

RADIATION EFFECTS ON THE GROWTH RATE
AND CELL POPULATION KINETICS OF
ACTIVELY GROWING AND DORMANT ROOTS
OF *TRADESCANTIA PALUDOSA*

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ABSTRACT

Actively growing and dormant roots of *Tradescantia paludosa* were exposed to x-rays to compare the radiosensitivity of an actively proliferating tissue with that of one which is not active but is potentially proliferative. The level of effect was ascertained by the degree of change in the rate of root growth 4 days after exposure. Cell population kinetics were measured in control and in irradiated roots to determine whether or not a change was produced either in the number of proliferating cells or in the mitotic cycle duration which was sufficient to explain the altered rate of root growth. Nuclear volumes were also measured to provide an estimate of the relative total target size in actively growing *vs.* dormant roots. Tritiated thymidine was used to measure the cycle duration and the proportion of cells synthesizing DNA. The results showed that 184 and 305 r respectively were required to reduce the linear root growth rate to 37 per cent of that of the control for actively growing and dormant roots. Mitotic cycle duration, measured 4 days after x-ray exposure, was the same as in the control. The number of proliferating cells, however, was reduced. The rate of cell production in the irradiated roots was reduced to approximately one-half that of the controls. The average nuclear volumes of active and dormant roots were 733 and 491 μ^3 respectively; thus the difference in the number of roentgens required to reduce growth to 37 per cent of that of the control can be attributed to the different average nuclear volumes. Therefore, the experiments suggest that part if not most of the differences in sensitivity between an actively dividing and an essentially non-dividing meristematic cell population resides in their different average nuclear volumes. Thus the law of Bergonie and Tribondeau needs to be reinterpreted, since the basic reason for the differences is secondary to whether or not the meristematic cells are proliferating.

INTRODUCTION

Studies on comparative cellular radiosensitivity have often demonstrated that the results are a reflection of changes in the cell population or the physiological state of the cells being irradiated. The Bergonie and Tribondeau (1) law expresses this concept in rather general terms by stating that the sensitivity of cells to irradiation is in direct

proportion to their reproductive activity and inversely proportional to their degree of differentiation. Ever since this law was first formulated, research has been conducted in an effort to elucidate and elaborate on the finer details of the problem of cellular radiosensitivity. The experiments described here may also be considered to be efforts

designed to add further understanding to the problem of cellular radiosensitivity, with particular emphasis on the response of an actively proliferating tissue as compared with a tissue which has the potential but is not proliferating.

Actively growing roots and dormant aerial roots of *Tradescantia paludosa* were utilized. Comparative radiosensitivity was ascertained by measuring the rate of root growth. These observations were related to the original irradiated cell populations, whose characteristics are described by histological and autoradiographic analyses. Possible residual radiation effects on the duration of the mitotic cycle were ascertained by the use of tritiated thymidine and autoradiographic methods on the 4th day after a D_{37} dose, *i.e.*, a dose which reduced the linear growth rate to 37 per cent of the control.

MATERIALS AND METHODS

Cuttings of *Tradescantia paludosa* (Sparrow's clone B2-2) having aerial root protuberances (Fig. 1) were maintained in continuous light at $20^{\circ} \pm 1^{\circ}\text{C}$ in aerated Hoagland's nutrient solution. The aerial root protuberances were considered to be the dormant form in this study. Dormancy is broken when stem cuttings with protuberances are immersed in the nutrient solution. At the temperature used in these experiments, approximately 3 days pass before the protuberance is transformed into an actively growing root.

Irradiation

Dormant roots were irradiated immediately after cutting; actively growing roots, 3 days after cutting. Irradiation was performed at a distance of 50 cm with a General Electric Maxitron x-ray machine at 250 kvp, 30 ma, with a 1-mm Al filter. At least 5 cuttings, each with several roots, were exposed to different amounts of irradiation.

Root Growth Measurements

Actively growing roots were measured immediately after irradiation. Succeeding measurements were made at the same time daily. The first root to show signs of growth was measured. The length was determined with a rule having 1-mm divisions. Dormant roots were measured in the same manner except that 3 days intervened between irradiation and commencement of measurements.

Cell Counts

The method used was the same as that previously utilized in experiments with *Pisum* (11). To obtain cell counts, the root meristem was defined as that

portion which showed a vivid Feulgen stain. The meristem defined in this manner includes differentiated cells such as those of the root cap as well as proliferating cells. The demarcation between deeply stained and lightly stained portions was determined by placing the root on frosted glass which was illuminated from beneath. The deeply stained portion was removed and placed in the well of a concave slide and macerated in 1 N HCl. Cell counts were made from these suspensions with a hemocytometer.

