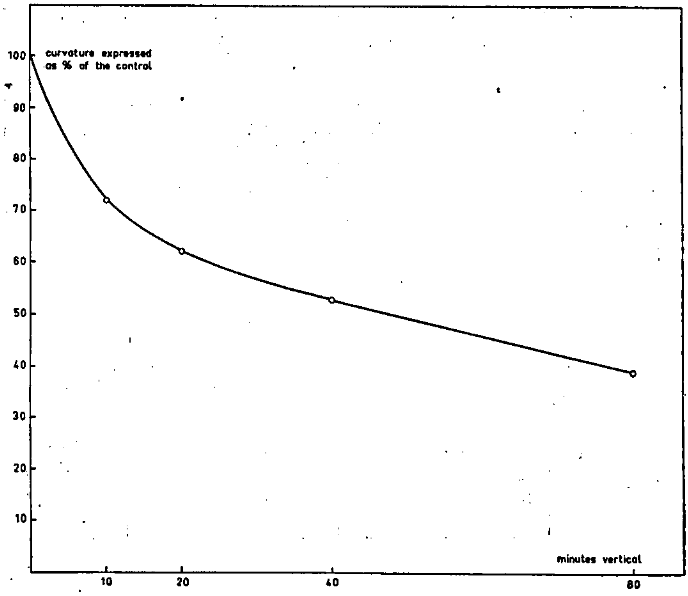

Fig. 5. Curvature of decapitated coleoptiles first kept vertically in a solution containing 1.0 mg IAA/l and then horizontally in a solution containing 0.1 mg IAA/l for 75 minutes; curvature expressed as a percentage of that of the controls. Controls: coleoptiles first kept vertically in water and then horizontally in the growth-substance (0.1 mg IAA/l) for 75 minutes.

(2) *Non-decapitated coleoptiles*

The same kind of experiments was performed with non-decapitated coleoptiles. The growth substance was added in various concentrations, in some experiments while the coleoptiles were kept vertically and in others while they were kept horizontally. The growth substance now did not enter the coleoptile via a cut surface, as in the experiments with decapitated coleoptiles, but could only enter through the cuticle. In the previous experiments the latter way of entrance, of course, also occurs; however, it was shown by ANKER (1954) that at the low concentration of 0.1 mg IAA/l the curvatures of non-decapitated coleoptiles are equal to those, shown by non-decapitated coleoptiles kept in water. This means that with this concentration the amount of IAA passing through the cuticle in the experimental period of 75 minutes is negligible. There is no possibility of a supply of growth substance via the basal cut surface since it is a well-established fact that auxin transport in the *Avena* coleoptile is strictly polar, from tip to base only.

Another difference with the former experiments in which decapitated coleoptiles were used, is that the effect of growth substance absorbed from outside is now superimposed on the action of natural auxin produced in the tip. A complication will occur if the growth substance supplied from outside enters into the tip and then interferes with the auxin production.

For the above-mentioned reason, viz. that during the relatively short period of 75 minutes there is no appreciable (in any case no observable) effect of the entrance of growth substance through the cuticle, in the present experiments higher concentrations of IAA were used, viz. 0.2 mg/l, 0.5 mg/l, 0.6 mg/l and 1.0 mg/l. The coleoptiles were kept horizontally in the solutions for 75 minutes, and the curvatures were compared with those of non-decapitated coleoptiles kept horizontally in water for the same period. The results are shown in Table V.

TABLE V

Curvature of non-decapitated coleoptiles kept horizontally for 75 minutes in growth-substance solutions of different concentrations. Controls: non-decapitated coleoptiles kept horizontally for 75 minutes in water.

Concentration of IAA in mg/l in the experimental series	Curvature in degrees		Curvature expt. as % of the controls
	Experimental coleoptiles in growth-substance solution	Control coleoptiles in water	
0.2	20.2 ± 1.21	20.5 ± 1.56	99
0.5	16.8 ± 0.98	14.8 ± 0.77	114
0.6	14.6 ± 1.11	12.3 ± 0.89	119
1.0	15.2 ± 1.32	17.6 ± 1.07	86

From the table it is clear that even in a solution of 0.2 mg/l the curvature carried out under the influence of the natural auxin is not affected by the IAA absorbed from outside. In the higher concentrations of IAA, namely of 0.5 and of 0.6 mg/l, on the other hand, the curvatures were larger than those of the controls. In the highest concentration used, 1.0 mg/l, the curvature showed a slight decrease and in some coleoptiles even positive curvatures were recorded. These results support the estimation of ANKER (1956) that the inner auxin concentration in coleoptiles is very near to the optimal one for the geotropic response.

In another series of experiments non-decapitated coleoptiles were first kept vertically in solutions of different IAA concentrations for various lengths of time and then horizontally in water for a further 75 minutes. In the control series, non-decapitated coleoptiles were kept vertically in water for the same length of time that the coleoptiles of the experimental series were kept vertically in the growth-substance solution, and then horizontally for 75 minutes in water. The results are given in Tables VI, VII and VIII.

These results are different from those recorded in Table V. In the latter instance decreases as well as increases of the curvature were observed, depending on the IAA-concentration of the outer solution. In the present experiments the curvatures of the experimental coleoptiles are all smaller than those of the controls. The difference may find its explanation in the experimental procedure. The fact that the curvatures arose during or after the supply of growth substance could have been of paramount importance.

TABLE VI

Concentration of growth-substance solution 0.1 mg IAA/l. Non-decapitated coleoptiles first kept vertically in the growth-substance solution for various lengths of time and then horizontally in water for 75 minutes. Controls: non-decapitated coleoptiles kept vertically and horizontally in water for the same lengths of time as the experimental coleoptiles.

Minutes in vertical position	Curvature in degrees		Curvature in experimental series as % of those of controls
	Experimental coleoptiles	Control coleoptiles	
10	18.2 ± 0.48	20.5 ± 0.56	89
20	18.0 ± 1.21	20.2 ± 1.03	89
40	16.3 ± 0.85	18.9 ± 1.75	86
80	15.8 ± 0.65	17.7 ± 0.61	89

TABLE VII

Concentration of growth-substance solution 0.5 mg IAA/l. Non-decapitated coleoptiles first kept vertically in the growth-substance solution for various lengths of time and then horizontally in water for 75 minutes. Controls: non-decapitated coleoptiles kept vertically and horizontally in water for the same lengths of time as the experimental coleoptiles.

Minutes in vertical position	Curvature in degrees		Curvature in experimental series as % of those of controls
	Experimental coleoptiles	Control coleoptiles	
10	14.7 ± 0.79	16.1 ± 0.70	91
20	13.1 ± 0.50	14.8 ± 0.46	89
40	12.5 ± 0.44	14.9 ± 0.52	84
80	11.6 ± 0.42	14.8 ± 0.28	78

TABLE VIII

Concentration of growth-substance solution 0.6 mg IAA/l. Non-decapitated coleoptiles first kept vertically in the growth-substance solution for various lengths of time and then horizontally in water for 75 minutes. Controls: non-decapitated coleoptiles kept vertically and horizontally in water for the same lengths of time as the experimental coleoptiles.

Minutes in vertical position	Curvature in degrees		Curvature in experimental series as % of those of controls
	Experimental coleoptiles	Control coleoptiles	
10	13.9 ± 0.85	16.1 ± 0.43	86
20	13.9 ± 0.49	14.2 ± 0.56	98
40	10.2 ± 0.53	13.3 ± 0.91	77
80	10.2 ± 0.81	12.8 ± 0.91	80

It is difficult to assume that the decreases in the curvature, for example those shown in table VI, where the concentration of the growth-substance solution was only 0.1 mg/l was due to an over-dosage of IAA since the geotropic response in this solution and even in one of double the concentration (0.2 mg/l, see above) is not impaired. If there were an over-dosage of IAA one would expect the decreasing effect exercised by the outer solution on the curvature to increase with the time the coleoptiles are immersed in the solution.

A possible explanation is that the decreased curving rate is due to under-dosage of growth substance. This means that the coleoptile tip produces less auxin in a growth-substance solution than in water. A depressing effect exercised by small amounts of growth substance diffusing through the epiderm of the tip on the auxin production would be in accordance with the inhibition of the regeneration of the physiological tip by growth substance absorbed from outside.

CHAPTER IV

EFFECTS OF SHORT AND OF ANTAGONISTIC HORIZONTAL EXPOSURES

When geotropically stimulated coleoptiles are placed vertically or are rotated along the horizontal axis of a klinostat, the geotropic curvature continues to increase. After a while, however, the process is reversed and finally the coleoptiles are again more or less perfectly straight. To obtain this result, it is not necessary to continue the lateral exposition to gravity until the curvature becomes visible. RUTTEN-PEKELHARING (1910) found that such a short exposure as 4 to 5 minutes results in a perceptible curvature.

