All the evidence obtained during leg 2 of DSDP points, however, to an early to middle Eocene age for the reflector identified here as horizon A. At site 8 turbidites are as much a part of the horizon A chert as the Radiolaria-rich siliceous ooze. But sites 9 and 10, which are farther from the continent, show little or no evidence for turbidite origin of the horizon A chert. The conclusion seems to be, therefore, that siliceous ooze sedimentation is really the factor determining the nature of horizon A and that turbidite association is only a function of proximity to the continent.

Ewing (*8*) has suggested that horizon A splits into 2 reflectors on the Bermuda Rise, the upper one being mid-Eocene in age. Indeed, a middle Eocene reflector is developed at site 9, but the lower reflector seems to be represented only by some siliceous radiolarian sediments of Upper Cretaceous age. The lower reflector is absent at site 10.

Alternatively, it is possible that horizon A actually was never cored in the outcrop area, and the Upper Cretaceous sediments recovered there come from a stratigraphic level lower than horizon A. Lack of chert or siliceous ooze in any of the piston cores taken in the outcrop area may support this view or may be due entirely to coring difficulties.

The existence of an early to middle Eocene reflector (horizon A), synchronous in a broad sense and consisting of one or more chert beds, seems well established. This reflector appears continuous over very large parts of the western North Atlantic, and possibly over parts of the East Atlantic as well. This reflector shows the least amount of chertification at site 10, a site that seems not to have been below the carbonate dissolution level at any time and that is farthest from continental influences. The most pronounced development of the reflector is at sites 8, 9, and 12, all of which were below the carbonate dissolution level at the time this reflecting layer was deposited and one of which (site 8) also received turbidite sediments.

STEFAN GARTNER, JR.
*Rosenstiel School of Marine and Atmospheric Sciences, Miami, Florida*

**References and Notes**

1. M. N. A. Peterson *et al.*, *Initial Reports of the Deep Sea Drilling Project* (Government Printing Office, Washington, D.C., 1970), vol. 2.
2. J. R. Heirtzler and D. E. Hayes, *Science* **157**, 185 (1967).
3. The age assigned to the *Orbulina* datum in (*1*) is 18 million years, but W. A. Berggren [*Nature* **224**, 1075 (1969)], on the basis of recent radiometric dates, has revised the age to 14 million years.
4. J. Ewing and M. Ewing, *Science* **156**, 1590 (1967).
5. J. D. Hays and N. D. Opdyke, *ibid.* **158**, 1001 (1967).
6. J. Ewing, J. L. Worzel, M. Ewing, C. Windisch, *ibid.* **154**, 1125 (1966).
7. T. Saito, L. H. Burckle, M. Ewing, *ibid.*, p. 1173.
8. M. Ewing, in C. L. Drake, *ibid.* **163**, 596 (1969).
9. Supported by NSF grant GA-1603. Contribution No. 1241, Rosenstiel School of Marine and Atmospheric Sciences, University of Miami.

20 July 1970

## Racemization of Amino Acids in Sediments from Saanich Inlet, British Columbia

Abstract. *In sediments spanning the last 9000 years from Saanich Inlet* L *enantiomers of amino acids are most abundant, but the percentages of* D *enantiomers increase with age, apparently because of partial racemization. Of the amino acids measured, glutamic acid and alanine show the greatest degree of racemization; leucine, isoleucine, and valine show the least.*

Core samples of sediments ranging from 0 to 34.8 m deep from Saanich Inlet, British Columbia, contain a number of amino acids. With exceptions such as glycine and $\beta$-alanine, these amino acids have at least one asymmetric center and, therefore, can exist as enantiomers generally designated D and L. As in living systems, the L enantiomers are most abundant in these sediments, indicating biological precursors. With depth, and consequently with age, the abundance of D enantiomers relative to L enantiomers increases slightly, apparently a reflection of partial racemization during the last 9000 years.

