are of biological origin but have entered the ancient sediments during modern times.

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12. Ages are estimated from information given in (*3*) and from a limited number of radiocarbon ages provided by Dr. A. W. Fairhall, Univ. of Washington, Seattle, obtained on samples from the same core on which this study was based.
13. We thank Mr. G. E. Pollock for providing the gas chromatographic columns for this work and for his counsel; we also thank Mrs. N. Shaltiel, UCLA, who performed amino acid analyses by ion exchange chromatography.

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Woody Plants: Changes in Survival in Response to Long-Term (8 Years) Chronic Gamma Irradiation

Abstract. *The number of plant deaths which occurred over 8 years of chronic gamma irradiation (20 hours/day) of 11 species of woody plants indicated a decline in the rate of death with increasing exposure time. This suggests that a highly effective repair system may develop, at least in the range of exposure which reduces survival by 50 percent. The inverse relationship previously found between interphase chromosome volume and radiosensitivity for single 16-hour exposures was confirmed for chronic exposures by construction of appropriate regressions. Radiosensitivity of a species can be predicted from these regressions if the interphase chromosome volume is known. The distributions of interphase chromosome volumes and predicted sensitivities are given for 215 species of woody plants.*

Observations on 11 species of woody plants made over 8 years have shown that the rate of death (reflected by the exposure required to reduce survival by 50 percent, the LD_{50}, expressed in average roentgens per 20 hour day) decreases with time. Earlier work (*1*) has established the inverse correlation between radiosensitivity (measured as LD_{50}) after single 16-hour cobalt-60 gamma irradiations and interphase chromosome volume for 28 species of woody plants. The extension of this correlation to woody plants exposed to daily gamma irradiation for up to 8 years has now been made.

Eleven species of woody plants (six angiosperms and five gymnosperms, Fig. 1), either seedlings or clonal propagations 2 to 5 years old, were transplanted into a field (*2*) on isodose arcs at various distances from a large (about 3800 curies) cobalt-60 gamma source. Average daily rates of exposure for each year were calculated for a year beginning 1 June and ending 31 May. We calculated accumulated exposures by multiplying the average daily rate of exposure at specified distances from the gamma source for any particular year by 365, and then adding these products.

Each year, in late spring or early summer after growth had begun, all new plant deaths were recorded. Various survival end points were determined from the survival curves constructed for each species for each year of chronic irradiation (*3*); only LD_{50} values are considered here. We determined the interphase chromosome volumes by calculating nuclear volumes from measurements of nuclei in sectioned shoot meristems and dividing the mean nuclear volume by the chromosome number characteristic of that species (*1*).

In some species, survival between the 1st and 2nd year or between the 2nd and 3rd year (or both) decreased appreciably. For this and other reasons (*3*), 3rd-year values were used to express relative radiosensitivities. The regressions for LD_{50} in average roentgens per day and accumulated kiloroentgens on interphase chromosome volume (both not significantly different from a -1 slope at the 5 percent level) are shown in Fig. 1. There is a clear inverse correlation between interphase chromosome volume and LD_{50} which accounts for the higher radiosensitivity of gymnosperms compared to angiosperms. The average LD_{50} for the five species of gymnosperms is 13.1 r/day, whereas that for the six angiosperms is 96 r/day. The two groups differ in sensitivity by a factor of over 7. Comparable relationships have been shown for woody plants given a single 16-hour radiation exposure (*1, 3*).

The most sensitive species examined had an LD_{50} of 7.2 r/day and an LD_{10} of 4.1 r/day. However, a reduction in total plant growth and, hence, in the amount of potential photosynthetic tissue occurred at much lower rates of exposure. Considerable reduction in growth at exposures less than half the LD_{50} occurred in a number of species. After 4 years of chronic irradiation the LD_{50} exposure for *Picea glauca* was 12.0 r/day, and the LD_{10} was 7.5 r/day. Exposures of 5.6 and 2.9 r/day, the latter less than half the LD_{10}, considerably reduced overall growth (Fig. 2). A severe reduction in photosynthetic leaf tissue resulting in slow starvation has been suggested as a possible cause of death in chronically irradiated trees (*4, 5*).

From comparable regressions determined for each year of irradiation, it became evident that the LD_{50} values based on roentgens per day changed more rapidly during the first 3 years than during later years of chronic irradiation. The average LD_{50} decreased by 30 percent by the 3rd year but by only 16 percent more during the next 5 years. Although the number of species available for computation of the regressions is smaller for the 7th and 8th years, the pattern of decrease is quite consistent. The regressions based on accumulated kiloroentgens, however, change considerably over the 8 years and change in the opposite direction. Thus, it becomes apparent that care must be taken when exposures are accumulated over a long period, since an exaggerated indication of the resistance of the species being examined may be given; that is, the longer the exposure period, the higher the accumulated exposure required to produce an LD_{50}.

