THE EFFECTS OF KINETIN ON THE GROWTH AND MITOTIC ACTIVITY OF EXCISED ROOTTIPS OF RYE IN VITRO

by

Da-ping Yang

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SUMMARY

The effects of kinetin on the elongation and mitotic activity of excised roottips of rye have been studied *in vitro*. 10-mm roottips of diploid (2n = 14) and autotetraploid (2n = 28) rye, *Secale cereale* var. Petkus, were incubated in a modified Burström medium with various concentrations of kinetin, indoleacetic acid (IAA), or both, ranging from 0.001 to 1 mg/l.

It was observed that at 1 mg/l, kinetin retards the linear growth of the main roots and causes complete inhibition of the initiation of lateral roots, while at 0.1 mg/l, it inhibits the elongation of lateral roots alone.

In a medium containing 3% dextrose, a low concentration of kinetin (0.001 mg/l) enhances the elongation of main roots of diploids, while in a medium of 2% dextrose it shows a tendency to inhibition.

IAA at 0.1 mg/l retards the elongation of main roots, and the initiation and the linear growth of lateral roots.

Kinetin can counteract the inhibition caused by high concentration of IAA (0.1 mg/l). This "antiauxin" effect of kinetin is more pronounced in tetraploids in which interactions are found between kinetin and IAA, while in diploids the combination of the two phytohormones produces additive effects in the elongation of main roots.

At 5 mg/l, kinetin enhances the mitotic index (MI) during the initial 6 hours of the treatment. Then, the MI decreases as the treatment is prolonged, and finally, after 72 hours, the

mitotic activity stops. At low concentration of kinetin, the pattern of cell multiplication is similar to that of the control roots, which show a sigmoid growth curve.

Cytological abnormalities and chromosomal aberrations, including excessively contracted chromosomes, breakages, bridges in anaphases and telophases, and binucleate cells were observed at concentrations starting from 1 mg/l of kinetin.

In conclusion, the results support the hypothesis that a multiple hormonal system controls the growth of roots. Furthermore, the inhibition caused by high concentration of kinetin in the elongation of roots after treatment for more than 27 hours is considered to be due to induction of abnormal mitosis and retardation of cell division.

INTRODUCTION

Growth of excised roots *in vitro* stands as the very first success in culturing plant organs and tissues (cf. White 1954). Tissue and organ cultures have the advantage of eliminating the complex influences of the intact plant, hence they provide a powerful tool for the study of cell division under precisely controlled conditions. Since the early thirties, when nutrient media for both excised roots and for cambium callus tissues were formulated (White 1934a, Gautheret 1934), many investigators have searched for better methods and media to grow more tissues *in vitro* and have applied the method to solving various biological problems. Prominent among the latter have been the study of growth, the morphogenesis of normal tissues or organs, and the ontogeny of plant tumors which show great similarities to cancers in animals (de Ropp 1947).

The search for a possible trigger mechanism responsible for the initiation of cell division has been a subject of interest for many years. Although several hypotheses have been postulated concerning this trigger mechanism, none is generally satisfactory (cf. Glass 1962). The discovery of kinetin (6-furfurylaminopurine) by Miller et al. (1956) shed new light on the speculation that endogenous growth factors might initiate cell divisions in plant. It was found that kinetin in combination with indoleacetic acid (IAA) stimulated cell division in tobacco callus tissue grown on a modified White's nutrient agar medium (Das, et al. 1956). Similar stimulating effects were also observed in many other unorganized

tissues grown in vitro, such as carrot root tissues, soybean cotyledon callus, pea root callus and cocklebur callus.

Intact and excised roots were also used to test the effect of kinetin on mitotic activity of the meristems of roottips and on elongation of roots. Kinetin effects ranged from slight stimulation at low concentrations to marked inhibition at higher ones. Fries (1960) reported that kinetin at 3×10^{-7}M enhanced the initiation of lateral roots of the intact seedlings of a strain of Lupinus hartwegii. Wittwer and Dedolph (1963), who studied the effects of kinetin on growth and flowering of intact tomato, cucumber, and pea plants by incorporating the chemical in culture solutions bathing the roots, found that kinetin at 10^{-7}M inhibited the over all growth of tomato and cucumber plants; while at concentrations from 10^{-7}M through 3×10^{-6}M, kinetin stimulated the growth of the roots of pea. Using excised segments of pea roots, Torrey (1962) demonstrated that kinetin at low concentration, 0.01 ppm (approximately 5×10^{-8}M), increased the number of lateral roots in the presence of a stimulatory concentration of IAA (5×10^{-5}M) and completely inhibited the initiation of laterals at 5 ppm (approximately 2.5×10^{-5}M). The inhibitory effects of kinetin on the linear growth of the main axis of excised tomato roots was reported by Butcher and Street (1960). However, at higher sucrose concentration, 30 grams per liter, kinetin promoted the linear growth and prolonged the duration of meristematic activity. The cause of the kinetin inhibition was attributed to decreasing the number of new cells in the meristematic zone. In other words, kinetin

slowed down the mitotic rate of the meristematic cells of the tomato roots. It was also reported that kinetin inhibited cell multiplication of the excised wheat roots *in vitro* (Murstrom 1960) and the mitoses of intact onion roots (McManus 1960).

A great many other materials have been used for detecting the biological effects of kinetin (cf. Miller 1961), and some of these concern its cytological effects on the mitotic division. Guttman (1956) treated intact onion seedling roots with kinetin and found a variety of cytological abnormalities, including binucleate cells, pycnotic nuclei, and polyploid cells in the meristematic mitoses at 3 and 5 ppm. At lower concentrations kinetin shortens the relative duration of prophase and lengthens the duration of the telophase. On the other hand, Haber and Luippold (1960), who studied the effects of kinetin on mitotic activity in dormant lettuce seeds, reported that at 5×10^{-5}M (approximately 10 ppm) kinetin stimulated mitotic division in the radicles of nongerminated seeds, but it neither changed the relative frequencies of phases in mitosis nor induced any cytological aberrations. Though most of the researchers agree that kinetin does initiate cell divisions under certain conditions, still the actual mode of action of kinetin in cell division is obscure. It seems that kinetin reacts differently upon different tissues. In tobacco pith callus, kinetin combined with IAA stimulates cell divisions and induces DNA synthesis and doubling (Das, et al, 1956, Patau, et al. 1957); while in pea root callus (Torrey 1961) kinetin stimulates mitosis of endomitotic cells already present in the cortex of the root, and has no connection with DNA synthesis. Even on similar tissues, the root

meristems, for example, contradictory reports have been published. There is no doubt that further investigation on the cytological effects of kinetin on cell division is needed for its clarification.

The experiments reported here are concerned with the effects of kinetin in combination with IAA on excised roots of rye *in vitro*. It consists of two parts. I. The effects of kinetin and IAA on the linear growth of excised roots of rye, in which a diploid rye and its autotetraploid were used with the hope that quantitative genetical differences in response to the kinetin treatment may be detected. II. The cytological effects of kinetin on the meristematic mitosis of the excised rye roots.

MATERIALS AND METHODS

I. For the Investigation of the Growth of the Roots.

1. Plant Materials

Diploids (2n=14) and tetraploids (2n=28) of Secale cereale var. Petkus were used as sources of excised roottips for the experiments. Seeds were sterilised by soaking in a 1 percent solution of a commercial detergent (Sur) for one minute, followed by 0.1 percent aqueous mercuric chloride for 20 minutes (tetraploid seed) or 15 minutes (diploid seed). The mercuric chloride was removed by five washes of sterile distilled water, each for about one minute.

Nine seeds were planted aseptically in a sterile 11-cm Petri dish, containing a Whatman No. 1 filter paper moistened with sterile distilled water. The seeds were arranged with their embryos pointing to the centre of the paper to assure good growth of the seminal roots. Seeds were allowed to germinate in darkness at 25±1°C in a constant temperature room. After 60 hours, when most of the roots reached about 20 to 30 cm, they were ready to be excised and inoculated into the culture medium.

2. Culture Media

After preliminary studies on several commonly used culture media (White 1954, Roberts and Street 1955, Burström 1941), a modified Burström medium was chosen which contained the following components per liter of solution:

$Ca(NO_3)_2 4H_2O$	93.60 mg
KNO_3	20.22 mg
$KH_2PO_4 \cdot H_2O$	44.00 mg
$MgSO_4$	24.10 mg
$Fe_2(SO_4)_3$	1.00 mg
$MnCl_2$	1.00 mg
$ZnSO_4$	0.02 mg
KI	0.02 mg
H_3BO_3	0.02 mg
Molybdic acid	0.21 mg
Nicotinic acid	0.50 mg
Thiamin	0.10 mg
Pyridoxine	0.50 mg
L-tryptophane	0.44 mg
Dextrose	20 grams

This final concentration was obtained by mixing and diluting several 10x stock solutions. Double distilled water was used in all the media.

Since it was reported (Butcher and Street 1960) that maximum growth of excised roots of rye was obtained when yeast extract was added to the medium, 30 mg Difco yeast extract per liter was used instead of L-tryptophane to serve for comparison in the experiments with tetraploid rye. This variant was called the "yeast control".

In one of the experiments on diploid rye thirty grams

of dextrose was used rather than the usual twenty grams to test the influence of sugar content on the effects of kinetin.

3. Culture Glassware

125-ml Pyrex Erlenmeyer flasks were used as culture vessels. They were cleaned first by Fisher's Sparkleen detergent solution, rinsed in running tap water overnight and then rinsed again with distilled and double distilled water. Stoppers were made of non-absorbent cotton wrapped in cheese cloth.

4. Experimental Design

Five concentrations of kinetin (6-furfurylaminopurine), 0, 0.001, 0.01, 0.1 and 1.0 mg/l, and four concentrations of indoleacetic acid (IAA), 0, 0.001, 0.01, and 0.1 mg/l, were used in a 5 x 4 factorial, completely randomized experimental design. There were twenty treatments altogether for all the possible combinations of the two factors. In the experiments on tetraploids, basic medium plus yeast extract was used for comparison. Fifty millilitere of the medium was distributed to each flask. The medium was adjusted to pH 5.5±0.05 with 1N HCl and 1N NaOH before the various concentrations of kinetin and IAA were added. After addition of the two phytohormones, the pH was checked again, and there was either no change or the pH decreased less than 0.2 unit. No further adjustment was made. The flasks containing the media for each treatment were autoclaved at 15 lbs per square inch for 20 minutes and stored in the laboratory at room temperature for not more than a week before inoculation.

Kinetin and IAA were prepared by dissolving them in a few drops of 1N HCl and alcohol respectively; then they were diluted by double distilled water to form a concentrated stock solution.

Into each flask four 10-mm excised roottips, healthy in appearance, were inoculated. Each treatment was done in five replications, so that twenty roots would be obtained at the end of experiment provided there was no loss by contamination.

5. Inoculation and culturing of the Roots

10-mm healthy seminal roottips were cut off aseptically by a sharp scalpel. Roottips were transferred by forceps sterilised by immersing in 70% alcohol and flaming over a Bunsen burner. Before handling the roots, the heated forceps was dipped into sterile medium to cool it.

Since the liquid medium provides superior availability of nutrients to the root surface and the growth of roots is not limited by oxygen supply in it (Day 1943, Street 1957), the inoculated flasks were placed in a constant temperature room at 25±1°C in darkness without forced aeration. After 14 days of incubation, the length of the main roots, the number of the lateral roots and the total length of the laterals were measured and recorded for statistical analysis.

6. Statistical Methods

Both the separate effects of kinetin and IAA and their interaction were analysed statistically. Approximately, 17% of the cultures were lost by fungal and/or bacterial infection, hence

unequal numbers of replications were obtained at the end of the experiments. Methods of analysis of variance for unequal numbers of replications were applied to correct this (Snedecor 1957). In the analysis of separate effects, when the F test was significant, least significant difference (LSD) for unequal replication was used to compare the means (Steel and Torrie 1960).

II. For the Cytological Studies of the Mitosis of Rootting

1. Treatment of the Roots

Diploid roots of Secale cereale var. Petkus were used for all cytological studies in this research. Procedure and methods for obtaining and culturing the excised roottips were exactly the same as reported in the experiment for the studies of growth. To the basic medium, 0.001 mg/l IAA was added. Kinetin, at 0, 0.001, 0.01, 0.1, 1.0, 3.0 and 5.0 mg per liter were added to test their effects on meristematic mitosis.

Ten 10-mm excised roottips were aseptically inoculated into each flask. Then they were kept in darkness at 25±1°C in a constant temperature room. After 3 hours, 6 hours, 12 hours or 27 hours of incubation, ten roottips from each treatment were fixed immediately in Carnoy's acetic alcohol (1:3). Roots inoculated into flasks with double distilled water were fixed at the same time for comparison. In each of eighteen other flasks four roottips were incubated for 72 hours. They consisted of six treatments: 0, 0.001, 1.0, 3.0, or 5.0 mg/l of kinetin in the basic medium, and another in double distilled water alone. Twelve

roottips from three flasks of each treatment were also fixed in acetic alcohol immediately after the incubation.

2. Methods of Cytological Examinations

After fixation for more than 12 hours, four roottips were chosen at random from each treatment, and slides were made for cytological examination. The Feulgen squash method was used. Roottips were hydrolised in 1 N HCl at 60°C from 4 to 8 minutes to assure good staining of the chromosomes and separation of the cells. It was found that higher kinetin concentrations or longer treatments required longer hydrolysis for separation of cells.

After hydrolysis the roottips were put on a piece of bibulous paper for a few seconds to remove the excess HCl. The roots then were stained in Feulgen reagent for about 15 minutes. When the roottips were turning purple, one root was put on a clean slide, about 0.5 mm of rootcap, which usually showed no cells in division, was cut off, and the next 1 mm of meristematic tissue was used for the squash. A drop of acetocarmine was added and a clean coverglass was put on it. Gentle tapping by the eraser-tipped end of a pencil was applied on the coverglass to loosen the tissue. Optimum time of hydrolysis ensured easy spreading of the cells. Then the slide was pressed by the thumb under a few layers of papers. Slides were either made semipermanent by putting two drops of a glycerine acetic acid mixture (2 parts glycerine plus 8 parts 45% acetic acid) at the edges of the coverglass or made permanent by the quick freezing methods (Conger and Fairchild 1953).

3. Counting of the Mitotic Cells

All the slides were coded before examination to eliminate subjective bias. From each slide five fields observed under a 10x ocular and a 10x objective, were chosen at random and the number of interphases, metaphases, anaphases and telophases were counted and pooled together for each slide. The prophases recorded here are those cells which show long, distinctive chromosomal strings in a coiling and twisting state. In anaphases two groups of chromosome are moving to the poles, and their ends are seperated.

