ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XLIV, nr 4
1975

The effect of the edein complex on the activity of apical root meristems of *Allium cepa* L.

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(Received: June 5, 1975)

Abstract:

The effect of a mixture of edeins in water solution on the meristematic activity of *Allium cepa* L. root tips was studied during the incubation and post-incubation periods.

Reduction of the elongation growth of the root tips and a strong decrease in the number of mitoses was observed what, as cytochemical and autoradiographical studies have shown, is due to the inhibition of DNA synthesis. DNA synthesis is completely inhibited after 24 hrs of incubation. Changes caused by 25 ppm concentration of the solution are reversible during postincubation. The concentration of 50 ppm produces irreversible damage. The main direction of cytochemical changes caused by the action of edeins on the meristematic cells is in good accordance with those recorded for bacterial cells (Kuryło-Borowska 1962, Borowski and Chmara 1968).

INTRODUCTION

Edeins (antibiotic) are polypeptides produced by *Bacillus brevis* Vm 4 consisting of four components, the most important being edein A ($C_{33}H_{58}O_{10}N_{10}$) and edein B ($C_{33}H_{60}O_{10}N_{12}$). Both of them represent the same mechanism of activity (Borowski and Chmara 1968). The two other components C and D are present in very small quantities. The mixture of edeins shows a wide range of activities: growth inhibition of bacterial colonies, fungial and cancer cells in some animal species.

A detailed study of the activity of edeins was performed on the model bacteria *Escherichia coli* (Kuryło-Borowska 1962). The bacteriostatic dose of edein causes rapid inhibition of DNA synthesis, stimulation of RNA synthesis, and it has no influence on the synthesis of proteins (Kuryło-Borowska 1962, Borowski and Chmara 1968). Full

inhibition of protein and RNA synthesis is brought about by edein concentration higher than bacteriostatic. The effect of a sublethal dose of edein is reversible: DNA synthesis and cellular divisions are restored when the antibiotic is removed.

It is suggested from the investigations on the mechanism of edein activity that they do not affect DNA itself only its replication system, most probably the DNA polymerase (Kuryło-Borowska 1964). It has not been found yet if this inhibition of DNA synthesis is caused by changes in polymerase activity through inhibition of formation of the DNA-polymerase complex, or through changes of the rate and proportions of DNA synthesis (Hierowski, Kuryło-Borowska 1965).

Considering the above data from research on microorganisms, the effect of edeins on cells of higher plants seemed to be worth a more detailed study. Investigation of the effects of edeins on the test cells of the root tip meristems of *Allium cepa* and the answer to the question if the main direction of cytochemical changes is similar to that noted in the microorganisms, were the purpose of this study. Special attention was devoted to changes of the DNA level.

MATERIALS AND METHODS

The meristematic cells of root tips of *Allium cepa* L. were used as material. An edeins complex (of uncertain composition) received from the Tarchomin Pharmaceutical Works "Polfa" was used as the reacting agent. The influence of two concentrations of edeins: 25 ppm and 50 ppm (solutions in tap water) was studied. Incubation and postincubation times are given in the table below.

