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*ACCELERATED DNA SYNTHESIS IN ONION ROOT MERISTEM DURING X-IRRADIATION**

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It has been demonstrated recently that deoxyribonucleic acid (DNA) contents of X-irradiated mammalian cells continue to rise to the premitotic level in the absence of mitosis.[1-3] This is in contrast to the widespread belief that interference with DNA biosynthesis is one of the primary actions of radiation.[4-7] The present report deals with the effects of X-rays on cell proliferation, DNA synthesis, and the nucleohistone complex in onion root tips. As it had been found to do in animal materials (*loc. cit.*), DNA duplication continues to occur in these plant cells after irradiation; moreover, it will be demonstrated that DNA synthesis is actually stimulated during the process of irradiation.

Material and Methods.—Seed onions with roots $1^1/_2$ to 2 inches long were irradiated with 200 r, 800 r, 2000 r, and 3,200 r at a dose rate of 50 r per minute (at 250 kV and 15 mA, using 0.25 mm Cu and 1.0 mm Al filters). During irradiation, roots were completely submerged in ordinary tap water or in tap water containing tritiated thymidine (0.4 μc/ml; spec. act.: 1.9 C/mM). Immediately after irradiation, roots were allowed to grow in tap water with or without added tritiated thymidine. Parallel controls with nonirradiated roots were also run. Roots were fixed for six hours in Baker's neutral formalin or in acetic acid alcohol fixatives at frequent intervals starting from zero and continuing up to 48 hours post-radiation. Formalin-fixed tissues were washed in running water for about six hours. All samples were stored in 70 per cent ethanol.

Twelve-micron paraffin sections of formalin-fixed tissues were used for microspectrophotometric determinations of DNA and histone in the same individual nuclei. Sections from all tissues were hydrolyzed together for eight minutes in 1 *N* trichloroacetic acid (TCA) at 60°C and stained with TCA-Feulgen.[8] DNA contents of randomly selected 1,230 uncut interphase, 20 late telophase, and 20 early prophase nuclei were measured at 6,000 Å. After the completion of DNA measurements, the sections were stained with alkaline fast-green[9] for photometric measurements of histone (also at 6,000 Å) in some of the same individual nuclei whose DNA contents had been determined and which had been mapped prior to measuring. Mitotic counts were also made on these sections. Acetic alcohol-fixed squash preparations of about 1 mm tips (excluding root caps) were used exclusively for making autoradiographs by the stripping film technique and for further mitotic counts. The frequency of radioactive nuclei was determined, and the number of silver grains over many randomly selected interphase nuclei were counted. In order to correlate the incorporation of tritiated thymidine with the synthesis of DNA, photometric Feulgen-DNA determinations and grain counts on the same individual interphase nuclei (711) were also made on some squash preparations.

Results.—(a) *Nuclear DNA and histone contents in control and irradiated cells:* The photometric DNA measurements of late telophase and early prophase nuclei

from nonirradiated control roots yield two distinct DNA classes (2C and 4C) in a ratio of 1 : 2. Three hundred and thirty interphase nuclei from 33 control roots show, in addition to these two classes, many intermediate DNA values representing nuclei in the process of DNA synthesis prior to their entrance into mitosis. The