The years of chronic irradiation were plotted against the ratio to the 1st-year

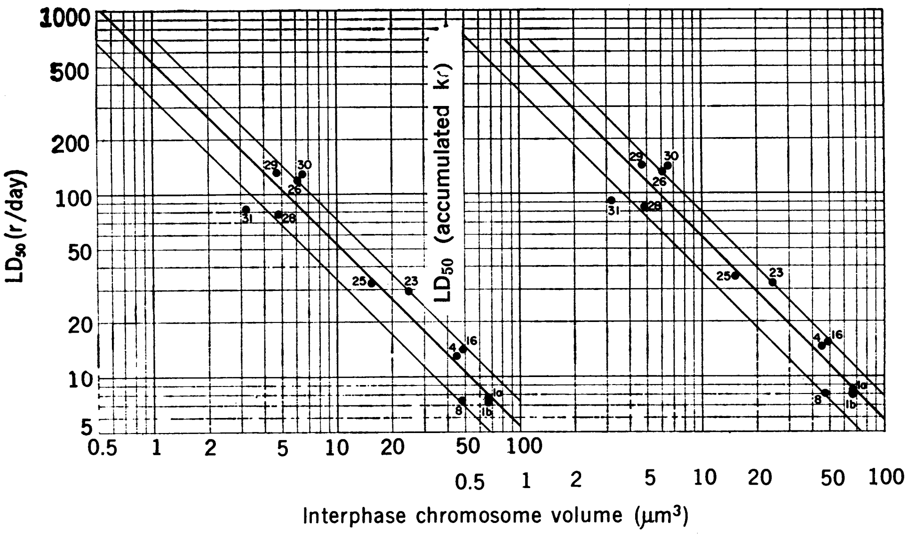

Fig. 1 (left). Regressions of 3rd-year LD$_{50}$ values in roentgens per day and accumulated kiloroentgens on interphase chromosome volume for 11 species of woody plants irradiated chronically. Slopes = −1. Parallel lines are the mean standard deviations. (A) Angiosperm; (G) gymnosperm. Listed in order of increasing interphase chromosome volume: 31, *Betula lutea* Michx. f. (A); 29, *Fraxinus americana* L. (A); 28, *Acer saccharum* Marsh. (A); 26, *Quercus borealis* Michx. f. var. *maxima* (A); 30, *Acer rubrum* L. (A); 25, *Viburnum dilatatum* Thunb. (A); 23, *Thuja occidentalis* L. (G); 4, *Picea glauca* (Moench) Voss (G); 8, *Pinus resinosa* Ait. (G); 16, *Larix leptolepis Gord.* (G); 1, *Pinus strobus* L. (G).

LD$_{50}$ for both average roentgens per day and accumulated kiloroentgens. Over 8 years, the LD$_{50}$ in average roentgens per day decreased by 46 percent; the decrease from the 2nd to the 8th year averaged only about 3.3 percent per year, whereas the change in accumulated kiloroentgens for the 8-year period is 4.3 times the 1st-year value. It is significant that after the 2nd year of irradiation, the rate of decrease in daily exposure to produce an LD$_{50}$ becomes progressively smaller, so that between the 7th and 8th year of irradiation there is virtually no change in survival (53 and 54 percent of the 1st-year value for 7th and 8th years, respectively). Whether an equilibrium between injury and repair has been reached such that the plants surviving after 8 years of irradiation will continue to survive by compensating in some manner for the radiation damage, or whether a slow decline in survival with additional years of irradiation will occur later cannot be stated definitely.

The relationships described above do make it possible to predict from the interphase chromosome volume the probable sensitivity (LD$_{50}$) of woody plants to chronic gamma irradiation. Such data for 215 species of gymno-

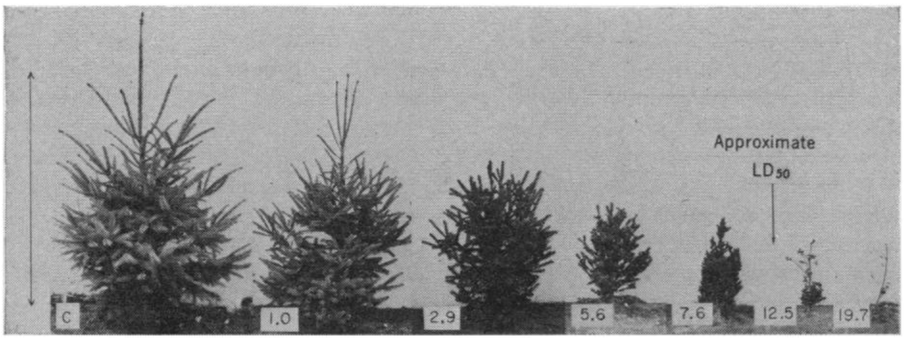

Fig. 2. Composite photograph of representative trees of *Picea glauca* after 4 years of chronic irradiation. Arrow at left indicates 1 m.

