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The response of the different parts of the cell elongation zone in root to external β-indolylacetic acid

by

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The problem dealt with in this paper has arisen in connection with Burström's (1957) theory of root elongation. According to this theory the manner of the response of the elongating cell in the root to β-indolylacetic acid (IAA) depends on the developmental phase of the cell.

Burström distinguished two phases of elongation: in the first elongation was favoured and in the second retarded by IAA.

As is known root cells cease to elongate at a certain distance from the tip. There must be, thus, a level in the root corresponding to the final moment of cell elongation and so to the end of the second phase postulated by Burström. Since the phase ends at a definite distance from the tip, it must also begin at certain distance from the tip. It seems therefore that in the root there may be zones corresponding to Burtström's elongation phases and with analogical properties. Within one zone elongation would be stimulated by IAA and within the other, situated further away from the tip, IAA would inhibit elongation.

In the literature on the influence of IAA on the growth of various parts of the root (Fiedler 1936, Goodwin 1959) the present author has found no data confirming the above supposition. On the contrary, the available experimental results indicate that the response to IAA has the same character throughout the elongation zone consisting in the inhibition of elongation. However, differences have been recorded in the sensitivity of various parts of the elongation zone. The results that Goodwin obtained by photographing the surface of growing *Phleum* roots show that the most sensitive to IAA is the basal part of the elongation zone. With the rise of the IAA concentration the length of the zone in which growth is inhibited increases. This result can be conformable with Burström's theory when only the second phase of cell growth is considered. However, there is no indication in Goodwin's report that nearer the tip there is a zone responding to IAA by more rapid growth.

The present report describes the response of wheat roots to different concentrations of IAA and discusses the arising implications from the viewpoint of Burström's theory.

MATERIAL AND METHOD

The observations were made on roots of intact seedlings of wheat var. *Eroica* Weibull. Soaked seeds were germinated in the way described in an earlier report (Hejnowicz 1959) at 20°C. The seedlings were planted into the holders of culture vessels made from transparent perspex (ten plants per vessel) 24 hours after germination. The vessels were then placed in front of a 40 W daylight fluorescent tube (fig. 1).

The nutrient solution was: KNO_3 10^{-3}M, $Ca(NO_3)_2$ 2×10^{-3}, KH_2PO_4 10^{-3}M, Na_2HPO_4 2.5×10^{-4}M, $MgSO_4$ 5×10^{-5}M, Fe-versenate 2×10^{-5}M. The nutrient was well aerated by continuously bubbling air through the solution in the culture vessels. The temperatures of the solution and the air were 22.5 and 23°C respectively, and were kept constant during the culture period. After 24 hours the nutrient was renewed; the fresh nutrient had the same temperature and the same composition.

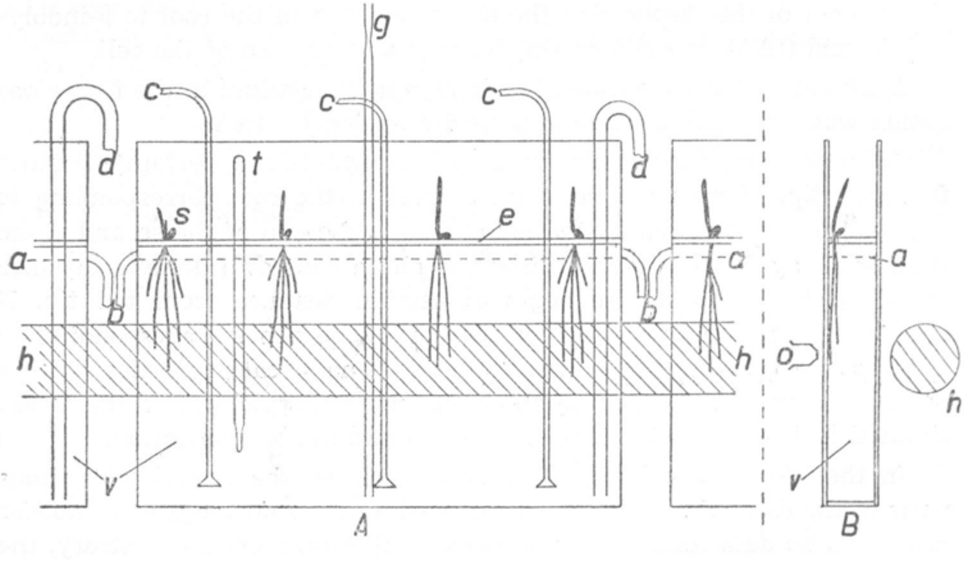


Fig. 1. Schematic diagram of the experimental equipment: *A* — front view; *B* — side view; *V* — culture vessel. *a* — surface of nutrient; *b* — tubes for leading away excess of nutrient; *c* — tubes for bubbling air; *d* — siphon for emptying the vessel; *e* — holder with openings; *g* — tube feeding the nutrient to the vessel from the reserve tank; *h* — 40 W fluorescent tube; *o* — objective of horizontal microscope; *s* — seedling; *t* — thermometer

The seedlings grew in four similar vessels placed in front of a fluorescent tube in a stream of air from two ventilators. The ventilation was provided to avoid local differences of temperature. On the fourth day after germination (about 40 hours after planting the seedlings in the vessels) the first photographs of the surface of selected roots growing vertically near the front wall of the vessel were made. A horizontal microscope with a camera was used. It is to be stressed that during the time the photographs were made the conditions in which roots grew remained unchanged, except that the stream of bubbles was stopped for about 5 minutes in the photographed vessel. In each vessel three roots were chosen for photographing. Each root was photographed with a sequence of 6 overlapping pictures using 36 mm. Adox KB film, the exposure time being 1 sec. The roots were photographed in rotation so that every root was photographed again at intervals of about 30 minutes. The time when the photographs were made was recorded in each case.