For each slide, the mitotic index (percentage of the dividing cells) and the proportion of each mitotic phase among the dividing cells was calculated. Data from four slides of each treatment were used for further statistical analysis.

In the meantime, the morphological changes of chromosomes and the aberrations of the mitosis were also examined. Since the frequency of these was not high, no numerical datum has been recorded.

4. Statistical Methods

The percentage of the mitotic cells and the proportions of prophase, metaphase, anaphase and telophase of the mitotic cells were transformed into their arcsines before statistical analysis. At each time interval, for each phase, analysis of variance was calculated. In case of a significant F value, Duncan's multiple range test was applied to test the differences among the means.

EXPERIMENTAL RESULTS

I. The Morphological Effects of Kinetin and IAA

Morphologically, both diploid and tetraploid roots responded similarly. In basic medium without any growth substance and in the treatment at lowest concentrations they grew normally and were white in color. As IAA was added, at 0.01 mg/l, about twenty percent of the roots were slightly swollen a few millimeters behind the apical meristems; at 0.1 mg/l, the swollen part was elongated to 6 to 8 mm and became more conspicuous. The number of swollen roots was also increased and the roots became brittle when they were handled for measurement.

Kinetin up to 0.01 mg/l showed no influence on the appearance of the roots. At 0.1 mg/l the roots became thick, strong, and straight; slightly brownish pigments might be seen along the main axis, and there were many root hairs. The lateral roots were also much thicker and stronger than those grown in lower concentrations of kinetin. At 1.0 mg/l the above phenomena were much pronounced. The pigmentation was deeper and the root hairs were numerous.

When kinetin and IAA were combined at high concentrations, the effects of both of them would show up.

II. The Effects of Kinetin and IAA on Elongation of the Roots

A. The Response of the Tetraploid Rye

To test effects of kinetin and IAA on the growth of the root of tetraploid rye, the length of main root, number of lateral roots and total length of laterals were measured (Table 1).

Analysis of variance of the separate effects, their interactions, and comparisons among means were calculated.

1. The Effects of Kinetin and IAA on the Elongation of the Main Root

Kinetin at 1.0 mg/l inhibits greatly the growth of roots and shows a significant difference from all other treatments (Table 2, Figure 1). There are no significant differences among the other treatments. It may be stated that 1 mg/l is a critical concentration of kinetin for the growth of main roots, at or above which elongation is greatly retarded.

The same measurements were made for IAA treatments (data, Table 1; statistical analysis, Table 3). The F value is highly significant, and comparison of the means shows that IAA inhibits the growth of the roots significantly at higher concentrations. These are graphed, together with standard errors, in Figure 1. It may be seen that at lower concentrations, both kinetin and IAA show a tendency to stimulate growth, but above certain levels they inhibit the growth. IAA exhibits a stronger inhibitory effect than kinetin.

2. The Effects of Kinetin and IAA on the Number of Lateral Roots

Kinetin at 1 mg/l produces complete inhibition of the initiation of lateral roots, while at 0.1 mg/l the number of laterals is markedly decreased (Figure 3). A significant difference is found between 0.1 mg/l and the control (Table 4).

IAA at lower concentrations appears to have no effect on

the initiation of lateral roots (Figure 3), they are almost equal in number to the controls. This is proved by statistical analysis which shows no significant difference among them (Table 5). At 0.1 mg/l, IAA almost completely inhibits the formation of laterals, for only 6 were found in 20 roots.

3. The Effects of Kinetin and IAA on the Elongation of Lateral Roots

The effects of kinetin and IAA are shown in Table 1 and 6, and Figure 5. At 0.1 mg/l kinetin greatly retards the elongation of lateral roots and differs significantly from all other treatments. Similarly, IAA at 0.1 mg/l severely inhibits the linear growth of laterals.

4. Interactions between Kinetin and IAA

Interactions between kinetin and IAA on the elongation of main roots the number and the elongation of laterals have been found (Tables 1, 7, 8, and 9). Although both kinetin and IAA either show a tendency to stimulation or no effect at lower concentrations and an inhibition at higher ones in the elongation of main roots (Tables 2 and 3), their individual effects are not additive when the two substances are combined together. On the contrary, the two together act antagonistically. For example, kinetin and IAA both showing a tendency to stimulation separately at concentrations of 0.01 and 0.001 mg/l respectively, when coupled together they inhibit the elongation of main roots, which attains only 72.75% of the control. This decrease in linear growth has been proved by

a significant t value of 2.62. A more striking point is that the remarkably inhibitory effect of IAA at 0.1 mg/l may be counteracted to a great degree when various concentrations of kinetin are combined with it (Figure 2). For instance, IAA alone at 0.1 mg/l restricts growth to only 27.11% of control; when kinetin is added at a concentration of 0.01 mg/l, the linear growth increases to 83.41% of control. Similar results in the number of lateral roots and the elongation of laterals are found (Figures 4 and 6).

T tests have been carried out to compare the treatment of IAA at 0.1 mg/l with the treatments of IAA (0.1 mg/l) and kinetin (0.001, 0.01, 0.1 and 1.0 mg/l) in combination in the elongation of the main root (Table 10). All combinations show significant differences from IAA alone. T values are all significant and even when the highest concentration of kinetin, 1 mg/l, is added, the difference is still significant at the 5% level.

B. The Response of Diploid Rye

Generally speaking, the main roots of diploids are longer than those of tetraploids after treatment with kinetin. The elongation of the diploid control here is equal to that of the tetraploid yeast control. Like the tetraploid, linear growth of main roots of diploids is severely inhibited by 1 mg/l of kinetin. IAA also inhibits linear growth of the main roots proportionally to concentration, with a threshold between 0.01 and 0.001 mg/l.

The effects of kinetin and IAA upon the number of lateral roots of diploids are also similar to those upon the number

of lateral roots of tetraploids, with inhibition being proportional to concentration. At the lower concentrations, however, the inhibition is not significant, and in the case of IAA there may even be a tendency to stimulation (Table 15). Similar effects are seen on the elongation of the lateral roots. In combination, the effects of kinetin and IAA upon elongation of tetraploid roots were antagonistic. When diploids were used, there was no interaction between these two phytohormones on the elongation of the main root. In regard to initiation and elongation of lateral roots, however, combinations of the two phytohormones tended to be stimulating, even at concentrations which inhibited the laterals when the phytohormones were applied separately. Details of these experiments in diploid rye and their statistical analysis are presented in Tables 11, 12, 13, 14, 15, 16, 17, 18 and 19, and in Figures 7, 8 and 9.

III. The Effects of Kinetin on the Elongation of Main Roots, Lateral Roots and Number of Lateral Roots of Diploids in Presence of 3 Percent Dextrose

Butcher and Street (1960) reported that in a medium of high sucrose content (3 percent), kinetin enhanced the linear growth of excised tomato roots and prolonged the meristematic activity. A similar experiment was conducted on the roots of diploid rye. Three percent dextrose was added to the medium instead of the usual 2%. The results of this experiment are shown in Table 20 and Figures 7, 8 and 9. These figures show that the effects obtained with increasing concentrations of kinetin on the length of main roots, the number of lateral roots, and the length of lateral

roots are similar to those obtained with roots grown in 2% dextrose. The average values, however, are generally lower in 3% dextrose than those in 2% dextrose, except that kinetin at 0.001 mg/l has a tendency to stimulation.

Analysis of variance for the effects of kinetin, sugar content and their interaction have been made on the elongation of main roots and lateral roots, and the number of lateral roots. The results are shown in Tables 21, 22, and 23. No significant interaction has been found between the two. The effect of kinetin itself is highly significant in all three cases; at 1 mg/l it retards the linear growth of the main roots, while at 0.001 mg/l, it shows a stimulation which reaches 133.66% of its control. The average sugar effect is significant in the elongation of lateral roots but not in the linear growth of main roots, nor in the number of lateral roots. However, when the minor effects are studied, significant differences appear: a) the linear growth of the main roots is higher in 2% dextrose than in 3% dextrose without kinetin i.e. two controls; b) the number of lateral roots is higher after 0.001 mg/l kinetin treatment in 3% dextrose than that in 2% dextrose; c) the length of lateral roots is higher after 0.01 mg/l kinetin treatment in 2% dextrose than in 3% dextrose. Those are proved by significant t values (2.35, 2.75 and 2.50 respectively) at the 5% level.

IV. The Effects of Kinetin on Meristematic Mitosis

1. Cytological Abnormalities

In control medium, neither chromosomal aberration nor mitotic abnormality was found. With kinetin at 0.001 mg/l for three hours, most of the prophases showed loosely associated chromatids (Figures 11 and 12), which were rarely seen in the control. Figure 13 represents a cell in which the chromosomes are well separated and showing completely untwisted chromatids. This is very similar to Figure 14 of Venkateswaran and Spiess (1963), who treated the cells of Vicia faba with sodium deoxyribonucleate. They interpreted their figure as an arrested anaphase. Since the chromatids in Figure 13 are still attached at the centromeres, and since many similar cells were found, none of which showed separated daughter chromosomes, we considered that they were late prophases or early metaphases, showing the despiralization effects of kinetin. With high concentrations of kinetin, 0.01 mg/l and 0.1 mg/l, or longer time of treatment, these effects were not so pronounced.

Metaphases under kinetin treatment gave deeply stained and widely scattered chromosomes; at 0.001 mg/l for 3 hours, the chromosomes were slim (Figure 14); while at 1 mg/l for 3 hours, they were greatly contracted (Figure 15). It seems that the spindles have been damaged for the figures resemble colchicine mitoses.

Occasionally, breakages could be found under low concentrations of kinetin (Figure 16). Dicentric bridges and fragments were frequently seen in anaphases at 1 mg/l for three hours

treatment (Figures 17, 18).

As the concentrations and time of treatment increased so did the number of telophases with bridges. Figures 19 and 20 reveal two cells treated at 5 mg/l for 27 hours, showing both dicentric and acentric bridges.

Binucleate cells were first found under kinetin treatment at 1 mg/l for 12 hours (Figure 21). The number of binucleate cells increased with the concentrations and the period of treatments. After 3 mg/l for 27 hours, more than ten were found in the five fields chosen on each slide. One of the slides treated at 5 mg/l for 27 hours gave a maximum of 16. Figure 22 shows one of them.

A few highly fragmented cells were found after 1 mg/l for 3 hours (Figure 23). They are possibly abortive anaphases. Since this kind of cell was very rare and none was found at higher concentrations, it is doubtful that kinetin has induced them.

2. The Frequency of Mitoses

a) The Effects of Kinetin on Number of Cell in Division

Mitotic index (MI) is used to test the effects of kinetin on the frequency of mitoses. The number of dividing cells and the total number of cells were counted and statistically analyzed (Table 24).

At three hours, the control gave the lowest mitotic index (1.64) while kinetin enhanced the MI at all concentrations tested, and more so at the highest ones, 5.09 for 3 mg/l, and

7.64 for 5 mg/l (Figure 10 and Table 24); even the excised roottips treated in double distilled water showed a higher number of mitoses. This is not difficult to understand since the excised roots have been put into a completely new environment, and drastic changes in their metabolic patterns are bound to happen. Instead of getting the nutrients by way of sieve tube of phloem, they have to absorb all the nutrient from the medium. Necessary metabolites and growth factors from other parts of the seedling may be cut off. A lag period in mitotic activities for adaptation seems to be a natural phenomenon. However, this lag period can be broken by treating the root with kinetin. In the case of the roots treated in double distilled water, the observed effect might be due to the fact that they do not have to adjust to a new medium and would thus continue to divide at the expense of their nutrient reserves.

After 6 hours' growth, kinetin at 5 mg/l still gives the highest mitotic index (7.71). The mitotic index of the control (3.45) increases to be the second highest one observed. This indicates that the roots have passed the lag period of cell division after six hours' growth in the medium. Besides the treatment under 5 mg/l of kinetin, roots in pure water, the control, and lowest concentration of kinetin show comparatively high numbers of mitotic cells to the others. However, there is not much difference among the intermediate concentrations.

For the 12 hour incubation, the control, kinetin at 5 mg/l and 0.001 mg/l give the three highest mitotic indexes. At intermediate concentrations, rather low indexes are obtained.

Mitoses in cells treated with double distilled water fell to only 0.49 percent, perhaps because of lack of nutrients (Duncan's test for 12 hour treatment in Table 24).

As the time of the incubation increases, the proportions of mitotic cells gradually increase in the controls and in low concentrations of kinetin; while at high concentrations, the dividing cells decrease. After 27 hours the two extremes meet at more or less the same level, hence no significant difference can be found (Figure 10). As the time is further prolonged, the mitotic figures in kinetin treatments above the concentration of 1 mg/l decrease continuously, then finally stop; and at the control and low concentrations the mitotic activities reach their maxima and then continue their normal growth thereafter. This may be seen from the results after 72 hours' incubation. No mitoses have been found after treatment above 1 mg/l: the mitotic activity stops completely. The control reaches its maximum (5.62), as does kinetin at 0.001 mg/l (4.51). Nevertheless, there is no significant difference between these two.

b) <u>The Effects of Kinetin on the Relative Frequencies of Mitotic Phases</u>

After 3 hours' incubation, the proportion of metaphase was increased significantly over that of the control. Variations in the proportions of prophase were found after 6 hours' incubation; however, only the treatment of 3 mg/l showed slight difference from the control (Table 25).

DISCUSSION AND CONCLUSIONS

I. The Morphology of the Roots

The morphology of the roots grown *in vitro* varies considerably. Usually the roots look normal and healthy, but thick, brown roots as well as tumor like swellings proximal to the root tips were observed. Similar main roots have been reported by Street (1954) for tomato treated with IAA, or grown in a medium of high sugar content and termed as an "aging" phenomenon. In our experiments, we found that the brownish pigmentation was much more pronounced in kinetin treatment than in IAA. Bamberger and Meyer (1960) reported that kinetin induced formation of pink pigment on the cotyledons of seedlings of *Amaranthus retroflexus* in the dark and found that the pigment was not an anthocyanin. It is not possible to say whether our result is an "aging" phenomenon induced by kinetin, since no further examination has been made. However, this result is noteworthy.

The morphology of the observed tumor like swellings at the proximal region of the roottips resembled closely the C-tumors in onion roots induced by colchicine, indolylbutyric acid, naphthalene acetic acid, phenyl propionic acid (Levan 1938, 1939), and IAA (McManus 1960). In this respect, the roots of rye seem more sensitive to IAA than do onion roots. Tumors in rye roots were increasingly frequent in treatment with IAA concentrations from 0.001 mg/l upwards, whereas McManus found them only after treatment

with 1 mg/l IAA. This variation, however, may also be attributed to the differences in age, source or culture technique of the tissue being tested.

