THE AUXIN PRODUCTION OF THE PHYSIOLOGICAL TIP OF THE AVENA COLEOPTILE AND THE REPRESSION OF TIP REGENERATION BY INDOLEACETIC ACID (NOT BY NAPHTHYLACETIC ACID AND 2,4-DICHLOROPHENOXYACETIC ACID)

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SUMMARY

The physiological tip of a decapitated *Avena* coleoptile is equivalent to the tip of the intact coleoptile as far as the regulation of the growth and the geotropic reaction is concerned. The regeneration of the physiological tip is completely inhibited by indoleacetic acid, even in con· centrations below 0.01 mg/l. The auxin analogues naphthylacetic acid and 2,4-dichloropheno-xyacetic acid do not repress the auxin synthesis.

1. INTRODUCTION

In coleoptiles the production of auxin is confined to the uppermost cells of the tip. Removal of the tip (decapitation) causes a rapid decrease of the growth rate of the remaining part. Two hours after the decapitation the distal cells of the stump start producing auxin (regeneration of the physiological tip), which permits continuation of growth. This remarkable occurrence is not only an interesting example of changing function because cells that were reacting to auxin now start to produce it, but the regeneration is also a unique opportunity to study the development of the faculty of auxin synthesis.

The first part of this investigation is related to the question whether the regenerated tip is inferior to the natural one or not. This question has been raised earlier by other investigators but the answers obtained were different owing to the method of investigation. In most cases the physiological tip seemed inferior to the natural one, notably when its activity was determined by comparing the growth rates of decapitated and intact coleoptiles (SÖDING 1925, 1929; DOLK 1926; HEYN 1931; THIMANN & BONNER 1933). A disadvantage of this method is that the results are much influenced by the extent of decapitation (1, 2, or more mm removed), the age of the coleoptile at the moment of decapitation and variations in duration of the experiments. In spite of these complications this method was chosen in the present investigation, but this time the growth rates of intact and decapitated coleoptiles were related to the maximum growth rates attainable by these organs with externally supplied auxin, which differ greatly. The equality of the relations (1:3) supports the equivalence of the physiological tip to the natural one. A second support is the observation that in both cases the auxin

production is just sufficient to secure the maximum rate of geotropic bending.

In the second part of this investigation it was found that the auxin production by the tip of the stump can be inhibited completely by IAA in concentrations as low as 0.01 mg/l, which supports the hypothesis that IAA is the natural repressor of its own synthesis. Naphthylacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D), added in growth-stimulating concentrations were not active in this respect.

2. MATERIAL AND METHODS

The general procedure of the investigation has been described repeatedly in earlier publications but most extensively in ANKER (1954). All experiments were done this time with 18 mm apical sections cut from 2.5–3 cm long coleoptiles. Before sectioning a tip of 1 mm had been removed. For each experiment 12 sections were submerged in aerated solutions of the required composition, or in water, at 23 °C. During germination and growth the coleoptiles received light from a 40 W bulb, filtered through red selenium glass. In order to prevent untimely breaking through of the primary leaf the seedlings were put in the dark on the fourth day. The experiments were done on the fifth day in the same red light. Further details of the method will be given where necessary.

3. RESULTS AND DISCUSSION

3.1. The growth rate of coleoptiles with a regenerated tip

It is difficult to find a reliable method for comparing the auxin production by the physiological tip with the production by the normal tip. An obvious method used by most authors is to compare the growth rates of intact and decapitated coleoptiles after the latter have grown a physiological tip. A difficulty of this method is that a reduced growth rate of the decapitated coleoptiles can be ascribed to a slower production of auxin but it is feasible as well that the capacity to react to auxin has decreased by decapitation. In the present investigation an attempt has been made to take the latter possibility into account by relating the growth rates of intact and decapitated coleoptiles with the maximum growth rate attainable by these organs after addition of exogenous auxin.