The aim of the photographs was to assemble data for determining the growth rate of various parts of the root. The first three sequences of photographs were intended to determine the normal rate of growth. Then the nutrient was changed. The new nutrient had the same composition, temperature, and degree of aeration, but contained additionally a freshly prepared solution of IAA. Three concentrations of IAA were applied: 2×10^{-9} M, 3×10^{-8} M, and 2×10^{-7} M, each concentration in a different vessel. In the fourth vessel the nutrient after the exchange contained no IAA. For exchanging the nutrient the old solution was siphoned off and then the fresh solution was poured into the vessles. After the exchange of solutions the roots were photographed every 30 minutes for another 6 hours. In the vessel where the concentration of IAA was 2×10^{-7} M the roots were photographed once again 14 hours after the application of IAA.

At the time the photographs were made a continuous flow of the nutrient was maintained through the culture vessel from a container placed at a higher level. The rate of the flow was 0.7 litres an hour.

Photographic enlargements 76 × were matched to reconstruct the root surface photographed at a given moment. There were always some characteristic points of the cell wall net present on all the photographic reconstructions of a root which made possible the determination of the elongation rate in different parts of the root.

Not all the roots chosen for observations grew straight enough throughout the experiment to be suitable for photography. The number of roots for which complete results were obtained was 3 in the 2×10^{-9}M concentration of IAA, 2 in the 3×10^{-8}M concentration, 3 in the 2×10^{-7}M concentration, and one in the control nutrient where the IAA concentration was 0.0.

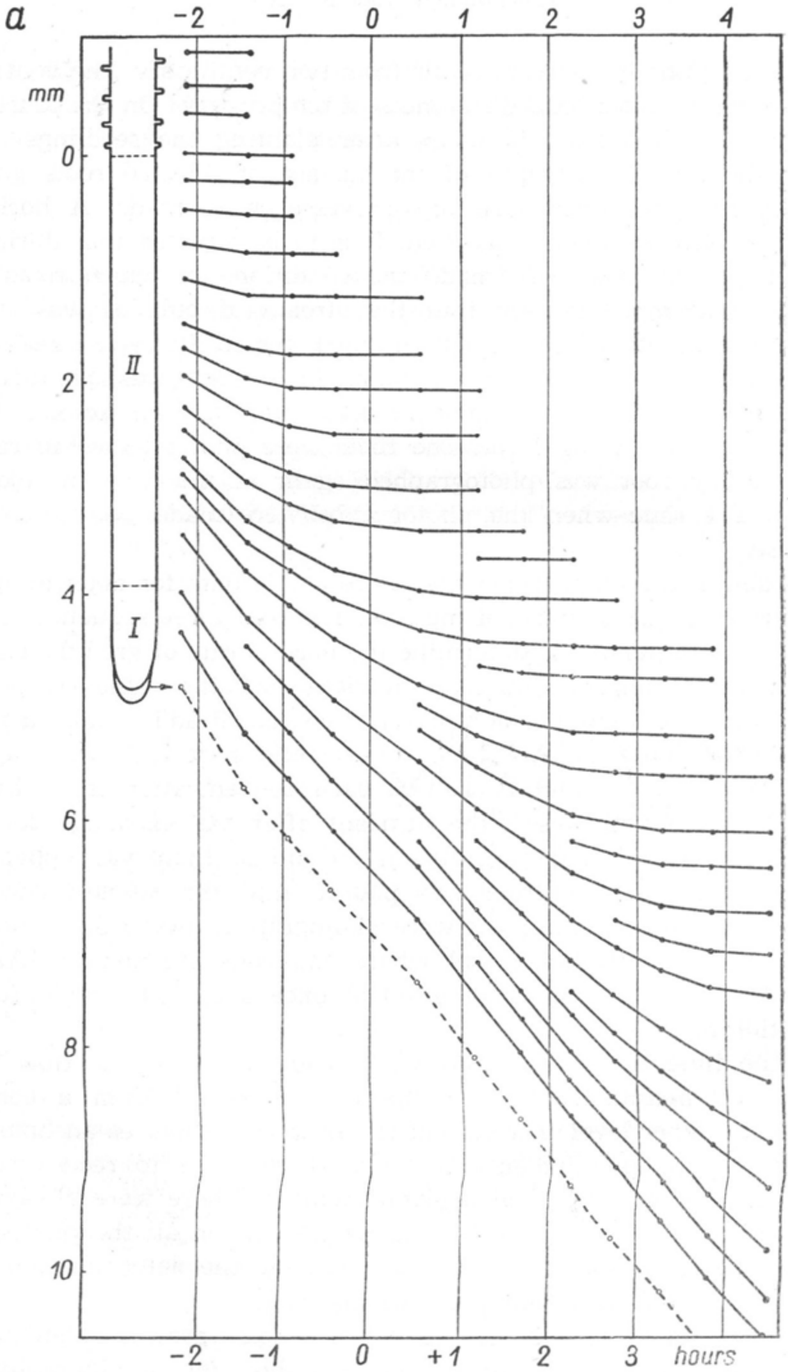
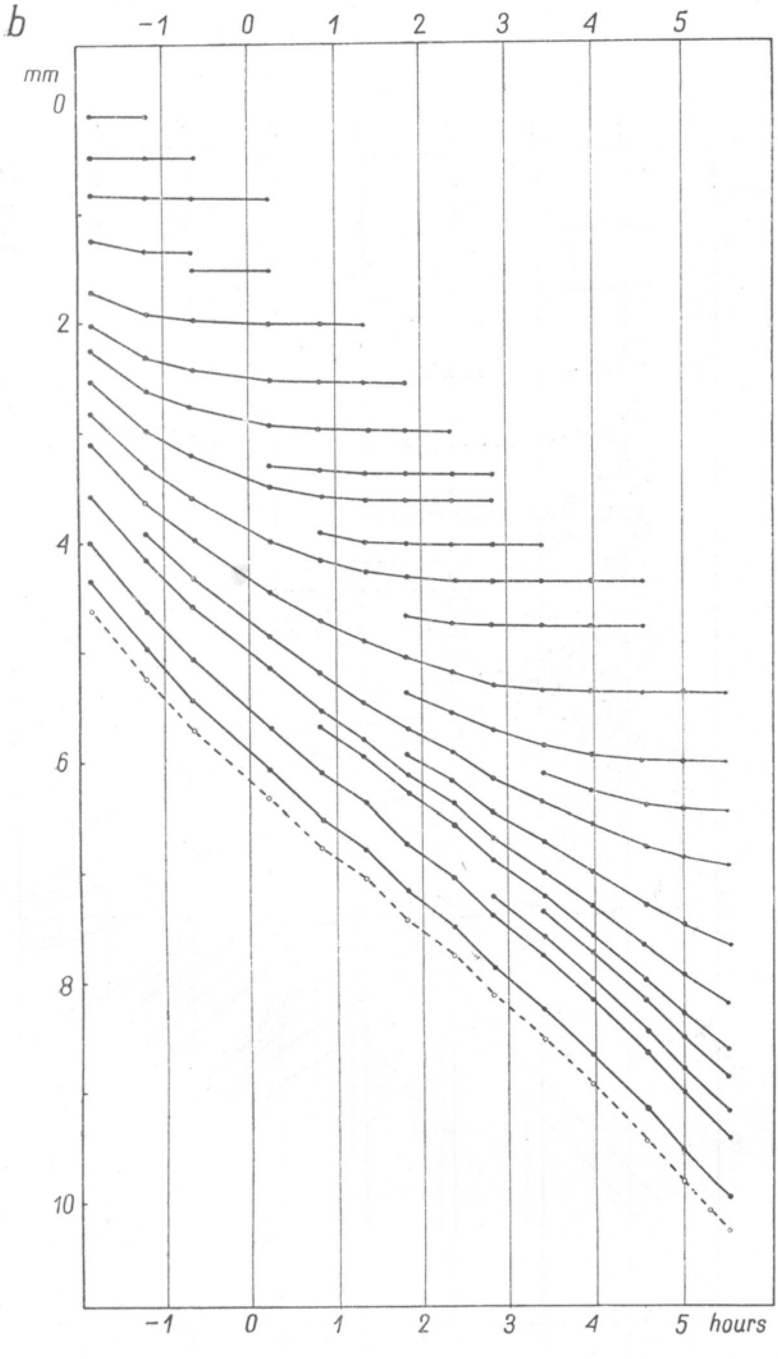