II. The Elongation of the Roots

It is presently generally accepted that the growth of roots is controlled by a specific balance of a number of growth factors or substances. A change in any one of these factors will disturb this balance and will have a definite effect on root growth. These ideas have gained further support in our experiments on root elongation, using varying concentrations of IAA and kinetin in the media.

Since excised roots of rye have apparently a low content of endogenous auxin, externally supplied auxin will enhance linear growth. The same result is found when using tryptophane, a precursor of IAA (Thimann 1935), at 400x the optimal molarity for IAA in the medium. No direct evidence has been found that the presence of IAA in a tryptophane supplemented medium is responsible for the observed stimulatory effects (Roberts and Street 1955). In this experiment, the basic medium used contains tryptophane at 0.44 mg/l, 400x the optimal concentration for IAA. Since autoclaving of media containing tryptophane may induce auxin activity (Kulescha and Gautheret 1949), the possibility exists that in our experiment autoclaving may have converted some tryptophane into IAA or its precursors. As a result, when exogeneous IAA was added to the medium, the level of IAA might have become too high so that the linear growth of main roots was inhibited. At the lowest concentration of IAA (0.001 mg/l), there is no significant difference from the control, at which a stimulating effect has been found by

Roberts and Street (1955).

Inhibitory effects of kinetin on the linear growth of roots have been reported in tomato, Brassica campestris, Allium cepa, and Allium sativum (cf. Miller 1961, Kittwer and Dedolph 1963). At low concentration, kinetin may enhance the linear growth of roots of Lactuca sativa (Dandkwardt-Lilliestrom 1957). We found that kinetin greatly retards the growth of main roots of rye at the concentration of 1 mg/l. Slightly stimulating effects are suggested at intermediate concentrations.

Skoog and Miller (1957) indicated a quantitative interaction between kinetin and IAA in the regulation of growth and differentiation of tobacco stem callus. They stated, "Interaction between IAA and kinetin and between these and other factors appear to exert decisive influences in each case. Both types of chemical seem to be required for growth. Low levels of one with high levels of the other and vice versa lead to opposite morphological end-results". A balance among growth factors (auxin, gibberellic acid, and kinetin) as a mechanism controlling the growth of roots has been postulated (Dawson and Street 1959, Butcher and Street 1960, and Street and Winter 1963). In the experiments to test the effects of growth substance on plant growth, their endogenous content also plays a very important role (Torrey 1962).

It is found in the present experiments that when kinetin and IAA are applied at the same concentration, or when a low level of IAA is combined with a higher level of kinetin, inhibition occurs,

although each alone is either stimulating or no effect. This suggests that the ratio of these two substances does affect the elongation of the root.

The multiple hormonal system controlling the growth of roots postulated by Butcher and Street (1960) is inferred from the "antiauxin" effect of kinetin and the different effects of kinetin in media of various sugar contents. Our experiments indicated that kinetin is able to counteract the inhibition caused by high concentration of IAA (Figures 2 and 6) and kinetin in the media of different sugar content reacts differently at 0.001 mg/l and 0.01 mg/l in the initiation and linear growth of lateral roots respectively. These findings are similar to that of Butcher and Street (1960).

It is possible that these effects of the phytohormones reported above and hereafter may not be due only to their direct action on the cells but also due to the physical-chemical changes, like pH, etc., in the culture medium induced by these compounds. Such changes, however, were not studied.

III. The Initiation of Lateral Roots

In studies on lateral roots, Roberts and Street (1955) found that IAA at concentration from 0.01 mg/l to 0.001 mg/l increased the initiation of the lateral roots of rye. In this study IAA at 0.1 mg/l almost completely inhibited the initiation of lateral roots, and there was no effect at lower concentrations. The disagreement here may be only apparent, for the tryptophane supplement in our medium may have resulted in increased level of IAA or its precursors after autoclaving, as discussed above.

Kinetin also inhibits the initiation of lateral roots at almost all the concentrations tried. However, low concentration of kinetin (0.001 mg/l) can counteract the inhibition caused by high concentration of IAA (0.01 mg/l). This result is similar to that of Torry (1962), who reported that the effectiveness of IAA at fairly high concentration (5×10^{-5}M, approximately 8.7 mg/l) could be further promoted by a low concentration of kinetin. The fact that a high IAA/kinetin ratio favours the initiation of lateral roots of rye supports Skoog and Miller's (1957) suggestion that a specific balance in the auxin-kinin system is involved in the initiation of lateral roots.

IV. Comparison between Diploid and Tetraploid Roots of Rye in Response to Kinetin and IAA Treatment

Hereditary factors controlling growth of excised roots were first studied by Skinner (1952, 1953), who worked on four geographic strains of groundsel and concluded that the growth rates of the excised roots were determined by a small number of major genes subjected to the action of a large number of modifiers. Schaeffer and Smith (1963) reported that auxin and kinetin had only a little effect on the growth of the tissue from the tumorous Nicotiana glauca-langsdorffii hybrid, but a high level of these two substances was needed for rapid growth of the non-tumorous mutant of the hybrid.

It is quite understandable that the genotype is basic to the response of the root to the various treatments with growth substances. Similarly, an autopolyploid may show a different response to the

same treatment than its diploid component. An autopolyploid plant usually has greater vegetative growth, larger cellular and nuclear volumes, larger size of leaves and flowers, etc., than its diploid (cf. Darnham 1962). Since the genotype was adapted to the diploid condition, the increased quantity of genes by chromosome doubling may result in loss of efficiency in the organism, as seen in physiological reactions.

Nevertheless, the results of the present experiments show that the elongation and initiation of diploid and tetraploid roots of rye generally follow a similar pattern. However, some differences were found, and these can be summarized as follows:

1. Linear growth of main root of diploids is generally greater than that of the tetraploids after the treatment of kinetin, while the situation is reversed in elongation of laterals.

2. The tetraploid roots are less sensitive to the inhibitory effects of both kinetin and IAA.

3. The antigonistic effect between high concentration of IAA and kinetin is more pronounced in tetraploids than that in diploids.

It seems that kinetin and IAA have a less inhibitory effect on the tetraploid than on the diploid roots. Assuming that endogenous growth substances play their roles in controlling growth of roots in vitro, the different behaviour of diploid and tetraploid roots to auxin and kinetin treatments may be due to their different endogenous contents of the two substances. On

the other hand, the possible inefficiency due to a somewhat unbalanced genotype of the autotetraploid may have induced a different degree of reactivity to similar concentrations.

V. Cytological Effects of Kinetin on Mitosis

It is a well known fact that kinetin stimulates cell divisions in various tissues (Das et al. 1956, Guttman 1956, Haber and Luippold 1960, Walker and Pietrich 1962). Some evidence for this was obtained in our experiments. High concentrations of kinetin showed an initial high mitotic index (MI). Low concentrations of kinetin induced a higher MI than the control only during the first 3 hours. At 6 hours and later the MI was found to be equal to or lower than the control at 0.001 mg/l and 1 mg/l of kinetin treatment respectively. As can be seen from Figure 10, the MI curve of the control starts with a low MI, but then gradually reaches much higher values. Evidence has been obtained (Das et al. 1956, Fatau et al. 1957) that both auxin and kinin are required for DNA synthesis, mitosis and cytokinesis. The fact that in our material the control started with such a low MI may be due to the drastic change of environment caused by excising and culturing the roottips. It may have been that sufficient kinetin or kinin were not readily available for these roots, so that either exogenous kinetin or a period of adaptation for the synthesis of kinetin was required for the initiation of normal mitosis.

The change in relative duration of the mitotic stages induced by kinetin was explained by Guttman (1956) as being the

result of a change in the rate of spiralization and despiralization of the chromosomes. The clear double-stranded chromosomes in Figures 11 to 14 (0.001 mg/l of kinetin treatment for three hours) may be an indication of this effect. However, there is no change in relative duration of prophase nor telophase, on which Guttman's inference was based. After 3 hours' treatment, the proportions of metaphase of our experimental treatments have significantly increased over that of the control. Since there is no change in the relative frequency of the mitotic stages beyond 6 hours, kinetin cannot possess a persistent effect on the duration of mitotic phases.

In addition to the effects of kinetin discussed above, a diversity of structural changes in chromosomes has been found at or above 1 mg/l kinetin treatment. These include excessive contraction of chromosomes at metaphase, chromosomal bridges in anaphase and telophase, breakages, and binucleate cells. These chromosomal aberrations are also frequently found in other plant tissues grown in a medium with complex growth substances like yeast extract, coconut milk or some other chemical substances (Straus 1954, Torrey 1959, Mitra et al, 1960, Venkateswaran and Spiess 1963). The excessively shortened and widely scattered chromosomes in metaphases after kinetin treatment are reminiscent of the effects of colchicine (Levan 1938).

The occurrence of bridges and fragments as a result of kinetin treatment have not been reported before. It is well known that changes in chromosomal structure can be easily induced by ionizing

radiations (cf. Lea 1946) or by various radiomimetic substances (Wilson 1960). Reunion of chromosomal fragments may give rise to new chromosomal complements (Davidson 1959), which can survive several generations under certain cultural conditions (Vanketeswaran 1963). From such a culture a new strain of aneuploid cells has been established. The ability of kinetin to induce chromosomal aberrations, as we observed in our experiments, may have given rise to various aneuploids in certain tissue cultures (Torrey 1959).

At 5 mg/l, kinetin induces many bridges at telephase. The nuclei in these cells are pycnotic (Figure 19 and 20). These bridges are probably the result of stickness rather than of structural rearrangements of the chromosomes (inversion, translocation). Levan (1951) considered them as an indication of toxic and lethal concentration of chemicals on chromosomes. Therefore the nuclei at this high concentration of kinetin gradually degenerated until finally the mitotic activity stopped after 72 hours of treatment. This also might be one of the main reasons why kinetin in high concentrations inhibits the growth of rye roots.

Binucleate and polyploid cells are frequently encountered in plant tissue cultures (cf. Nartanen 1963) and in the root meristem under kinetin treatment (Guttman 1956). In this experiment, only binucleate cells were found, no polyploid cells were recorded. Binucleate cells were first observed after treating the root for 12 hours with 1 mg/l of kinetin, and their number increased with the concentrations and duration of treatment.

Binucleate cells were never seen in division. It is probable that during the completion of mitosis of the original cells, the high kinetin concentration prevented cell wall formation.

In conclusion, it is considered that our results have given further support to the following findings of the earlier workers:

a) The growth (elongation and cell division in our case) of excised roots *in vitro* is not controlled by a single auxin but by a balance among a number of phytohormones, as postulated by Street and Winter (1963).

b) Kinetin inhibits the elongation of roots at relatively high concentration (Butcher and Street 1960, cf. Miller 1961, and Wittwer and Dedolph 1963).

c) The "antiauxin" effect of kinetin in the linear growth of roots (Butcher and Street 1960) is confirmed.

d) Kinetin stimulates cell division (Das et al. 1956, Guttman 1956, Haber and Luippold 1960, Halder and Dietrich 1962).

e) Kinetin affects the spiralisation of the chromosomes, and induces the formation of pycnotic nuclei and binucleate cells (Guttman 1956).

The new findings of the author are:

a) The metaphases after kinetin and colchicine treatment appear to be similar, suggesting an effect of kinetin on the formation of the spindle.

b) At high concentration (at or above 1 mg/l), kinetin induces chromosomal fragments and bridges in mitosis.

c) The tetraploid rye roots are less sensitive to the inhibitory effects of both kinetin and IAA than its diploids. The antagonistic effect between high concentration of IAA and kinetin is more pronounced in tetraploids than in diploids.

Table 1. The effects of different combinations of kinetin and IAA on length of main root, number of lateral roots and total length of lateral roots of tetraploid rye.

Kinetin mg/l	IAA mg/l	Number of Roots Examined	Length of Main Root (mm) Mean±S.E.	Length of Main Root (mm) Percent of Control	Number of Lateral Roots Mean±S.E.	Number of Lateral Roots Percent of Control	Total Length of Laterals (mm) Mean±S.E.	Total Length of Laterals (mm) Percent of Control
0 (Yeast Control)	0	16	110.31±3.82	118.45	19.12±2.23	124.89	333.75±45.20	169.52
0.		16	93.13±5.58	100.00	15.31±1.91	100.00	196.88±29.24	100.00
0.001		16	89.93±5.51	96.56	17.56±1.73	114.70	230.87±24.85	117.26
0.01	0	12	105.08±2.17	112.83	14.92±2.57	97.45	249.75±52.32	126.85
0.1		16	90.31±5.27	96.78	4.68±0.46	30.57	41.56± 8.37	21.11
1.0		12	58.42±8.47	62.73	0.00±	0.00	0.00±	0.00
0.		12	106.92±5.10	114.81	15.33±2.29	100.13	317.50±58.47	161.27
0.001		20	105.31±3.45	113.07	13.85±1.95	90.46	171.65±25.22	87.21
0.01	0.001	8	67.75±7.97	72.75	7.86±1.94	51.47	105.75±35.37	53.71
0.1		20	74.30±5.57	79.78	4.35±0.62	28.41	47.70± 9.03	24.23
1.0		16	61.00±5.92	65.50	0.00	0.00	-	-
0.		12	75.50±10.28	81.07	15.08±4.37	98.50	218.83±64.32	111.15
0.001		12	73.25± 7.75	78.65	13.50±3.13	88.18	157.33±47.44	79.91
0.01	0.01	16	52.25± 9.30	56.10	6.38±2.49	41.67	92.19±40.42	46.83
0.1		16	41.50± 6.45	44.56	1.82±0.65	11.89	13.81± 7.01	6.00
1.0		20	24.95± 3.25	26.79	0.00	0.00	-	-
0.		20	25.25± 1.60	27.11	0.30	<0.001	-	-
0.001		12	56.33± 8.41	60.49	6.83±1.94	44.61	84.41±39.50	42.87
0.01	0.1	16	77.68± 4.09	83.41	11.31±2.11	73.87	109.94±22.70	55.84
0.1		16	61.63± 8.67	66.16	4.43±1.22	28.94	38.87±11.44	19.74
1.0		20	36.90± 4.40	39.62	0.00	0.00	-	-

Table 2

Analysis of Variance of Kinetin Effects on the Elongation of Main Roots of Tetraploids

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F
Kinetin	5	21,098.24	4,219.65	4.89**
Among Flasks	16	13,811.21	863.45	
Within Flask	66	24,890.50	377.13	
Total	87	805,000.00		

** Significant at 0.01 level F 1% = 4.14

LSD Test (5% level)

Treatment*					
1.0 mg/l	0.1 mg/l	0.001 mg/l	Control	0.01 mg/l	Yeast Control
58.42	89.93	90.13	93.13	105.08	110.31

