THE IMMEDIATE EFFECTS OF LOW DOSES OF X-RADIATION ON THE FREQUENCY OF SEVERAL MITOTIC STAGES IN THE ALLIUM ROOT TIP

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Many studies have been made of the effects of radiations on mitosis in plant cells, but the design of the experiments has been such that they could not be compared readily with effects on animal cells. In the present study an attempt has been made to deal with plant material in the same manner as has been frequently used for animal tissues, namely, (1) to maintain the cells at a constant temperature before and after irradiation, (2) to make accurate dosage measurements at each treatment, (3)

TABLE I

The onion root tip cell mitotic cycle at 32° C.

Stage	Criteria by which the beginning of each stage is distinguished in the fixed, stained cell as observed with 4 mm. objective and 10× ocular	Relative frequency
Interphase	Densely granular appearance of nucleus caused by crowding of chromatin threads; nucleoli visible	.7546 (.7552)
Prophase		
Early	Nucleus enlarged; chromatin threads larger and less crowded; granules larger; nucleoli less distinct	.1672 (.1692)
Late	Chromosomes thicker and better separated; nucleoli absent	.0184 (.0182)
Prometaphase	Nuclear membrane absent	.0105 (.0112)
Metaphase	Chromosomes in equatorial plane	.0133 (.0124)
Anaphase	Proximal ends of chromatids separated	.0154 (.0102)
Telophase	Newly-formed cell plate visible; chromosomes less distinct	.0211 (.0211)

Figures in parentheses from Laughlin (1919).

to subdivide certain of the longer mitotic stages, so that more detailed information on the mitotic effect could be obtained, (4) to define carefully the terminology used to designate the different stages (Table I), and (5) to make regular and frequent counts of both control and treated cells.

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Materials and Methods

Ease in handling and in control of mold as well as bacterial infection of the roots dictated the use of seeds of *Allium cepa* in preference to bulbs. Preliminary experiments showed no significant differences in mitotic frequency between root tips of different seeds.

Ultraviolet radiation was used to inhibit mold growth during germination. Preliminary tests indicated that the dose of ultraviolet used had no effect on the germination time of the seeds or the rapidity of root growth. Seeds were agitated continuously for a period of six minutes in a quartz flask at a distance of about four inches from a four-watt General Electric germicidal lamp. This was done in a closed, sterilized chamber, which was also used to make subsequent transfers. The seeds were then transferred, aseptically, to sterile petri dishes containing filter paper dampened with distilled water. The petri dishes and filter paper had been sterilized by dry heat and the water had been sterilized by ultraviolet radiation.

A darkened incubator maintained at 26° C. was used to assure constant temperature during germination.[3] After twenty-four hours the seeds were removed from the incubator long enough to receive a second ultraviolet treatment for one minute to further retard the growth of any mold that had succeeded in contaminating the dishes. No roots were in evidence at this time.

After sixty hours, the roots had reached a length of 9 to 15 mm. At this time forty germinated seeds were transferred to each of two petri dishes. One of these sets was used as a control. The other was irradiated with either 128 r or 512 r of x-rays at the same dose rate, *i.e.*, 86 r/min.[4] The 128 r and 512 r doses were chosen because they were not large enough to cause death of the cells but sufficiently large to give easily measurable mitotic inhibition. Irradiation was done with a Coolidge tube (122 kv.p., 5 ma., with a 0.28-mm. aluminum filter). The dose rate was determined with a Victoreen dosimeter before each treatment.

Immediately following x-irradiation, roots of two of the irradiated and two of the control seeds were fixed in a solution of three parts absolute alcohol, one part glacial acetic acid and one part chloroform for six hours. The remaining germinated seeds were immediately placed in an incubator maintained at 32° C. and kept there throughout a five-hour period. During this time control and treated root samples were removed and fixed at one-half-hour intervals.

The material was stained with Feulgen's reagent and made into squash preparations according to a method worked out by Dr. Mary Esther Gaulden of the Oak Ridge National Laboratory (personal communication).

The numbers of the different mitotic stages occurring in ten fields at the center of each root tip squash preparation were recorded. A Howard disc was used to avoid any overlapping of these fields. The number of each of the seven mitotic stages recorded in all tips at a given half-hour interval was summated to give obser-

[3] Preliminary experiments, in which the roots were allowed to grow in darkness at a constant temperature, showed that the diurnal mitotic rhythm, as described by Kellicott (1904), only occurs when *Allium* is allowed to grow in an environment where light is present. These experiments confirm that which was inferred by Gray and Scholes (1951).