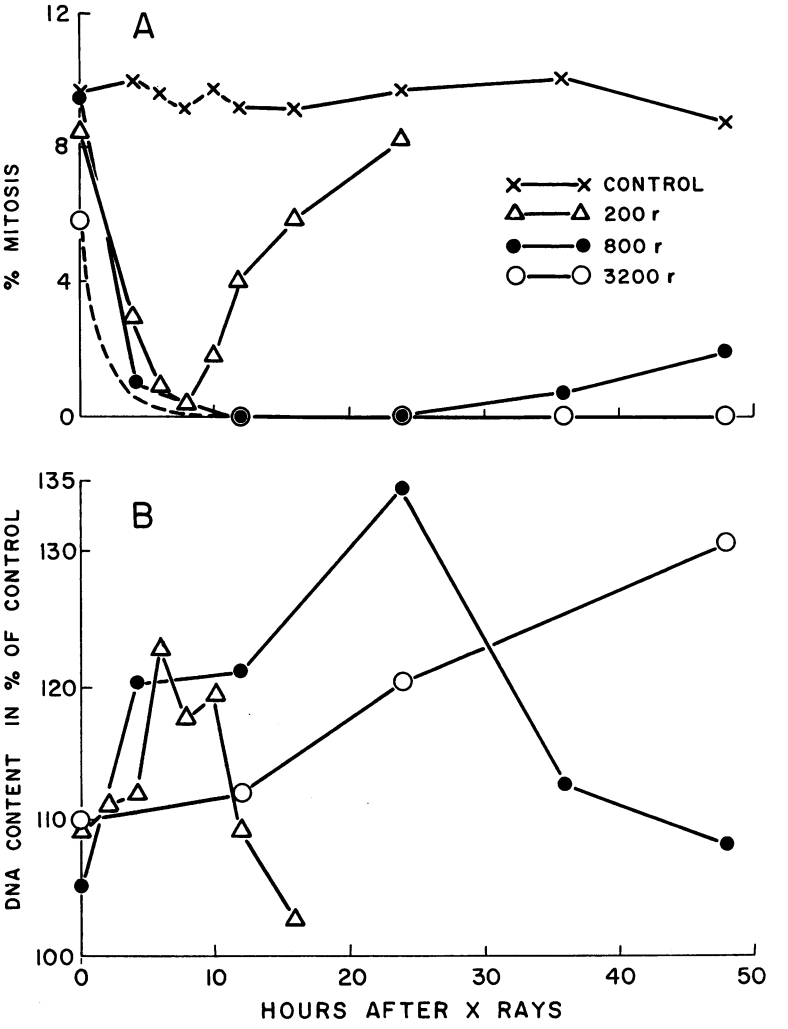

FIG. 1.—Changes in mitotic rates (A) and in average Feulgen-DNA contents (B) as a function of time. A: Number of roots per point = 3 (except 2 at 10 hours after 200 r and at 24 and 48 hours after 800 r) with a mean number of 387 cells scored per root. B: Control value (i.e., base line) obtained from 330 nuclei in 33 roots; number of roots per point is same as in (A); for each point, from 30 to 60 nuclei were measured.

frequency of 2C interphase nuclei (50%) in these roots is higher than that of 4C (37%).

The frequencies of 4C nuclei in tissues collected immediately after irradiation (i.e., at time zero) with 200 r, 800 r, and 3,200 r are 57, 47, and 48 per cent, respectively. This change in the distribution of DNA values toward the higher class raised sig-

nificantly the average DNA contents of the populations of irradiated nuclei up to about 10 per cent over the control value (see Fig. 1). The initial increase in DNA values of irradiated roots does not seem to be due to an immediate block of mitosis, because at time zero the mitotic rates after 200 r(8.4 ± 1.01) and 800 r(9.4 ± 0.49) are approximately the same as in simultaneous controls (9.6 ± 1.22). It is, however, lower after irradiation with 3,200 r(5.9 ± 1.06), a dose which required a sufficently long administering time (64 minutes) for the mitotic block to take effect.