The presence and distribution of amino acids in cores of modern marine sediments have been documented (*1*). The degree of racemization of amino acids has been followed by enzymatic techniques in fossil *Mercenaria* shells from three ages—Recent, Upper Pleistocene, and Miocene (*2*). The percentages of D enantiomers increased from 0 in Recent shells to 20 to 40 percent in Upper Pleistocene shells to 50 percent in Miocene shells. The object of the present work was to find out whether by gas-liquid chromatographic techniques, the degree of racemization during diagenesis of marine sediments can be detected within the first several thousand years.

Saanich Inlet is a stagnant fjord of Puget Sound on the coast of British Columbia where sediments are accumulating (*3*). A comprehensive geochemical study has been made of this inlet (*4*). Amino acids were one of a number of classes of compounds investigated. Four samples were selected for the determination of enantiomers of amino acids (Table 1). Core 4 (0 to 0.15) is from the top 15 cm of a 2.5-m surface core. Core 3B (7.9 to 8.2), core 3B (17.1 to 17.4), and core 3B (34.5 to 34.8) are from a deep core; the sample name includes the depth in meters below the sediment-water interface at which the sample was taken (*4*).

Sediment samples were first extracted with a mixture of benzene and methanol (7 : 3). The residue was dried and extracted twice with 1*N* HCl to remove free amino acids; these free amino acids were not examined for ratios of enantiomers. Subsequently, the bound amino acids were isolated by hydrolyzing the sediment in 6*N* HCl for 22 hours at 110°C. The acid hydrolyzate was separated from the sediment residue by filtration. The HCl was removed from the hydrolyzate at reduced pressure on a rotary evaporator. The residue was dissolved in citrate buffer and chromatographed on a Dowex 50 ($H^+$) column which was eluted with $H_2O$ followed by 0.2*N* $NH_4OH$. The ammonium hydroxide fractions, containing amino acids, were combined, concentrated, and chromatographed on a Dowex 1 ($OH^-$) column which was eluted with $H_2O$ followed by 0.1*N* HCl. The hydrochloric acid fractions, containing amino acids, were combined, concentrated, and evaporated to dryness in a vacuum desiccator at 40°C. Portions of each sample dissolved in citrate buffer were analyzed on an automated amino acid analyzer (Beckman 120C), and the concentration of each amino acid (Table 1) was calculated from the resulting chromatograms.

Although 20 amino acids were found in the samples only 13 are reported here; these are the amino acids whose presence was easily confirmed by gas-liquid chromatographic techniques. The enantiomeric distribution of seven of these amino acids was estimated. For

Table 1. Percentages of D amino acid enantiomers and concentrations (nmole/g) of amino acids in Saanich Inlet sediments. The approximate age and weights of the cores are: core 4 (0 to 0.15), < 100 years, 23.5 g; core 3B (7.9 to 8.2), 2000 years, 50.0 g; core 3B (17.1 to 17.4), 4500 years, 43.1 g; core 3B (34.5 to 34.8), 9000 years, 78.4 g. Percentage of D amino acids (%D) includes the effects of the L-2-butanol impurity of the derivatives (except for alloisoleucine) and possible 2 to 4 percent racemization from acid hydrolysis (except for isoleucine).