* Significant difference exists between treatments lying on different lines

Table 3

Analysis of Variance of IAA Effects on the Elongation of Main Roots of Tetraploids

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F
IAA	4	89,985.99	22,496.50	39.00**
Among Flasks	14	8,074.80	576.77	
Within Flask	57	20,843.21	365.67	
Total	75	118,904.04		

** Significant at .01 level F 1% = 5.02

LSD Test (5% level)

Treatment				
0.1 mg/l	0.01 mg/l	Control	0.001 mg/l	Yeast Control
22.33	75.50	93.13	106.92	110.31

Table 4

Analysis of Variance of Kinetin Effects on the Number of Lateral Roots of Tetraploids

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F
Kinetin	4	2,042.15	510.54	3.23*
Among Flasks	14	2,207.98	157.71	
Within Flasks	57	1,463.50	25.68	
Total	75	5,713.63		

*Significant at 5% level F 5% = 3.11

LSD Test (5% level)

Treatment				
0.1 mg/l	0.01 mg/l	Control	0.001 mg/l	Yeast Control
4.68	14.92	15.31	17.56	19.12

Table 5

Analysis of Variance of IAA Effects on the Number of Lateral Roots of Tetraploids

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F
IAA	4	5,036.98	1,259.25	9.39**
Among Flasks	14	1,818.47	129.89	
Within Flasks	65	3,480.50	53.55	
Total	83			

** Significant at 1% level F 1% = 5.03

LSD Test (5% level)

Treatment				
0.1 mg/l	0.01 mg/l	Control	0.001 mg/l	Yeast Control
0.3	15.08	15.31	15.33	19.21

Table 6

Analysis of Variance of Kinetin Effects on the Elongation of Laterals of Tetraploids

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F
Kinetin	4	727,482.31	181,870.58	4.62*
Among Flasks	14	550,813.69	39,343.84	
Within Flask	57	669,575.00	11,746.93	
Total	75	1,947,871.00		

*Significant at 5% level F 5% = 3.11

LSD Test (5% level)

Treatment				
0.1 mg/l	Control	0.001 mg/l	0.01 mg/l	Yeast Control
41.56	196.88	230.87	249.75	333.75

Table 7

Analysis of Variance of Kinetin x IAA Interaction on the Elongation of Main Roots of Tetraploids

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F
Kinetin	4	24,858.87	6,214.72	
IAA	3	84,546.74	28,182.25	
Kinetin x IAA	12	90,832.96	7,569.41	16.25**
Error	57	26,566.78	466.08	
Total	76	226,805.35		

** Significant at 1% level F 1% = 2.53

Table 8

Analysis of Variance of Kinetin x IAA Interaction on the Number of Lateral Roots of Tetraploids

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F
Kinetin	3	3,110.62	1,036.87	
IAA	3	1,825.20	608.40	
Kinetin x IAA	9	2,590.93	287.88	3.24**
Error	44	3,907.77	88.81	
Total	59	11,434.52		

** Significant at 1% level F 1% = 2.84

Table 9

Analysis of Variance of Kinetin x IAA Interaction on the Elongation of Lateral Roots of Tetraploids

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F
Kinetin	3	829,188.59	276,396.20	
IAA	3	552,873.77	184,291.26	
Kinetin x IAA	9	743,328.76	82,592.08	4.55**
Error	44	799,115.74	18,161.72	
Total	59	2,924,506.86		

** Significant at 1% level F 1% = 2.84

Table 10

T Tests for the Interaction between Kinetin and IAA on the Elongation of Main Roots of Tetraploids

Treatment	Mean ± S.E.	t value
IAA 0.1 mg/l + no kinetin	25.25 ± 1.60	
IAA 0.1 mg/l + 0.001 mg/l kinetin	56.33 ± 8.41	4.60**
IAA 0.1 mg/l + 0.01 mg/l kinetin	77.68 ± 4.09	9.13**
IAA 0.1 mg/l + 0.1 mg/l kinetin	61.63 ± 8.67	3.25**
IAA 0.1 mg/l + 1.0 mg/l kinetin	36.90 ± 4.40	2.45*

** Significant at 1% level
* Significant at 5% level

Table 11. The effects of different combinations of kinetin and IAA on length of main root, number of lateral roots and total length of lateral roots of diploid rye.

Kinetin mg/l	IAA mg/l	Number of Roots Examined	Length of Main Axis (cm)		Number of Lateral Roots		Total Length of Laterals (cm)	
			Mean±S.E.	Percent of Control	Mean±S.E.	Percent of Control	Mean±S.E.	Percent of Control
0.		20	110.50±6.19	100.00	13.44±3.51	100.00	191.35±42.69	100.00
0.001		20	93.35±9.08	84.48	7.70±2.29	57.46	131.55±40.90	68.75
0.01	0	12	108.00±7.30	97.74	7.75±1.93	57.84	237.33±67.47	124.03
0.1		20	115.75±8.07	104.75	2.35±0.88	17.54	29.90±15.41	15.63
1.0		12	54.42±6.7%	49.25	-	-	-	-
0.		20	97.15±3.81	87.92	16.15±2.37	120.52	247.21±50.15	129.19
0.001		20	99.25±7.89	89.92	6.55±1.85	48.86	141.14±51.62	73.26
0.01	0.001	16	74.44±9.71	67.37	5.50±1.48	41.04	133.88±47.59	69.97
0.1		16	99.56±9.55	90.10	1.25±0.40	9.33	13.13± 3.75	6.86
1.0		20	52.70±6.02	47.69	-	-	-	-
0.		20	65.35±8.32	59.14	11.60±2.66	86.57	183.85±44.23	96.08
0.001		20	84.93±8.06	76.86	15.06±2.71	112.39	240.44±45.69	125.65
0.01	0.01	16	55.06±8.46	49.83	5.25±0.97	39.18	68.12±16.11	35.60
0.1		16	67.44±10.20	61.03	0.31±0.25	2.31	3.06± 2.35	1.60
1.0		16	29.81± 4.30	26.98	-	-	-	-
0.		12	18.00±2.42	16.29	1.67±0.60	12.46	6.33± 2.72	3.31
0.001		20	16.85±2.17	15.25	2.00±0.57	14.93	5.75± 2.64	3.00
0.01	0.1	20	20.15±2.52	18.24	0.70±0.39	5.22	3.40± 2.16	1.78
0.1		16	14.44±1.88	13.07	0.13± .12	0.97	0.56± 0.56	0.29
1.0		16	13.88±0.95	12.56	-	-	-	-

Table 12

Analysis of Variance of Kinetin Effects on the Elongation of Main Root of Diploids

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F
Kinetin	4	33,711.26	8,427.82	4.59*
Among Flasks	16	29,398.05	1,837.38	
Within Flask	63	65,170.35	1,034.44	
Total	83			

*Significant at 5% level F 5% = 3.01

LSD Test (5% level)

Treatment				
1.0 mg/l	0.001 mg/l	0.01 mg/l	Control	0.1 mg/l
54.42	92.35	108.00	110.50	115.75

Table 13

Analysis of Variance of IAA Effects on the Elongation of Main Roots

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F
IAA	3	72,110.54	24,036.79	24.47**
Among Flasks	14	13,750.58	982.19	
Within Flask	54	48,331.52	895.03	
Total	71	134,192.44		

** Significant at 1% level F 1% = 5.56

LSD Test (5% level)

Treatment			
0.1 mg/l	0.01 mg/l	0.001 mg/l	Control
18.00	63.35	97.15	110.99

Table 14

Analysis of Variance of Kinetin Effects on the Number of Laterals of Diploids

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F
Kinetin	3	1,221.48	407.16	4.95**
Among Flasks	14	933.30	66.66	
Within Flask	54	4,558.50	84.42	
Pooled Error	68	5,491.80	80.76	
Total	71	6,713.28		

** Significant at 1% level F 1% = 4.10

LSD Test (5% level)

Treatment				
1.0 mg/l	0.01 mg/l	0.001 mg/l	0.01 mg/l	Control
0.00	2.35	2.65	7.25	13.40

Table 15

Analysis of Variance of IAA Effects on the Number of Lateral Roots of Diploids

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F
IAA	3	1,662.05	554.02	4.97**
Among Flasks	14	1,303.57	93.11	
Within Flask	54	6,267.25	116.06	
Pooled Error	68	7,570.82	111.34	
Total	71			

** Significant at 1% level F 1% = 4.10

LSD Test (5% level)

Treatment			
0.1 mg/l	0.01 mg/l	Control	0.001 mg/l
1.67	11.60	13.40	16.15

Table 16

Analysis of Variance of Kinetin Effects on the Elongation of Lateral Roots of Diploids

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F
Kinetin	3	410,228.44	136,742.81	4.57**
Among Flasks	14	272,963.67	19,497.41	
Within Flask	54	1,760,090.50	32,594.27	
Pooled Error	68	2,033,054.17	29,897.86	
Total	71	2,443,282.61		

** Significant at 1% level F 1% = 4.10

LSD Test (5% level)

Treatment			
0.1 mg/l	0.001 mg/l	Control	0.01 mg/l
29.90	131.55	191.35	237.33

Table 17

Analysis of Variance of IAA Effects on the Elongation of Lateral Roots of Diploids

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F
IAA	3	452,003.83	150,667.94	4.29**
Among Flasks	14	469,399.17	33,528.51	
Within Flask	54	1,917,919.00	35,517.02	
Pooled Error	68	2,387,318.17	35,107.62	
Total	71	2,839,322.00		

** Significant at 1% level F 1% = 4.10

LSD Test (5% level)

Treatment			
0.1 mg/l	0.01 mg/l	Control	0.001 mg/l
6.33	183.85	191.35	267.21

Table 18

Analysis of Variance of Kinetin x IAA Interaction on the Number of Lateral Roots of Diploids

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F
Kinetin	3	755.57	251.86	
IAA	3	2,117.07	705.69	
Kinetin x IAA	9	1,272.79	141.42	2.95**
Error	54	2,587.78	47.92	
Total	69	6,733.93		

** Significant at 1% level F 1% = 2.78

Table 19

Analysis of Variance of Kinetin x IAA Interaction on the on the Elongation of Lateral Roots of Diploids

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F
Kinetin	3	774,433.99	258,144.66	
IAA	3	880,377.24	293,445.80	
Kinetin x IAA	9	638,437.24	70,937.47	4.85**
Error	54	790,018.69	14,629.98	
Total	69	3,083,267.39		

** Significant at 1% level F 1% = 2.78

Table 20. The effects of kinetin on the growth of main, root, number of laterals, and total length of lateral roots of diploid rye in presence of 3 percent dextrose.

Kinetin mg/l	Number of Roots Examined	Length of Main Axis (mm)		Number of Laterals		Total Length of Laterals (mm)	
		Mean ± S.E.	Percent of Control	Mean ± S.E.	Percent of Control	Mean ± S.E.	Percent of Control
0.	20	82.55±8.72	100.00	13.70±1.93	100.00	132.85±29.60	100.00
0.001	20	110.50±8.30	133.86	15.50±2.89	113.14	216.25±49.68	162.78
0.01	12	89.58±10.00	108.52	6.83±2.34	49.85	80.17±25.17	60.35
0.1	20	95.50±12.26	115.69	2.00±0.06	14.60	14.00± 4.57	0.11
1.0	12	49.25± 5.20	59.66	0.00	-	0.00	-

Table 21

Analysis of Variance of the Effects of Kinetin and Sugar content on the Elongation of Main Roots of Diploids.

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F
Sugar	1	5,060.02	5,060.02	3.57
Kinetin	4	50,980.08	12,745.02	9.00**
Sugar x Kinetin	4	12,361.58	3,090.40	2.18
Error	158	223,790.22	1,416.39	
Total	167	292,191.90		

** Significant at 1% level

LSD Test for Kinetin Effect in 3% Dextrose (5% level)

Treatment				
1.0 mg/l	Control	0.01 mg/l	0.1 mg/l	0.001 mg/l
49.25	82.55	89.58	95.50	110.50

Table 22

Analysis of Variance of the Effects of Kinetin and Sugar content on the Number of Lateral Roots of Diploids.

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F
Sugar	1	144.00	144.00	1.78
Kinetin	3	3,026.33	1,008.77	12.48**
Sugar x Kinetin	3	471.56	157.19	1.95
Error	136	10,900.67	80.81	
Total	143			

** Significant at 1% level F 1% = 3.94

LSD Test for Kinetin Effect in 3% Dextrose (5% level)

Treatment			
0.1 mg/l	0.01 mg/l	Control	0.001 mg/l
2.00	6.83	13.70	15.50

Table 23

Analysis of Variance of the Effects of Kinetin and Sugar Content on the Length of Lateral Roots of Diploids

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F
Sugar	1	161,806.92	161,806.92	6.83*
Kinetin	3	586,400.78	195,466.93	8.25**
Sugar x Kinetin	3	111,701.47	37,233.82	1.57
Error	136	3,220,838.24	23,682.63	
Total	143	4,080,747.31		

* Significant at 5% level F 5% = 3.92 1% = 6.84
** " " 1% " F 1% = 3.94

LSD Test for Kinetin Effect in 3% Dextrose (5% level)

Treatment			
0.1 mg/l	0.01 mg/l	Control	0.001 mg/l
14.00	80.17	132.85	216.25

Table 24. The effects of kinetin on number of cells in division.

Treatment			Number of Cells			
Substance	Concentration mg/l	Hours	Division	Total	Mitotic Index % ± S.E.	F^1
H_2O			161	5166	3.12±0.10	
Kinetin	0.		60	3606	1.64±0.11	
"	0.001		98	3563	2.81±0.40	
"	0.01	3	102	3964	2.61±0.21	20.56**
"	0.1		121	4648	2.59±0.15	
"	1.0		166	4754	3.48±0.03	
"	3.0		243	4682	5.09±0.37	
"	5.0		336	4673	7.64±1.01	
H_2O			157	4676	3.47±0.66	
Kinetin	0.		152	4270	3.48±0.26	
"	0.001		129	4261	3.13±0.37	
"	0.01	6	71	3479	2.06±0.34	14.35**
"	0.1		134	6177	2.22±0.43	
"	1.0		95	5078	1.91±0.34	
"	3.0		109	5363	2.02±0.36	
"	5.0		284	3760	7.71±0.78	
H_2O			24	4040	0.49±0.22	
Kinetin	0.		197	4054	4.73±0.61	
"	0.001		155	3926	4.02±0.43	
"	0.01	12	104	4520	2.32±0.17	21.96**
"	0.1		140	4207	3.31±0.37	
"	1.0		69	3465	2.02±0.17	
"	3.0		80	3428	2.44±0.31	
"	5.0		276	5204	5.26±0.64	
H_2O					0.01	
Kinetin	0.		161	3915	4.83±0.41	
"	0.001		124	3853	3.28±0.55	
"	0.01	27	154	4565	3.36±0.35	1.32
"	0.1		130	3938	3.32±0.10	
"	1.0		156	3467	4.60±0.46	
"	3.0		161	5163	3.05±0.74	
"	5.0		198	4842	4.20±0.72	
Kinetin	0.		172	3088	5.62±0.51	
"	0.001	72	133	2943	4.51±0.52	
"	1.0		79	3151	2.26±0.80	
"	3.0[2]		0	-	-	6.11**
"	5.0[2]		0	-	-	

Table 24 cont'd.