The changes in mitotic indices and in average DNA values of nuclear populations with time after irradiation are graphically represented in Figure 1. The increase

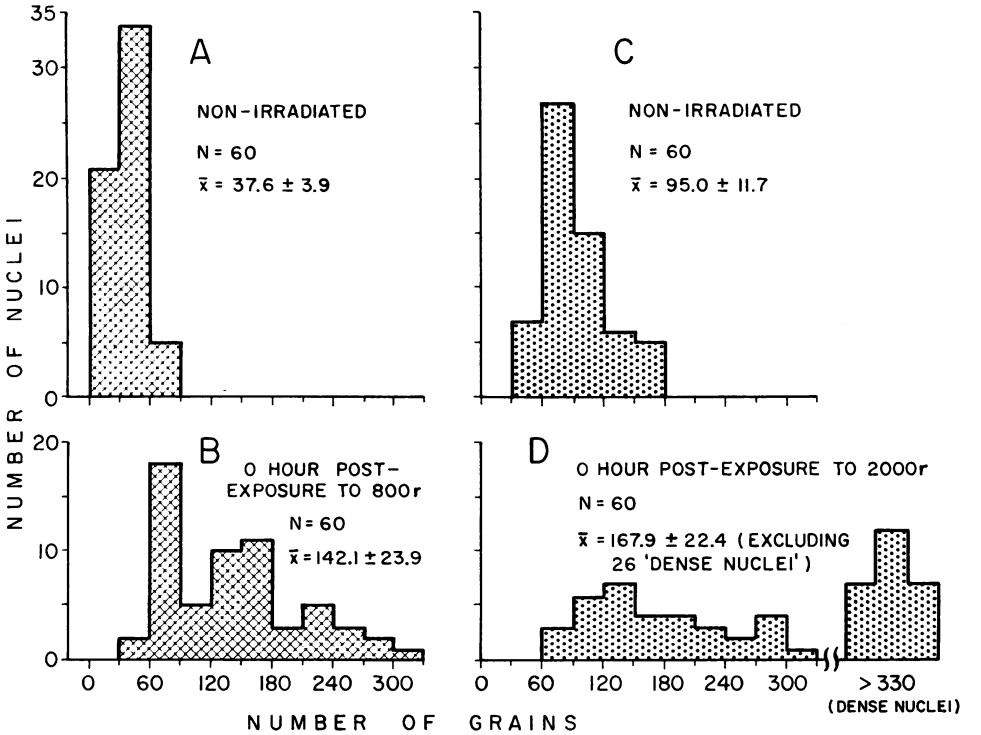

FIG. 2.—Distribution of silver grains over irradiated and non-irradiated nuclei. During irradiation (at 50 r/min) roots were submerged in tap water with 0.4 μc/ml of thymidine-H^3. Exposure to thymidine-H^3: A and B for 16 minutes; C and D for 40 minutes. All autoradiographs exposed for six weeks. N = number of nuclei (20/root); $\bar{x}$ = mean grain number/nucleus.

and eventual decrease (toward control levels) of DNA values in roots irradiated with 200 r and 800 r correspond directly to the inhibition and recovery of the mitotic rates. Roots exposed to 3,200 r, in which mitosis does not recover, also exhibit permanently increased DNA values; the slower rate of DNA increase in this last group is possibly due to the death of many 2C cells in this population.

Fast-green measurements of histone in 20 mitotic and 20 interphase nuclei from control tissues, whose DNA contents had been determined previously, show a DNA-histone ratio of 1.03 ± 0.01. Forty nuclei from roots collected 6 and 16 hours after irradiation with 200 r and 40 from roots sampled 12 and 48 hours after exposure to 3,200 r give DNA-histone ratios of 1.03 ± 0.01 and 1.01 ± 0.01, respectively.

These ratios do not differ from that of control roots. Hence, the nucleohistone-complex of the irradiated nuclei exhibits normal cytochemical characteristics.

(b) *Incorporation of tritiated thymidine:* It has been mentioned above that the average DNA values increase during irradiation. Since the mitotic rates in these tissues immediately after irradiation are about the same as those of the unirradiated controls, it suggests that DNA synthesis is stimulated during irradiation. Additional experiments have, therefore, been performed to determine whether there is an increased incorporation of tritiated thymidine into DNA during irradiation. It can be seen in Figure 2 that roots which are irradiated in the presence of tritiated thymidine for 16 and 40 minutes (total doses 800 r and 2000 r, respectively) and collected immediately after irradiation show a very high increase, compared to controls, in the incorporation of tritiated thymidine. In the former, the number of silver grains over an average nucleus is up to four times more than in the control (Fig. 2 A and B); in the latter, it is about two times more, even after excluding 16 (out of 60) nuclei which produced such high densities of silver grains that exact counts could not be made (Fig. 2 C and D).

