Studies on the inhibitory action of auxin on root growth

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It is well known that auxin causes inhibition of root growth when present in nutrient solution (Åberg 1957). In the previous paper (Hejnowicz and Erickson 1968) based on continuous recording of elongation of the roots treated with indoleacetic acid (IAA) it has been shown that removal of auxin from the nutrient solution resulted in a rapid resumption of elongation, unless the auxin treatment was shorter than 60 min. If it was shorter, the recovery was delayed. The time lag was such that the inhibition which set in rapidly after beginning of the treatment and to which the lag phase contributed, lasted for about 1 hour at 25°C, independently of the treatment duration unless the latter was longer than 1 hour. A hypothesis has been put forward that root elongation is controlled by a radial gradient of auxin concentration in the root and that externally administered auxin infringes this gradient bringing about inhibition. The elongation of the root tip would be possible when the concentration of auxin in root elongation zone increases with the distance from the surface, or to put it shortly, when there is a positive radial gradient of auxin in the root. If auxin treatment is not long enough to achieve a steady state of diffusion of auxin into the root, a negative gradient of the absorbed auxin exists at the moment of ending the treatment and such a gradient will occur in the deeper part of the root for a certain time. Consequently, the root will remain inhibited for this period. However, if the treatment is longer than 1 hour the null gradient of diffusible auxin, characteristic for the steady state, changes rapidly on treatment ending in a positive gradient due to the leakage of the auxin absorbed from the root washed by nutrient solution. Considering this hypothesis one may ask: how will the root elongation zone behave if auxin is supplied to it in another way than to its surface? What is the temperature coefficient of duration of the inhibition phase elicited by short auxin treatment, does it support the hypothesis that physico-chemical process of diffusion is involved?

METHODS

1. Application of auxin to various parts of root

The construction of the experimental device used for growing roots is shown in Fig. 1. The device consisted of two parts with an independent flow of solution in each. The lower part was a piece of thick-walled glass tube, 8 cm long, with an inner

diameter of 1.5 mm. It was fastened to a piece of plexiglass and covered in front by non-fluorescing immersion oil and a strip of cover glass so that in microphotography the surface of the root tip growing inside was unobscured by the cylindrical external surface of the tube. Its lower end was connected with a Teflon tube which provided a flow of a nutrient solution upward at a rate of 0.5–1.0 cm per sec. The

Fig. 1. Chamber for growing a root.

1 — Teflon tube providing solution to the basal part of root; 2 — upper tube; 3 — lower tube; 4 — cover glass attached by means of non-fluorescing immersion oil; 5 — Teflon tube providing solution to the apical part of root.

solution left the tube at the junction between the two parts and dripped down its side. The upper part was a piece of thin-walled glass tube, 4 cm long, with lumen diameter 3 mm. Its upper end fitted a hole in a projection of the plexiglass, which was a sort of nest for corn seedling. Along its side a Teflon tube was inserted which provided a downward flow of solution.

The elongation of a root growing in the tubes was recorded by means of the streak photography method described in the previous paper (Hejnowicz and Erickson 1968). A root marked with small grains of zinc sulphide (tracer powder) producing yellow fluorescence when excited by violet light was grown in front of a microscopic camera with photographic film which moved transversely to the root axis. A yellow filter in the camera transmitted the fluorescing light only so that the grains on the root surface produced streaks on the film. The shutter in the camera

was closed automatically for 1 min. at 13.5 min. intervals so the streaks were interrupted and in this way they were marked in time. In this method the slope of a streak on the streak photograph is proportional to the rate of elongation occurring at the given moment in the root region basal to the grain which produced the streak. The apical streaks in particular show the course of elongation of the root.

Corn seedlings with root length of about 6 cm were selected for the experiment. The root was powdered with the tracer powder and subsequently inserted in the tubes so that its tip was initially 2 cm below the junction. It was left to grow in nutrient solution which flowed through both the tubes, and when the tip was about 3 cm below the junction the recording of growth started. The upper Teflon tube was connected to a reservoir containing nutrient solution with auxin about 1 hour later. If necessary auxin solution was directed also to the lower tube containing the root tip at a suitable time.