| Amino acids | | Core 4 (0 to 0.15) | | Core 3B (7.9 to 8.2) | | Core 3B (17.1 to 17.4) | | Core 3B (34.5 to 34.8) | | Difference between %D of core 3B (34.5 to 34.8) and core 4 (0 to 0.15) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | %D | Conc. | %D | Conc. | %D | Conc. | %D | Conc. | |
| α-Alanine | (Ala) | 6.6 ± 0.3 | 232 | 8.2 ± 0.4 | 221 | 9.8 ± 0.3 | 233 | 13.5 ± 0.5 | 46 | 6.9 ± 0.8 |
| Valine | (Val) | 6.0 ± 0.4 | 228 | 7.0 ± 0.5 | 93 | 7.7 ± 0.5 | 154 | 7.9 ± 0.4 | 49 | 1.9 ± 0.8 |
| Isoleucine | (Ileu) | 3.8 ± 0.2* | 184 | 3.5 ± 0.3* | 64 | 3.6 ± 0.5* | 117 | 3.7 ± 0.4* | 47 | ~ 0 |
| Leucine | (Leu) | 6.6 ± 0.4 | 275 | 5.7 ± 0.3 | 118 | 7.0 ± 0.2 | 178 | 8.5 ± 0.3 | 66 | 1.9 ± 0.7 |
| Threonine | (Thr) | | 112 | | 40 | | 36 | | 3 | |
| Proline | (Pro) | 6.9 ± 0.4 | 249 | 5.8 ± 0.3 | 92 | 9.0 ± 0.5 | 124 | 9.8 ± 0.4 | 27 | 2.9 ± 0.8 |
| Serine | (Ser) | 8.1 ± 0.3 | 265 | 8.7 ± 0.5 | 54 | | 34 | | 3 | |
| Aspartic acid | (Asp) | | 490 | | 75 | | 24 | | 3 | |
| Phenylalanine | (Phe) | 6.0 ± 0.5 | 114 | 6.4 ± 0.6 | 74 | 7.2 ± 0.5 | 105 | 9.3 ± 0.6 | 33 | 3.3 ± 1.1 |
| Glutamic acid | (Glu) | 8.1 ± 0.9 | 387 | 8.9 ± 1.2 | 106 | 10.8 ± 1.2 | 10 | 18.2 ± 1.0 | 11 | 10.1 ± 1.9 |
| Alloisoleucine | (Aileu) | 1.9† | 4‡ | 1.9† | 1‡ | 2.0† | 2‡ | 3.3 ± 0.6† | 2‡ | 1.4 ± 0.6 |
| Glycine | (Gly) | | 472 | | 293 | | 285 | | 46 | |
| β-Alanine | (β-Ala) | | tr | | 4 | | 10 | | 4 | |

* (Peak at D-Ileu position)/[(Peak at D-Ileu position) + (L-Ileu)] × 100; measured on chromatograms from XE-60 column. † (D-Aileu)/[(D-Aileu) + (L-Ileu)] × 100; measured on chromatograms from XE-60 column. Only single determinations were made for three samples. ‡ Calculated from percentage of D-Aileu, using concentrations of Ileu from this table.

this work diastereomeric derivatives of the enantiomeric amino acids were made by esterification with D-2-butanol and acylation of amino and hydroxyl groups with trifluoroacetic anhydride (*5*). Subsequently the *O*-trifluoroacetyl groups were removed by methanolysis and the resulting hydroxyl groups were acylated with acetyl chloride (*6*). Previous work with this gas chromatographic technique (*5*) established that the order of peak elution for diastereomers of amino acids is DD and LL superimposed and LD and DL superimposed. The first letter of a pair indicates the configuration of the amino acid, and the second letter the configuration of the alcohol. Because the D-2-butanol contained about 4 percent L-2-butanol, diastereomers containing each enantiomeric alcohol were formed. Gas-liquid chromatography of the amino acid derivatives was carried out for all samples on three capillary columns, each coated with a different liquid phase—UCON 75H-90,000, XE-60, and Carbowax 20M. Under the gas-liquid chromatographic conditions used, no single column completely resolved the mixture of amino acid derivatives. By the use of three different gas chromatographic liquid phases, however, most amino acid derivatives could be resolved from each other and from unidentified compounds, thereby increasing the reliability in peak assignments for amino acid derivatives. Figure 1 shows chromatograms of amino acid derivatives obtained from the top and from the bottom of the sediment core.

The percentage of each D amino acid relative to the total amount of that amino acid present in each sample was determined by measurement of peak heights on two to seven chromatograms obtained with different sample concentrations and averaging these results. Table 1 shows these averages and the ranges of average values. The applicability of this method of measurement was verified on gas-liquid chromatograms of derivatives of alanine and leucine having known stereoisomeric compositions. The percentages of D amino acids listed on Table 1 include contributions from the 4 percent L-2-butanol impurity and contributions from possible partial racemization during acid hydrolysis of the samples. A measure of this partial racemization can be estimated from the percentages of D amino acids determined in the top sample, core 4 (0 to 0.15), if the assumption is made that the amino acids in this <100-year-old sample have undergone no measurable natural racemization.