Autoradiography

PROPORTION OF CELLS SYNTHESIZING DNA IN DORMANT ROOTS: The dormant root is aerial and does not have the same absorption capabilities as an actively growing root. In order to determine the number of cells synthesizing DNA in the dormant root, the following procedure was used. The protuberances representing roots were excised from the plant in a manner which allowed each protuberance to remain attached to a small wedge of stem tissue. The excised roots were then immersed for 2 hours in Hoagland's nutrient solution containing $4 \mu\text{c}/\text{ml}$ tritiated thymidine ($\text{H}^3\text{-T}$) obtained from New England Nuclear Corporation ($5.3 \text{ c}/\text{mmole sp. act.}$). Samples were removed from the solution, fixed with Carnoy's solution, stained by the Feulgen method, and squashed. The squash preparations were coated with Kodak NTB liquid emulsion and exposed for 7 days at 4°C .

MITOTIC CYCLE TIME MEASUREMENTS WITH $\text{H}^3\text{-T}$: The method used was described by Quastler and Sherman (6) and for *Tradescantia* specifically by Wimber (15). $\text{H}^3\text{-T}$ labels a block of cells in interphase, and the cycle time can be estimated from the rhythmic increase and decrease of the number of these labeled cells in mitosis with time. Generally the mitotic cycle duration is taken to be the interval of time between two successive ascending portions of such a curve. In our experiments, the time interval between the 30, 35, or 40 per cent intercepts was used to estimate cycle duration.

Duration of Mitosis (Prophase, Metaphase, Anaphase, and Telophase)

The increase in the number of labeled prophases as they appeared in mitosis following a pulse label with $\text{H}^3\text{-T}$ was determined and the curve was extrapolated to the abscissa. The increase in the number of labeled metaphases as they appeared in division was also determined and this curve also was extrapolated to its intercept with the abscissa. The difference between the time intercepts for the prophase and metaphase cells is the minimum prophase dura-



FIGURE 1 Photograph of aerial dormant roots on a *Tradescantia* plant. The arrows indicate the location of the dormant roots.

TABLE I
Linear Regression Models for Growth Rate of
Actively Growing Roots Following
Exposure to X-Rays

Exposure	Linear regression model	Correlation coefficient
<i>r</i>		
Control	$Y = -0.275 + 0.031 X^*$	0.998
	$Y = 0.040 + 0.028 X$	0.999
50	$Y = -0.100 + 0.024 X$	0.997
100	$Y = 0.000 + 0.013 X$	0.998
150	$Y = +0.240 + 0.014 X$	0.994
200	$Y = +0.480 + 0.011 X$	0.993
250	$Y = +0.410 + 0.007 X$	0.978
300	$Y = +0.420 + 0.006 X$	0.974
400	$Y = +0.530 + 0.005 X$	0.986
500	$Y = +0.390 + 0.006 X$	0.939

* *Y*, length in cm; *X*, time in hours.

tion. The proportion of prophase cells to total mitotic figures was determined in 6 meristems. With these data, the duration of mitosis can be determined as follows:

$$\frac{\text{Proportion of prophase cells}}{\text{Proportion of mitotic cells}} = \frac{\text{Duration of prophase}}{\text{Duration of mitosis}} \quad (1)$$

The duration of mitosis and the total mitotic cycle time of active and dormant roots were measured on the 4th day after a D_{37} exposure to x-rays. At this time the roots were immersed for 30 minutes in nutrient solution containing 0.5 $\mu\text{c/ml}$ $\text{H}^3\text{-T}$ (sp. act. 5.3 c/mmole, obtained from New England Nuclear Corp., Boston), washed, returned to the non-radioactive nutrient solution, and sampled for 30 hours. After sampling, these roots were handled in the same manner as the dormant roots (see above under Autoradiography) except that the emulsion was exposed for 14 days rather than 7 days.

The proportion of cells in mitosis was determined by scoring 1000 cells in Feulgen-stained squash preparations from 3 separate root tips on the 4th day after a D_{37} dose of x-rays.

Nuclear Volume

The average nuclear volumes of active and dormant roots were obtained from root meristems that were killed, fixed in Craff III (3:2:4:1 respectively, v/v , of 1 per cent chromic acid, 10 per cent acetic acid, water, and formalin), dehydrated, infiltrated

TABLE II
Linear Regression Models for Growth Rate
of Roots Exposed to X-Rays When Dormant

Exposure	Linear regression model	Correlation coefficient
<i>r</i>		
Control	$Y = -0.210 + 0.032 X^*$	0.999
50	$Y = -0.220 + 0.037 X$	0.997
100	$Y = -0.210 + 0.029 X$	0.999
150	$Y = -0.160 + 0.031 X$	0.998
200	$Y = -0.110 + 0.020 X$	0.999
300	$Y = -0.210 + 0.016 X$	0.999
400	$Y = +0.090 + 0.004 X$	0.992

* *Y*, length in cm; *X* time in hours.

with paraffin by the use of a tertiary butyl alcohol series, and stained with safranin and fast green. The diameters of interphase nuclei of meristematic cells just above the root caps were measured with a Zeiss ocular micrometer. The nuclei of two actively growing and two dormant roots were measured, and the average nuclear volumes calculated.