In this chapter the results are reported of an investigation in which the above-mentioned phenomenon was studied more closely. The experiments were carried out with decapitated coleoptiles in an IAA-solution of 0.1 mg/l and with non-decapitated coleoptiles in water.

A. CURVATURE OF COLEOPTILES THAT HAVE BEEN PLACED VERTICALLY AFTER A GEOTROPIC EXPOSURE

Non-decapitated coleoptiles were kept horizontally in water for a certain length of time and then placed vertically, also in water. Decapitated coleoptiles were kept horizontally in a growth-substance solution (0.1 mg IAA/l) for a certain length of time and then vertically either in the same solution or in water. The length of the period of geo-induction varied from 30 to 90 minutes; the results, shown in Fig. 6, may be regarded as typical of the general behaviour of coleoptiles in this type of experiment. Whether the coleoptiles were kept horizontally for short or for long periods, the behaviour was the same; there were, of course, differences in the absolute values of the curvatures.

It appears that non-decapitated coleoptiles start curving 20 to 30 minutes after being placed horizontally. Upon being placed vertically, the geotropic curvature continues for another 30 minutes and then a straightening of the coleoptile takes place rather rapidly. The straightening proceeds from the base towards the tip until the coleoptile is once more straight. In the case of decapitated coleoptiles kept both horizontally and vertically in a growth-substance solution, exactly the same happens. Therefore, there is a striking conformity between the reactions of the non-decapitated coleoptiles and the decapitated ones kept in the IAA solution of 0.1 mg/l. In the case of decapitated coleoptiles kept horizontally in the growth-substance solution and verti-

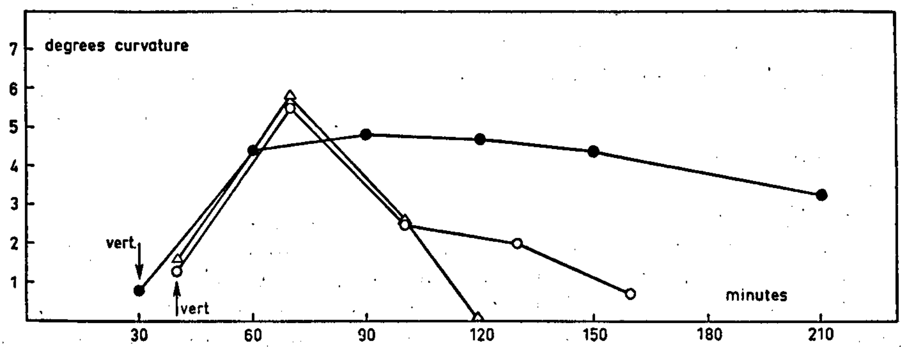

Fig. 6. Geotropic curvatures of coleoptiles carried out in the vertical position after periods of 30 or 40 minutes in the horizontal position. Triangles: non-decapitated coleoptiles, horizontal and vertical in water; circles: decapitated coleoptiles, horizontal and vertical in a growth-substance solution (0.1 mg IAA/l); dots: decapitated coleoptiles, horizontal in a growth-substance solution (0.1 mg IAA/l), vertical in water. Arrows indicate when the coleoptiles were placed vertically.

cally in water, however, the curvature increases during the first 30 minutes after the coleoptiles have been placed vertically in the same way as in the previous cases and then here too the curving comes to a standstill but there is no appreciable straightening of the coleoptiles.

From these results one must conclude that the changes brought about in the coleoptile by an exposure to gravity disappear within 30 minutes and that in the meantime reactions are initiated to restore the original situation. In this process of restoration growth substance is evidently involved since a straightening reaction did not occur when decapitated coleoptiles were placed vertically in water.

This lack of restoration in the absence of growth substance is of a similar nature as what might be called the absence of a "preparatory or introductory process" when coleoptiles are placed horizontally in water and then vertically in a growth-substance solution. One could suppose that in a horizontal position in the absence of growth substance, the decapitated coleoptile changes internally. Such an "introductory process" could consist of a change either in the sensitivity or in the reactivity of the coleoptile. If it should occur, the coleoptile would show a curvature when placed vertically in a growth-substance solution after a horizontal exposure in water. That such an introductory process does not exist is clearly demonstrated by the following experiment in which decapitated coleoptiles were first kept horizontally in water for periods of 30 or 40 minutes, during which time no curvatures were registered, and then vertically in a growth-substance solution (0.1 mg IAA/l). No geotropic curvatures could be ascertained. This again shows that the presence of growth substance in the coleoptile is required in order that a geotropic induction may take place.

This explanation would have to be discarded if it should appear that an introductory process actually occurs in the horizontal position. In that case it would have to be assumed that it is abolished in the vertical position before the effect of the growth substance becomes apparent. In all the preceding experiments it has been found that this effect only becomes visible after 20 to 30 minutes.

The following experiments do not yield any evidence for this explanation.

B. The curvature of coleoptiles during antagonistic exposures to gravity

In a way the effect of antagonistic exposures to gravity on the curvature has already been illustrated by the experiments under (A). The counterinduction obtained by curved coleoptiles being placed vertically, however, is very small. To study some aspects of the resulting curvatures more quantitatively, the coleoptiles were kept horizontally for a certain length of time, first in one position and then they were rotated through 180°, so that the former upper side became the lower

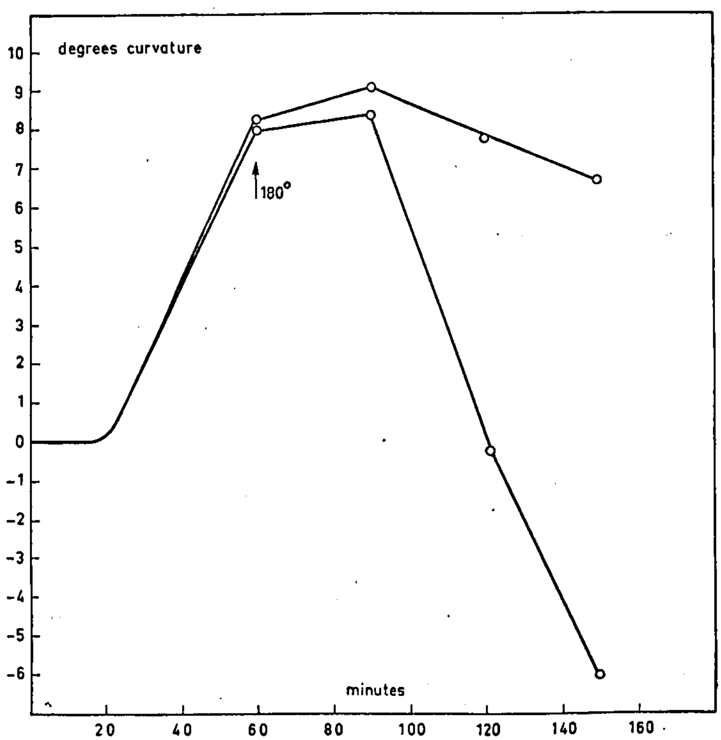

Fig. 7a. Curvature of decapitated coleoptiles kept horizontally in a growth-substance solution (0.1 mg IAA/l), first in one position for 60 minutes and then rotated through 180°. Upper line: kept in water after rotation; lower line: left in the growth-substance solution after rotation.

side. The curvature induced in the latter position would then be in a direction opposite to the initial one.

In preliminary experiments, decapitated coleoptiles were kept horizontally in a growth-substance solution (0.1 mg IAA/l) for 60 or 90 minutes and then rotated through 180°, after which they were kept either in water or remained in the growth-substance solution. The curvatures exhibited by such coleoptiles are shown in Fig. 7*a* and in Fig. 7*b*.