Fig. 2. Lines of the displacements of various points on the root with regard to the fixed base. Vertical axis — distance in mm., horizontal axis — time in hours. The exchange of the nutrient was assumed as 0 time. Before exchange the normal nutrient was used. After exchange: diagram *a* — normal nutrient; diagram *b* — nutrient + 2 × 10^{-9}M IAA



The extrapolated sections of lines and the lines corresponding to the displacement of the blunt apical point are marked by broken lines. The schematic diagram on the left side of the graph *a* shows the root tip drawn to scale with the graph and in the position corresponding to the original position of the points on the root. *I* — zone of cell divisions, *II* — elongation zone

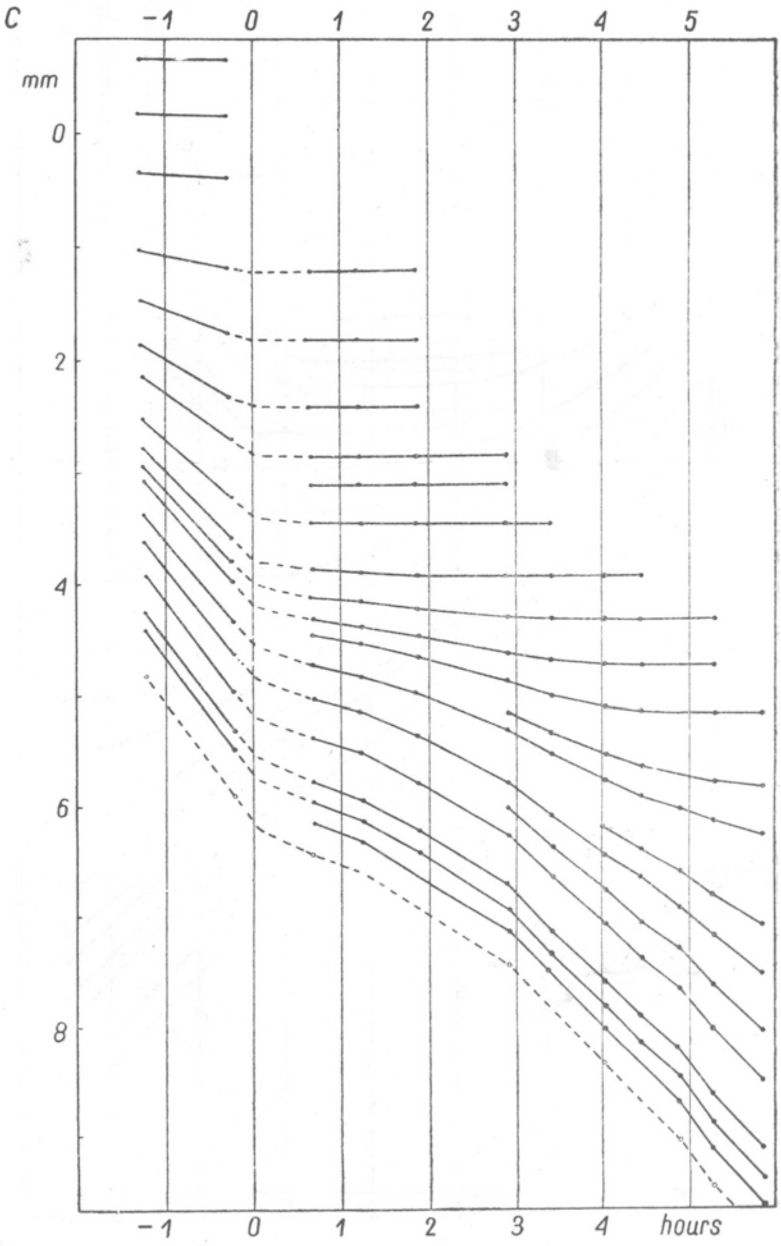
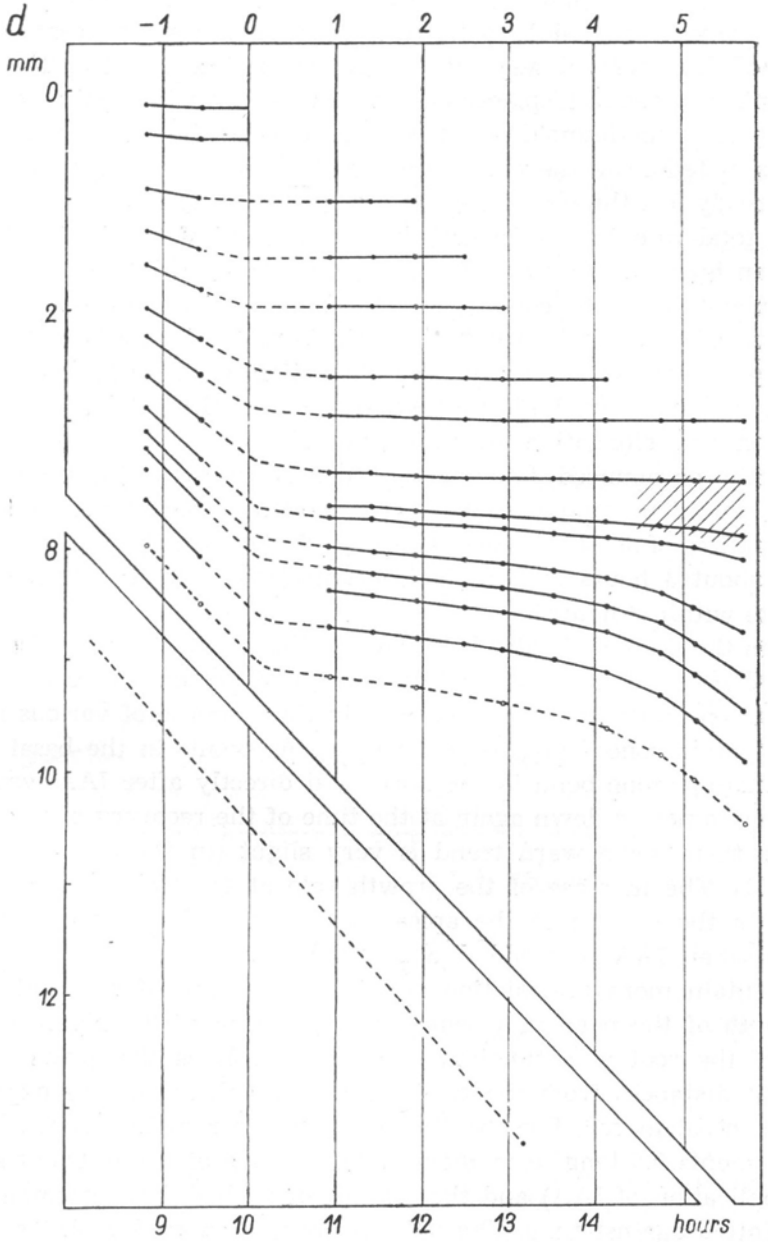


Fig 3. The same as fig. 2, but: c — 3×10^{-8}M IAA; *d* — 2×10^{-7}M IAA. The area of



oblique lines on graph *d* corresponds to the part of the root where the swelling is formed

RESULTS

The manner in which roots grew in various IAA concentrations is illustrated in a general way by the graphs in figs. 2 and 3. The graphs represent the lines of displacements with time of various points on a root with regard to the immobile root base. Obviously, the displacement of the points situated near the tip is the greatest, whereas the points which have already left the elongation zone are not displaced.

The total rate of root growth in the nutrient without IAA is about 1 mm. an hour and the length of the zone of growth is about 5 mm., of which about 0.8 mm. corresponds to the cell division zone (this last figure was obtained in an earlier investigation, Hejnowicz 1959). The remainig 4.2 mm. correspond to the elongation zone. As is to be seen on the graphs, the two higher concentrations of IAA have a retarding effect on the elongation of roots, though the retarding influence is differently pronounced for different concentrations. The inhibition of growth caused by supplying IAA lasts a rather short time. In the IAA concentration of 3×10^{-8}M the recovery of the growth rate is complete within about 3 hours and in the concentration of 2×10^{-7}M it is nearly complete within 6 hours.