The basic composition of nutrient solution was: $\text{Ca}(\text{NO}_3)_2$ $2.5 \times 10^{-3}\text{M}$ KCl $1.2 \times 10^{-2}\text{M}$, $\text{Ca}(\text{H}_2\text{PO}_4)_2$ $0.5 \times 10^{-3}\text{M}$, MgSO_4 10^{-3}M . The solution contained freshly prepared indole-3-acetic acid (IAA) or naphthalene-acetic acid (NAA) if necessary. pH was about 4.8, temperature 25°C . The roots were illuminated with weak violet light from a mercury bulb through a Corning 7-59 filter with a peak transmission between 360-380 $\text{m}\mu$ and a cell filled with water.

2. Experiments on the effect of temperature

The method of growing roots was similar to that described above but the lower part of the chamber was attached to the wall of a glass cell from inside. Water of temperature controlled by an ultrathermostat flowed through the cell. The room temperature was maintained at about the same level as that of the bath. Root growth was followed visually by means of a horizontal microscope in weak white light. The displacement of some natural marks on the root cap against the scale in the ocular was recorded at 5-min. intervals.

RESULTS

1. Effect of auxin supplied on basal part of the root

IAA in 10^{-7} , 10^{-6} , 10^{-5} and 10^{-4}M concentrations in flowing nutrient solution was applied to the basal region of the root extending from about 3 cm beyond the tip to root base, while the apical region 3 cm long remained in flowing nutrient solution without IAA. Five to ten roots were investigated for each concentration, their growth was recorded for about 1 hour before and 5 hours after starting the treatment. The roots grew at a rate of about 1 mm per hour before application of IAA. IAA in 10^{-7} and 10^{-6}M concentration had no visible effect on root elongation over a 5-hour period (Fig. 2 a, b). 10^{-5}M caused inhibition of elongation which

became noticeable about 30 min. after starting the treatment. This inhibition lasted for 40–60 min. and later the root recovered and elongated at the initial rate (Fig. 2 c). IAA in 10^{-4} M concentration caused inhibition which began about 30 min. after starting the treatment and lasted for the next 100–150 min. Afterwards the apical part of the elongation zone recovered and elongated rather rapidly while the basal part of the previous elongation zone did not grow (Fig. 2 d). This irre-

Fig. 2. Streak photographs of corn roots.

A–D roots which were treated with different concentration of IAA in their basal parts: A – 10^{-7} M IAA, B – 10^{-6} M, C – 10^{-5} M, D – 10^{-4} M. In A–C the camera was lowered at the start of the treatment. In D the treatment started at the beginning of growth recording. The treatment is shown by heavy horizontal line. E – as C but 10^{-5} M NAA; F – root which during the recording period was continuously in contact with 10^{-4} M IAA in its basal part (about 5 hours after starting the treatment on the basal part of root and about 2 hours after the recovering of elongation) and to which 10^{-7} M IAA was administered for 10 minutes at the moment shown by arrow. G – as F but not treated with IAA on its basal part.

versibly inhibited part of the elongation zone swelled subsequently as it ordinarily happens after auxin treatment. The elongation rate after recovery was somewhat lower than before treatment, presumably because the elongation zone was shortened.

For comparison, the effect of NAA supplied in a 10^{-5} M or 10^{-4} M concentration to the basal part of root was investigated on few roots. Its effect was the same as that of IAA in these concentrations (Fig. 2 e), i.e. there was a temporary inhibition after which the apical part of the elongation zone resumed elongation.

Six roots in contact with 10^{-4} M IAA in their basal parts but which had already recovered from inhibition were treated additionally with 10^{-7} M IAA on the apical

part for 10 min., 5–6 hours after starting the treatment on the basal part. The purpose of the experiment was to see if roots adapted to basally supplied auxin behave in respect to auxin supplied directly on the elongation zone in the same way as do the control roots. Treatment on the apical part of the root resulted in inhibition of elongation lasting for about 60 min. It means that the pretreated roots behaved similarly as did the nonpretreated ones (Fig. 2 f, g).