Photometric DNA measurements and silver grain counts on the same nuclei reveal that the increased incorporation of tritiated thymidine during irradiation does not just represent increased thymidine utilization but corresponds to increased synthesis of Feulgen-stainable DNA. The estimation of the ratio R of the average amounts of DNA synthesized in time t in X-rayed samples to the average amounts in control samples can be made from the following equation:

$$R = 1 + \frac{\bar{X}_t - \bar{X}_c}{\bar{X}_t},$$

where $\bar{X}_t$ and $\bar{X}_c$ are the mean Feulgen-DNA values of irradiated and control samples respectively, and $\bar{X}_t$ is the fraction of DNA synthesized by an average control nucleus in time t. To obtain the value of $\bar{X}_t$, it is necessary to know the class value of 2C nuclei and the time necessary for such a nucleus to complete its DNA duplication. The 2C class value is obtained by measuring DNA of telophase nuclei, and the time required for an average onion root tip nucleus to undergo DNA doubling appears to be about six hours, like that of *Vicia faba* root tip nuclei [5] in

TABLE 1

A CORRELATION OF GRAIN COUNTS WITH DNA SYNTHESIS IN THE SAME INDIVIDUAL NUCLEI

	Treatment (50 r per min)	No. of roots	No. of nuclei	Mean grain no. per nucleus	Mean DNA content per nucleus	Grain ratio (X-rayed to control)	Ratio* of newly synthesized DNA (X-rayed to control)
I	Control	1	42	20.4	35.67	1.7	2.0
	2,000 r	1	39	35.2	38.12		
II	Control	3	86	11.5	48.18	5.2	4.5
	800 r	3	134	59.9	51.68		

* Calculated from $R = 1 + \frac{\bar{X}_t - \bar{X}_c}{\bar{X}_t}$, the ratio of average amounts of DNA synthesized in time t in X-rayed samples to average amounts in control samples (see text). Squash preparations were stained with Feulgen; in experiment (I), grain counts preceded DNA measurements; in (II), DNA measurements preceded application of stripping film. Autoradiographs were exposed for 1 and 4 weeks, respectively. DNA values in column 5 are given in arbitrary units of Feulgen-dye content, which, for technical reasons, differed in the 2 experiments.

which the durations of the total mitotic cycle and of the post-synthetic interphase period are similar to onion. Table 1 reports the results of two experiments in which grain ratios and ratios of newly synthesized DNA of irradiated nuclei to those of control nuclei were compared by making both types of measurements on the same nuclei. In each of the two experiments, these ratios agree fairly well with one another. The six-hour total synthesis time used in this calculation is likely to be a minimal estimate; if DNA synthesis takes longer, the values of R would increase and become larger than the grain ratios. Factors other than net synthesis would only have to be invoked, however, if the grain ratios were appreciably larger than the corresponding R values.

An analysis of frequencies of radioactive nuclei in nonirradiated and irradiated tissues reveal that these frequencies are significantly increased in tissues collected immediately after exposure to X-rays (Fig. 3). When such irradiated tissues are allowed to grow in tap water containing tritiated thymidine for an additional hour, these frequencies become similar to those from the corresponding controls.