When the lines of the displacements of the points on the roots growing in IAA concentrations of 3×10^{-8} and 2×10^{-7}M are examined carefully it is to be seen that there are differences in the response of various parts of the elongation zone. The lines connecting the points in the basal part of the elongation zone bend to the horizontal directly after IAA was added and they do not go down again at the time of the recovery of the growth rate, or their down ward trend is very slight (in the concentration of 3×10^{-8}M). The increase of the growth rate at the time of the recovery consits in the growth of the apical part of the elongation zone where growth when IAA was added was still slow.

To obtain more information about the response of the root to IAA the length of the particular segments (consisting of the given group of cells) of the root as a function of time as well as the growth rate in different distances from the tip in successive phases of the experiment have been calculated. For the first purpose the root has been „divided" into segments 0.5 long in moment of the change of the nutrient solution (the application of IAA) and the lengths of such defined segments were then plotted against time. The data are presented on Fig. 4. As is to be seen IAA has little influence on growth of the segment which at the moment of nutrient change was located between 0.5 and 1 mm from the tip. In futher away from the tip located segments IAA (higher concentration of it) caused immediately distinct inhibition or retardation of growth. This effect (inhibition or retardation of growth) was temporary in segments which at the moment of IAA (10^{-8} M and 10^{-7} M) application were located in apical part of the elongation zone and was irreversible (irreversible inhibition of growth) in segments located at this moment in the basal part of the elongation zone. The length of irreversibly inhibited part of the elongation zone and the rate of adaptation to IAA in apical part of elongation zone depended on IAA concentration — were longer and lower respectively in the highest of used concentrations of IAA.