2. Effect of temperature on duration of the lag phase of inhibition

IAA (10^{-7} M) was supplied in nutrient solution for 10 min. to the apical part of the roots growing at various temperatures (10 – 22.5°C). This treatment resulted in rapid inhibition which lasted for some time. Thereafter the root recovered from

Fig. 3. Diagram showing the effect of temperature on the duration of the inhibition phase evolved by short IAA treatment given on apical part of corn root — full circles, and on the initial growth rate — open circles. The lines were fitted by means of least squares method.

inhibition rather abruptly. The time course of root elongation was similar as in Fig. 2f, g. The duration of the inhibition phase, T_i , at various temperatures was determined. The start of auxin treatment was assumed as the beginning of the inhibition phase and the moment at which the root recovered 50% of the steady elongation rate after treatment was assumed as its end. Thirteen roots were investigated. T_i de-

creased as temperature increased, it was 140 and 80 min. at 10 and 22.5°C, respectively. In order to obtain the temperature coefficient Q_{10} for the reciprocal of T_i (see discussion) $1/T_i$ or logarithm $1/T_i$ was plotted against temperature (Fig. 3) and the point on the graph were fitted to a straight line by the least-squares method. (The logarithmic graph corresponds to the assumption that $1/T_i$ is an exponential function of temperature, i.e. $1/T_i = kQ'_{10}$). For comparison also the rate of root elongation before the treatment was plotted against temperature. A temperature coefficient Q_{10} for the reciprocal of duration of the inhibition phase in the temperature interval 10–22°C was 1.8 and 1.6 as obtained from the linear and logarithmic graph, respectively, and Q_{10} for the elongation rate in the same interval was about 4. The Q_{10} value was obtained as ratio of the interpolated values at 20°C to that at 10°C on the linear graph or from the slope of the interpolated line on the logarithmic graph.

DISCUSSION

There is little reason to doubt that the inhibition of elongation caused by auxin applied to the basal part of root is due to the fact that the auxin is translocated to the root elongation zone. An indication that the applied auxin reached the elongation zone was given by the swelling of the irreversibly inhibited part of the elongation zone, which is a typical response to auxins. In view of the relatively high rate of translocation of auxin noted in the roots studied, we may assume that the auxin was translocated to the root tip in phloem. The elongation zone ended, namely, in the experimental roots about 0.6 cm beyond the tip, the zone in the basal part of the root to which IAA or NAA was supplied was 3 cm from the tip, hence the distance along which auxin was translocated longitudinally was not less than 2.4 cm. The time lag before inhibition appeared was about 30 min. after starting the treatment. During this time the auxin moved first radially into the root and thereafter longitudinally to the elongation zone. Assigning a part of that time for the radial movement, we come to the conclusion that the postulated longitudinal transport was faster than 5 cm per hour. This is already within the range of phloem translocation rate. The assumption of rapid transport of auxin in phloem is consistent with the preliminary observation that the duration of the lag phase before inhibition starts after supply of auxin to the basal part of the root, did not depend significantly upon the distance between the elongation zone and the treated zone.

Eliasson (1961, 1963) observed inhibition of root growth and swelling of the inhibited portion of root after application of various auxins to the hypocotyl of pea seedlings and to the leaves of aspen cuttings. He also considered this effect as evidence in favour of auxin transport to the elongation zone and he assumed also that the transport occurred in phloem.

Bonnet and Torrey (1965) demonstrated directly an acropetal transport of auxin in roots by using labelled IAA and root segments. They found for this transport in segments of *Convolvulus* root a rate of 1 cm per hour. This rate lies within the range usually observed in segments of various tissues (Hertel and

Leopold 1963). It is much lower than the rate found for the roots in the present study. However, we must take into account that the excised segments are in an unnatural environment and probably are devoid of some forces which regulate the transport in the intact organs (see Whitehouse and Zalik 1967).

The response of the root elongation zone to auxin supplied to the basal part of the root differed from that induced by auxin given directly on the elongation zone. The first was temporary while the second lasted as long as the treatment unless the latter was shorter than 1 hour (Hejnowicz and Erickson 1968). Temporary inhibition was also observed by Eliasson (1961, 1963) in experiments with application of IAA or NAA to cotyledons of pea seedlings or leaves of aspen cuttings. We see two possible explanations why the inhibition caused by the application of auxin beyond the root elongation zone is temporary: 1. the auxin is inactivated somewhere in the tissue, 2. the infringement of the radial gradient of auxin in elongation zone is temporary only. The first explanation fits the classical theory of auxin in roots which claims that an auxin is present in supraoptimal concentration in intact roots and that any addition of auxin from outside should result in an inhibition of elongation, unless the external auxin is inactivated metabolically. Galston and Dalberg (1954) showed that cells in the region of maturation possess a higher IAA-oxidase activity than do meristematic cells, and that the production of this enzyme increases in response to its artificially elevated level in the tissue. One can assume therefore that the auxin supplied beyond the elongation zone set in motion some process by which the absorbed auxin is inactivated. If the process starts with a certain time lag only the first portion of absorbed auxin will be able to inhibit elongation. It may also be assumed that when auxin is applied directly on the elongation zone, the process of auxin inactivation does not keep pace with auxin uptake so that the auxin is able to inhibit elongation as long as it is present in the external solution. If so, however, why does the root which has recovered the ability of elongation, being continuously provided with auxin in its basal part, i.e. adapted to auxin, react to short auxin treatment given directly on its apical part in the same way as a non-pretreated root?