When the effect of the 4 percent L-2-butanol is subtracted, the percentages of D amino acids that may be attributed to partial racemization during hydrolysis range from about 2 to 4 percent. This general magnitude of partial racemization has been observed previously during the acid hydrolysis of proteins (*7*). Therefore, partial racemization of amino acids in sediments during acid hydrolysis seems reasonable.

The percentages of D amino acids listed in Table 1 generally increase with increasing depth. Two exceptions are leucine and proline, for which the concentrations of the D enantiomers decrease slightly for core 3B (7.9 to 8.2) but increase at greater depths. Another exception is isoleucine. The percentage of D-isoleucine apparently averages about 4 percent. The constancy of these measurements is readily explained by the fact that L-isoleucine does not racemize to D-isoleucine but rather to D-alloisoleucine. This racemization of L-isoleucine has been demonstrated earlier in other geological samples (*2*). The gas-liquid chromatographic peak labeled "D-isoleucine position" corresponds to the derivative made from L-isoleucine and the 4 percent L-2-butanol impurity. This peak, therefore, can be used as a measure of the amount of L-2-butanol in the D-2-butanol derivatizing reagent. The gas-liquid chromatographic peak corresponding to D-alloisoleucine is obscured by L-alanine on Fig. 1.

Figure 2, however, shows gas chromatograms of amino acid derivatives from the bottom core sample obtained on two gas-liquid chromatographic columns of different liquid phases. A peak corresponding in retention time to D-alloisoleucine is indicated on both chromatograms. The percentages of D-alloisoleucine were estimated from peak height measurements of D-alloisoleucine and L-isoleucine on chromatograms obtained from the XE-60 chromatographic column. Only single determinations were made for three samples which were chromatographed at high concentrations in order to pro-

duce a measurable peak for D-alloisoleucine. Accurate measurement of the percentage of D-glutamic acid in core 3B (34.5 to 34.8) is questionable because of low concentrations and peak broadening.

Although all percentage D values in Table 1 do include contributions from the L-2-butanol and from partial racemization, the difference between these values for any amino acid should cancel these contributions and provide a measure of racemization occurring during diagenesis of the sediments. Some differences do not appear to be significant because of the spread of ± values. There is a trend, however, of increasing differences with depth. A very significant difference in percentage of D amino acids is found between the top and the bottom of the core. The last column of Table 1 shows that the increase in percentage of D amino acids from the top to the bottom of the core varies from about 1 to at least 7 percent. These changes in percentages of D amino acids are believed to reflect the amount of racemization that has taken place over about 9000 years. Because racemization involves first-order kinetics, half-lives of racemization can be estimated from data on Table 1. If the extreme values from the last column are used, these estimates range from 28,000 to 220,000 years, and racemization would be essentially complete in ten half-lives or between about $3 \times 10^5$ and $2 \times 10^6$ years. Actual times for complete racemization probably differ from these estimates because of the variability of factors such as temperature, *p*H, and catalysts. Amino acids, at least in the sediments of Saanich Inlet, apparently undergo racemization processes early in the diagenetic history of the sediment. Of the amino acids that could be measured, $\alpha$-alanine and glutamic acid appear to racemize most quickly during mild alkaline treatment (*8*) and, therefore, the slightly alkaline conditions of these sediments may promote racemization.

Besides distributions of amino acid enantiomers, the varying concentrations of amino acids are also of interest. Glycine and $\beta$-alanine, having no asymmetric center, produce only one peak each on the gas chromatograms (Figs. 1 and 2). The concentration of glycine decreases with depth, while the concentration of $\beta$-alanine increases from a trace to 10 nmole/g at 17 m and then decreases. The content of asymmetric amino acids generally decreases with depth, but the decrease is irregular. Serine, threonine, and aspartic acid diminish dramatically. This decrease with depth or age, especially of threonine and serine, has been noted in other geological samples (*2*). The diminishing concentration of serine with depth is accompanied by an apparent rapid increase in the percentage of D-serine. The rapidly diminishing total concentration of this acid, however, prevented accurate determination of the percent of D-serine at depth.