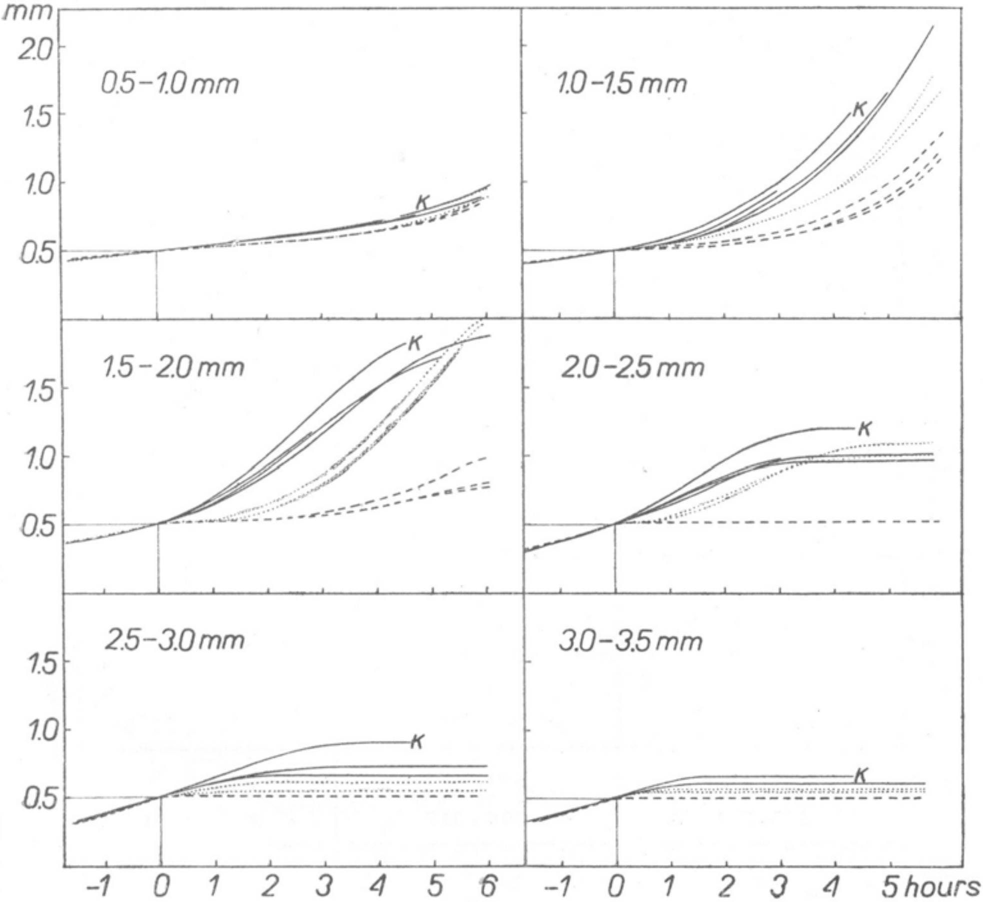


Fig. 4. The courses of growth of different root segments distinguished on the photographic series and which at the moment of nutrient change (0 time) were all 0.5 mm long. On one graph the courses of growth of segments situated with respect to the tip in the same way at 0 time are showed. The position at 0 time is given by the figures in left corner of the graph. Continuous lines correspond to the segments from roots growing in normal solution (the line marked with K) and in solution containing 2×10^{-9}M IAA.: Dotted lines and broken lines correspond to segments from roots growing in solution with 3×10^{-8} and 2×10^{-7} M IAA respectively.

To obtain the picture of effect of IAA on distribution of elongation rate in the root the following calculations have been made. The increment of the distance $\triangle l$ between two points separated by the distance l (about 0.2 mm.) is calculated by comparing two consecutive series of photographs of zone root. When $\triangle l$ is divided by the mean of the distances l at the time the two series of photographs were made and the results is recalculated to one hour and then multiplied by 100, the relative elon-