A first indication of the equivalence of the physiological tip to the natural one is the fact that in both cases the auxin production is sufficient for the growth to occur with one third of the highest rate possible (0.6:1.6 and 0.4:1.1). Other observations from *fig. 1*, though less relevant to the present problem, are the great differences of the maximum growth rates of intact and decapitated coleoptiles, and the IAA concentrations required to attain them. It appears that decapitation reduces the maximum growth rate to 70% of that of the intact coleoptile (1.1:1.6), but it is attained at 4% of the concentration to be supplied to intact coleoptiles. The latter difference is certainly connected with the slow permeation of IAA through the cuticle (ANKER 1971), but more puzzling is the considerable reduction of the top speed of elongation caused by the removal of
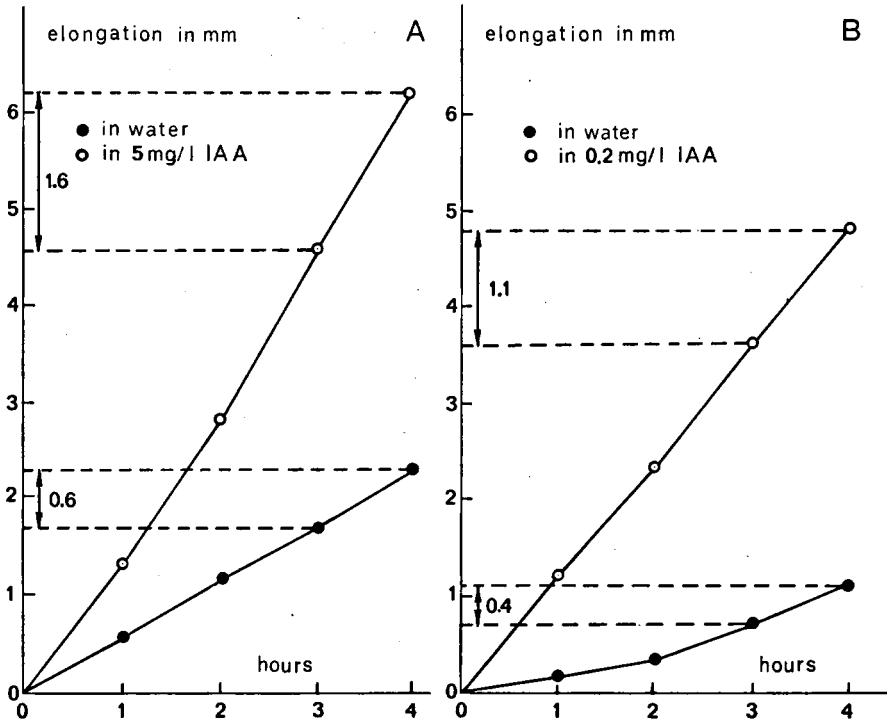
Fig. 1. The growth rate of intact (A) and decapitated (B) sections in relation to the maximum growth rate attainable in auxin solutions.

only a 1 mm tip. Two explanations at first sight seeming obvious, 1) the effect of injury and 2) the removal of a part that could have contributed to some extent to the elongation of the stump, become less conclusive after determining the distribution of growth over the coleoptile, done by AVERY & BURKHOLDER (1936). They found that growth takes place throughout the length of the coleoptile, and not until the coleoptile nears maturity does growth become relatively greater near the tip. Hence it seems of theoretical interest to examine the third possibility, viz. that substances from the extreme tip – substrates and/or hormones (apart from IAA) – are essential for optimal growth.