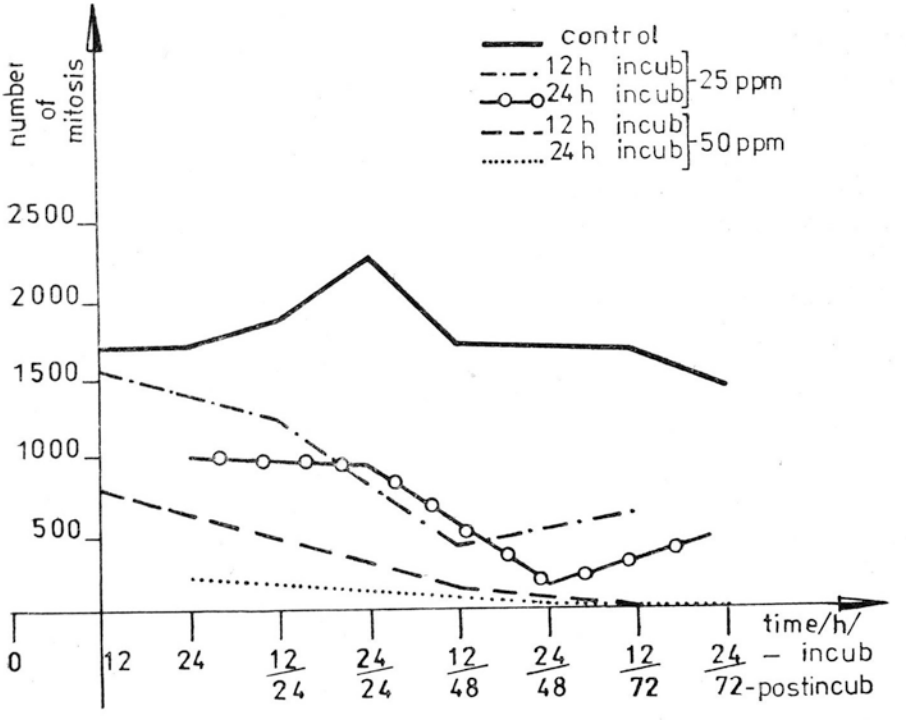
Concentration ppm	Incubation hrs	Postincubation hrs		
25	12	24	48	72
	24	24	48	72
50	12	—	48	72
	24	—	48	72

The control material for all experimental series was grown in the same conditions as the test onions. Root tips were fixed in acetoalkohol (AA) 1 : 3 (by volume), in CrAF 0.5-1-20 and in acetoformalin (AF) 0.5-20 (per cent ratio). Squash (after AA) preparations stained with acetoorcein and microtome preparations (after CrAF and AF) — stained by the Feulgen method, toluidine blue pH 3.4 and fast green were made. All reactions were executed as described by Pearse (1957).

Disturbances in cellular divisions were studied and the number of mitoses was estimated on the squash preparations. Mitoses were counted on the whole preparation made from a root tip 2 mm long. Additionally the autoradiographical method was used to determine the course of DNA synthesis. Radioactive ^{3}H-thymidine was administred at concentrations of 2 μCi per 1 ml of the solution; the incubation time being 2 hours. The course of DNA synthesis at both concentrations of edeins was studied after 1 and 2, 11 and 12, 23 and 24 hours of incubation and in the second series 24 hrs of incubation and 1 and 2, 5 and 6, 11 and 12, 23 and 24, 47 and 48, 71 and 72 hrs of postincubation, respectively.

RESULTS

Edeins have a strong inhibitory effect on the elongation growth of roots. This effect is proportional to the concentration of the solution and to the incubation time. A slight acceleration of growth is observed in the postincubation period following 12 hrs incubation in the 25 ppm solution of edeins. Primarily it is probably caused by elongation of the cells and after 48 hrs by the increase in the number of mitoses. After 24 hrs of incubation (at 25 ppm) the course of root elongation is similar, only the rate is much slower.



Inhibition of root growth is connected with significant changes in the number of cellular divisions in relation to the control. The numerical data obtained from the experiments are illustrated by the graph.

The graph shows, that the action of edeins causes a sudden decrease in the number of mitoses, proportional to the applied concentration of edeins and incubation time. Lowering of the number of mitoses continues in the postincubation period; it falls to zero after higher concentration of edeins. At lower concentration it declines during the first 48 hrs, later an increase is observed (see graph).

The edeins solution does not induce significant disturbances in the course of mitosis: no figures indicate disturbances in the formation and functioning of the mitotic spindle and phragmoplast. A strong contraction of chromosomes was, however, observed already after 6 hrs of incubation. Its intensity was directly proportional to the concentration of the solution and incubation time (after 24 hrs of incubation at a concentration of 50 ppm it reached 70% in relation to the control). It is characteristic and astonishing that this shortening of chromosomes persists during the 72 hrs of postincubation.