RESULTS

Growth Rate Measurements

Buchanan (2) has shown that root growth can be expressed by the simple equation

$$L = kt + C \quad (2)$$

where *L* is the length of the root, *k* is the rate of root growth (centimeters per unit time), *t* is time, and *C* is the length of the root at time zero (t_0). The plot of $L - C$ versus time produces a straight line. Van't Hof and Ying (14) have shown that *k* in equation 2 is related to rate of new cell production as follows:

$$k = \frac{N_{ct}}{CT} E \quad (3)$$

where N_{ct} is the number of cells in the mitotic cycle, *CT* is the average mitotic cycle time, and *E* is a proportionality constant with units of centimeters per cell. From equation 3 it is seen that *k* will decrease with either an increase in *CT* or a decrease in N_{ct} , or a combination of an increase in *CT* and a decrease in N_{ct} . (For a more detailed discussion of these relationships see reference 14.)

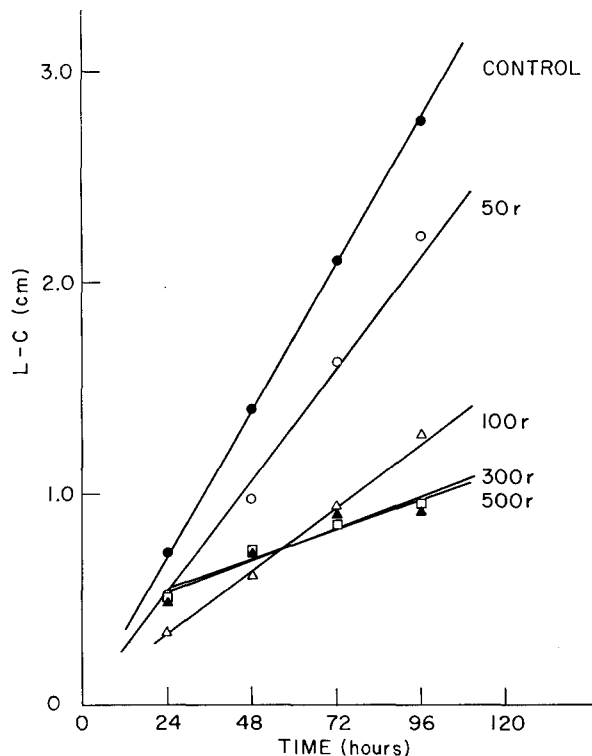


FIGURE 2 Increase in length of *Tradescantia* roots with time following exposures to 0 to 500 r of x-rays while actively growing. L , length of root; C , length of root at time zero.

In Tables I and II are shown the linear regression models and the correlation coefficients for the growth of active and dormant roots after irradiation. They represent the average growth of roots from 5 cuttings per x-ray exposure per time interval and are the statistical expression of equation 2. Some of these regression lines are displayed in Figs. 2 and 3 for active and dormant roots respectively. The expected linearity was observed and the validity of the linear regression model was supported by the correlation coefficients. A D_{37} dose was determined from the linear regression model of log per cent effect (Y) versus exposure (X). The data concerning growth rate for active and dormant roots are shown in Fig. 4. Based on growth rate, the D_{37} dose for actively growing roots was 184 r; for dormant roots it was 305 r.

Cellular and Cell Population Characteristics at the Time of Irradiation

The H^3 -T studies on dormant roots indicate that very few cells are synthesizing DNA as compared with the active root (Table III). The presence of mitotic figures in dormant roots was somewhat unexpected at first. Presumably they repre-

sent either a small number of cells which divide for maintenance purposes, *i.e.* cellular replacement of surface cells which are sloughed off or become desiccated, or cells which remain in division for an indefinite period of time.

The average nuclear volumes also differed between active and dormant roots. The nuclei in cells of active roots averaged $733 \pm 25 \mu^3$ as compared with $491 \pm 31 \mu^3$ for the dormant roots. Given the dose required to reduce growth to 37 per cent of the control and the average nuclear volume, the amount of energy required to produce equivalent effects in actively growing and dormant roots can be estimated. For every roentgen, 1.77 ionizations occur per cubic micron of wet tissue, and for every ion pair, 32.5 electron volts are deposited in the tissue (5). Therefore, the product of nuclear volume (in μ^3), 1.77, 32.5, and the dose required to reduce growth 37 per cent will provide an estimate of the amount of energy required to produce this effect. The numbers of electron volts required for actively growing and dormant roots are 8.6×10^8 kev and 7.8×10^8 kev respectively. Radiobiological, histological, and cytological dif-