[4] This was the dose rate as determined with the dosimeter lying on a wooden table at the same level at which the petri dishes were placed during treatment. The scattering effect of the glass, as determined by measurements made with the dosimeter lying on a petri dish, added approximately 1.3% to these doses and dose rates.

vational totals.[5] All observations were made with a 4 mm. objective and 10 × oculars.

A one-tailed chi-square test was run to determine when the experimental percentages differed, at the 5 per cent level of significance, from the control percentages. Using one degree of freedom, $P > 3.84$ indicates this significance. The results of these tests will be found in Tables II and III.

Results

Results of 128 r x-ray treatment

The effects of 128 r of x-rays on the different mitotic stages are shown in Table II. Interphase was the only stage in which the frequencies of cells showed no significant differences from control frequencies within five hours following x-raying.

Table II

Ratios of experimental frequencies to control frequencies for 128 r

Hours after x-raying	Stage						
	Interphase	Early prophase	Late prophase	Prometa-phase	Metaphase	Anaphase	Telophase
0.0	1.01	0.98	0.95	0.81	0.63	1.12	1.11
0.5	1.02	0.97	1.29	*0.53*	0.77	0.84	0.92
1.0	1.02	0.96	1.16	*0.46*	*0.55*	0.84	0.89
1.5	1.00	*1.15*	1.29	*0.47*	0.60	*0.51*	0.54
2.0	1.02	1.12	0.96	0.65	*0.32*	*0.33*	0.68
2.5	1.04	*1.17*	*0.43*	*0.23*	*0.13*	*0.25*	*0.32*
3.0	0.98	*1.39*	*0.59*	*0.33*	*0.14*	*0.29*	*0.29*
3.5	1.00	*1.44*	*0.20*	*0.05*	*0.07*	*0.14*	*0.13*
4.0	1.00	*1.46*	*0.14*	*0.00*	*0.00*	*0.06*	*0.13*
5.0	1.01	*1.36*	*0.17*	*0.00*	*0.00*	*0.00*	*0.00*

The italicized values indicate that the experimental percentage differs from the control percentage at the five per cent level of significance.

In all other stages, a period of "normal" mitotic activity ranging from a half-hour duration in prometaphase to between two and two and one-half hours in late prophase and telophase was followed by a significant change in frequency. The phrase, "normal mitotic activity," is used to denote the period in which no observable changes in mitotic activity in relation to the control cells was evident.

Early prophase cell frequencies increased significantly beginning one and one-half hours after x-raying, and remained significantly higher than the early prophase control cell counts throughout the five-hour sampling period.

X-rayed late prophases showed no significant differences from the controls for two hours, after which they gradually fell to a minimum at four hours.

[5] These totals may be found in the thesis from which this paper is condensed, in the University of Tennessee library. It is from these data that the ratios in Tables II and III were calculated. Totals at each time interval range from 1296–2420 cells for the controls and 1767–2910 cells for the treated root tips in the 128 r experiment, and from 1340–1742 cells for the controls and 1431–2510 cells for the treated in the 512 r experiment.

Prometaphase, metaphase, anaphase, and telophase treated-to-control ratios decrease more or less gradually, virtually reaching zero three to four hours after treatment.

Results of 512 r x-ray treatment

The effects of 512 r of x-rays on the different mitotic stages are shown in Table III. Following one and one-half hours of normal frequency, treated interphase cells increased in number and maintained a level significantly higher than the controls from two to four hours after treatment.

TABLE III

Ratios of experimental frequencies to control frequencies for 512 r

Hours after x-raying	Stage						
	Interphase	Early prophase	Late prophase	Prometa-phase	Metaphase	Anaphase	Telophase
0.0	1.01	0.96	1.29	1.00	0.89	1.19	1.08
0.5	1.02	0.98	0.91	0.67	1.10	0.95	0.98
1.0	0.99	1.04	1.50	0.86	0.99	0.85	0.97
1.5	1.03	1.03	0.99	*0.31*	*0.43*	*0.50*	0.65
2.0	*1.04*	1.12	0.91	*0.05*	*0.12*	*0.23*	*0.48*
2.5	*1.06*	1.08	*0.53*	*0.05*	*0.00*	*0.09*	*0.26*
3.0	*1.05*	*1.20*	*0.38*	*0.00*	*0.00*	*0.03*	*0.09*
3.5	*1.05*	*1.23*	*0.23*	*0.00*	*0.00*	*0.00*	*0.06*
4.0	*1.06*	*1.21*	*0.16*	*0.00*	*0.00*	*0.02*	*0.00*
5.0	1.02	*1.38*	*0.12*	*0.00*	*0.00*	*0.00*	*0.00*

The italicized values indicate that the experimental percentage differs from the control percentage at the five per cent level of significance.