It should be mentioned that the survival for the majority of the roots in the 50 ppm edein solution is 24 hrs, after this period of time only few of them are viable when transferred to water.

Cytochemical changes. It was observed, that edeins inhibit cell divisions and cause a strong contraction of chromosome arms. Considering the data given above and the literature, quantitative changes of both nucleic acids (DNA and RNA) but mainly the former, and proteins during incubation and postincubation should be expected.

Stain reactions (Feulgen method and toluidine blue pH 3.4) and the autoradiographic method were applied in order to study the DNA level and its synthesis. Conclusions were based mainly on the latter method. It has been concluded, that during the first 1 and 2 hrs of incubation in the 25 ppm solution of edeins DNA synthesis remains at nearly the same level as in the control, but after 11 and 12 hrs a significant lowering can be observed, especially in the periblem. In the terminal hours of incubation (23 and 24 hrs) only traces of synthesis can be seen. The course of synthesis in the 50 ppm solution is analoguous to that observed for the 25 ppm solution, but the significant depression is observed as early as after 1 and 2 hrs, and after 23 and 24 hrs of incubation incorporation of ^{3}H-thymidine is completly inhibited. During the 72 hrs of postincubation DNA synthesis is not recovered in these roots (if they survive). But after previous incubation for 24 hrs in the less concentrated solution (25 ppm), incorporation of thymidine was not observed until the period of 48—72 hrs after transferring the roots to water, where it never reached the level of the control.

Quantitative changes of the RNA level in the cytoplasm and the nucleolus were determined on preparations stained with toluidine blue at pH 3.4. The RNA level in the cytoplasm was observed to rise during incubation at both concentrations of edeins. It seems to be independent from the length of the incubation period. During the first 48 hrs of postincubation the quantity of RNA stabilizes at the level reached during the incubation period, that is higher than in the control. After 72 hrs of postincubation a return to the control level is observed for the lower concentration of edeins. Preparations for the higher concentration of edeins are hardly legible, most of the cells are dead.

No changes were observed in the staining of the nucleoli in any of the variants of the experiment; colour intensity was equal as in the control.

The level of acid and basic proteins during the whole period of root growth was also studied. No changes were observed in the level of acid proteins in the cells. Significant quantitative differences were found as regards the basic proteins, both in the nucleus and the cytoplasm. The direction of these changes is the same as in the case of RNA. An increase of the quantity of basic proteins in the nucleus and the cytoplasm takes place at both concentrations of edeins. These changes are reversible in the postincubation time; the control level is reached after 48 hrs in the cytoplasm and after 72 hrs in the nucleus.

DISCUSSION

The water solution of a mixture of edeins is toxic for the meristematic cells. Cell degeneration takes place already after 24 hrs of action of the 50 ppm solution (roots decay in postincubation).

Edeins cause a strong contraction of chromosomes, proportional to the concentration of the solution and incubation time. The contraction of chromosomes is usually accompanied by changes in their chemical constitution, enrichment in RNA being one of them. The RNA is probably of nucleolar origin.

As mentioned in the introduction, Kuryło-Borowska (1962) studies on the effects of edeins on the cells of *Escherichia coli* have shown that edeins inhibit DNA synthesis during incubation, but recovery takes place at once when the antibiotic is removed from the medium.