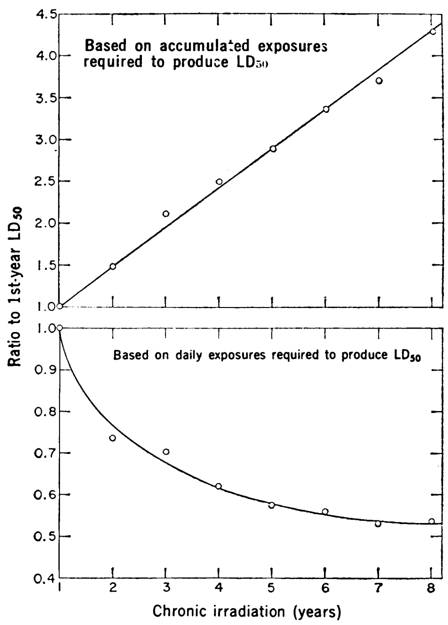

Fig. 3. The changing relationships between roentgens per day and accumulated kiloroentgens at LD$_{50}$ for 8 years of chronic irradiation. All values are expressed as ratios of the 1st-year LD$_{50}$ (= 1.0).

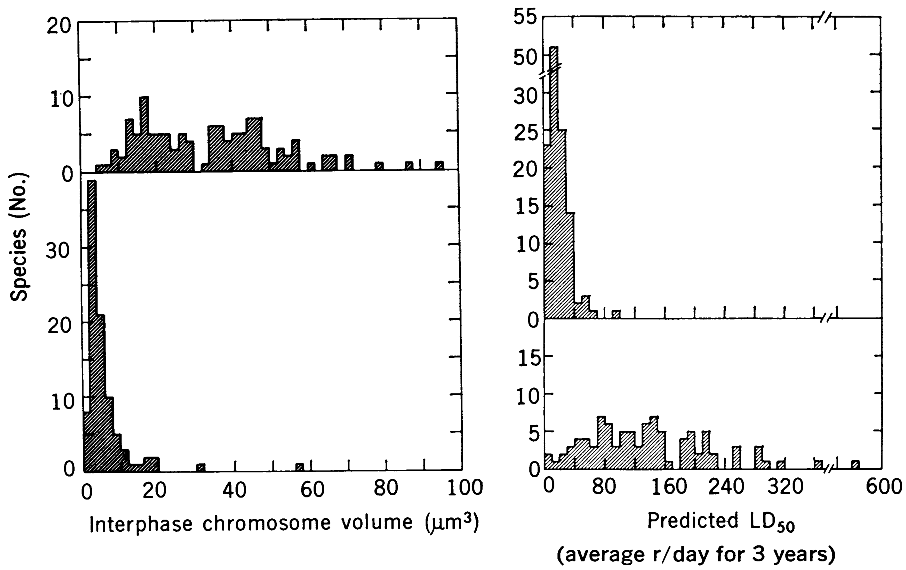

Fig. 4 (left). Histograms of the distribution of interphase chromosome volumes (*1*) and predicted LD$_{50}$'s (in average roentgens per day for 3 years of chronic irradiation) for 120 species of gymnosperms and 95 species of woody angiosperms (increments of 2 μm^3 and 10 r/day).

sperms and angiosperms are given elsewhere (*1*) and have been used to predict the expected sensitivity to chronic irradiation. Because most of the gymnosperms examined so far have relatively large interphase chromosome volumes compared to most of the angiosperm species, the predicted sensitivities for both groups show very little overlap (Fig. 4). Whereas 94 percent of the gymnosperms have predicted 3rd-year values of 40 r/day or below, only 8 percent of the angiosperms have predicted values this low. Of the predicted gymnosperm values, 75 percent are between 11 and 40 r/day; of the angiosperm values, 63 percent are between 101 and 570 r/day. The ability to make such predictions for previously unirradiated species should be useful in the planning of various kinds of experiments including those in which woody plants of economic importance are irradiated to produce useful mutations.