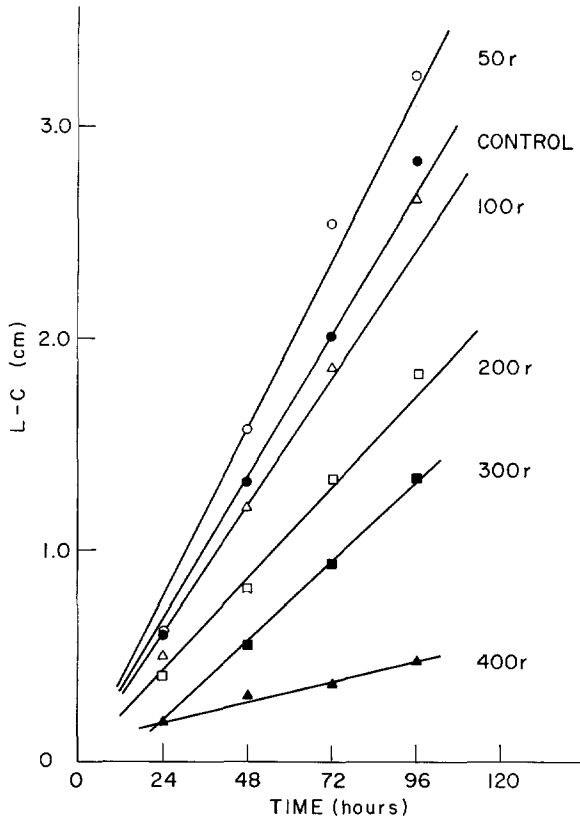


FIGURE 3 Increase in length of *Tradescantia* roots with time following exposures to 0 to 400 r of x-rays while in the dormant state. *L*, length of root; *C*, length of root at time zero.

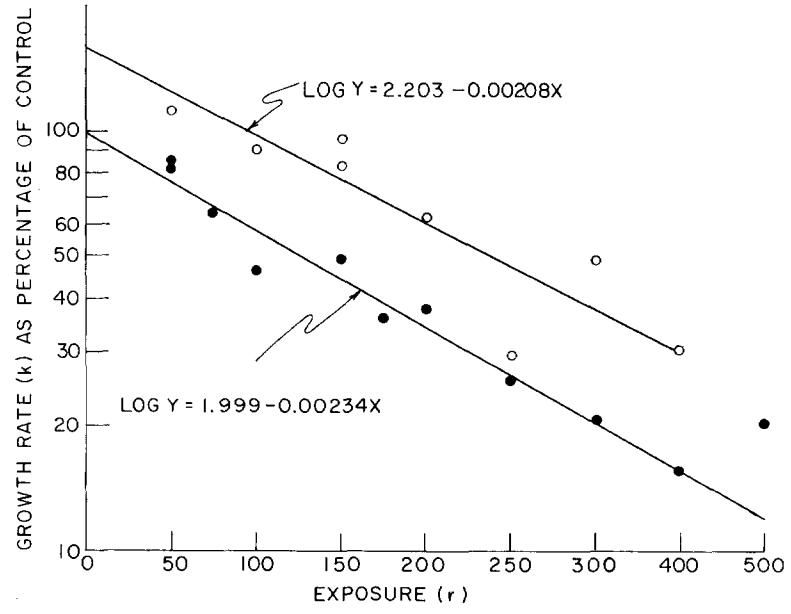


FIGURE 4 The effect, as percentage of control, of various x-ray exposures on roots irradiated while actively growing (closed circles) or while dormant (open circles). Equations are the linear regression models; *Y* is percentage of control; *X* is exposure.

ferences between dormant and actively growing roots are summarized in Table III.

Cell Kinetic Studies 4 Days after a D_{37} Growth Rate Dose

Equations 2 and 3 approximate the manner in which root growth rate, mitotic cycle duration, and the number of cells in the cycle are related, and were derived on the basis of simplifying assumptions. Irradiation was shown to decrease the growth rate, and therefore it remained to be determined which variables were altered. Van't Hof and Ying (14) have suggested that the number of cells in the cycle (N_{ct}) can be calculated using the following equation:

$$N_{ct} = N_T M \frac{CT}{D_m} \quad (4)$$

has been obtained by Van't Hof *et al.* (12) and Van't Hof and Ying (13). In the first study, a small tetraploid population was followed through three sequential cell divisions with no increase in the population size after the completion of each mitotic cycle. If exponential kinetics were involved, the population should have doubled after each division. In the second study, the number of potential tetraploid cells was measured and compared with the number of tetraploids observed after one complete mitotic cycle. The comparison showed that the number of tetraploid cells was one-half the number of potential tetraploids, indicating that after each division one daughter cell, on the average, differentiates and the other continues to proliferate.