As usual, the coleoptiles started curving about 20 minutes after the moment they were placed horizontally. After they had been rotated through 180° they kept on curving in the initial direction for another 20 to 30 minutes. Then the coleoptiles left in the growth-substance solution began to curve rapidly in the opposite direction; it looked even as if the "back curvature" proceeded more rapidly than the initial curvature. In the case of coleoptiles kept in water after rotation through 180°, these also started curving in the opposite direction after 30 minutes. The "back curvature", however, was much slower than the original

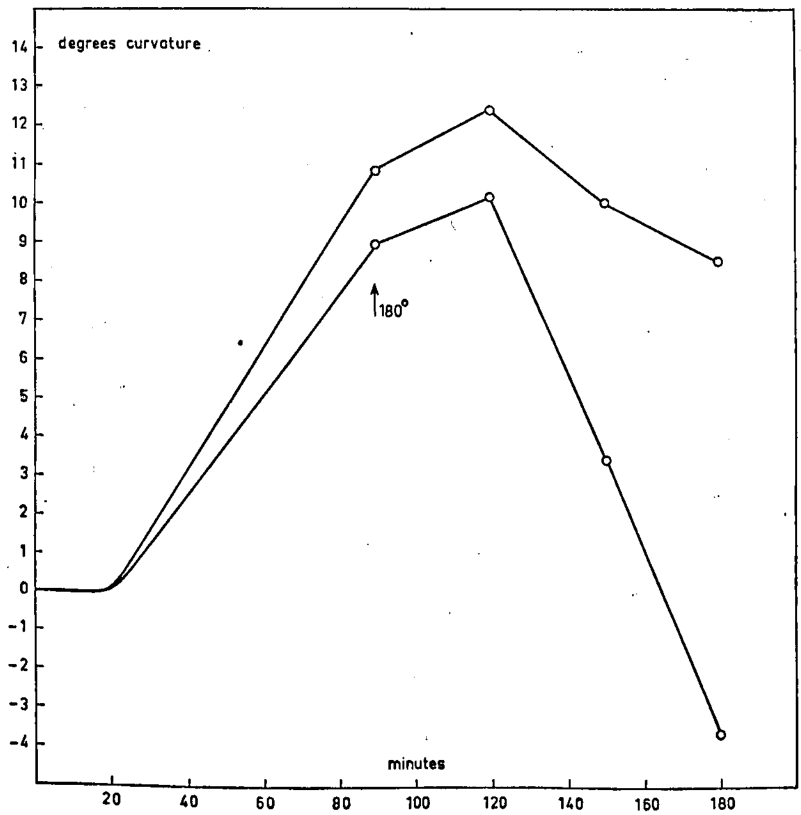

Fig. 7b. Curvature of decapitated coleoptiles kept horizontally in a growth-substance solution (0.1 mg IAA/l), first for 90 minutes in one position and then rotated through 180°. Upper line: kept in water after rotation; lower line: left in the growth-substance solution after rotation.

curvature. Here again it is observed that growth substance must be immediately available for the development of a geotropic curvature. As soon as the supply is discontinued the curving rate diminishes and, as in the present case, the effect of the antagonistic exposure is small. Complete absence of a back curvature was not found; apparently there is some auxin left to be utilized. The bulk of the growth substance, however, is bound—probably used up in the growth process during the first curvature—at any rate, it is not available for the back curvature.

The behaviour of non-decapitated coleoptiles in water (not shown in the figures) was exactly the same as that just described for decapitated coleoptiles kept in a solution of growth substance before and after rotation.

There were indications that the back curvature was more rapid than the original one, and to investigate this possibility, decapitated coleoptiles were kept horizontally in a growth-substance solution (0.1 mg IAA/l) for periods of 10, 20 and 90 minutes and then rotated through 180°, after which they were still kept in the same solution. To ensure that sufficient growth substance had been taken up for a proper geotropic reaction after 10 and 20 minutes, several experiments were also done in which the coleoptiles were pretreated with growth substance in the vertical position: they were kept vertically in the growth substance for 30 minutes before they were placed horizontally. An example is shown in fig. 11. This shows that the pretreatment had no effect. Growth substance was therefore not a limiting factor in these experiments. To compare the rates of curvature, control coleoptiles were kept horizontally in the growth-substance solution for the whole of the experimental

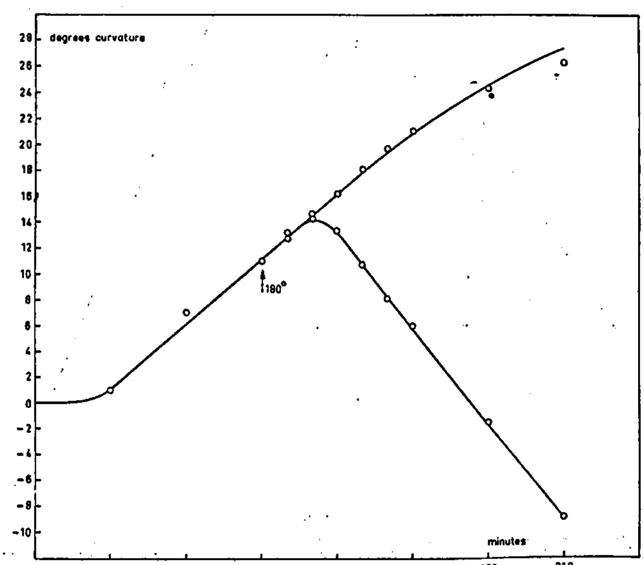

Fig. 8. Curvature of decapitated coleoptiles in a growth-substance solution (0.1 mg IAA/l). Upper line: controls kept in the same position for the entire experiment; lower line: rotated through 180° after 90 minutes.

period, and ~~not rotated through 180°. The same was done~~ with non-decapitated coleoptiles kept in water: after 10, 20 and 90 minutes they were rotated through 180°, while the control coleoptiles were kept in the same position for the entire experimental period.

The curvatures were measured at short intervals so that the curving rate could be determined accurately. Each experiment was repeated four or five times, and the values given in the results are the averages of these experiments. The results are shown in Figs. 8, 9, 10, 11, 12, 13 and 14.

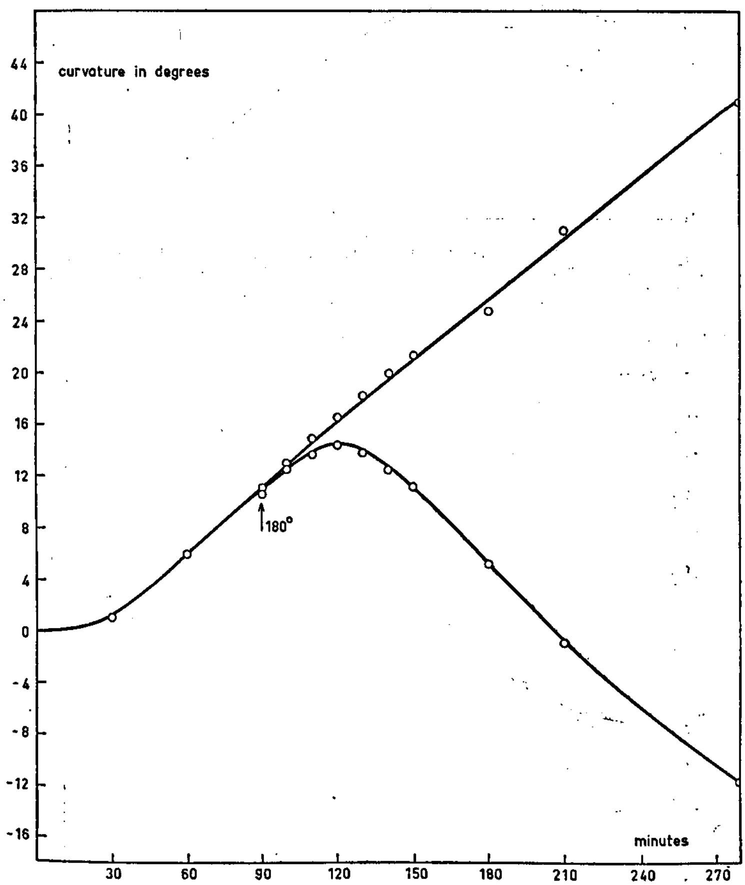

Fig. 9. Curvature of non-decapitated coleoptiles in water. Upper line: controls, not rotated through 180°; lower line: rotated through 180° after 90 minutes.

From the results the rates of curvature were calculated, starting from that point in the curves where the progress curve becomes a straight line. For example, the data from Figs. 8 and 9 (coleoptiles rotated after 90 minutes) yield the following figures:

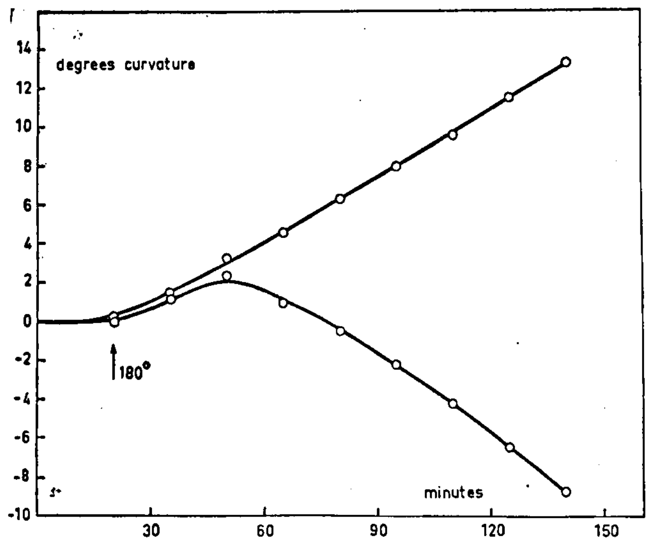

Fig. 10. Curvature of decapitated coleoptiles kept in a growth-substance solution (0.1 mg IAA/l). Lower line: rotated through 180° after 20 minutes; upper line: controls, not rotated through 180°.