The second possible explanation of the temporary inhibition is based on our hypothesis that a positive centripetal gradient of auxin, i.e. a decreasing auxin concentration from the centre towards the root surface is a prerequisite for root elongation. If only along a part of the radius the gradient is not centripetally positive elongation will be inhibited. Let us consider on this basis the effect of auxin supplied to the basal part of the root and transported to the elongation zone by phloem. At first, before attaining a steady state inflow of auxin into the elongation zone, auxin concentration near the phloem will be higher than in the centre. A manifestation of this will be an inhibition of elongation. We may predict however, that the infringement of the gradient will be temporary only, it will occur until the steady state is attained. In the steady state phase when internal inflow of auxin into the elongation zone occurs at constant rate, the central part of the root becomes saturated with auxin. The pattern of metabolizing auxin, which maintains a positive radial gradient in normal roots, and the possible leakage of auxin on the root surface

washed by nutrient solution reestablish the positive gradient. A manifestation of this will be a recovery of the elongation ability.

The latter explanation assigns a certain role in reestablishing the positive gradient to the leakage of auxin at the root surface. Eliasson (1959a) concluded that some inhibitory substances are effectively removed from the root surface by the nutrient solution continuously passing over the roots or by activated carbon particles sticking to the surface. Although he was not able to draw any definite conclusions regarding the nature of the inhibitory substances, he showed that antiauxins counteract the inhibition what would indicate that the substances are native auxins. The same author (1959b) also showed that the roots of pea seedlings were not so sensitive to 2,4-D application to the hypocotyl when they were continuously washed with fresh nutrient solution, as when they were grown on filter paper. He assumed that 2,4-D leaked out from the roots and was removed by the solution washing the roots. Outward movement of auxin was observed also by Bennet, Clark and Wheeler (1959): rapid initial uptake of IAA by potato disks was succeeded by extrusion of absorbed IAA into the external solution.

The next problem is the lag phase before elongation returns to normal after short auxin treatment given directly on the apical part of the root. We have two possible explanations of the lag phase (Hejnowicz and Erickson 1968). 1. The root tip requires some time to form the auxin oxidase or to develop another mechanism of auxin metabolization. It has been shown in a previous paper (Hejnowicz and Erickson 1968) that this explanation does not fit well the behavior of the root after auxin treatment of varying duration or after two short treatments given at different intervals. Another doubt is raised by the reported here results of the experiment on duration of the inhibition phase at various temperatures. If this explanation were valid the duration of the inhibition phase should be temperature-sensitive in a degree characteristic for metabolic processes, while in fact the sensitivity was clearly lower. 2. Basing on the hypothesis of the significance of the radial auxin gradient we may assume that the duration T_i of the inhibition phase induced by the short auxin treatment is the same as the duration T_d of the phase of unsteady state of diffusion of auxin into the root during which a negative gradient of auxin occurs somewhere along the radius in the elongation zone, i.e. $T_i = T_d$. From the solution of Fick's equation it follows that T_d is inversely proportional to the diffusion coefficient D , i.e. $T_d = a/D$ where a — proportionality constant. If our hypothesis is correct, the reciprocal of T_i would be related to temperature in the same way as the diffusion coefficient, i.e. its Q_{10} should be low (about 1.3). The observed Q_{10} is 1.6–1.8. Its value is higher than Q_{10} for diffusion but is closer to the latter than to Q_{10} of a metabolic process. It lies in the range expected for a combination of physical and metabolic processes what may be explained by the assumption that the diffusion of auxin into the root is not quite "free", but that probably there exist some barriers of selectively permeable cytoplasm. The total "thickness" of these barriers would be, however, small in comparison with the "thickness" which is traversed by physical diffusion. It would be much more difficult to explain why

Q_{10} for $1/T_i$ is so much lower than Q_{10} for a metabolic process if we consider the lag phase as a manifestation of some metabolic process.