The curves in Figs. 5 and 6 provide an estimate of the average mitotic cycle time, which is approxi-

TABLE III
Radiobiological, Histological, and Cytological Data from Dormant and Actively Growing Roots

	Dormant roots	Actively growing roots
Calculated nuclear volume	491 ± 31*	733.41 ± 25
Percentage of cells in mitosis	1.89 ± 1.66	7.23 ± 0.84
Percentage of cells synthesizing DNA ‡	1.89 ± 1.66	28.82 ± 3.52
X-Ray exposure (r) required to reduce growth rate to 37 per cent	305	184
Energy absorbed (kev) per nucleus at the D_{37}	8611 ± 539	7759 ± 261

* This volume relates very well with those of Swift (10) for cells of *Tradescantia* with a 2 C amount of DNA.

‡ For dormant roots this value represents the labeled interphase cells observed after 120 minutes in 4 $\mu\text{C}/\text{ml}$ $\text{H}^3\text{-T}$; for actively growing roots it represents the number of labeled cells observed after 30 minutes in 0.5 $\mu\text{C}/\text{ml}$ $\text{H}^3\text{-T}$.

where N_T is the total number of cells in the meristem, M is the proportion of cells in mitosis, CT is the average total cycle time, and D_m is the duration of mitosis.

Equation 4 considers only the entrance and exit rates of cells into the meristem compartment and not the distribution of cells within the mitotic cycle. In view of Johnson's (4) theoretical treatment of cell distribution within the cycle, it appears that *Tradescantia* root meristems are tissues wherein differentiation takes place late in interphase. Also, since the root meristem is not an exponentially expanding population, the exact nature of the distribution of cells in interphase is still unknown. Evidence supporting the notion that the root meristem is a tissue with steady state cell population kinetics and not an exponentially expanding population

mately 20.50, 19.40, 20.25, and 18.25 hours respectively for the active root control, the dormant root control, the irradiated active roots, and the irradiated dormant roots. The dormant roots were analyzed 4 days after growth was initiated by submersion in nutrient solution. These curves also provide estimates of the interphase periods (Table IV).

The curves in Fig. 7 provide information necessary for determining the duration of mitosis (D_m). The upper curve shows an increase in the number of $\text{H}^3\text{-T}$ -labeled prophase cells as they appear in division. Extrapolation of this curve to the abscissa gives an estimate of the minimum G2 duration. This minimum is 0.87 hour. The lower curve in Fig. 7 shows an increase in the number of labeled metaphase cells with time. Extrapolation of this

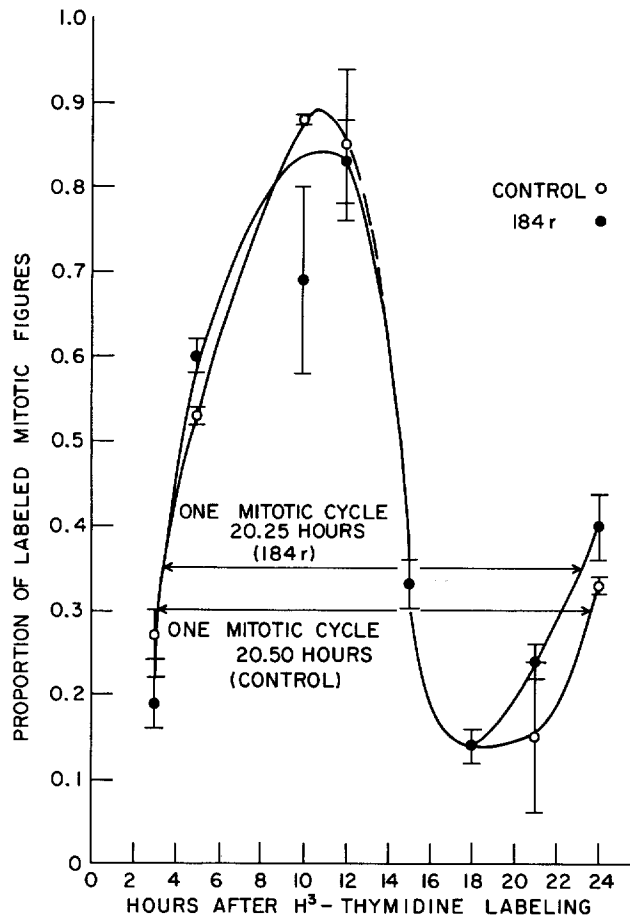


FIGURE 5 Mitotic cycle duration of control and irradiated actively growing roots.

curve indicates that it intercepts the abscissa at 2.07 hours. The difference between 0.87 hours and 2.07 hours is the minimum prophase duration, 1.2 hours. Cytological examination of 6 slide preparations indicated that approximately 0.60 of the mitotic figures were prophases, thus:

$$\frac{D_m}{1.2 \text{ hr.}} = \frac{1.0}{0.6}$$

The duration of mitosis (D_m) was thus determined to be 2 hours.