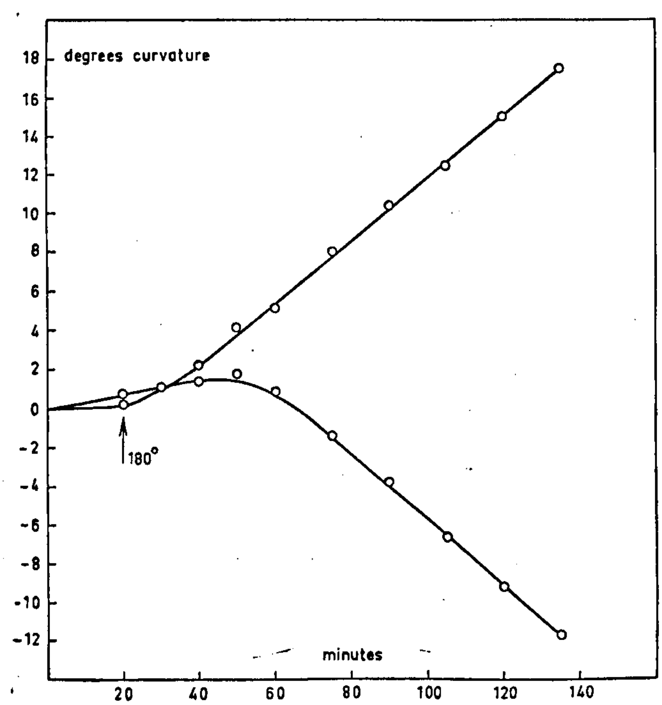

Fig. 11. Curvature of decapitated coleoptiles kept horizontally in a growth-substance solution (0.1 mg IAA/l). Upper line; controls, kept in the same position for the entire experiment; lower line: coleoptiles first kept vertically in the growth-substance solution for 30 minutes and then placed horizontally, first in one position and then rotated through 180° after 20 minutes.

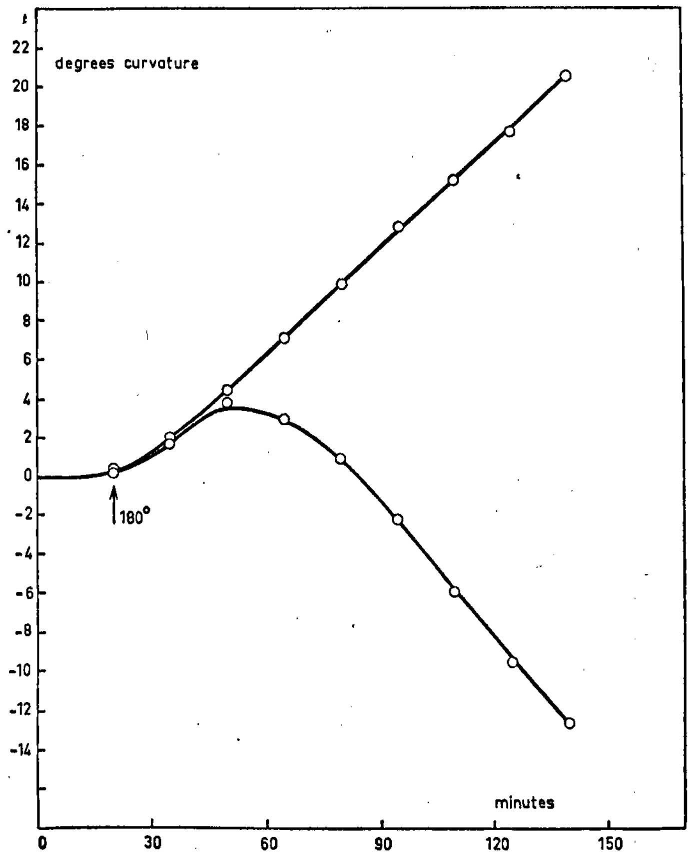

Fig. 12. Curvature of non-decapitated coleoptiles kept horizontally in water. Upper line: kept in the same position for the entire experiment; lower line: rotated through 180° after 20 minutes.

Decapitated coleoptiles:

Back curvature:

Curvature after 120 minutes . . . . : 13.2°
,, ,, 180 ,, ... : -1.3°
Rate of curvature . . . . : 14.5 / 6
: 2.4°/10 min.

Original curvature:

Curvature after 60 minutes . . . . : 7.2°
,, ,, 180 ,, ... : 24.4°
Rate of curvature . . . . : 17.2 / 12
: 1.4°/10 min.

Non-decapitated coleoptiles:

Back curvature:

Curvature after 130 minutes . . . . : 13.8°
„ „ 180 „ . . . . : 5.2°
Rate of curvature . . . . : 8.6
5
: 1.7°/10 min.

Original curvature:

Curvature after 60 minutes . . . . : 5.9°
„ „ 180 „ . . . . : 24.8°
Rate of curvature . . . . : 18.9
12
: 1.6°/10 min.

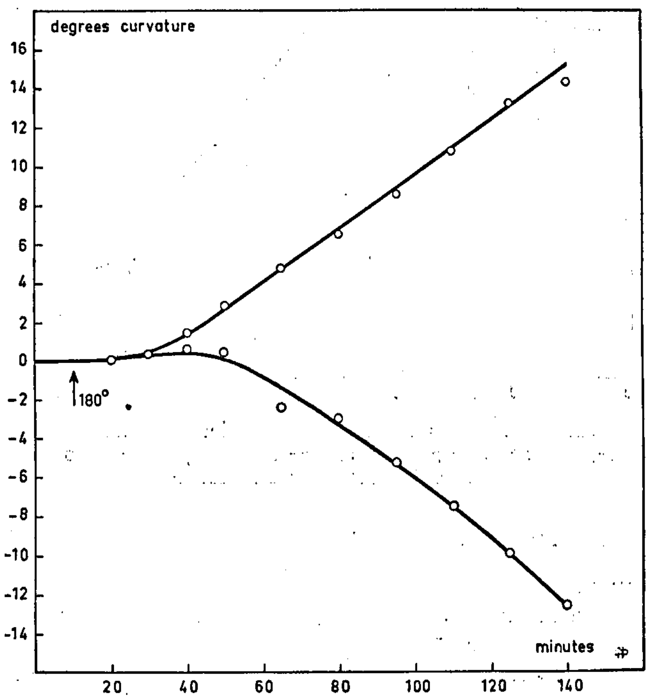

Fig. 13. Curvature of decapitated coleoptiles kept horizontally in a growth-substance solution (0.1 mg IAA/l). Lower line: rotated through 180° after 10 minutes; upper line: controls, kept in the same position for the entire experiment.

The rate of curvature in all experiments was calculated in the same way, and is given in Table IX.

From this table it can be seen that even under the same conditions the rate of curvature varies considerably. In the controls, the rate of curvature in the original direction of decapitated coleoptiles varied from 1.1°/10 minutes to 1.5°/10 minutes while in the case of non-decapitated coleoptiles the variation went from 1.6°/10 minutes to 2.4°/10 minutes. In all cases the rate of curvature of non-decapitated coleoptiles was higher than that of decapitated ones. The reason for these variations in the rate of curvature is not clear; they are most probably due to slight differences in the physiological condition of the coleoptiles.

In the case of decapitated coleoptiles there was an increase in the difference between the rate of the original curvature and the rate of the curvature in the opposite direction with the time that the coleoptiles had been kept in the first position. In the case of non-decapitated coleoptiles the largest difference was found with coleoptiles which had been kept horizontally for 20 minutes before rotation while with

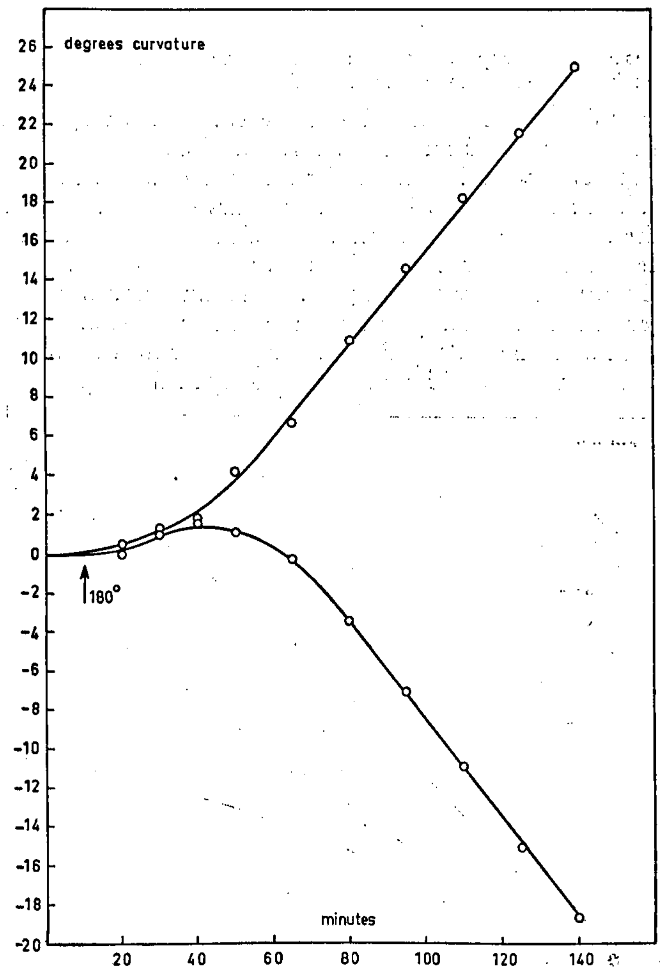

Fig. 14. Curvature of non-decapitated coleoptiles kept horizontally in water. Upper line: controls, not rotated through 180°; lower line: rotated through 180° after 10 minutes.