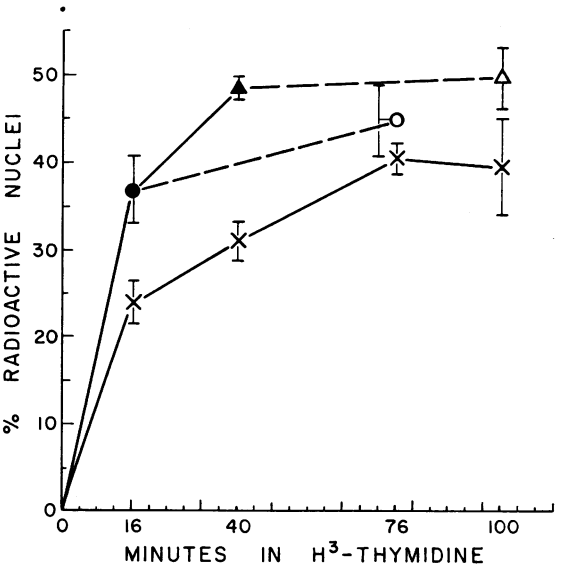

FIG. 3.—Frequency of radioactive nuclei from nonirradiated and irradiated (dose rate 50 r/min) roots as a function of time of exposure to thymidine-H^3. Each point represents 3 roots (except 2 at 1 hour after 800 r) with a mean number of 205 nuclei counted per root. ×: control samples; ●, ○: zero and one hour samples, respectively, after 800 r; ▲, △: zero and one hour samples, respectively, after 2,000 r. All autoradiographs exposed for six weeks.

Discussion.—In agreement with other studies, it is found that X-irradiation inhibits mitosis in these root tip cells. DNA synthesis, on the other hand, continues during a period of complete mitotic arrest. Howard[10] and Kelly[6] state that DNA synthesis may be relatively insensitive to radiation and that the decreased synthesis after exposure to X-rays could be a secondary consequence of mitotic arrest and cell death, since these will bring about a change in the cell population. The microspectrophotometric as well as autoradiographic data on DNA synthesis from the present study reveal not only that DNA replication does continue after irradiation, but also that the rate of synthesis is increased during irradiation. Furthermore, an additional number of 2C nuclei appears to be triggered into DNA synthesis during irradiation. It is not yet known how these effects are brought about by X-rays. That DNA synthesis should be accelerated in consequence of a treatment known to damage DNA is perhaps not too surprising in view of the finding that "damaged" DNA can be a very efficient primer of DNA synthesis in *in vitro* systems.[11]

It has been reported by many workers (see Stocken[7] for a recent review) that in cells which are supplied with labeled DNA precursors after X-irradiation a reduction

in precursor uptake into their DNA takes place. From the present results, it seems clear that such a reduction could be a consequence of the acceleration of DNA synthesis and the mitotic inhibition resulting from irradiation. These two effects in combination are bound to lead to a reduction in the frequency of 2C nuclei capable of entering DNA synthesis and consequently to reduced overall precursor uptake some time after irradiation. This could also explain the disappearance of intermediary Feulgen-DNA values from onion root tips collected several hours after X-irradiation, as reported by Swift.[12]

Rasch and Woodard[13] have recently demonstrated that the DNA-histone ratio remains constant in a variety of normal and pathological plant tissues. That this ratio in onion root tip cells remains unaltered after X-radiation has been shown in the present study. Pycnotic changes which produce a changed Feulgen/fast-green ratio in nuclei of damaged animal cells[14] do not occur in these root tips within 48 hours after irradiation.

The present results, thus, demonstrate that X-ray inhibition of mitosis in onion root tip cells is not mediated through a blockage on DNA synthesis or reflected in changes in the nucleohistone complex. It is not clear yet whether the accelerated DNA synthesis that takes place during X-ray treatments is one of the causes for this inhibition or whether it is due to an entirely different process.

Summary.—The effects of various doses of X-rays on synthesis of DNA and on the nucleohistone complex in individual onion root tip cells has been studied using both microspectrophotometric and autoradiographic methods.

Cell proliferation is inhibited by X-rays. The process of DNA synthesis, on the contrary, is stimulated during irradiation and continues to the premitotic level in a period of complete mitotic inhibition. The normal cytochemical properties of the nucleohistone complex appear unchanged even after heavy irradiation.

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