The early prophase frequency does not differ significantly from the control frequency for two and one-half hours. Subsequently it shows a significantly higher frequency, which is maintained through the five-hour sampling period.

Late prophase, prometaphase, metaphase, anaphase, and telophase frequencies, on the other hand, gradually decrease after one to two hours at the normal level. Late prophases have virtually disappeared at the end of five hours; prometaphases, metaphases, anaphases, and telophases at the end of two to three hours after irradiation.

DISCUSSION

It has been found in a variety of materials, both animal and plant, that if tissues are exposed to ionizing radiations, a decrease in the number of prometaphases, metaphases, anaphases, and telophases follows, and if the dose of ionizing radiations is great enough to reduce the number of cells in these stages to zero, the order of disappearance of these stages will be the same as the order in which cells pass through them (Carlson, 1954). Ionizing radiations tend to cause a blockage in one of the mitotic stages. The cells that have passed this stage continue to progress through the mitotic cycle. Hence, it is obvious that the first stage after the blockage point

will be the first stage vacated by the cells in mitosis, and each subsequent stage would be vacated in the order in which they occur. The more or less gradual progression of cell frequencies toward zero in these stages, as determined in this paper, seems to be in complete agreement with Carlson's statement.

Investigators working with *Vicia* root tips have reported different intervals of time between treatment with ionizing radiations and minimal mitotic activity. Deufel (1951) found that if the broad bean root tip was irradiated with 150 r of x-rays at a dose rate of 5 r/minute, and the tips were kept at 18° C. between treatment and fixation, the low point of mitotic activity was reached after 12 hours. Mottram (1936) states that the minimal mitotic activity of the root tip cells was reached nine hours following a 210 r dose of gamma-rays. According to the work of Juengling and Langendorff (1930), the minimal mitotic rates occur (1) fifteen hours following a 175 r dose of x-rays, (2) eighteen hours following treatment with 420 r of x-rays, and (3) thirty-three hours after irradiation with 550 r of x-rays, but there is no change in mitotic rate after treatment with doses of 40 or 80 r of x-radiation. All of Juengling and Langendorff's work was done with a dose rate of about 20 r/minute.

Working with *Allium* root tips, Darlington and La Cour (1945) wrote that minimal mitotic activity occurs (1) about ten hours after treatment with 150 r of x-rays when the root tips are kept at a temperature of 24° C. after treatment, and (2) about twenty-four hours after x-irradiation with the same dosage if the root tips are kept at 16° C. after treatment. Marshak (1937), however, states that minimal mitotic activity occurs in *Allium* root tips three hours after treatment with either 70 or 220 r of x-rays at a dose rate of 20 r/minute. This is confirmed by Gray *et al.* (1940) using a small dosage of gamma-rays and maintaining a temperature of 25° C. following treatment. Gray, in a personal communication to Carlson (1954), however, states that "the true value could easily have been as late as six hours since the minimum tends to be rather flat."

It has been concluded by Carlson (1942) that the low point of mitotic activity in the grasshopper neuroblast, at 26° C., occurs one hundred minutes after treatment with 31 r of x-radiation. Carlson, Snyder and Hollaender (1949) found that the low point of mitotic activity in the grasshopper neuroblasts is reached about sixty-six minutes after treatment with 32 r of gamma-rays when the material is maintained at 38° C. following treatment. It may be the effect of temperature on the time required for irradiated cells to complete mitosis that accounts for the apparent wide discrepancies in the results cited above.

My studies show that minimal mitotic activity of *Allium* root tip cells occurs between three and four hours after treatment with 128 r of x-rays, and between two and one-half and three hours after treatment with 512 r of x-radiation. The material was maintained at a temperature of 32° C. between treatment and fixation.

Concurrence exists among investigators that, if the treatment dosage is sufficiently large, cells in interphase at the time of treatment may be prevented from entering early prophase. These findings are identical with those in the present study (see Tables II and III).

Differences of opinion, however, exist concerning the immediate reaction of prophase cells to irradiation. Koller (1943), working with *Tradescantia* pollen grains, is among those who believe that prophase is not very sensitive to x-radiation since cells in prophase decrease in frequency following irradiation. These prophase cells,

according to him, complete mitosis with little or no delay as do the cells in prometaphase through telophase. Koller, therefore, concludes that cells in interphase are the most sensitive to ionizing radiations.

By dividing the grasshopper neuroblast prophase stage into five sub-stages, Carlson (1940) concluded from statistical calculations that middle prophase is the stage most sensitive to mitotic inhibition. This same publication also indicated that cells treated while in middle and early prophase revert to earlier stages. This reversion of cells in middle and late prophase was later confirmed by direct observation of living cells (Carlson, 1942). St. Amand (1956) also confirmed this from studies of living cells. Deufel (1951) describes the slowing down of prophases in the *Vicia* root tip after irradiation.