DISCUSSION

Cytological and autoradiographic analyses of the actively growing and dormant roots showed that neither the number of cells in mitosis nor the number of DNA-synthesizing cells at the time of irradiation could be correlated with the difference in radiosensitivity (Table III).

On the other hand, the experimental results show clearly that the difference in radiosensitivity between actively growing and dormant roots is related to the unequal nuclear volumes of the meristematic cells (Table III). Sparrow *et al.* (9) showed that actively growing and dormant shoots of *Pinus strobus* displayed different radiosensitivities which could be related to the different nuclear volumes of the shoot meristem cells. The ratio of the change in average nuclear volumes for active cells to that for dormant cells was approximately 1.8. The tolerance of the dormant plant was approximately 1.5 times that of the actively growing plant. For *Tradescantia paludosa* the ratio of the nuclear volumes is about 1.5; the tolerance of the dormant roots is about 1.6 times that of the actively growing roots (Table III). The very close agreement of these ratios for two species offers further evidence supporting the concept that radiosensitivity is a reflection of nuclear size.

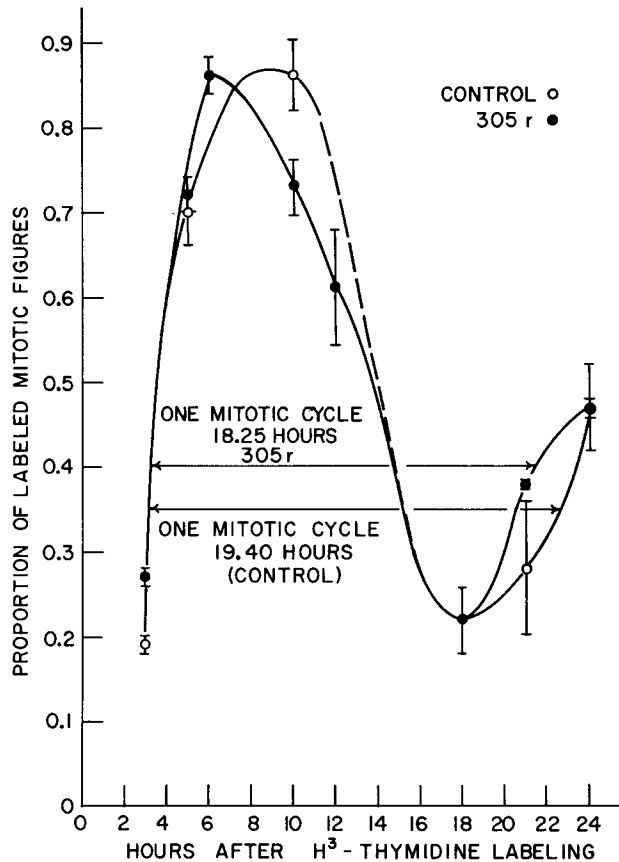


FIGURE 6 Mitotic cycle duration of control and roots irradiated while in the dormant state.

Recently the concept that radiosensitivity is the reflection of nuclear size has been restated and another notion added (8). The addendum states that although radiosensitivity is a function of nuclear volume (where no variation in chromosome number exists), the amount of energy deposited within a nucleus required to produce a given effect approaches a constant. Thus, in calculating the amount of energy absorbed (in electron volts) required to reduce growth to 37 per cent of the control, the values for actively growing and dormant roots were found to be similar (Table III).

Because the proliferative capacities of the dormant and actively growing roots at the time of irradiation were vastly different and because the amount of energy absorbed which produced an approximately equivalent effect in these roots was almost the same (Table III), an addition to the Bergonie-Tribondeau law seems necessary. The addition should state that embryonic or meristematic cells will reflect, in their radiosensitivity,

differences in nuclear volume which exist at the time of irradiation.

Although differences in nuclear volume offer a satisfactory explanation for the increased tolerance of dormant over actively growing roots, there remains the problem of explaining the general phenomenon of reduced growth rate of roots. The question still to be answered is whether the reduction in growth rate brought about by ionizing radiation is the result of a decrease in the rate of cell proliferation or a decrease in cellular elongation or a combination of the two. In order to determine which of these possibilities was responsible for the reduced growth rate, three determinations must be made: (a) the growth rate must be shown to be linear as stated in equation 2; (b) the mitotic cycle duration must be measured; and (c) the number of cells in the mitotic cycle must be estimated. Linear growth rate is an assurance that the rate of cell production (N_{ct}/CT) and that of cell elongation are proceeding in a constant fashion. Figs. 2

TABLE IV
Duration in Hours of Total Cycle, Mitosis, and Interphase Period of Irradiated and Control Actively Growing and Dormant Roots

Roots	Interphase period*			Mitosis‡	Total cycle
	G1	S	G2		
Actively growing					
Control	5.8	10.1	2.6	2	20.50
184 r	5.6	10.5	2.2	2	20.50
Dormant§					
Control	4.4	11.4	1.6	2	19.4
305 r	3.25	11.4	1.6	2	18.25

* G1: Total cycle time - (S + G2 + M).