TABLE IX

Rate of curvature of non-decapitated coleoptiles in water and of decapitated coleoptiles in a growth-substance solution (0.1 mg IAA/l) before and after rotation through 180°.

Condition of coleoptile	Minutes horizontal before rotation through 180°	Rate of curvature in °/10 minutes	
		Controls	After rotation
Non-decapitated	10	2.4 ± 0.29	2.6 ± 0.18
	20	1.8 ± 0.09	2.3 ± 0.11
	90	1.6 ± 0.15	1.7 ± 0.09
Decapitated	10	1.2 ± 0.05	1.5 ± 0.09
	20	1.1 ± 0.07	1.2 ± 0.12
	20 *	1.5 ± 0.10	1.7 ± 0.12
	90	1.4 ± 0.07	2.4 ± 0.11

(* pretreated, i.e. coleoptiles kept vertically in the growth-substance solution for 30 minutes before being placed horizontally).

decapitated ones the largest difference was found when they had been kept horizontally for 90 minutes before rotation.

There are two possible reasons for this difference in the rate of curvature. The first is that, because of the curvature, the curving apical part of the coleoptile is not at an angle of 90° to the vertical when the coleoptile is rotated through 180°. It is possible that the induction is noticeably influenced by this angle and the induction would be greater in the second horizontal position. The other possibility is that the internal state of the coleoptile is changed during the first curvature in such a way that it would curve more easily in the opposite direction.

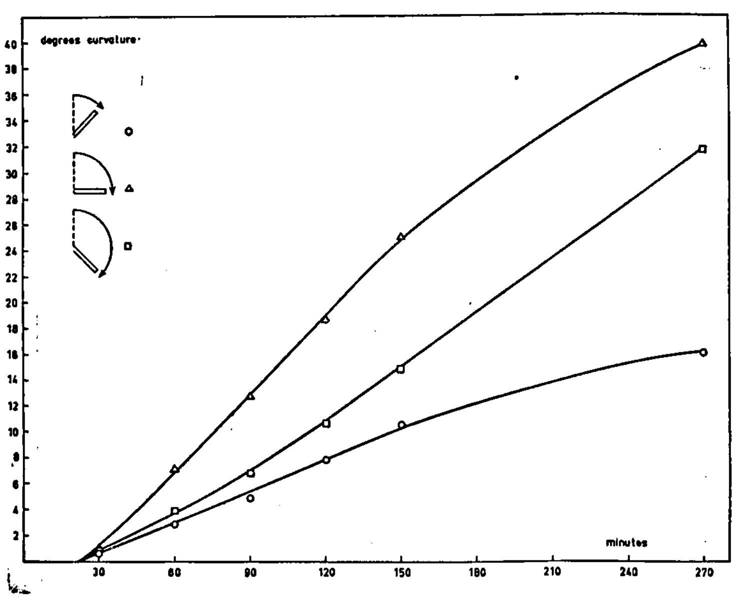

Fig. 15. Curvature of decapitated coleoptiles kept in a growth-substance solution (0.1 mg IAA/l) at angles of 45°, 90° and 135° to the vertical.

This is not the place to speculate on the different ways in which this might occur.

To investigate the first possibility, three sets of decapitated coleoptiles were kept in a growth-substance solution (0.1 mg IAA/l): one series at an angle of 45° to the vertical, the second series at an angle of 90° while the third series was kept at an angle of 135°. The three series were run simultaneously. The curvatures were measured at intervals of 30 minutes. The results are shown in Fig. 15. The same experiments were done with non-decapitated coleoptiles kept in water. The results of the latter are shown in Fig. 16.

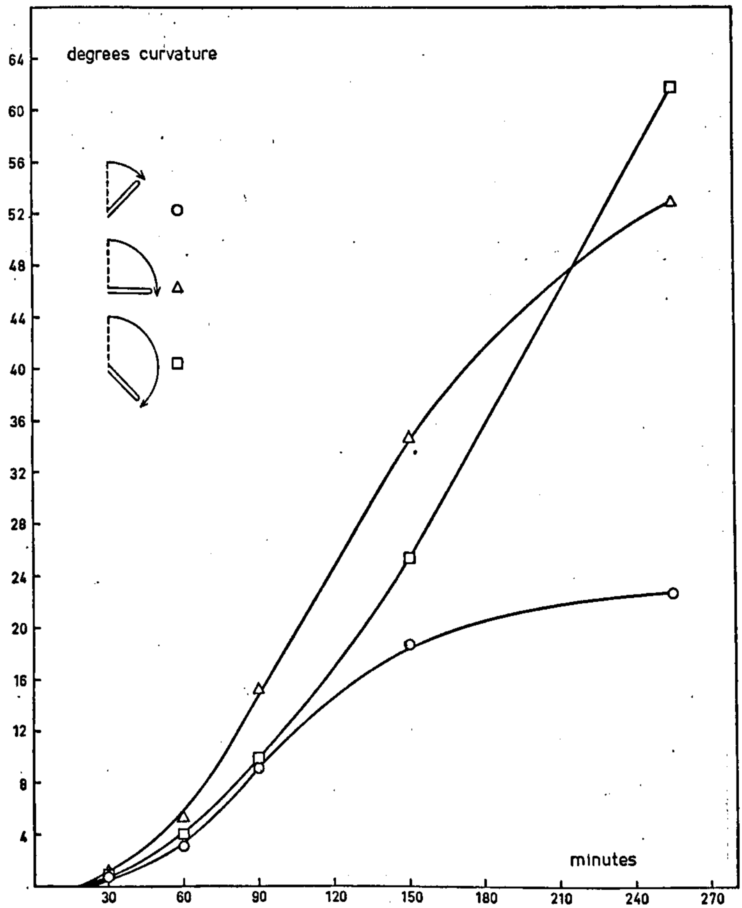

Fig. 16. Curvature of non-decapitated coleoptiles kept in water at angles of 45°, 90° and 135° to the vertical.

As was expected, the curvature of the coleoptiles that were placed at an angle of 90° to the vertical, was larger than that of the coleoptiles that were placed at an angle of 45° or of 135°. In the case of non-decapitated coleoptiles the curvature of those placed at 135° finally exceeded the curvature of those that were placed at 90°. This, however, did not take place before the former had reached a curvature of ± 45°, i.e. when they had reached the horizontal position. In the case of decapitated coleoptiles the curvature of those placed at 90° remained larger than that of the coleoptiles placed at 45° and 135° for the entire experiment. In view of the small differences between the positions before and after rotation in the experiments shown in Figs. 8—14, and the duration of those experiments, the increased rate at which the curvature in the opposite direction proceeds will not have been due to different angles to the vertical.

To investigate the second possibility, namely that the exposure to gravity had changed the reactivity of the coleoptile, the following experiments were performed: decapitated coleoptiles were kept horizontally in water for 60 minutes. After that, the experimental and the control series were placed in a growth-substance solution (0.1 mg IAA/l) but the experimental series was rotated through 180° while the control coleoptiles were left in the original position. The curvatures were recorded at intervals of 15 and 30 minutes and the results are shown in Fig. 17.