Table 24 cont'd.

1. Calculated from arcsine transformed data.
2. No mitoses has been found so there is no total cell count.

** Significant at 0.01 level.

Duncan's Test. (5% level)

Hours	Treatments*							
	K_0	K_3	K_2	K_1	H_2O	K_4	K_5	K_6
3	1.64	2.59	2.61	2.81	3.12	3.48	5.09	7.64
	K_4	K_5	K_2	K_3	K_1	H_2O	K_0	K_6
6	1.91	2.02	2.06	2.22	3.13	3.47	3.48	7.71
	H_2O	K_4	K_2	K_5	K_3	K_1	K_0	K_6
12	0.49	2.02	2.32	2.44	3.31	4.02	4.73	5.26
		K_5	K_1	K_3	K_2	K_6	K_4	K_0
27		3.05	3.28	3.32	3.36	4.20	4.60	4.83
						K_4	K_1	K_0
72						2.26	4.21	5.62

* 1. H_2O: distilled water, K_0: control, K_1: 0.001 mg/l, K_2: 0.01 mg/l, K_3: 0.1 mg/l, K_4: 1 mg/l, K_5: 3 mg/l, K_6: 5 mg/l.

2. Significant difference is found between means lying on different lines.

Table 25. The effects of kinetin on relative frequencies of mitotic stages.

Treatment			Proportion of Division			
Substance	conc. mg/l	Hours	Prophase	Metaphase	Anaphase	Telophase
H_2O			68.40	13.05	3.22	15.20
Kinetin	0.		86.63	5.50	4.30	3.57
"	0.001		68.07	21.87	2.15	10.91
"	0.01	3	77.86	15.55	0.76	5.84
"	0.1		67.05	20.85	6.53	5.57
"	1.0		72.09	18.97	2.00	6.94
"	3.0		70.31	22.53	2.03	5.13
"	5.0		74.56	18.55	0.50	6.39
H_2O			74.66	10.92	4.80	9.62
Kinetin	0.		71.56	11.96	2.71	9.17
"	0.001		82.66	10.86	1.46	5.09
"	0.01	6	59.79	19.79	8.98	11.57
"	0.1		57.36	22.60	5.50	14.54
"	1.0		67.09	10.43	3.04	19.44
"	3.0		53.21	16.41	1.98	17.39
"	5.0		80.49	8.71	0.58	10.22
H_2O			56.55	23.22	14.58	3.15
Kinetin	0.		53.67	16.56	6.37	20.37
"	0.001		68.05	16.54	1.26	14.15
"	0.01	12	74.82	9.80	4.80	10.58
"	0.1		63.99	15.71	3.59	16.71
"	1.0		63.95	10.22	3.12	22.71
"	3.0		52.58	15.74	2.08	29.70
"	5.0		66.98	18.97	0.57	13.48
Kinetin	0.		50.24	13.17	7.53	28.66
"	0.001		54.46	13.19	5.77	26.59
"	0.01	27	59.99	11.39	2.71	25.92
"	0.1		50.32	20.82	7.47	21.39
"	1.0		63.23	14.12	4.89	17.76
"	3.0		57.47	16.39	3.66	22.48
"	5.0		52.94	21.88	0.76	24.42
Kinetin	0.		46.79	20.32	5.49	27.40
"	0.001	72	36.57	27.95	3.96	31.52
"	1.0		54.36	17.07	2.71	25.86
"	3.0		-	-	-	-
"	5.0		-	-	-	-

Table 25 cont'd

Table 29 cont'd.

Duncan's Test. (5% level)

Hours	Mitotic Phase	Treatments*							
		K_0	H_2O	K_2	K_6	K_4	K_3	K_1	K_5
3	Meta-phase	5.50	13.05	15.55	18.55	18.97	20.85	21.87	22.53
		K_5	K_3	K_2	K_4	K_0	H_2O	K_6	K_1
6	Pro-phase	53.21	57.36	59.79	67.09	71.56	74.66	80.49	82.66

* See footnote of Table 24.

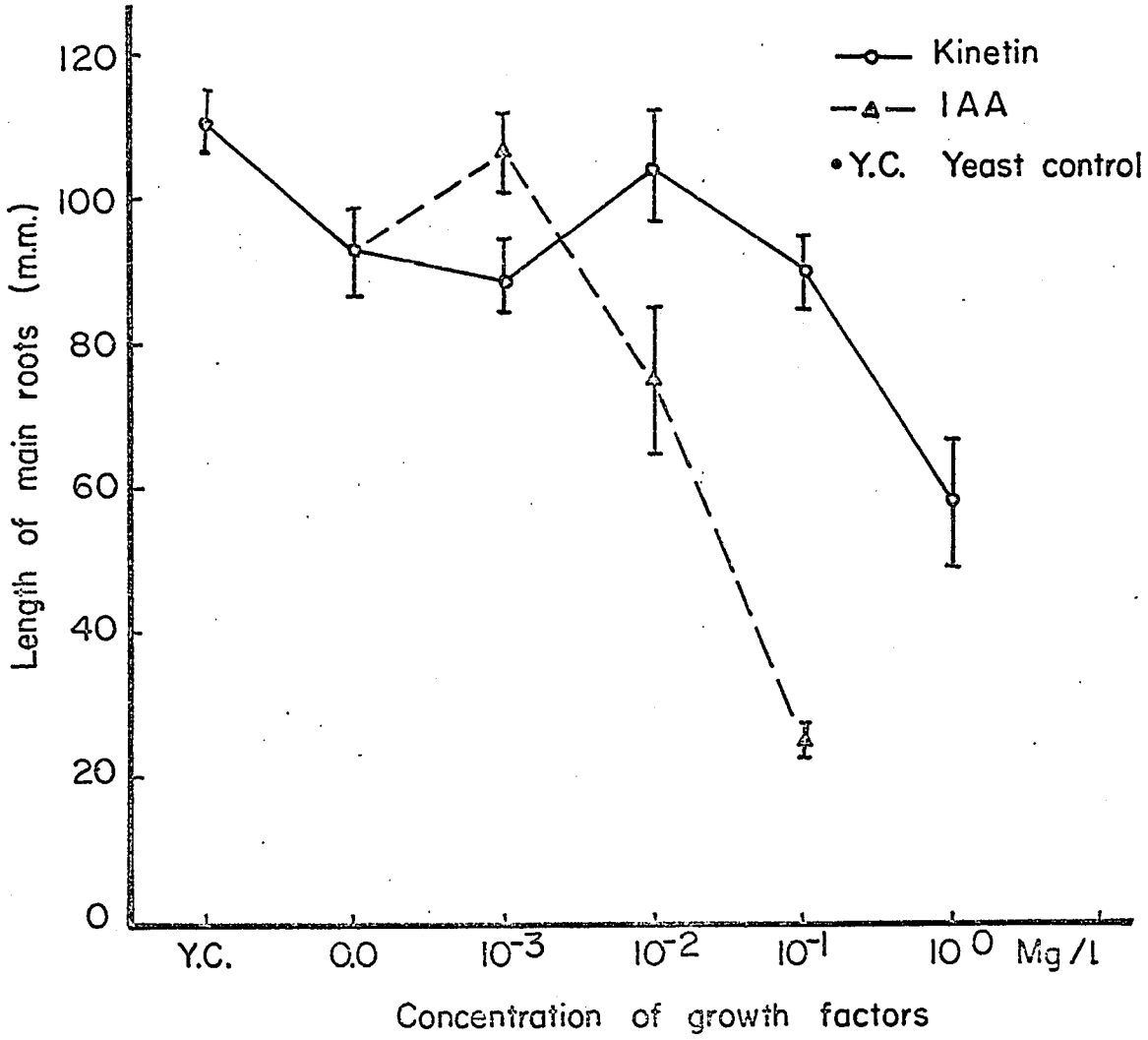

Figure 1

The effects of Kinetin and IAA on the growth of main roots of tetraploid rye. Vertical lines at each point represent twice the standard error.

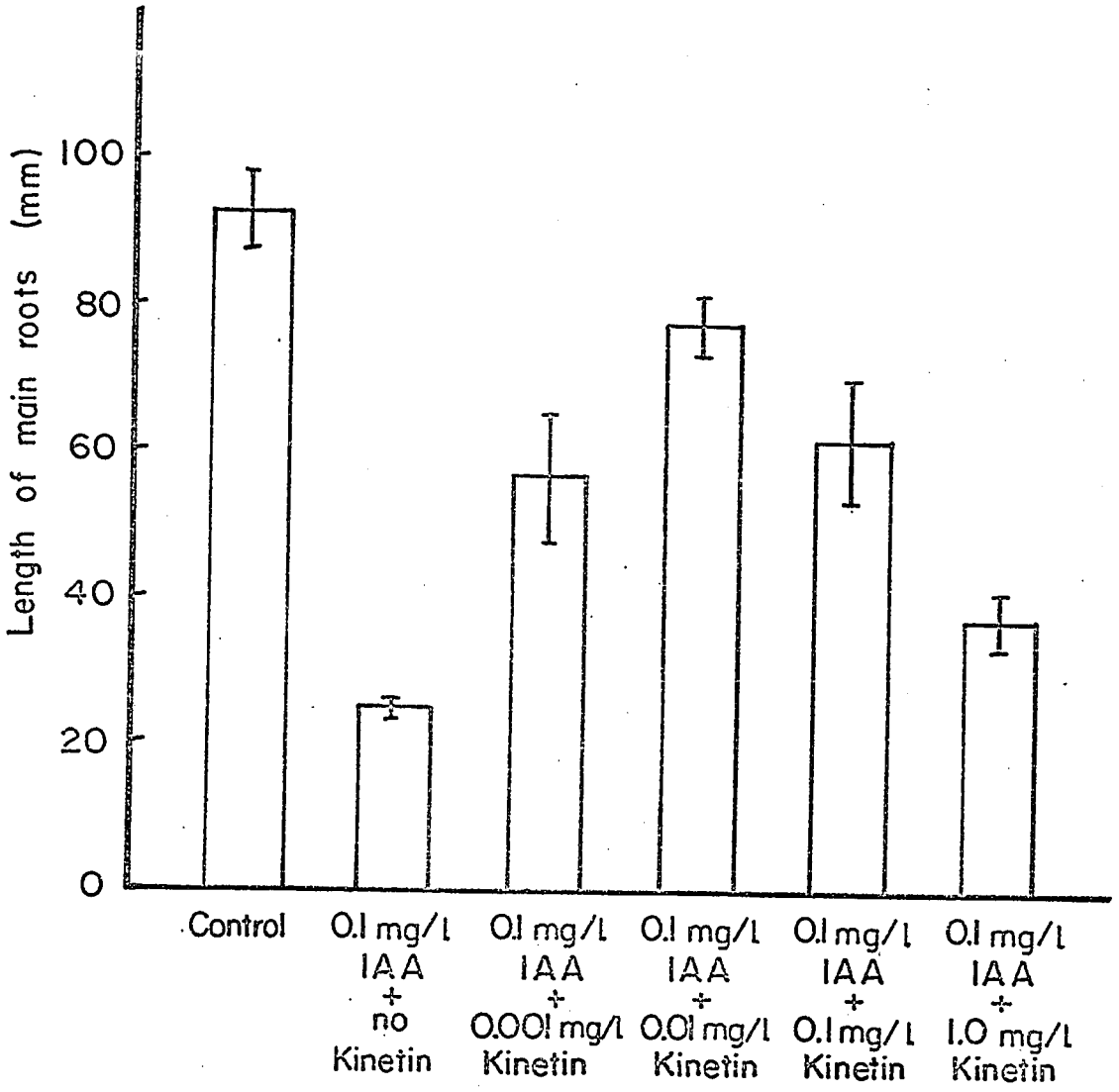

Figure 2

Interaction between Kinetin and IAA on the growth of main roots of tetraploid rye. Standard errors are indicated by the line symbols at the top of the hostogram.

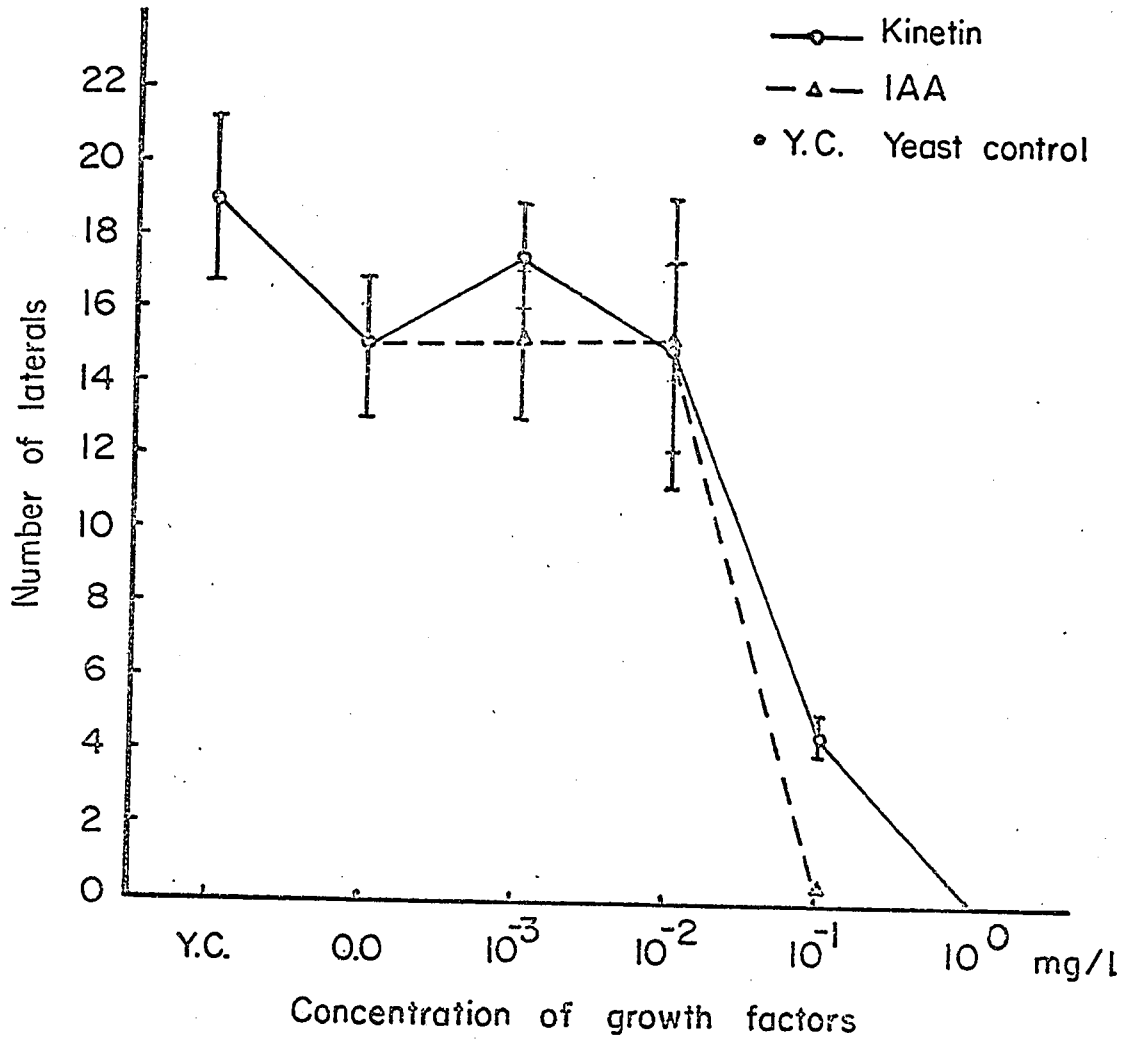

Figure 3

The effects of Kinetin and IAA on the number of lateral roots of tetraploid rye. Vertical lines at each point represent twice the standard error.