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Crystal and Molecular Structure of a Thymine Phototrimer

Abstract. *Thymine trimer was isolated from a frozen aqueous solution of thymine which was irradiated with ultraviolet light and was presumably formed through the rearrangement of an initial oxetane photoproduct. X-ray diffraction analysis of a single crystal has confirmed the trimeric diol structure and has established the stereoconfiguration of the molecule. The possible importance of the diol structure in photobiology is pointed out.*

Irradiation of [2-^{14}C]- or [5-methyl-^{3}H]-thymine in frozen aqueous solution with ultraviolet light (254 nm) produced two detectable products (*1–3*). These were designated as photoproduct of thymine No. 1 (PT_1) [R_F, 0.15 in a solution of n-butanol, acetic acid, and water (80 : 12 : 30) on a paper chromatogram] and photoproduct of thymine No. 2 [R_F, 0.29] (*2*). The latter was a mixture of *cis-syn*-thymine dimer and thymine-thymine adduct (T-T adduct) (*4*). The characterization of T-T adduct (*4, 5*) was essential for the characterization of PT_1 (*6*) with its intriguing properties (*7*). For structural proof, we analyzed a crystal of PT_1 by x-ray diffraction.

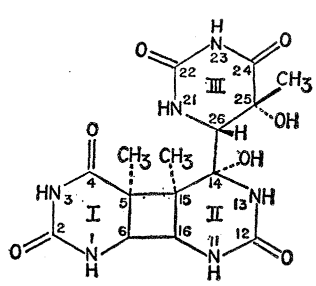

Fig. 1. Stereoconfiguration of the thymine trimer.

X-ray diffraction data for a single crystal of the thymine trimer were collected on a four-circle, fully automated diffractometer (Table 1). Because of the extremely small size of the crystal, only 38 percent of the total data possible within the CuKα 1.5418 Å sphere was obtained. The structure was solved directly by the symbolic addition procedure (*8*). Atomic coordinates and anisotropic thermal parameters were refined by least-squares methods, and all of the hydrogens except those on N-3 and one of the water molecules were located in a difference map. The final *R* factor (agreement between observed and calculated structure factors) for the observed data is 7.9 percent.

The thymine trimer (Fig. 1) shows similarities to both the T-T adduct (*5*) and the cyclobutyl-type dimers. The cyclobutane ring is only slightly puckered with a dihedral angle of 173.5°. This is significantly flatter than the four-membered rings in free dimers of the *cis-syn*-cyclobutyl type—for example, 155° in the uracil dimer (*9*) and 152° to 153° in the dimethylthymine dimer (*10*). However, it is not as flat as the 178° reported for the four-membered ring in the *cis-syn* intramolecular bridged dimer of 1,1′-trimethylenebisthymine (*11*). Rings I and II are rotated with respect to each other by torsion angles of 4.8° and 6.8° about the C-5–C-15 and C-6–C-16 bonds, respectively. Bond distances involving ring III and its substituents in the trimer are very similar to those involving ring I in the T-T adduct. The OH group on C-14 is axial to ring II while the methyl group on C-15 is equatorial to ring II. With respect to ring III, the methyl group on C-25 is axial and the hydroxyl group on the same carbon is equatorial. A water molecule crystallizes with the trimer and is included in the extensive system of hydrogen bonding in the crystal. There are also two intramolecular hy-

Table 1. Physical constants of PT_1.

Molecular formula	$C_{15}H_{20}N_6O_7 \cdot H_2O$
Molecular weight	414.38
Habit	Acicular
Crystal size (mm)	0.44 by 0.06 by 0.03
Space group	$P\bar{1}$
a	9.373 ± .004 Å
b	14.387 ± .004 Å
c	7.201 ± .003 Å
α	103.20°
β	100.00°
γ	91.80°
Molecules/unit cell	2
Density (calc.)	1.48 g/cm³
No. of independent reflections	1636

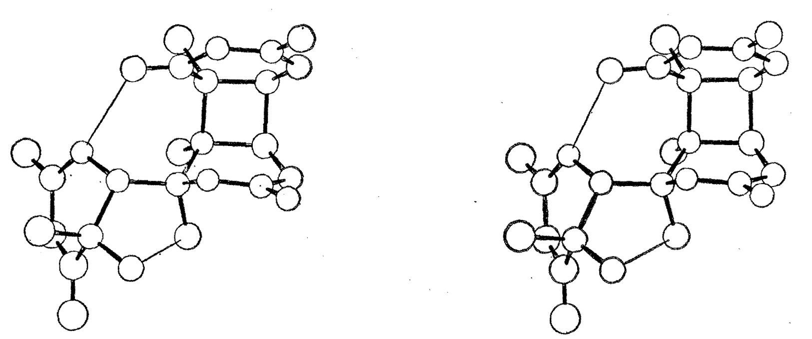

Fig. 2. Stereodiagram of the configuration of the thymine trimer as determined by x-ray analysis. This picture should be seen with a three-dimensional viewer for printed stereophotographs (*12*).