In the case of the coleoptiles which were not rotated through 180°,

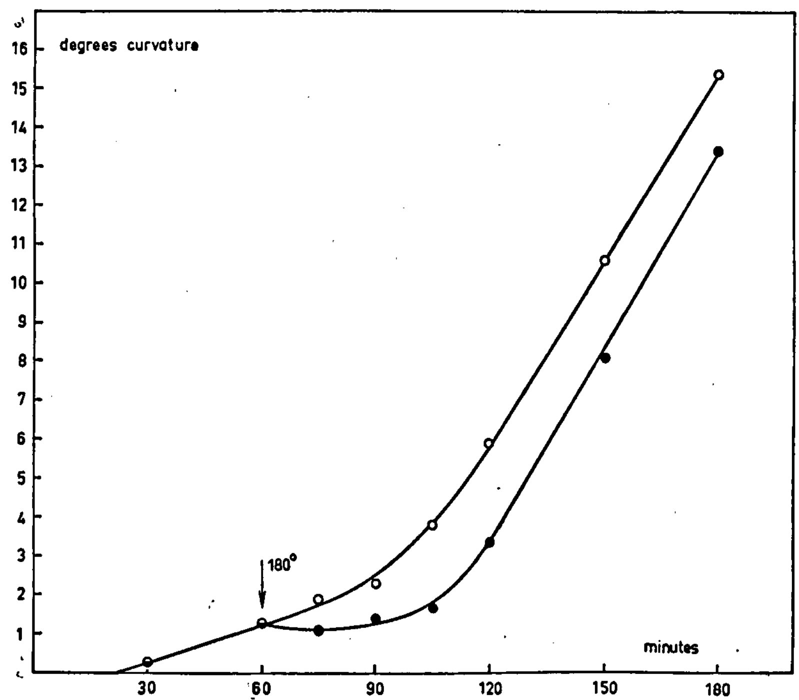

Fig. 17. Curvature of decapitated coleoptiles kept horizontally first in water for 60 minutes and then in a growth-substance solution (0.1 mg IAA/l) for the rest of the experiment. Circles: kept in the same position after addition of growth substance; dots: rotated through 180° after addition of growth substance. (In the latter case the progress curve has been reversed in order to facilitate comparison.)

the usual course of the development of the curvature was found. In the case of the coleoptiles which were rotated through 180° after remaining in water for 60 minutes, the curvature in the original direction was slight and lasted for 15 minutes only. The coleoptiles started curving in the opposite direction, and this curvature soon proceeded at the same rate as the control series and this rate too remained constant up to the end of the experiment. In this case there was definitely no increase in the rate of curvature of the coleoptiles rotated through 180° above that of the coleoptiles which were kept in the same position for the entire experiment. This leads to the conclusion that no changes took place in the coleoptiles during the time that they were kept horizontally in water, which would make them more receptive to the action of growth substance.

In another experiment, decapitated coleoptiles were kept in a growth-substance solution (0.1 mg IAA/l): for the first 90 minutes they were kept horizontally, and then for 30 minutes vertically. After that they were placed horizontally again, but in such a way that the former upper side then became the lower. The results are shown in Fig. 18.

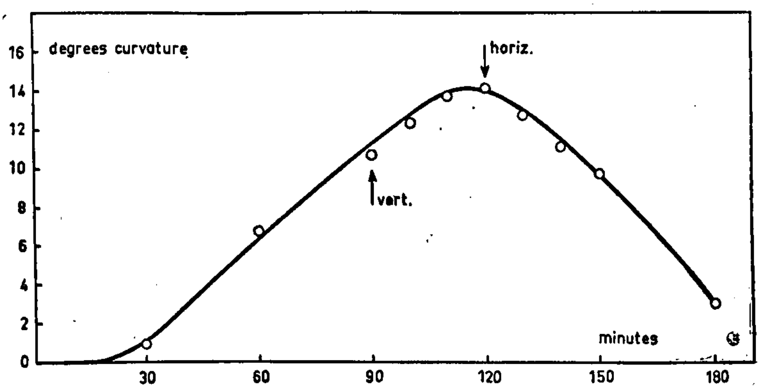

Fig. 18. Curvature of decapitated coleoptiles kept in a growth-substance solution (0.1 mg IAA/l). Coleoptiles kept horizontally for 90 minutes, then vertically for 30 minutes and then horizontally again in such a way that the former upper side became the lower side.

From these results the rate of the original curvature and the rate of the back curvature were calculated. The following was found: rate of original curvature: 1.6°/10 minutes; rate of the back curvature: 1.9°/10 minutes. The increased rate of the back curvature, therefore, is again apparent and its occurrence is not prevented by an interposed period in the vertical position.

The special point of the investigation in the latter part of this chapter was what might be the reason why the back curvature always proceeds more rapidly than the curvature in the original direction. Two possibilities were suggested and investigated: (i) the geo-induction is stronger when the coleoptiles are placed at an angle of more than 90° to the vertical and (ii) the internal state of the coleoptile is changed as soon as the coleoptile is placed horizontally, which changes make it more receptive and/or more reactive to an opposite induction.

The first possibility could be discarded (p. 34) and, as to the second possibility, one has to realise that the increased rate of curvature was only observed when it counteracted a curvature in the opposite direction. There was no increased rate of curvature when the coleoptiles had previously been in a horizontal position in which they could not take up growth substance and consequently did not receive any induction (Fig. 17).

This takes the problem from the field of geotropism into that of growth. The increased rate of curvature is apparently not caused by a factor of a specific geotropic nature but by the fact that the coleoptiles were curved. The mere fact that unequal growth has taken place in the opposite sides of the coleoptiles facilitates the process of straightening.

During the development of the back curvature the difference in the growth rate of both sides is larger than that present during the original curvature.

It is of no use to speculate here on the question whether this is due to an extra decrease of the ability to grow at the upper side or to an extra increase of this ability at the lower side. The solution has not to be sought in the direction of depletion of growth material only, since it must be taken into account that the rate at which the original curvature proceeds can remain constant for more than 3 hours (Fig. 9),

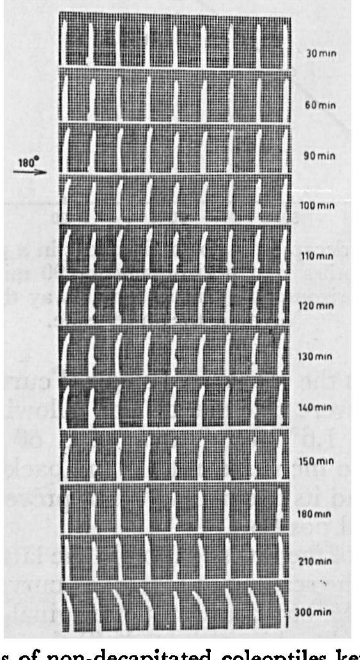

Fig. 19. Shadowgraphs of non-decapitated coleoptiles kept horizontally in water and rotated through 180° after 90 minutes. Note the straightening which proceeds from the base towards the tip and the complete absence of S-shaped curvatures.

and it only decreases, as it seems, by a decreased geo-induction due to a less favourable position of the apical part with respect to the direction of the gravitational force (Fig. 16).

Another feature which emerges from these experiments is the shape assumed by the coleoptiles—decapitated as well as non-decipatated ones—during the back curvature. Both the original and the back curvature start at the apical end and proceed towards the base. The back curvature, however, is preceded by a straightening of the coleoptiles, and this process starts at the base and proceeds towards the tip, just as in the case of geotropically curved coleoptiles when they are placed vertically (see p. 22). The back curvature does not start until the straightening of the coleoptiles has been completed. In only a few isolated cases an S-shaped curvature was found, that is, the tip of the coleoptile had started curving upwards before the coleoptile had completely straightened. The whole process of curvature in one direction, straightening and curvature in the opposite direction is shown in Fig. 19.

CHAPTER V

THE EFFECT OF ANTAGONISTIC GEOTROPIC EXPOSURES OF SHORT BUT EQUAL DURATION ON THE SUBSEQUENT DEVELOPMENT OF THE CURVATURE IN THE VERTICAL POSITION

In the preceding chapter the effect of antagonistic geotropic exposures on the rate of curvature was studied. After the coleoptiles had stayed in a horizontal position for some time, they were rotated through 180° and came under the influence of a geotropic exposure in the opposite direction. In those experiments the time of exposure in the two positions was not taken into account.

This chapter is concerned with the influence which two geotropic exposures in opposite directions for the same length of time exercise on the subsequent behaviour of the coleoptiles in the vertical position when they were placed in that position before any curvature became visible or at most had proceeded noticeably.

The procedure was as follows: non-decapitated coleoptiles were kept horizontally in water for exactly 10 minutes and then they were rotated through 180°. After 10 minutes in the second horizontal position, they were returned to the vertical and in that position shadowgraphs were made. A second experiment was performed, in which the coleoptiles instead of 10 minutes, stayed in the antagonistic positions for 20 minutes. The results are shown in Figs. 20 and 21 respectively.

The same experiments were also carried out with decapitated coleoptiles kept in a growth-substance solution (0.1 mg IAA/l). The times of exposure were here also 10 and 20 minutes respectively. A variation in this procedure was that the coleoptiles were kept vertically in the growth-substance solution for 30 minutes before the experiment started.

Here too this measure was taken in order to avoid that the amount of growth substance taken up by the coleoptiles during the 10 or 20 minutes before they were rotated through 180° would be insufficient. The results are shown in Figs. 21 and 22 respectively.

In the case of non-decapitated coleoptiles no curvatures were registered during the first 20 minutes. Only after the coleoptiles had been placed vertically the curvatures became visible. The coleoptiles began to curve in the direction of the first geotropic reaction; this curvature increased for about 20 minutes and then a counteraction set in; this

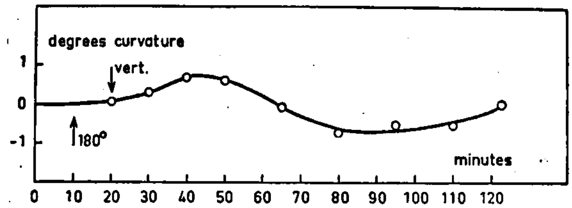

Fig. 20. Curvature of non-decapitated coleoptiles in water. Kept horizontally first for 10 minutes in one position and then rotated through 180°. After another 10 minutes returned to the vertical position.