3.2. The geotropic reaction of coleoptiles with a regenerated tip

A second support for the equivalence with respect to auxin production of the physiological tip to the natural one is the fact that in both cases the auxin production is sufficient for a maximum rate of geotropic curving. It was impossible to speed up the geotropic curvature by adding auxin. This is shown in *fig. 2*. In this figure the maximum rate of curvature (degrees per hour) of a given set of coleoptiles is indicated by a dotted line. It is seen that the dotted lines of the
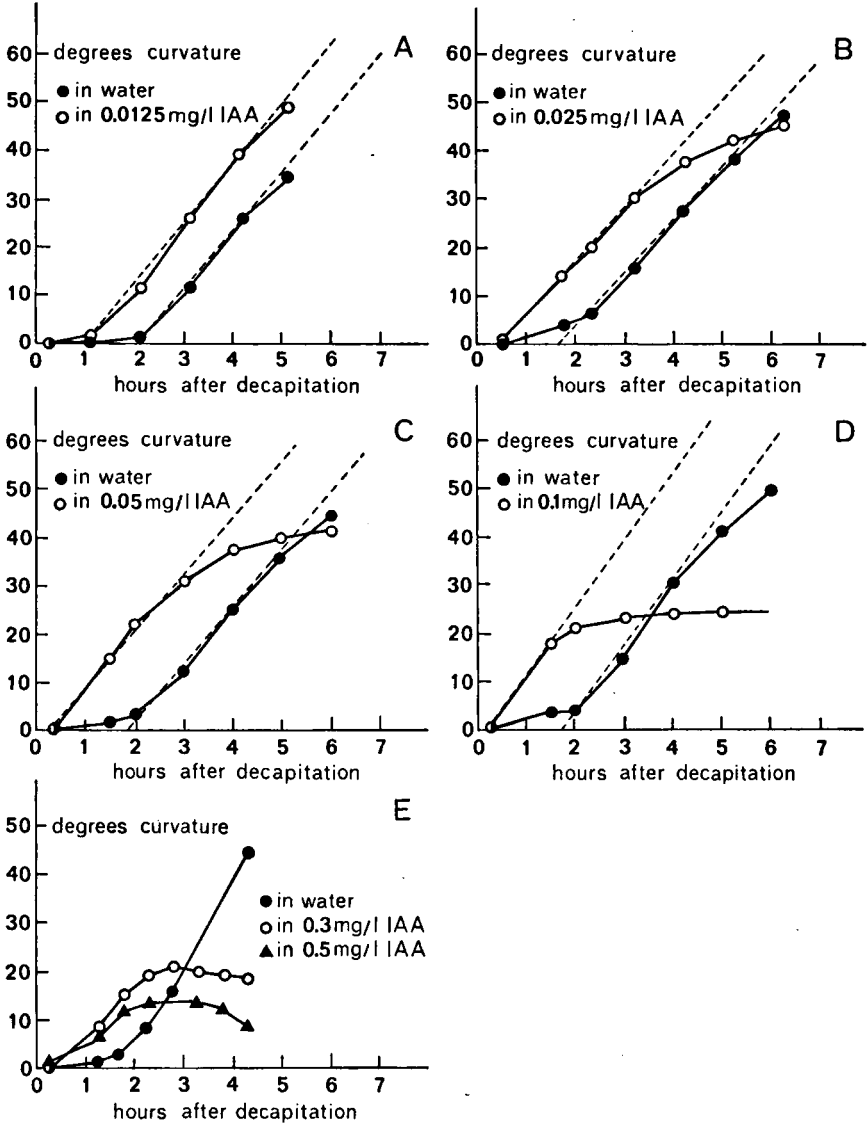
Fig. 2. Comparison of the development of the geotropic curvature of decapitated sections in water and in IAA solutions.

coleoptiles in water run parallel with those in auxin solutions of different concentration. In other words, the auxin production by the physiological tip guarantees a maximum rate of curvature.

This maximum is eleven degrees per hour and amounts to only one third of that of the non-decapitated coleoptiles (see ANKER 1971). The removal of the

tip, therefore, reduces the rate of geotropic response about twice as much as it does the rate of growth. The extra reduction is probably connected with the fact that the power to transport auxin in a lateral direction is already much reduced in the subapical region. The decreased lateral distribution of auxin in the lower parts of the coleoptile could also explain why auxin, even in very low concentrations (*fig. 2B*), inhibits the geotropic curvature considerably. Small increases of the IAA concentration cause earlier inhibitions (*fig. 2B-E*). On this great sensitivity of the geotropic reaction of a decapitated *Avena* coleoptile to the auxin concentration a test method can be based, particularly when a low auxin activity is expected.

3.3. The influence of IAA on the regeneration of the physiological tip

The possibility to inhibit the regeneration of the physiological tip with diffusates from plant parts was demonstrated by SÖDING (1925) and THIMANN & BONNER (1933). Their suggestion that the growth hormone itself is the inhibitor of the hormone production was supported by PLATT (1965). He found that 10^{-7} M IAA "cuts effective regeneration by more than half, and 5×10^{-7} M eliminates regeneration entirely".

With the present experiments the work of Platt was confirmed with a different method, and the question was raised whether IAA in natural concentrations, which are lower than those used by Platt, is still active in this respect. For this purpose the growth rate of decapitated coleoptiles in water (control) and in IAA solutions was followed over 7 hours. It is seen from *fig. 3* that the growth of the control coleoptiles showed the pattern already known from the early investigations of Dolk, Söding and others, i.e. a rapid decrease of the growth rate during the first hours after decapitation to a minimum, followed by an increase to a more or less constant level attained between the 3rd and the 4th hour.