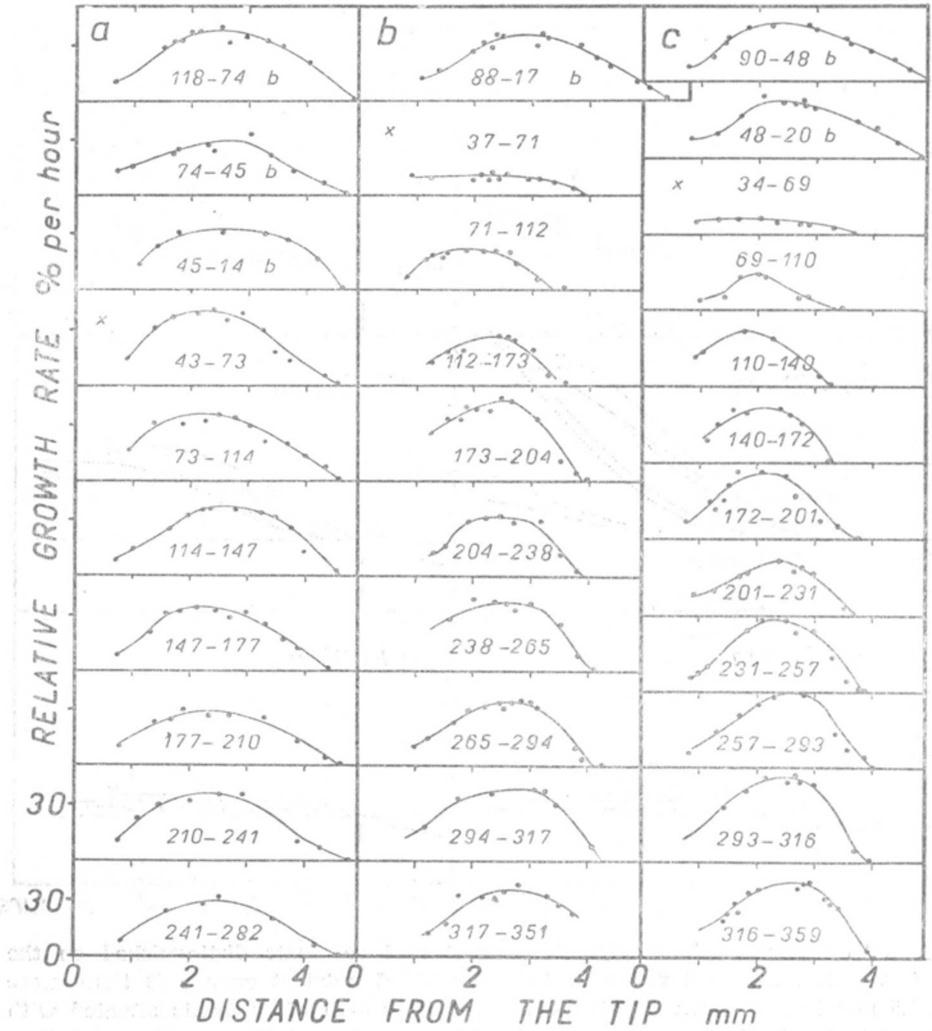


Fig. 5. Relative growth rate per cent per hour plotted against distance from the tip in successive intervals of the experiment. The intervals (given in minutes on graphs) before the exchange of the nutrient are marked with the letter *b*, the first interval after the exchange is marked with *x* in the left hand corner of the graph: *a* — nutrient without IAA; *b* and *c* — after exchange, nutrient + 3×10^{-8} M IAA

gation rate of the section 1 of root is obtained, expressed in percentages per hour of the length of the growing part. It is to be stressed that for the calculations only those pairs of points separated by the distance l were used which were situated on one photograph in each of the consecutive series. In this way the possible source of error caused by the inaccurate matching of the photographs from one series was eliminated.

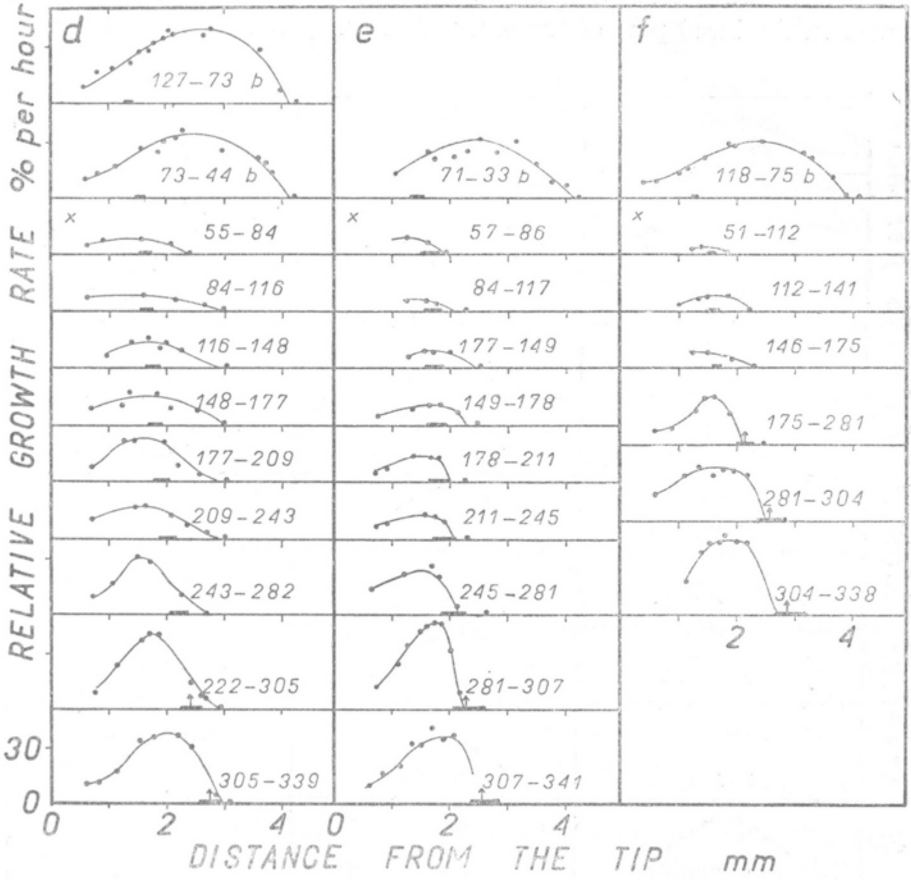


Fig. 6. The same as fig. 4, but the culture solution after exchange contained 2×10^{-7}M IAA. The heavy line along the x-axis marks the section of the root where the swelling will form. The vertical arrow marks the developing swelling

The relative rate of elongation of the region l is subordinated to the average distance from the tip to the middle of this region during the period of time under consideration; in this way the graphs in figs. 5, 6, and 7 have been obtained.

As is to be seen even in the nutrient without IAA there are some variations in the growth of the root. This variation is apparent in the control root (fig. 5a) for which the curve reflecting the relative rate of growth has a different height and a different shape at various periods. Although, the variation might be caused by errors in the determinations of the relative elongation rate, it is also very probable that the variation actually exists. However, the variation of the growth rate of the control root is very small as compared to the differences caused by IAA when its concentration is of the order of 10^{-8} and 10^{-7}M.