Chadwick and Burg (1967) have recently offered an explanation of the inhibition of root growth caused by an auxin which involves ethylene. They found that IAA induces the formation of ethylene gas in the root which inhibits elongation. All that can be said here is that this explanation is not contradictory with the hypothesis of significance of the radial gradient of auxin as both concern different levels of the mechanism controlling root growth. One thing is certain, viz. that auxin does not control root growth directly, but indirectly via some process or processes, one of which may be the formation of ethylene gas.

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SUMMARY

IAA or NAA was applied on the basal part of intact corn root in such a way that the basal part was in contact with the flowing nutrient solution containing the auxin while the apical part of the root was washed by flowing nutrient solution without auxin. The auxins in 10^{-4} M concentration brought about temporary inhibition of elongation which commenced about 30 min. after starting the treatment and lasted for 100—150 min.

The effect of temperature on the duration of the inhibition phase induced by short auxin treatment on the apical part of the root was investigated in the range 10—22.5°C. The temperature coefficient Q_{10} for the inhibition phase was 1.6—1.8.

The results are discussed against the hypothesis that the inhibitory action of auxin on root elongation is due to infringement of the radial gradient of auxin in the root elongation zone.

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Studia nad hamującym działaniem auksyn na wzrost korzenia

Streszczenie

W poprzedniej pracy wysunięto hipotezę, że hamujące działanie auksyny dostarczonej z zewnątrz na wydłużanie wierzchołka korzenia polega na zaburzeniu radialnego gradientu auksyny wewnątrz strefy wydłużania. Według tej hipotezy wydłużanie korzenia zachodzi wtedy, gdy koncentracja auksyn w strefie wydłużania wzrasta od powierzchni do wnętrza, czyli gdy istnieje tam dodatni gradient auksyny w kierunku dośrodkowym.

Przebieg wzrostu korzenia badano metodą fotografii smugowej. Podawano kwas indolo-3-octowy (IAA) albo naftylooctowy (NAA) na bazalną część korzenia siewki kukurydzy w ten sposób, że część korzenia zaczynając się w odległości 3—4 cm od szczytu i kończąc się na ziarniaku omywał strumień pożywki zawierającej auksynę, zaś apikalną część korzenia obejmującą strefę wydłużania omywał strumień pożywki bez auksyny. IAA względnie NAA, w koncentracji 10^{-4} M powodowało przemijające zahamowanie wydłużania zaczynające się 30—40 minut po rozpoczęciu podawania auksyny i trwające 100—150 minut. Taka reakcja wierzchołka na podaną bazalnie auksynę zgadza się ze wspomnianą hipotezą.

Krótkotrwałe działanie IAA (np. przez 10 minut) na apikalną część korzenia powoduje zahamowanie wydłużania trwające około 60 minut w temp. 25°C , podczas gdy wznowienie wydłużania po działaniu dłuższym niż 60 minut następuje natychmiast po usunięciu auksyny z pożywki. Wspomniana hipoteza wyjaśnia takie zachowanie się korzenia przebiegiem zmian profili koncentracji auksyny w korzeniu w czasie dyfuzji przed ustaleniem się stanu równowagi, a w szczególności wiąże zwłokę we wznowianiu wydłużania po krótkotrwałym działaniu auksyny z fazą w której wewnątrz korzenia istnieje negatywny gradient auksyny. Inne tłumaczenia muszą opierać się na jakiś procesach metabolicznych uruchamianych podaniem auksyny. W celu sprawdzenia, czy faza zahamowania wzrostu wywołana krótkotrwałym działaniem auksyny wiąże się z procesem dyfuzyjnym czy z procesem metabolicznym zbadano zależność między długością fazy zahamowania a temperaturą w przedziale $10-22,5^{\circ}\text{C}$. Okazało się, że współczynnik temperaturowy Q_{10} dla odwrotności okresu zahamowania wynosi 1,6—1,8. Ten stosunkowo niski współczynnik wskazuje na to, że udział procesów metabolicznych w określaniu długości fazy zahamowania jest mały i że hipoteza wyjaśniająca zwłokę we wznowieniu wydłużania po krótkotrwałym działaniu auksyny przebiegiem procesu dyfuzji auksyny, przy założeniu roli promienistego gradientu auksyny, jest prawdopodobna.