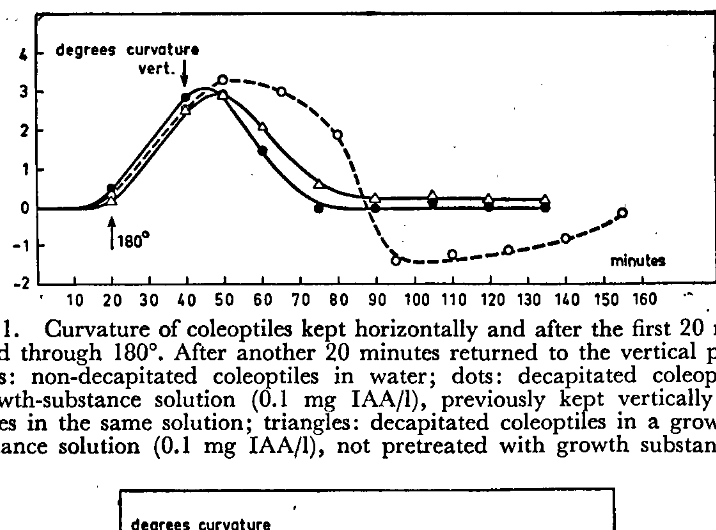

Fig. 21. Curvature of coleoptiles kept horizontally and after the first 20 minutes rotated through 180°. After another 20 minutes returned to the vertical position. Circles: non-decapitated coleoptiles in water; dots: decapitated coleoptiles in a growth-substance solution (0.1 mg IAA/l), previously kept vertically for 30 minutes in the same solution; triangles: decapitated coleoptiles in a growth-substance solution (0.1 mg IAA/l), not pretreated with growth substance.

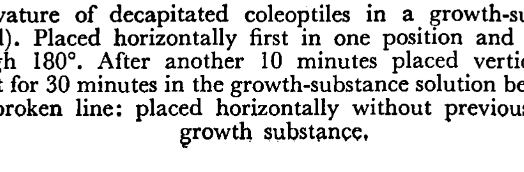

Fig. 22. Curvature of decapitated coleoptiles in a growth-substance solution (0.1 mg IAA/l). Placed horizontally first in one position and after 10 minutes rotated through 180°. After another 10 minutes placed vertically. Solid line: coleoptiles kept for 30 minutes in the growth-substance solution before being placed horizontally; broken line: placed horizontally without previous treatment with growth substance.

resulted first in a straightening of the coleoptiles and then in a curvature in the direction of the second geotropic reaction. Finally the coleoptiles became straight again. As could be expected, the curvatures were very small because in each position the geotropic exposure lasted only 10 minutes. It is evident, however, that both geotropic exposures caused a curvature, and that an opposing geotropic induction does not neutralize the reaction to the first exposure. When the geotropic inductions lasted 20 minutes, the same happened: during the 20 minutes that the coleoptiles were kept in the second position, they already started curving according to the original induction and after they had been placed vertically, this curvature still increased for about 10 minutes, after which the counteraction set in. After they had become straight, they also showed a slight curvature in the direction indicated by the second geotropic induction, and then they slowly became straight again.

In the case of decapitated coleoptiles, however, only a slight curvature in the original direction and a straightening of the coleoptiles were recorded. The coleoptiles did not curve according to the second geotropic exposure, not even when they had previously been kept for some time in the growth-substance solution.

The changes inside the coleoptile which are induced by the unilateral influence of gravity, are brought about within a relatively short time. This is proved by the fact that a curvature may make its appearance in the period in which an antagonistic geotropic induction has already set in. That the non-decapitated coleoptiles curved in both directions would seem to indicate that the changes are more rapidly induced by the gravitational force in the tip of the coleoptile than in the lower zones. In view of the fact that the tip is the production centre of auxin, and considering that the possibility of lateral distribution of this substance presumably decreases rapidly in the basal direction (ANKER 1956), the above-mentioned difference in the behaviour of decapitated and non-decapitated coleoptiles is perhaps not so surprising.

CHAPTER VI

GENERAL DISCUSSION

The experiments reported in the preceding chapters yield information on several questions concerning the regeneration of a physiological tip, the uptake and fixation of growth substance, and the mechanism of the geotropic response in *Avena* coleoptiles. The geotropic response of decapitated coleoptiles strictly depends on the amount of growth substance that is available to the reacting tissue; this appears unambiguously in all experiments concerned. Since, in order to obtain geotropic curvatures in such coleoptiles, the latter have to be kept in growth-substance concentrations that are sub-optimal for straight growth (ANKER, 1954, 1956), the development of such a curvature is a sensitive indicator for the presence (in a low concentration) of growth substance.

A. Regeneration of the physiological tip

It has been shown that even in decapitated coleoptiles which have been separated from the seed, a certain regeneration of a physiological tip takes place. In a horizontal position the effect of this regeneration becomes apparent 2 to 3 hours after decapitation for at that time a marked increase in the rate of development of the geotropic curvature is noted. This time interval is the same as that found by Söding (1925, 1929), Dolk (1926), Tendeloo (1927), Beyer (1928), Tsi-Tung Li (1930) and Went (1942) for the regeneration of a physiological tip in decapitated coleoptiles that have not been separated from the seed.

It is shown in this paper that growth substance taken up from solutions of sub-optimal concentration for straight growth (0.1 mg IAA/l), is fixed to, or utilized by the protoplasm within 30 to 45 minutes and cannot regulate the geotropism in a subsequent period. This means that, when isolated and decapitated coleoptiles start to curve geotropically in water after 2 hours, a slow production of growth substance continues at a constant level all the time. This result is well in line with Skoog's statement (1937) that in deseeded, decapitated coleoptiles of *Avena* a slight formation of auxin occurs. The same was observed with deseeded and decapitated *Avena* coleoptiles by Arnal and Gebhardt (1955). The amount of precursor present in such coleoptiles is apparently sufficient to yield enough growth substance for a geotropic curvature to continue for at least 5 hours at a constant rate.

Arnal and Gebhardt (1955) observed that even in an IAA solution (1. 0 mg/l) after 10—16 hours a sudden increase in the growth rate occurs. In coleoptiles that are kept in water a similar increase sets in at the same time. The authors ascribe this increase to the regeneration of a physiological tip. They further conclude that this regeneration is not synonymous with a production of IAA since it was not only found in coleoptiles that were kept in water but also in those that were provided with IAA. As the change in growth rate occurred much later than normally, the regeneration of the physiological tip taking place, as a rule, 2 to 3 hours after decapitation, it is not unconceivable that it is due to some other cause, e.g. to stimulation either by the products of an autolysis or by products of bacterial origin.

In any case, one can conclude from the present experiments that in a medium containing IAA no regeneration of a physiological tip occurs. In long-lasting experiments in an IAA solution (0.1 mg IAA/l) the geotropic curvature of horizontally placed decapitated coleoptiles proved to proceed at a rate that remained constant for several hours. If a regeneration of a physiological tip should take place, this rate would either increase (external concentration sub-optimal) or decrease (internal concentration becoming supra-optimal), and this is not so. This suppression of regeneration by IAA might indicate that IAA is the auxin actually produced in the regeneration process since the equilibrium

$$\text{precursor} \rightleftarrows \text{growth substance}$$

will shift to the left in the presence of growth substance. On the other hand, it is possible that during growth the precursor is used up in other processes, as, from the work of THIMANN *et al.* (1951) it is known that during growth a qualitative change in the cell-metabolism occurs, the nitrogen metabolism included (see also BLANK and FREY-WYSSLING (1941)). It must be left to further experiments with other growth substances to check which of these explanations is correct.

B. THE UPTAKE AND FIXATION OF GROWTH SUBSTANCE

The uptake of growth substance from the external solution by the tissue of decapitated coleoptiles proved to be a rapid process. When, for instance, such coleoptiles were kept in a solution of growth substance (0.1 mg IAA/l) for 5 minutes only, they showed a geotropic curvature appreciably higher than that of controls kept in water. It appeared that the amount of growth substance taken up by the coleoptile increases with an increase in the time of growth-substance supply, up to 30 minutes. It would seem that after about 30 minutes a constant ratio is established between the rate of uptake (and basipetal transport) of growth substance and its fixation to, or utilization by the protoplasm. At this steady state a certain amount of growth substance appears to remain available for further activity, *in casu* the regulation of a subsequent geotropic curvature; this is the "free auxin".