The concentration 2×10^{-7}M of IAA (fig. 6d-f) inhibits completely the growth in the basal part of the elongation zone, at least from the middle

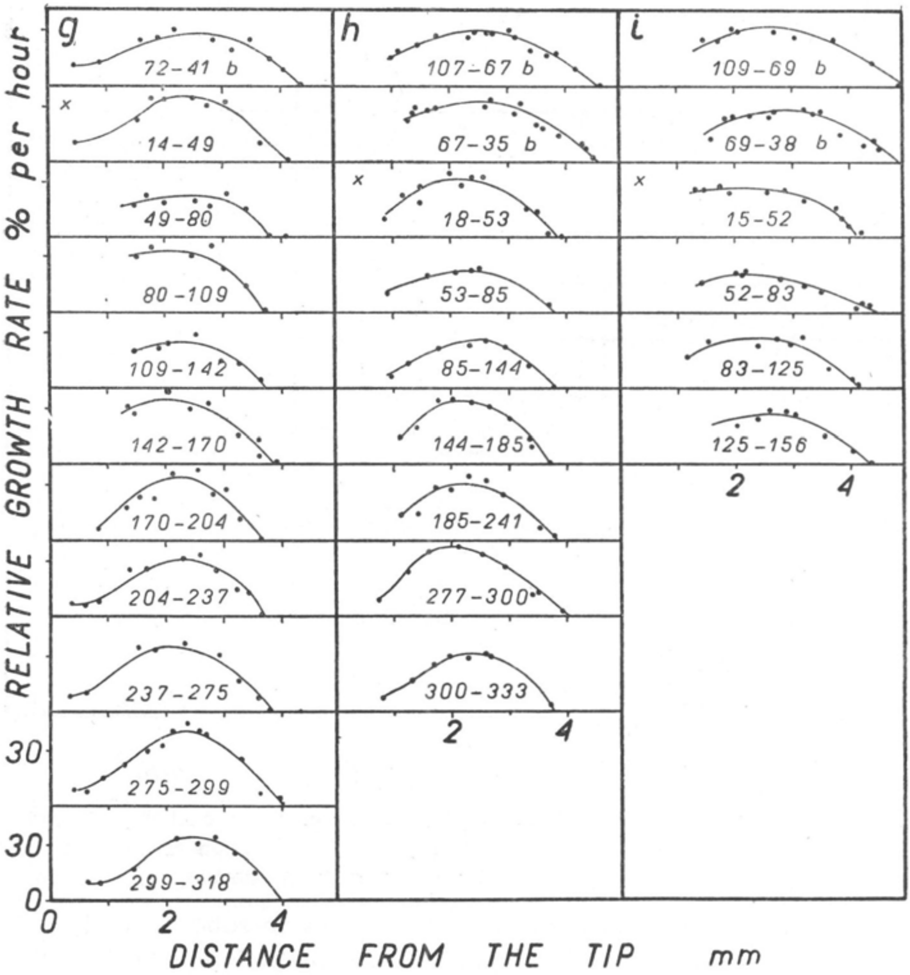


Fig. 7. The same as fig. 4, but the culture solution after exchange contained 2×10^{-9}M IAA

of this zone, and considerably reduces the rate of growth in the apical part of the zone. The retardation of growth in the region of the first millimetre of the elongation zone (the second millimetre of the root) is temporary, and 2 hours after IAA was applied the rate growth begins to increase gradually. The root section which will later be transformed into the characteristic swelling is situated at first more or less in the middle of the zone where the rate of growth increases. After some time this section of the root is no longer situated in the middle of the new elongation zone but in its basal part. This means that the recovery of the elongation does not spread to cells situated more basal in the root, and that the elongation in the region on the basal side of the future swelling ends rapidly. About 3 hours after the application of IAA the growth rate in the second millimetre of the root assumes the original value for this region of the root. However, the rate of growth continues to increase further beyond the original value. Consequently the curve representing the relative rate of elongation after adaptation to IAA is much steeper than before IAA treatment.

About 5 hours after the application of IAA a swelling of the root begins to be visible on the basal end of the new elongation zone. When the photographs made 5 and 14 hours after the application of IAA are compared it is to be seen that the transversal growth leading to the formation of the swelling lasts a short time. After 14 hours the swelling is not much thicker than after 5 hours. The diameter of the completely developed swelling is about 10 per cent greater than the diameter of the root. The length of the swelling along the root is about 0.5 mm. Its characteristic trait is that it is covered by dense root hairs.

IAA at the concentration of 3×10^{-8}M (fig. 5 b, c) causes the complete and irreversible inhibition of growth in the basal part of the elongation zone, reducing its length by about 1—1.5 mm., and a strong but brief reduction of the growth rate in the other part of the zone. Only in the most apical part of the elongation zone the growth rate, still very low at the time IAA was supplied, remains more or less unchanged at this concentration of IAA. The inhibition of growth at the end of the elongation zone is irreversible, but in the remaining part of the zone the recovery of the growth rate begins already an hour after the application of IAA. The length of that part of the root in which the recovery of the elongation rate takes place is greater than in the IAA concentration discussed above.

It is characteristic that, in spite of the intensively renewed growth, the new elongation zone after 6 hours of the experiment ended at distance 4 mm. from tip though during this time the increment of length of the root was about 4 mm. This means that IAA constantly retards the growth of cells which have moved to a certain distance from the tip.

In this concentration of IAA the distribution of growth in the apical part of the elongation zone after the recovery of the elongation rate is the same as before IAA treatment. The concentration was probably too low to stimulate the elongation rate in the apical part.

In the lowest of the applied concentrations of IAA (fig. 6) only a shortening of the elongation zone takes place, i. e. growth is inhibited in the basal part of the zone. In this case also the shortening of the elongation zone is maintained throughout the time of the observations.

The conclusion to be reached from what has been said above is that the response of roots to higher IAA concentration can be divided into two stages. In the first stage the elongation rate is reduced and in the case of the 2×10^{-7}M concentration this applies to the whole length of the elongation zone. The differences between the various parts of the elongation zone in this stage consist only in the various degrees of the retardation of growth. In the second stage qualitative differences between the apical and the basal parts of the elongation zone appear. The apical part has the ability to recover from the initial inhibition of growth, and when the concentration of IAA is 2×10^{-7}M even a certain stimulation of the growth rate is manifested, whereas in the basal part growth is irreversibly inhibited. It is noteworthy that the recovery of growth in the highest of the applied IAA concentrations is due to that short part of the elongation zone which, at the time IAA was applied, bordered on the cell division zone and still grew very slowly.

DISCUSSION

The present work has been undertaken in connection with Burström's theory, thus its results must be discussed first of all from the standpoint of this theory.