As is shown in Table II, *Allium* root tip cells, treated with 128 r of x-rays, exhibit no significant decrease in late prophase frequency until two and one-half hours after treatment. Obviously, they are not progressing into prometaphase because the frequency of cells in this stage has been decreasing, significantly, since the first one-half hour after treatment, and continue to do so until frequency reaches zero. Therefore, it must be concluded that late prophases have begun to revert, two and one-half hours subsequent to treatment, to early prophase. Early prophase has been increasing, significantly, since one and one-half hours after treatment. This was probably due (1) to an accumulation of cells passing into early prophase from interphase and being, at least partially, blocked from continuing to subsequent stages, and (2) to reversion of late prophases after two hours.

Treatment of the root tip cells with 512 r of x-rays causes this same effect (see Table III) but to a greater degree. Late prophase cells could not be progressing to prometaphase after the third hour, at the latest, as prometaphase frequency is zero after that time. Therefore, as their count is dwindling, late prophase cells must be reverting to early prophase. Some early prophase cells are reverting to interphase as witnessed by a significant experimental increase in interphase cell counts at, and following, the second hour after treatment. The reversion of early prophase cells is indicated by the fact that late prophase is decreasing at the hours when early prophase is showing no significant change. Hence, reversion of early prophase cells would counterbalance the influx of cells into that stage from late prophase. Interphase is increasing significantly at this time. Of course, some of the interphase increase is due to the cells passing from telophase into interphase, but this influx ceases before interphase increase stops.

If these hypotheses and conclusions are justified, they would indicate that late prophase is the most sensitive stage and early prophase is the second most sensitive stage to the effects of x-radiation. Since the criteria I used to designate late prophase seem to include the late and middle prophases of Carlson (1940, 1941, 1942) and St. Amand (1956), the stage sensitivities shown in this paper closely parallel the results reported by these two investigators. Reversion is not a limited phenomenon by any means. Beatty and Beatty (1954a and 1954b) have reported cases of it in *Tradescantia,* while Darlington and La Cour (1945) have noted evidence of reversion in *Trillium.* Reversion of early prophase to interphase may explain the long period in which no change in frequency is observed in early prophase.

The period in which no significant difference between experimental and control frequencies occurred, demonstrated by cells in late prophase, both after 128 r and 512 r doses, could be explained, possibly, in this manner. The critical period must

be in late prophase if reversion occurs as has been previously stated. This period could not be too far into late prophase, however, as no piling-up of cells occurs in this stage. Some cells may pass through this block following treatment to give the "normal" mitotic period in late prophase. This passage will also contribute to the "normal" mitotic periods in subsequent stages for a short time following treatment.

Piling-up in late prophase need not occur, even if the critical period is in late prophase, if all the cells very close to the critical period at the time of irradiation are so sensitive as to revert.

Summary

1. Root tip cells of germinated *Allium* seeds were given doses of 128 or 512 r of x-rays. The mitotic effects of this treatment were determined by making counts, in fixed preparations, of cells in several mitotic stages at regular intervals following x-raying.

2. After treatment with 128 r of x-rays virtual disappearance of cells in late prophase, prometaphase, metaphase, anaphase and telophase was observed between three and four hours. No observable change occurred in the interphase frequencies, but an increase was observed in early prophase one and one-half hours subsequent to treatment.

3. After receiving 512 r of x-radiation, late prophase, prometaphase, metaphase, anaphase and telophase cells fell to minimal frequency between one and one-half hours and three hours. The fall in frequencies after this dose was noticeably more rapid than following the 128 r dose. An increase was noted in interphase frequency two hours following treatment and in early prophase three hours following treatment. Again, late prophase frequency did not reach zero, but was at its lowest point five hours subsequent to treatment.

4. Minimal mitotic activity in the root tip cells of *Allium cepa* is reached between three and four hours subsequent to treatment with 128 r of x-rays, and between two and one-half and three hours following treatment with 512 r of x-rays. Between treatment and fixation all root tips were maintained at 32° C.

5. Reversion of cells from late prophase to early prophase is indicated when the cells are treated with 128 or 512 r of x-radiation and from early prophase to interphase when the cells are treated with 512 r of x-rays. It is probably due to this latter reversion that the minimal mitotic rate was reached in less time by the cells treated with the larger dose of x-rays.

6. Late prophase, which includes the latter portion of middle prophase of certain other investigators, seems to be the most sensitive and early prophase the second most sensitive stage to the mitosis-inhibiting effect of x-radiation.

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