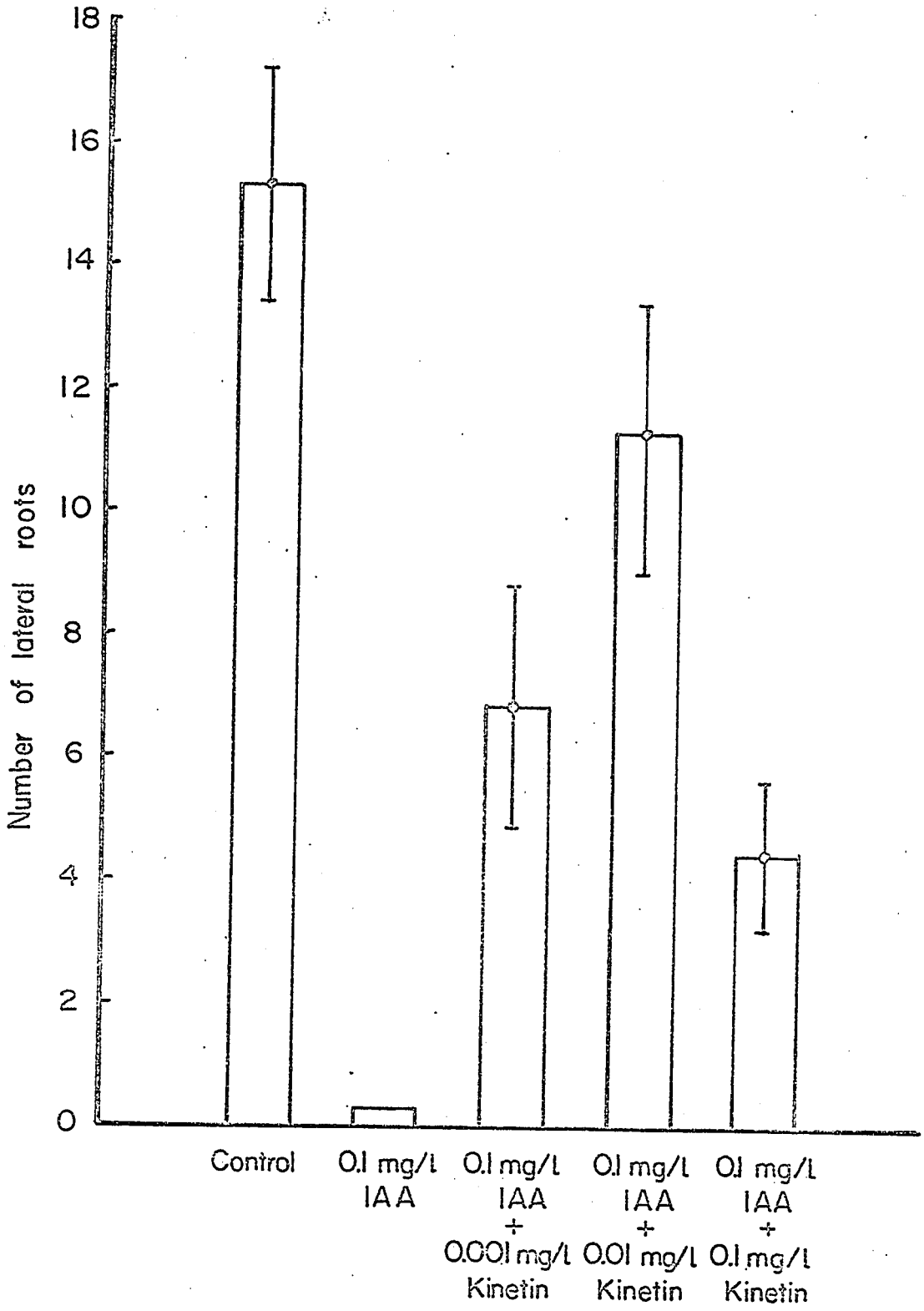

Figure 4

Interaction between Kinetin and IAA on the number of lateral roots of tetraploid rye. Standard errors are indicated by the line symbols at the top of the histogram.

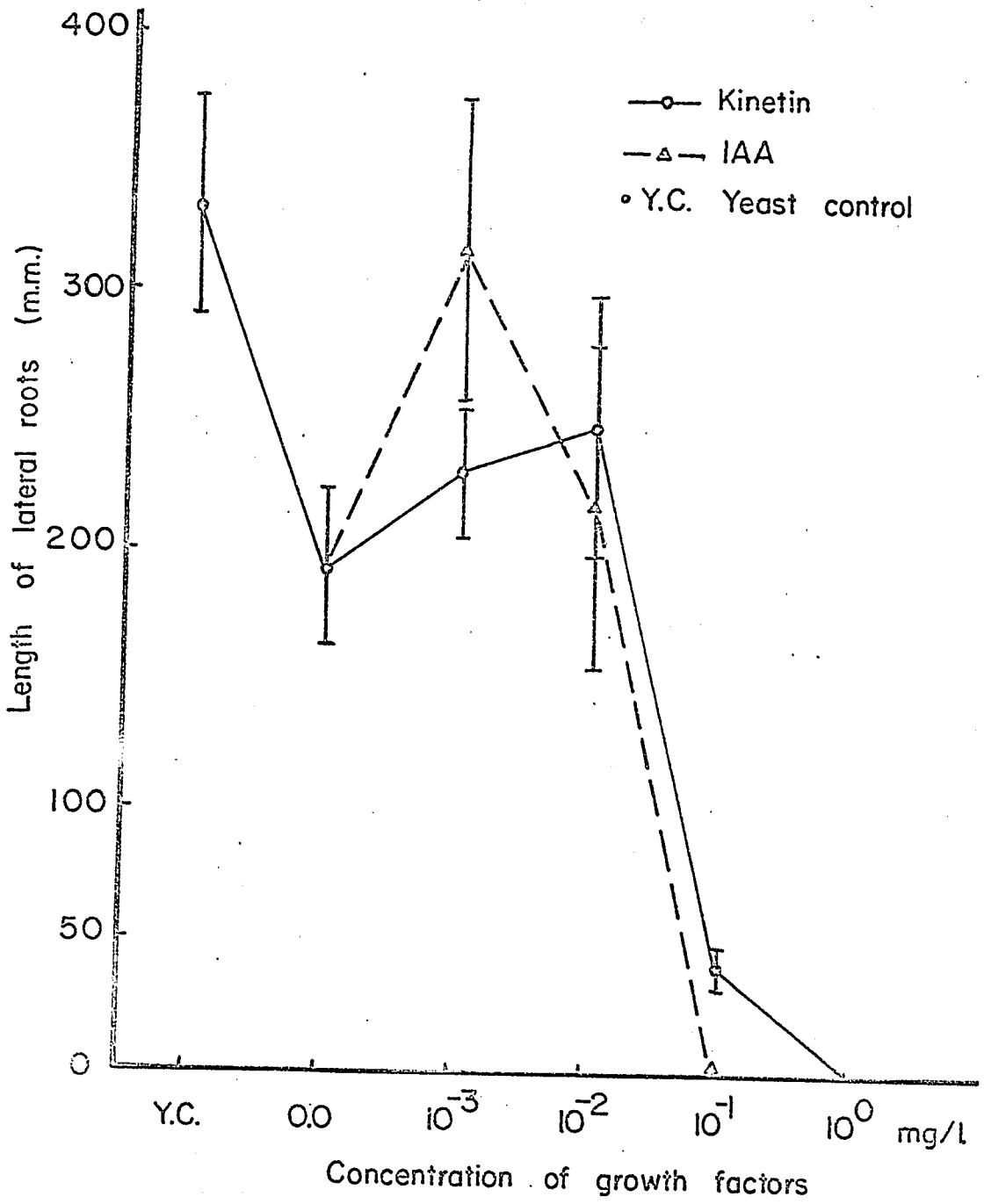

Figure 5

The effects of Kinetin and IAA on the total length of lateral roots per root of tetraploid rye. Vertical lines at each point represent twice the standard error.

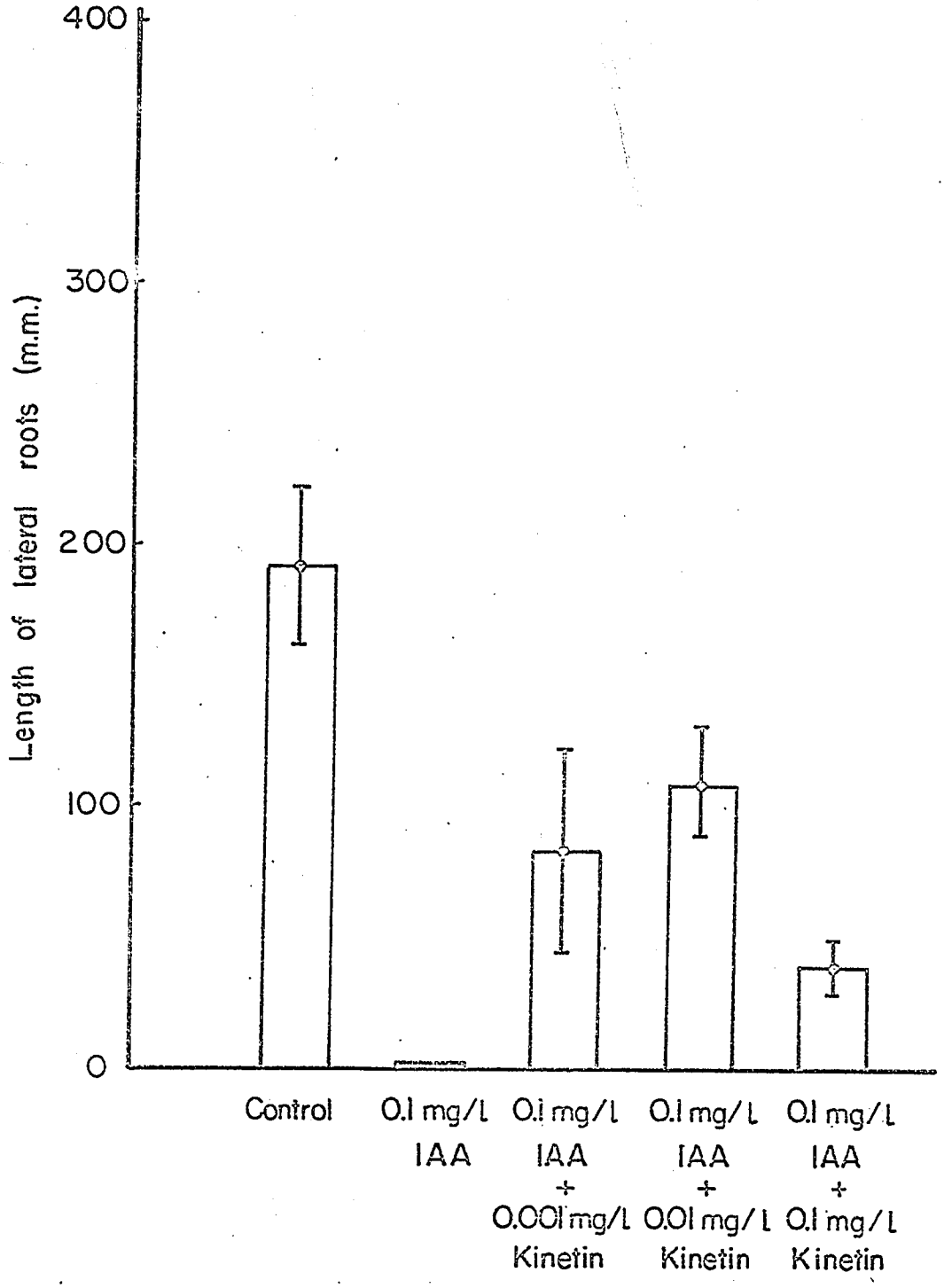

Figure 6

Interaction between Kinetin and IAA on the total length of lateral roots per root of tetraploid rye. Standard errors are indicated by the line symbols at the top of the histogram.