S: time span between the 0.5 intercepts of the rising and descending portions of the labeled mitotic figure curves (Figs. 5 and 6).

G2: time interval between time zero and the 0.5 intercept on the rising portion of the same curves minus the duration of mitosis.

‡ M: determined by use of Fig. 7 and equation 1.

§ Dormant roots were analyzed 4 days after growth was initiated by submersion in nutrient solution.

TABLE V
Summary of Cell Kinetic Data Four Days after a D_{87} Exposure

	Control roots		Irradiated roots	
	Active	Dormant	Active	Dormant
Proportion of cells in mitosis (M)	0.067	0.066	0.068	0.071
Number of cells in mitotic cycle (N_{ct})*	58,300	54,300	29,300	24,075
Duration of mitosis (D_m) in hours	2.0	2.0	2.0	2.0
Average mitotic cycle time (CT)	20.5	19.4	20.25	18.25
Rate of cell production (N_{ct}/CT)	2,840	2,800	1,445	1,320

* Calculated with the use of equation 4.

and 3 and Tables I and II show that a linear rate of growth does, in fact, occur at all exposures used in these experiments. Therefore, the mitotic cycle duration (CT) and the number of proliferating cells (N_{ct}) were determined on the 4th day after x-ray exposure. The data in Table V indicate clearly that the mitotic cycle duration of the cycling cells was unchanged by exposures to x-rays which substantially reduced the growth rate. However, the number of cells moving through the mitotic cycle did show a decrease, with the consequence that the rate of cell production was also reduced. It may be concluded, then, that the lower growth rate produced by irradiation was primarily due to a decrease in number of cells passing through the mitotic cycle. Moreover, since the rate of cell production was almost equally reduced by two exposures equal with respect to effect but unequal

with respect to dose, additional evidence was presented supporting the concept that the production of similar effects in different nuclei occurs when the nuclei have absorbed an equivalent amount of energy (8). The calculated amount of energy absorbed in dormant root nuclei was about 8.6×10^3 kev for a 305-r exposure; for actively growing roots it was about 7.8×10^3 kev for a 184-r exposure. (This difference is not statistically significant at the 0.05 level.) After these exposures, the rate of cell production dropped from the control level of 2.8×10^3 to 1.3×10^3 and from 2.8×10^3 to 1.4×10^3 cells per hour, respectively, for dormant and actively growing roots.

As pointed out in Table III, the average calculated nuclear volume for the dormant roots was $491 \mu^3$. This value is in close agreement with those of Swift (10) for telophase nuclei in *Tradescantia*

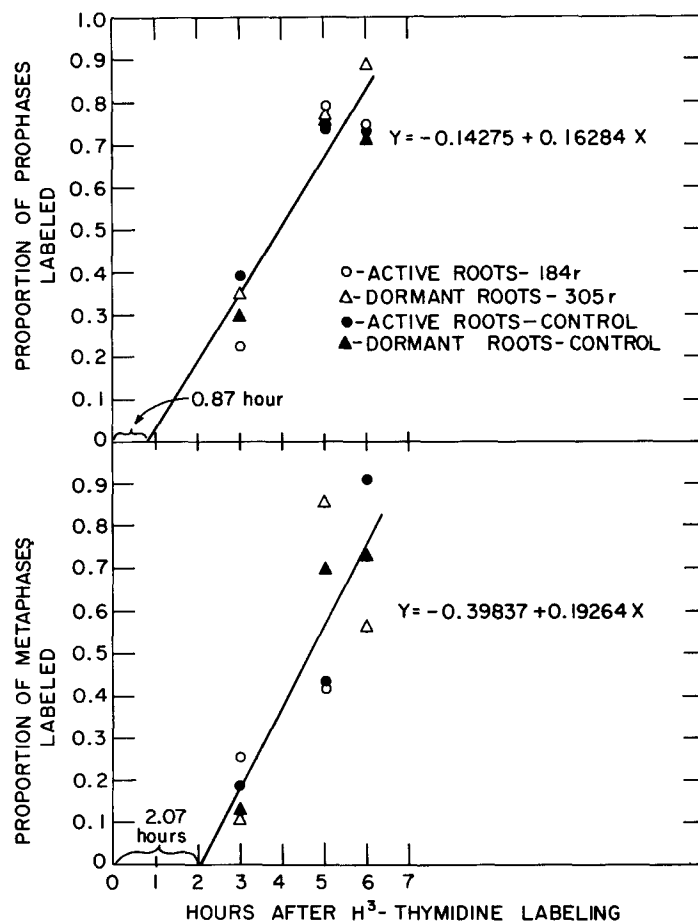


FIGURE 7 Upper curve: increase in number of labeled prophase cells with time. Lower curve: increase in number of labeled metaphase cells with time. Equations are the linear regression models; see text for explanation.

paludosa root meristems, and therefore represents cells with a 2 C amount of DNA. The very low number of cells synthesizing DNA and the very low mitotic frequency support this hypothesis.