As a matter of course, the growth of the experimental coleoptiles in IAA solutions showed a different pattern during the first hours. The point was, however, what effect the removal of IAA would have on the further course of the growth. According to expectation, the transfer of the coleoptiles from the IAA solution to water was not immediately followed by a decline of the growth. But
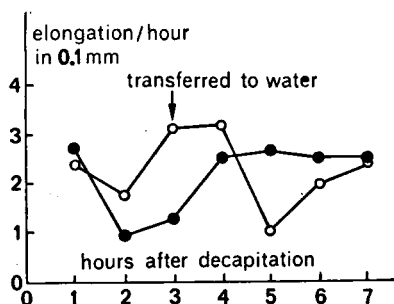

Fig. 3. Regeneration of the physiological tip in sections growing in water (black dots) and in sections pretreated with 0.01 mg/l IAA.

after the 4th hour the growth rate fell rapidly to the same level to which the growth of the coleoptiles in water had descended at the second hour. The most obvious explanation of this decline, immediately followed by an increase of the growth, is that the internal IAA concentration had lowered so much between the 4th and the 5th hour that the regeneration was no longer repressed and growth could be resumed with the auxin produced by the physiological tip. The above results were obtained with IAA in the concentration of 0.01 mg/l, but a complete repression of the auxin production was even found at half this concentration.

The absence at the 3rd hour of a physiological tip in coleoptiles treated with 0.01 mg/l IAA could be confirmed by a second decapitation at that time (*fig.4*). This should have reduced the growth considerably if a physiological tip had been present, but the actual decrease was 5–7%, a percentage that can be explained by the fact that about the same percentage of the section was removed with the second decapitation.

One may note further from *fig. 4* that the control coleoptiles in water were able to regenerate a second physiological tip.

3.4. No inhibition of the regeneration by 2,4-D and NAA

It was seen in the previous section that IAA is a potent inhibitor of the auxin synthesis in the tip of a decapitated coleoptile. To test whether this may be a case of end product inhibition in the field of hormone metabolism, the effect of auxin analogues was studied. For that purpose 2,4-D and NAA were added in various concentrations (0.005, 0.04, 0.1, 0.5 and 1.0 mg/l 2,4-D or 0.2 and 0.4 mg/l NAA). In no case an inhibition of the regeneration was observed (*fig. 5*).

This follows from the observation that the changes of the rate of growth of the coleoptiles in water and in the auxin solutions occurred simultaneously. For instance, the increase of the growth rate of the control coleoptiles after the second hour, due to the regeneration, is parallelled by a similar increase of the auxin-treated coleoptiles. In other words, the stimulation of growth by the added
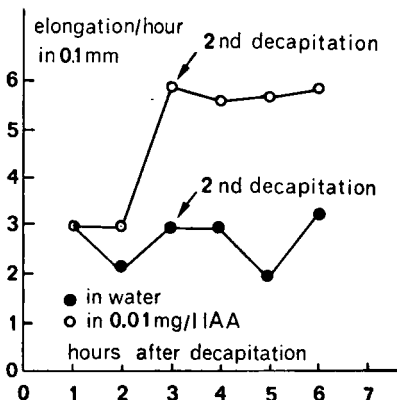

Fig. 4. Effect of the second decapitation on the growth of sections in water and in 0.01 mg/l IAA.
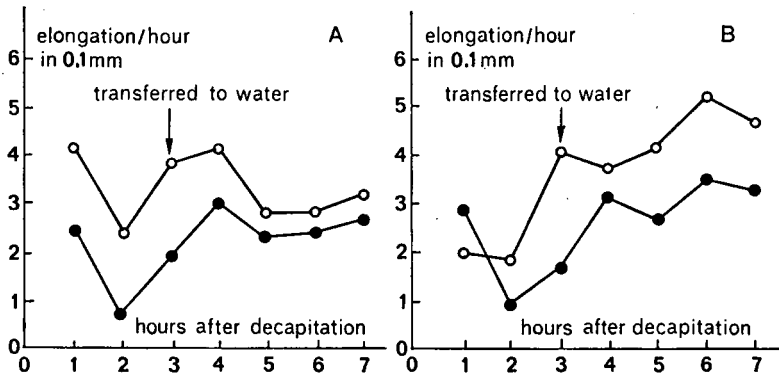
Fig. 5. The growth of sections transferred from 0.2 mg/l NAA (A) or from 0.5 mg/l 2,4-D (B) to water, compared with that of control sections in water.

auxin is superposed on the growth owing to the endogenous auxin. Accordingly, the transfer of the coleoptiles from the auxin solutions to water at the third hour was not followed by the decline of the growth occurring in the case of IAA, because the regeneration had already taken place before in the presence of the auxin analogue.

From these observations it furthermore follows that the regeneration is not conditioned by a minimum activity of growth but by a minimum concentration of IAA. The resemblance of NAA and 2,4-D to IAA apparently is not close enough to act at the same time as a growth stimulator and as a repressor of the synthesis of IAA.

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