According to Burström the phases of cell elongation differ, among other things, by the response to IAA, the difference being defined as follows: in the first phase IAA stimulates and in the second it inhibits elongation. The zones of the root corresponding to these phases should differ in the same way as the phases.

In the course of the present experiments no alleged differences between the various parts of the elongation zone have been observed during the first direct response to IAA. Throughout the whole length of the zone reacting to IAA the first response was the inhibition of elongation. It is true, however, that the inhibition was smaller at the apical end of the elongation zone. The same trend in the response to IAA throughout the length of the elongation zone is maintained only during the first stage of the response. After a certain time differences between the apical and the basal parts of the elongation zone appear. These differences fully correspond to the postulates of Burström's theory. In the basal region of the elongation zone the inhibition of elongation is permanent, while in the apical one the recovery of elongation takes place, and in higher IAA concentrations the recovery even leads to higher elongation rates than the original rate in this region of the zone. The circumstance that the curve representing the relative rate of growth in the renewed elongation zone is steeper than before the application of IAA is of a great significance for Burström's theory. At the same time the elongation zone is shorter. The differences in the distribution of growth cause corresponding differences in the course of cell growth (Hejnowicz, Brodzki 1960). It is, thus, clearly apparent that the change in the distribution of growth caused by IAA must be accompanied by an increase of the growth rate of cells in the first stage of their elongation and the shortening of the elongation time, i. e. the shortening of the second phase of cell growth. This is in agreement with Burström's theory.

In the experiments here reported the adaptation of roots to IAA was very rapid. A similarly rapid adaptation had been observed in preliminary experiments. The age of the roots as well as the applied IAA concentration have undoubtedly much significance for adaptation, since, as was demonstrated by Burström (1957), the sensitivity of the root to IAA decreases during the development of the seedlings regardless of whether IAA was supplied or not. In his report Burström presents data from which it results that one day the roots of seedlings a few days old responded strongly to IAA of the concentration 10^{-7}M and did not react at all the next day (in the test with the length of epidermal cells). In the present experiment the IAA concentrations were on the boundary of root sensitivity. If the influence of IAA was determined from measurements of the daily increment of root length it would be so small that it could easily be overlooked.

The possibility of an equally rapid and complete adaptation of the root in wheat to IAA of the concentration 5×10^{-7}M is also indicated by the results reported by Ekdahl (1953), though in his later experiments (1957) the adaptation was slower and incomplete.

In connection with the question of adaptation it is to be stressed that adapted roots, resembling control roots so far as the total elongation rate is concerned, grow in a different way, which was already pointed out by Burström (1957).

It also is noteworthy that only those cells become adapted to higher concentration of IAA which either had just left the cell division zone or were still in this zone at the time IAA was applied.

Moreover, mention must be made of the fact that these cells compose the same part of the root apex which, in Pilet's and Galston's (1955) experiments with roots of lentil, was characterized by a marked increase of IAA oxidase activity under the influence of external IAA or dichlorophenol. In the experiments of those workers the treatment with IAA caused a drastic increase of the activity of IAA oxidase (through the enhanced production of peroxides) in the apical part of the root. In the remaining part of the apex the IAA oxidase activity, high even under normal conditions, did not undergo major changes. This means that oxidase activity was shifted in the apical direction. According to Pilet and Galston the activity of IAA oxidase is a factor regulating the elongation of root cells by chaging the amount of IAA. The shift of the oxidase activity towards the tip would thus cause an earlier beginning of elongation, and the greater IAA oxidase activity in the cells coming out from the cell division zone would make their growth possible even in the presence of external IAA. The facts here reported fit well into this pattern. It also seems that the interpretation of the adaptation mechanism based on the changes in the activity of IAA oxidase can be reconciled with Burström's theory, even when all the objections against such an interpretation of adaptation raised by Burström himself (1957) are accounted for.

The swelling of the root formed in IAA concentrations of the order of 10^{-7}M also necessitates some coments. As is known the swelling is caused by the transversal growth of cortical cells (Cholodny 1932, Levan 1939), while the present experiments indicate that it develops at the boundary between the zone of inhibited growth and the zone where adaptation takes place. The transversal growth of cortical cells takes place when the elongation of the root section under consideration stops, i. e. when the growth of the cells from the central cylinder has finally ended (since these cells take no part in the formation of the swelling). The situation at this moment seem to be as follows: the cortical cells still grow transversally, whereas the growth of the cells of the central cylinder is already ended. The supposition, therefore, arises that the swelling corresponds to a shift of the boundaries between zones reacting differently to IAA in different types of cells. The cells from the cortex of the swelling would thus belong to the zone in which the recovery of the growth rate takes place; the boundary of the zone for that type of cells would be situated on the basal side of the swelling. Analogically, the central cylinder inside the swelling would belong to the zone with irreversibly inhibited growth and in the cylinder the boundary of the zone would be on the apical side of the swelling.

SUMMARY

For the experiments IAA concentrations making possible a rapid and complete adaptation of wheat roots to IAA were used. It has been found that in the response of the elongation zone to IAA two stages can be distinguished. In the first stage which starts immediately after IAA application the response consists in the inhibition of elongation. During this stage the differences between the response of various parts of the elongation zone are only quatitative; the sensitivity to the inhibitory influence of IAA on elongation increases with the distance from the tip.

In the second stage of the response qualitative differences between the apical and the basal parts of the elongation zone appear. In the apical part the recovery of elongation and, in the strongest of the applied concentrations, even a stimulation of the relative elongation rate take place in the presence of IAA. On the other hand the inhibition in the basal part remains irreversible. The boundary between these two parts depends on the IAA concentration and shifts towards the tip with an increase of the concentration. In higher IAA concetrations a swelling is formed on the root at the boundary between the two parts of the elongation zone.

The observed changes in the distribution of growth under the influence of IAA are in good agreement with Burström's theory on the existence of two phases in the elongation of cells which differ by their response to IAA.

The experimental part of this work was performed at the Department of Plant Physiology, University of Lund. The author wants to express his gratitude to Professor Hans Burström for valuable criticism.

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