Thus it is relatively sound to assume that dormant roots contain cells with a 2 C amount of DNA and actively growing roots contain cells with varying amounts of DNA ranging from 2 C to 4 C. The distribution of the DNA content per cell in an actively growing root can be estimated if the population is asynchronous and if the duration of the period of mitosis, G1, S, and G2 are known. Tables III and IV provide the necessary information about the period durations, and it is assumed that the dividing population is asynchronous. The total cycle time is equated to 1 and the duration of each

period or phase is expressed as some fraction of 1. These proportions provide an estimate of the distribution of cellular DNA levels in the active root (Table VI).

Because very few cells were synthesizing DNA in the dormant roots, an explanation for the difference in radiosensitivity between actively growing and dormant roots is that the latter are composed mostly of G1 cells, *i.e.* cells which have not replicated their DNA. Thus the target offered to the x-rays would be less than that in an actively dividing population. In fact, the difference between the average populations with respect to the average amount of DNA per cell is very near to 30 per cent, or, looking at it another way, the average DNA per cell of the actively growing roots is about

TABLE VI
*Estimates of the Relative DNA Content of
 Cells Occupying the Meristems of Actively
 Growing and Dormant Roots*

Root	Proportion of dividing population*	DNA level	
		2 C	4 C
Actively growing			
Prophase	0.058	—	0.058
Metaphase	0.039	0.039	—
Ana-telophase			
G1 period	0.283	0.283	—
S period	0.494	0.247	0.247
G2 period	0.126	—	0.126
Total	1.00	0.569	0.431
	Average (A) †	2.86	
Dormant			
Prophase	0.019	—	0.019
Metaphase	—	—	—
Ana-telophase			
G1 period §	0.962	0.962	—
S period	0.019	0.010	0.010
G2 period	—	—	—
Total	~1.00	~1.00	~0.00
	Average (D) †	2.0	

* The total cycle duration was equated to 1 and the duration of each period or phase was expressed as some fraction of 1.

† $A/D = 1.43$.

§ Most cells are considered to be in G1 period because (a) the nuclear volume averages are low, (b) very few cells are in S, and (c) Swift (10) has shown that the smaller nuclei in an asynchronous population have not replicated their DNA.

1.43 times that of the dormant root cells (Table VI). This value corresponds well to the 1.6 increase in radiation tolerance exhibited by the dormant roots.

The reduction in the number of cells in the mitotic cycle after irradiation even up to 4 days after exposure implies that the meristem as a tissue was unable to divert cells from the path of differentiation back into the mitotic cycle. If such a diversion should occur, a non-linear growth rate would be expected. The present question, however, is, what happened to those cells which were removed from the cycle by ionizing radiation? Examination of the regression equations in Tables I and II, as well as the curves in Figs. 2 and 3, reveals two interesting but somewhat nebulous

responses to ionizing radiation. The first is that the growth curves for the irradiated actively growing and dormant roots do not extrapolate back to zero. The second response which is of interest is the apparent stimulation of growth by irradiation of the dormant roots with low doses of x-rays.

The inability to extrapolate the growth curves back to zero probably reflects an interesting cellular response to ionizing radiation by cells which were proliferative but, because of radiation damage, now presumably differentiate and elongate. The latter phenomenon can occur because of its insensitivity to irradiation (3). The net results of such an event would be these: (a) an apparent increase in growth soon after irradiation due to additional cells entering the stage of elongation; (b) a concomitant decrease in the number of cells in the mitotic cycle; and (c) a constant but decreased growth rate following the exodus of cells from the cycle into differentiation. The positive values for the actively growing roots at time zero after most doses could be explained in this manner. Little can be said, however, about the initial events after irradiation of dormant roots, since 3 days intervened between exposure to x-rays and the commencement of root length measurements.

The apparent stimulation of growth rate following the exposure of dormant roots to low doses of x-rays requires a less precise and more speculative explanation. The stimulation of dormant roots with low doses of x-rays now joins observations made by others who irradiated dormant cells of seeds and bulbs and produced a stimulation of growth (7). Although the explanations of stimulation are at present unsatisfactory, they should eventually include alterations in the kinetic parameters discussed in this paper; that is, an increased growth rate must be due to either an increase in the number of cells in the mitotic cycle (N_{ct}), or a decrease in cycle duration (CT), or an increase in the average cell length at maturity. Once these parameters are evaluated, further investigations should be conducted to determine the system responsible for the changes observed.

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