From these data it follows that the rate of fixation or utilization of growth substance is slower than that of its uptake. At the steady state a certain amount of "free auxin" is available for further activity or for transport in a more basal direction. It further appears that the geotropic curvature after the coleoptiles have been transferred to water proceeds under such circumstances for 30 to 45 minutes. The development of this curvature is made possible by the growth substance already fixed in the horizontal position and by the "free" growth substance still available but also fixed within 30 to 45 minutes.

The work of REINHOLD (1954) indicates that in the uptake of auxin at least two components can be distinguished. BOTTELIER (1954), working with petioles of *Ageratum*, came to the conclusion that auxin, once it is taken up in the cells, is immediately fixed. JOHNSON and BONNER (1956), working on the uptake of 2,4-D (2,4-dichloro-phenoxy acetic acid), C^{14} carboxyl labelled, by coleoptile sections of *Avena*, came to the conclusion that two stages can be distinguished in the initial rapid uptake of 2,4-D, and that they are followed by a third kind of uptake. These stages are:

(1) a diffusion into the accessible part of the tissue, which is completed within 30 minutes. This part of the absorbed 2,4-D can freely diffuse out again;

(2) an exchangeable binding of 2,4-D, also completed within 30 minutes. This fraction cannot leach out from the tissue;

(3) a continuous uptake going on for hours. This 2,4-D cannot diffuse out and is not exchangeable.

As to stage (1) the present experiments yield no information. On the other hand, a time of 30 minutes was also found for the "binding" of the IAA, stage (2), but there are no data on the exchangeability of this binding. The fact that decapitated coleoptiles keep on curving when kept horizontally in a 0.1 mg IAA/l solution, whereas they stop curving after a while when they are transferred to water, is in favour of the continuous uptake, stage (3), described by Johnson and Bonner.

C. The mechanism of the geotropic curvature

As a variant on Went's almost classic slogan "without growth substance no growth" the phrase "without growth substance no geotropism" would be an apt expression of what the present experiments show. When it is realized that the faculty to develop a geotropic curvature is coexistent with that for straight growth, this is a trivial truth for the geotropic reaction.

Two kinds of experiments, however, clearly show that the unilateral gravitational force is "perceived" by the coleoptile only in the presence of growth substance. Decapitated coleoptiles, kept horizontally in water and then vertically in a growth-substance solution (p. 23) do not curve geotropically. This result is incompatible with that of an early experiment of Brauner (1923), in which decapitated coleoptiles were kept horizontally for 10 minutes and then provided with tips of coleoptiles which had been kept vertically; after that they developed a geotropic curvature. The results, however, are not very convincing. Similar experiments were made by Cholodny (1924) with decapitated roots and root tips, and in these experiments no curvatures were obtained. The results of Cholodny are well in line with those of the present experiments.

In other experiments (p. 13) decapitated coleoptiles were kept horizontally in water and then transferred in the same position to a growth substance solution in which they remained for 30 minutes. It appeared that nothing had been changed either in the receptivity or in the reactivity of the coleoptiles by the preceding exposure to gravity in the absence of IAA.

The experimental data, although indicating a direct dependence of the geotropic "perception" on the presence of growth substance in an available form, do not give any information on the nature of this process. An unequal lateral distribution of growth substance as a result of the unilateral action of gravity certainly offers an acceptable explanation, but leaves the question of the mechanism of this lateral shift of auxin unanswered.

Part of the present experiments indicate that growth substance once it has been taken up by the tissue is bound within 30 to 45 minutes and that it can no longer move freely about in the coleoptile. This is most clearly shown in the experiments in which decapitated coleoptiles had been kept horizontally in a growth-substance solution for a certain length of time, first in one position and then rotated through 180° (p. 24). In the latter position the reaction goes on for about 30 minutes in the original direction; this is the same time required for the curvature in the original direction to appear. Then only does the curvature in the opposite direction start. It appears therefore that the reaction made possible by the growth substance in the first horizontal position does not end at the moment of rotation through 180° and that the growth-substance molecules involved in this reaction cannot be mobilized in order to be utilized in a reaction according to the new orientation. This most clearly appears in the experiments where the coleoptiles were transferred to water at the moment of rotation. Therefore a curvature according to the new orientation can only be realized if free growth substance is available. This fact is not in contradiction with the theory of a lateral distribution of auxin by gravity, since the greater part of the growth substance is fixed and the free part is too small to cause a curvature. The fact, however, that the straightening of a curved coleoptile proceeds from the base towards the tip, should be explained in a different way and is perhaps due to the same type of growth-technical phenomena as mentioned below for the rate of the back curvature.

The effects of two short-lasting antagonistic exposures of equal duration (10 or 20 minutes) on decapitated coleoptiles kept in growth-substance solution and on non-decapitated coleoptiles kept in water, do not neutralize each other. In non-decapitated coleoptiles the curvature arising as a result of the second exposure is stronger than that which arises as a result of the first exposure. This, however, is in agreement with the conception that the tip of a coleoptile is more sensitive to "perceive" the gravitational force than the lower parts.

In these short-lasting exposures the exposure time approaches that which according to RUTTEN-PEKELHARING (1910) is needed for a "just visible" curvature (50 % of the coleoptiles curved) namely 4 to 5 minutes. These short exposures to unilateral gravity are sufficient to elicit a geotropic reaction. This means that the lateral shift of the auxin transport, which is generally thought to be a preliminary step in the development of the curvature, should have proceeded to such an extent that as a consequence of a difference in the rate of growth of the lower side and of the upper side of the coleoptile a curvature is found.

SCHRANK (1944*a*) calculated that a transversal migration of an auxin-ion in a coleoptile by electrophoretic transport would take about 6 minutes. It is strange that it should take another 20 minutes before the difference between the growth of the lower and upper side is large enough to yield a visible curvature. Therefore not all questions with regard to the unequal distribution of growth substance by unilateral gravity, and with regard to the mechanism by which it is brought about, have as yet been answered.

Finally the phenomenon has to be discussed that after an antagonistic geotropic exposure of curved coleoptiles, the curvature according to the second exposure, the back curvature, generally proceeds at a somewhat higher rate than the original curvature. This phenomenon does not seem to be due to differences in geotropic induction (experiments in which coleoptiles were kept at angles of 45° and 135° to the vertical) nor to differences in geotropic sensitivity (Fig. 17), but must be due to growth-technical factors. The nature of the latter is still unknown but they certainly are not related to geotropism.

SUMMARY

1. The experiments described in this paper were performed with decapitated and non-decapitated *Avena* coleoptiles separated from the seed. They were immersed in water or in a growth-substance solution (usually 0.1 mg IAA/1). The geotropic response was studied with the aid of an apparatus similar to that described by Anker (1954).

2. Even in deseeded coleoptiles a regeneration of a physiological tip takes place, whose activity attains a constant though low rate after 2 to 3 hours and continues for at least 5 hours. In the presence of growth substance (0.1 mg IAA/1) in the external solution no regeneration of a physiological tip takes place.

3. In deseeded and decapitated coleoptiles the geotropic reaction as well as the geotropic perception are dependent on the presence of a growth substance, in this case IAA.

4. The uptake of growth substance is a rapid process, whereas the fixation of the growth substance to the protoplasm proceeds at a much slower rate. 30 to 45 minutes after its uptake in the cells all growth substance is bound. This means that, in the presence of growth substance, after about 30 minutes a steady state is established, i.e. an equilibrium between the rate of uptake, basipetal transport and fixation of growth substance, so that a certain constant amount of "free" growth substance remains available in the tissue.

5. In the absence of IAA no "preparatory processes" are induced by the unilateral influence of gravity in decapitated coleoptiles kept horizontally in water. For, if transferred into a growth-substance solution (0.1 mg IAA/1) and placed vertically, they do not curve and, if left horizontally after addition of IAA, they do not curve faster than coleoptiles which at the moment of growth-substance addition are given an antagonistic geotropic induction.

6. If an antagonistic geotropic induction is given to curved coleoptiles, the back curvature proceeds at a higher rate than the curvature in the original direction. It appeared that this was not due to the higher value of the angle between the tip and the vertical at the moment of rotation through 180°, but is caused by "growth-technical" rather than by specific geotropic factors.

7. When horizontally placed coleoptiles (in growth substance) are either put vertically or rotated through 180° the development of the curvature according to the first geo-induction proceeds for ca. 30 minutes. This is apparently due to: (1) the IAA is fixed during the first induction and is no longer available and (2) the concentration of the free auxin is too low to cause a curvature in the opposite direction. The straightening of geotropically curved coleoptiles after an antagonistic induction starts at the base and proceeds acropetally.

8. Two short-lasting antagonistic exposures (10 or 20 minutes) do not neutralize each other. They induce two subsequent reactions. With non-decapitated coleoptiles the second reaction is stronger than the first and than that of decapitated coleoptiles in a growth-substance solution. This indicates a particular ability of the coleoptilar tip to regulate geotropism.

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