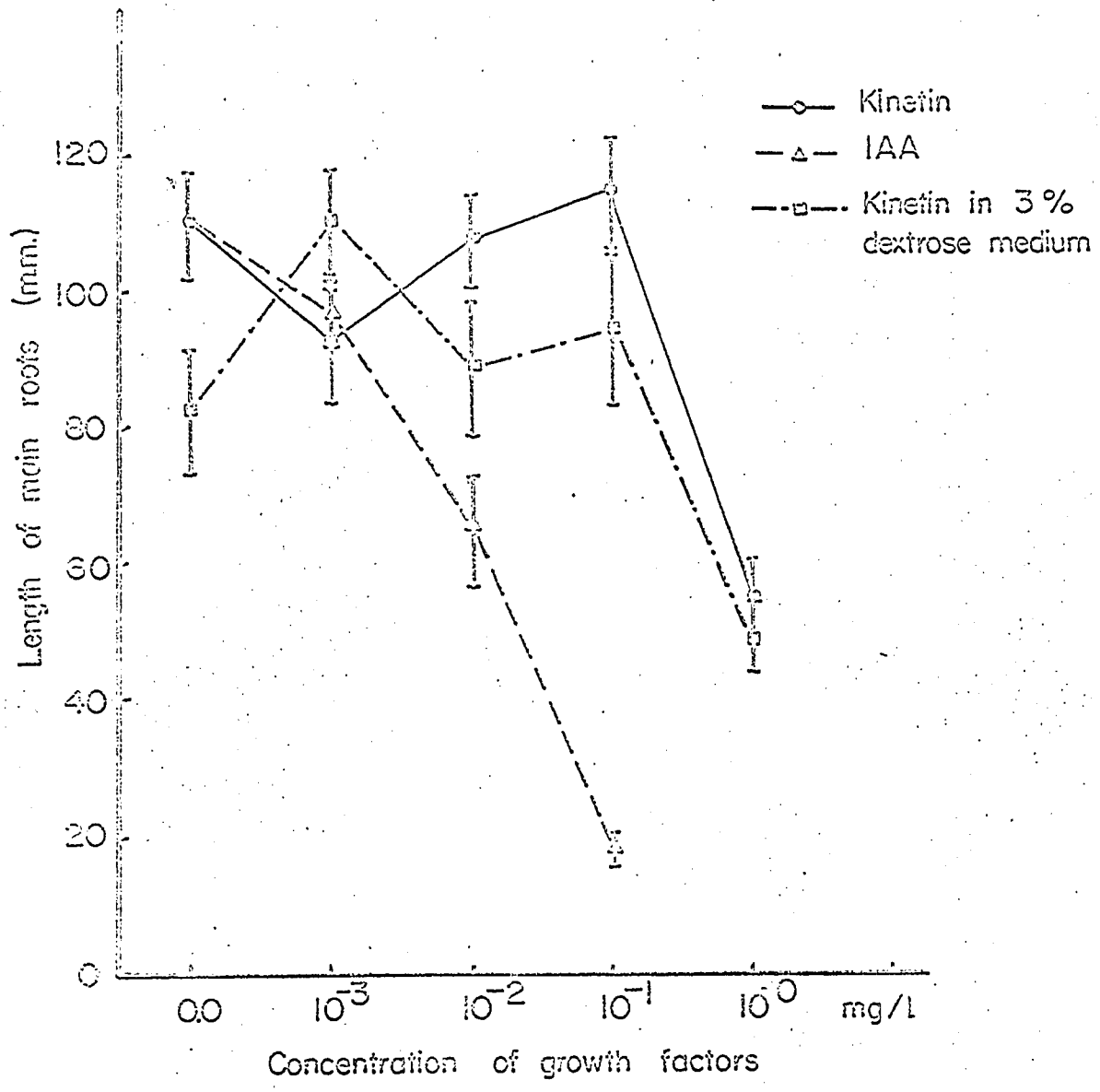

Figure 7

The effects of kinetin and IAA on the growth of main root of diploid rye. Vertical lines at each points represent twice the standard error.

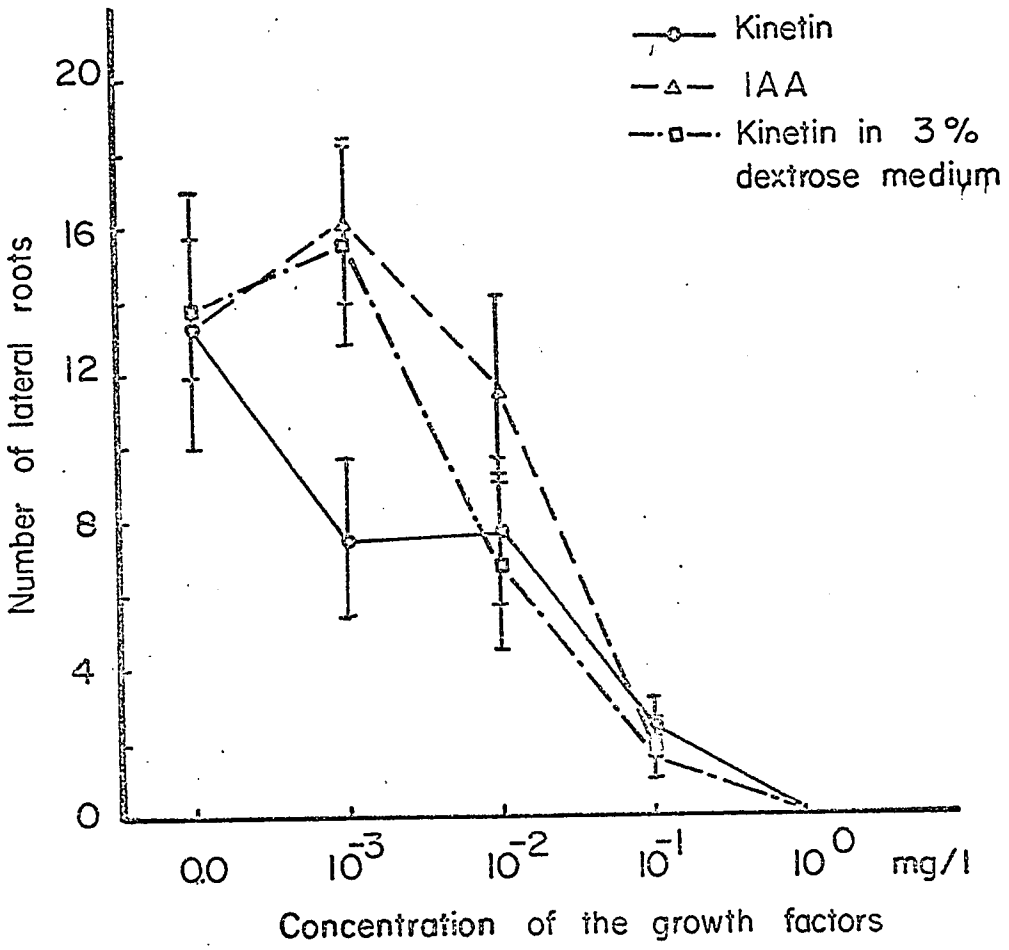

Figure 8

The effects of Kinetin and IAA on the number of lateral roots of diploid rye. Vertical lines at each point represent twice the standard error.

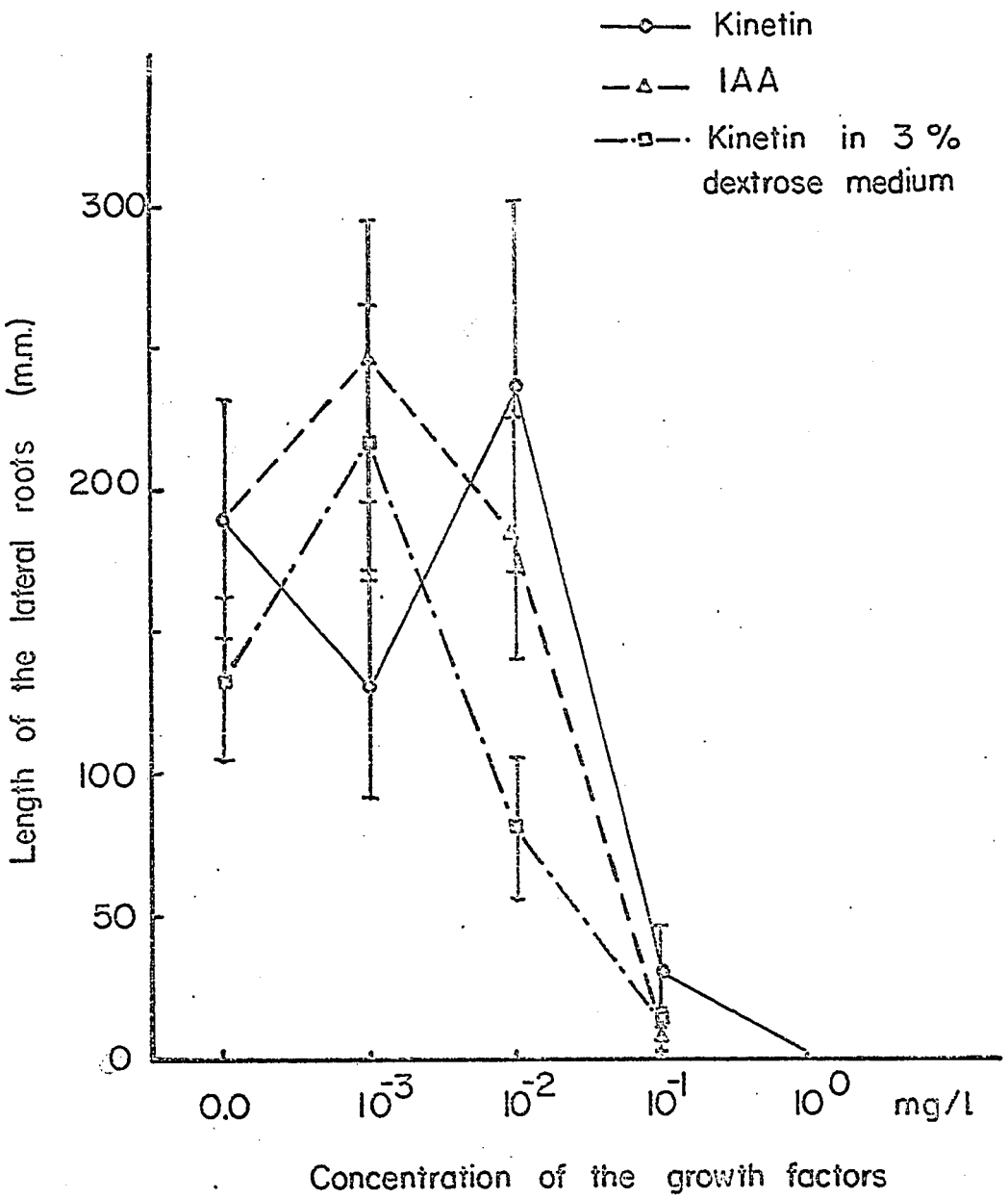

Figure 9

The effects of Kinetin and IAA on the total length of lateral roots per root of diploid rye. Vertical lines at each point represent twice the standard error.

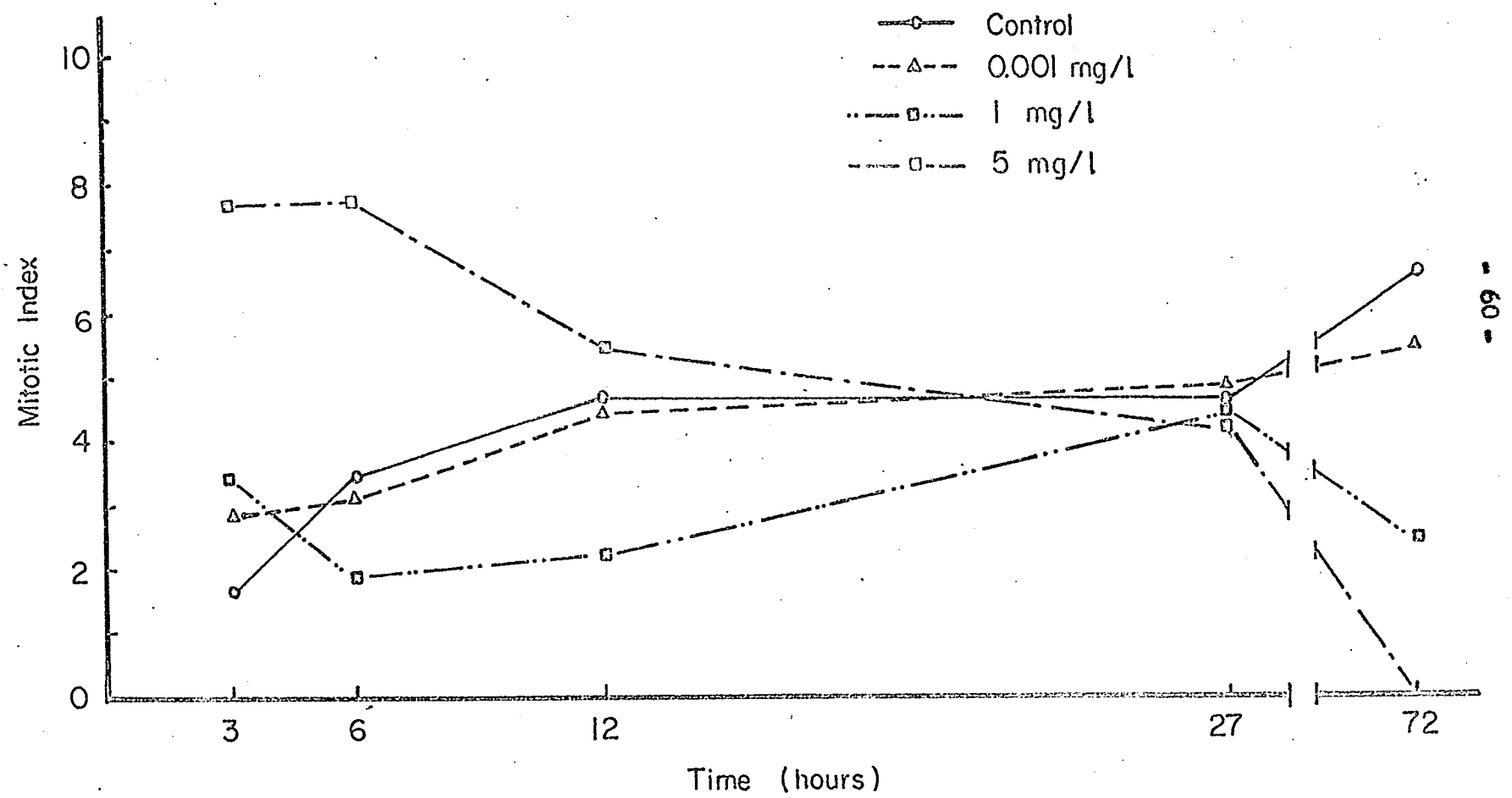

Figure 10

The effects of concentrations of Kinetin on mitotic index with respect to the duration of treatment.