The decrease in DNA synthesis during the incubation period was observed also in the meristematic cells of *Allium cepa* root tips. The higher the concentration of edeins applied, the sooner the effect appears (after 24 hrs only traces of incorporation of thymidine are observed). These changes are reversible for the lower (25 ppm) concentration of edeins after a long postincubation period (72 hrs). For the higher concentration

these changes are irreversible. They might be explained by the mechanism suggested by Kuryło-Borowska (1964); that is by the influence of edeins not directly on DNA, but on the system of enzymes (probably on DNA polymerase). The course of cell divisions started before the incubation remain unaltered and mitoses initiated during the incubation period, in the cells containing a double quantity of DNA may take place without any disturbances. The inhibition of DNA synthesis and the significant lowering of the number of mitoses connected with it occur probably after the end of these divisions as a result of the impairment of the enzymes. These effects are visible after a rather long time; near the end of the 24 hrs of incubation and the begining of postincubation (the mitotic cycle of *Allium cepa* lasts about 20 hrs). After 12 hrs of incubation in the more concentrated solution of edeins the synthesis in the meristematic cells is not restored during the 72 hrs of postincubation, what leads to the supposition, that the damage to the enzymes is irreversible. But after incubation in the lower concentration of edeins the DNA synthesis is not restored until after 48—72 hrs of postincubation; thus so long time is needed in order to reactivate the enzymes. This supposition is supported by the curves in the graph; after 24 hrs of incubation the number of mitoses rises for the concentration of 25 ppm and falls to zero for the concentration of 50 ppm.

The changes of the DNA level in the cells of *Allium cepa* are in good accordance with these reported by Kuryło-Borowska (1962) for bacteria. Different protein levels are, however, observed in each of these objects. In bacteria complete inhibition of protein synthesis does not take place at concentrations lower then sublethal. In *A. cepa*, where the levels of acid and basic proteins were studied separately, the results of the stain reactions show that the level of acid proteins is constant (equal with the control) both during incubation and postincubation. The quantity of basic proteins rises under the influence of edeins, but during the 72 hrs of postincubation it returns to the level of the control.

Acknowledgements

It is a pleasure to thank Prof. Dr J. Szuleta for suggestions and constructive criticism during the progress of experiments. The authors thank also inż. K. Dzięgielewski for the mixture of edeins.

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Wpływ kompleksu edein na aktywność merystemów wierzchołkowych korzeni Allium cepa L.

Streszczenie

Badano wpływ wodnego roztworu kompleksu edein na aktywność merystematyczną wierzchołków korzeni *Allium cepa* L. w czasie inkubacji w roztworze i postinkubacji w wodzie. Stosowano dwa stężenia edein: 25 ppm i 50 ppm. 24-godz. działanie edein o stężeniu 50 ppm jest letalne, po działaniu 25 ppm zmiany są jeszcze odwracalne. Stwierdzono hamowanie wzrostu korzeni na długość, spadek liczby mitoz, oraz silną kontrakcję chromosomów.

Badano zmiany poziomu DNA, RNA, białek kwaśnych i zasadowych stosując barwne reakcje cytochemiczne, a w przypadku DNA także autoradiografię przy użyciu ^{3}H-tymidyny. Otrzymane wyniki wskazują na spadek poziomu DNA proporcjonalny do stosowanego stężenia i czasu inkubacji. Włączanie tymidyny w 23—24-ej godz. w roztworze edein prawie całkowicie ustaje. Wznowienie syntezy DNA następuje w okresie 48—72 godz. postinkubacji po działaniu niższego (25 ppm) stężenia edein, a po działaniu stężenia 50 ppm nie zostaje wznowione. Poziom RNA i białek zasadowych wzrasta w czasie inkubacji, a w 72-godz. postinkubacji wraca do poziomu kontroli. Edeina nie wpływa na poziom białek kwaśnych.

Ogólny kierunek zmian ilościowych badanych związków w komórkach merystematycznych korzeni *Allium cepa* jest zgodny z opisanymi przez Kuryło-Borowską (1962) oraz Borowskiego i Chmarę (1968) w komórkach bakteryjnych *Escherichia coli.*

Otrzymane wyniki wskazują na istotną zależność hamowania wzrostu korzeni i spadku liczby mitoz od zmian cytochemicznych w komórkach merystematycznych.