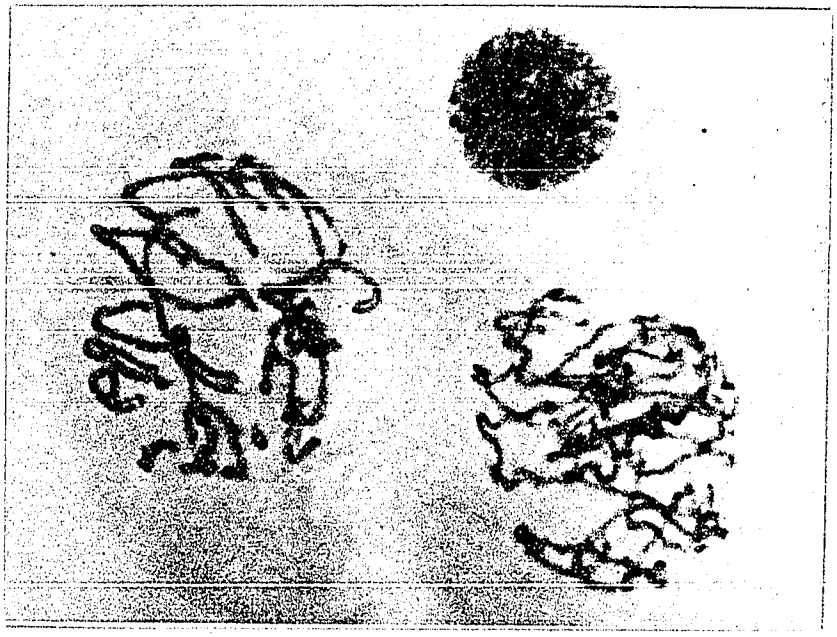

Figure 11. Two prophases after the treatment of kinetin at 0.001 mg/l for 3 hours. x600.

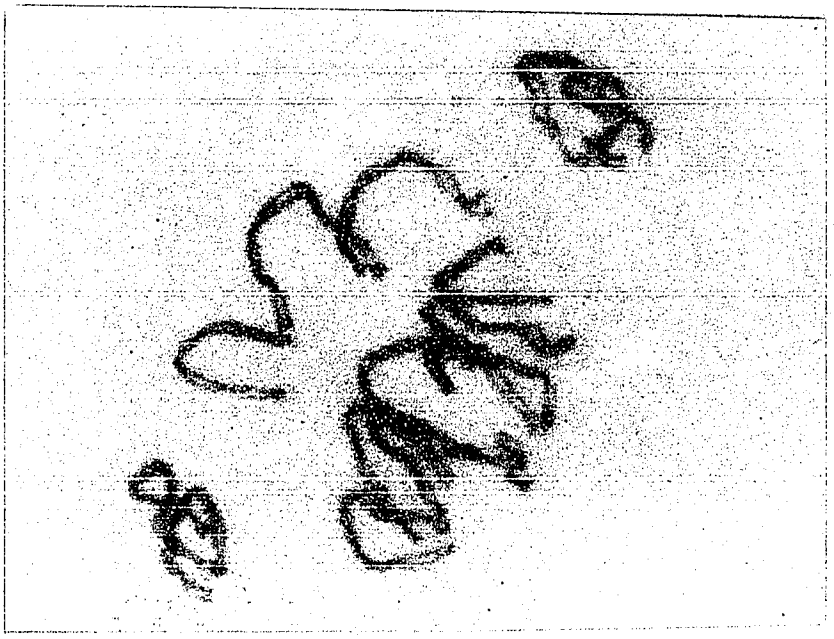

Figure 12. A late prophase after the treatment of kinetin at 0.001 mg/l for 3 hours. x600.

Figure 13. An early metaphase after the treatment of kinetin at 0.001 mg/l for 3 hours. x600.

Figure 14. A metaphase after the treatment of kinetin at 0.001 mg/l for 3 hours. x600.

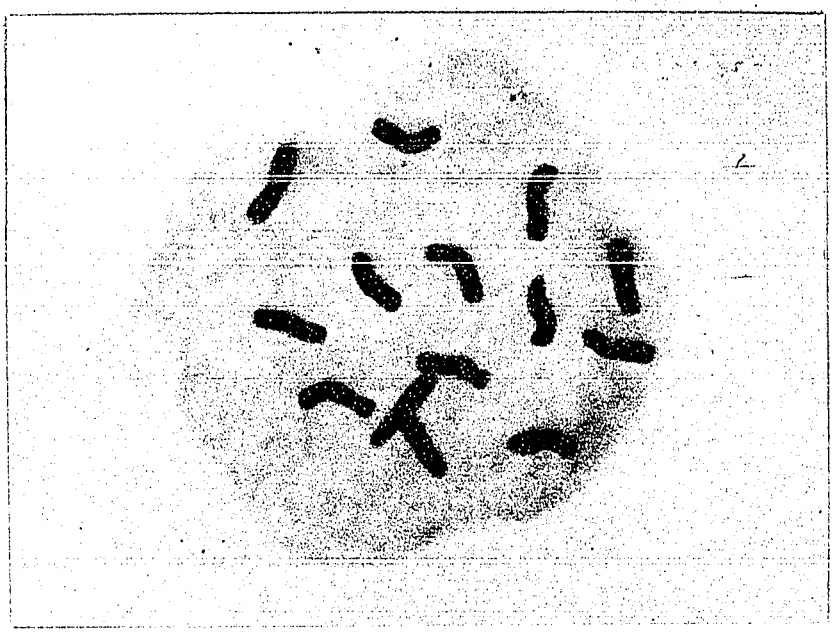

Figure 15. A metaphase after the treatment of kinetin at 1 mg/l for 3 hours. x600.

Figure 16. A prophase with breakage after the treatment of kinetin at 0.001 mg/l for 3 hours. x600.

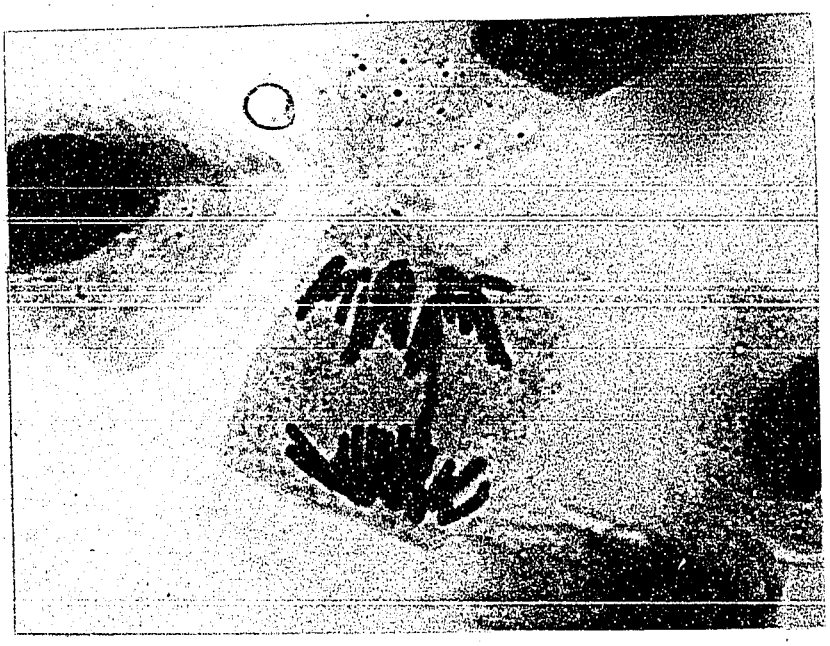

Figure 17. An anaphase with dicentric bridge after the treatment of kinetin at 1 mg/l for 3 hours. x600.

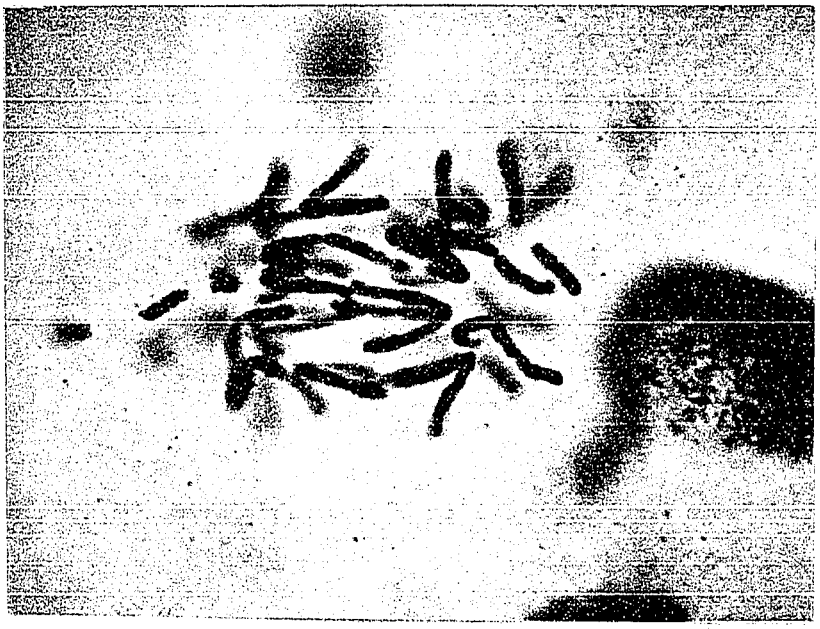

Figure 18. An anaphase with bridges and breakages after the treatment of kinetin at 1 mg/l for 3 hours. x1250.

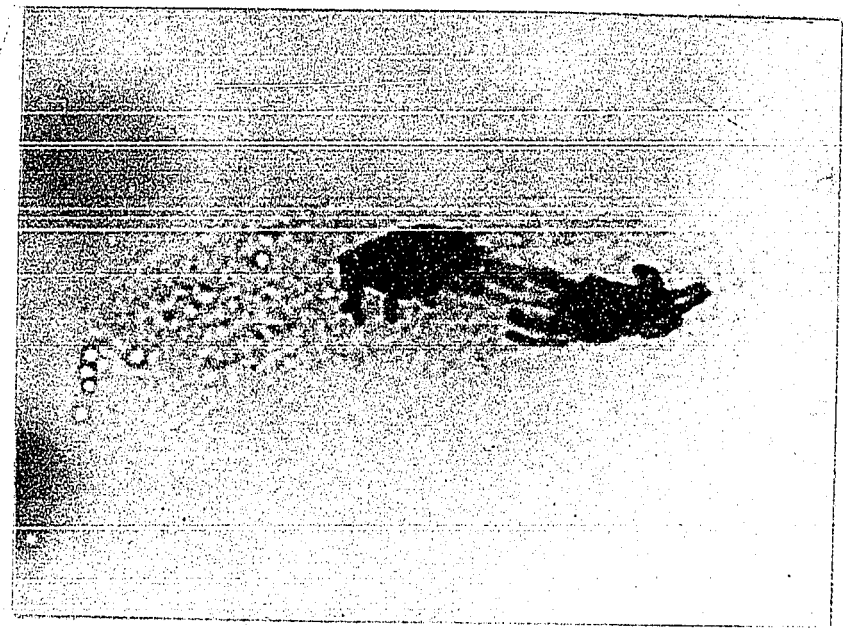

Figure 19. A telophase with chromosomal bridges after the treatment of kinetin at 5 mg/l for 27 hours. x600.

Figure 20. A telophase with acentic and dicentic chromosomal bridges after the treatment of kinetin at 5 mg/l for 27 hours. x1250.

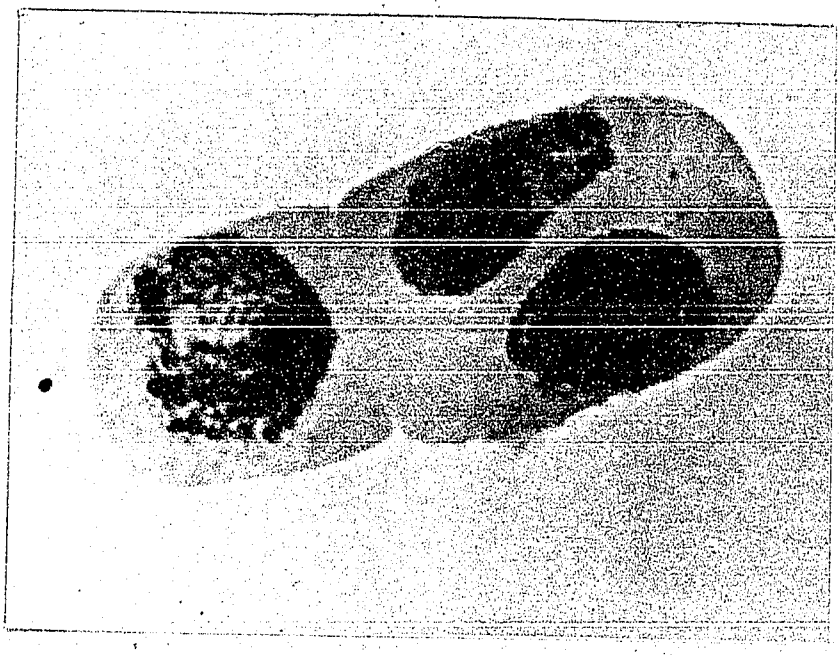

Figure 21. A binucleate cell after the treatment of kinetin at 1 mg/l for 12 hours. x600.

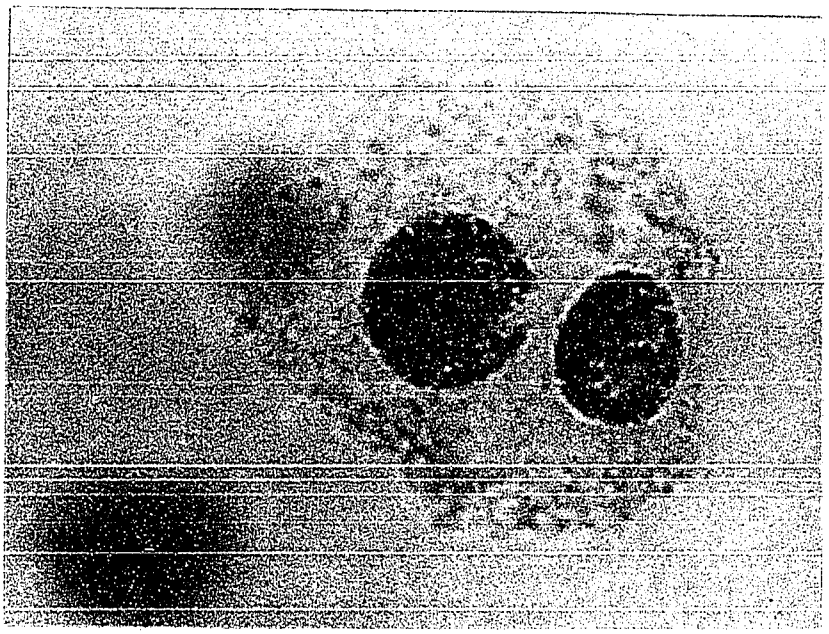

Figure 22. A binucleate cell after the treatment of kinetin at 5 mg/l for 27 hours. x600.

Figure 23. An abortive anaphase with many fragments after the treatment of kinetin at 1 mg/l for 3 hours. x1250.

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