The gas-liquid chromatographic techniques used here have been applied previously to amino acids in 3 billion-year-old Fig Tree chert (*9*) and in 60 million-year-old Green River Formation oil shale (*10*). Only L amino acids were detected in Fig Tree chert, whereas both D and L amino acids in unequal concentrations were found in Green River oil shale. We suggested that these populations of amino acids were either preserved in the rocks since the time of deposition or were due largely to incorporation in the rocks of modern biological materials. The L amino acids found by enzymatic techniques in 2 billion-year-old Gunflint chert were interpreted to be of recent origin (*11*). From this work on Saanich Inlet sediments, it is estimated that amino acid populations indigenous to sediments older than about 2 million years should be racemic. The fact that the amino acids in the ancient sediments mentioned above are not racemic suggests that many of these amino acids

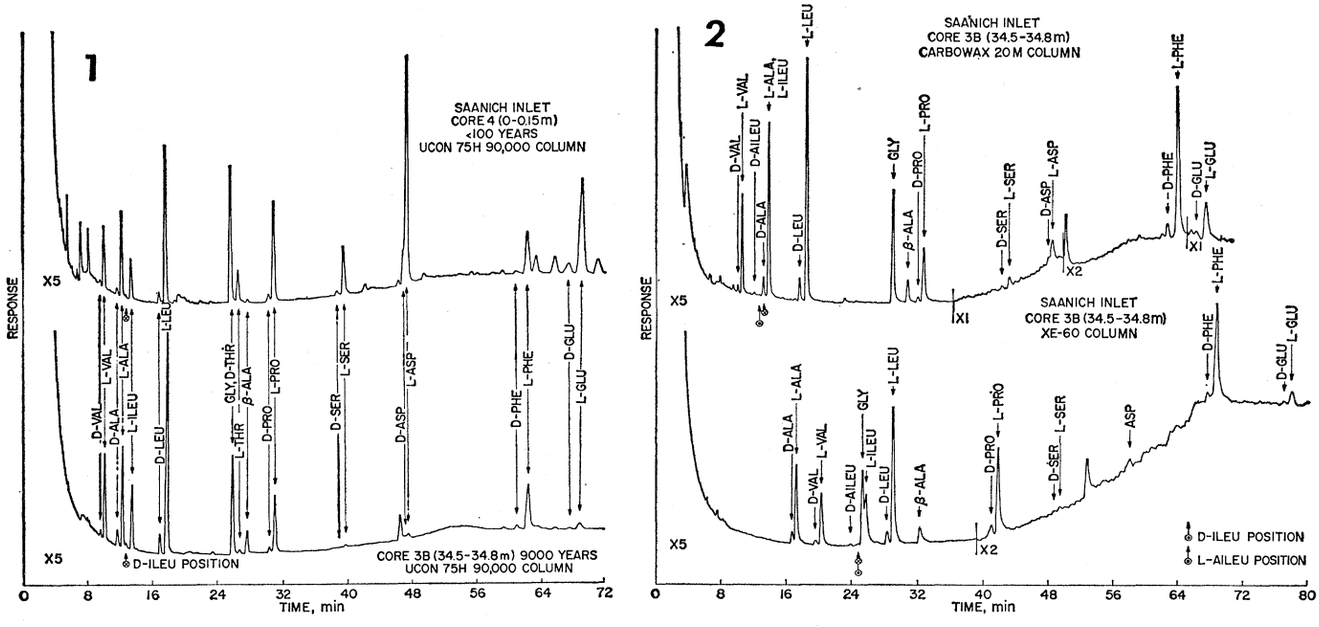


Fig. 1. Gas-liquid chromatograms of *N*-trifluoroacetyl-D-2-butyl esters and *O*-acetyl-*N*-trifluoroacetyl-D-2-butyl esters of amino acids in hydrolyzates from sediments of cores from Saanich Inlet. Abbreviations for amino acids are listed in Table 1. Column: 0.5 mm by 46 m, UCON 75H-90,000; Perkin-Elmer 881 gas chromatograph with flame ionization detector; He, 10 ml/min; temperature program 100° to 150°C at 1°C/min. Fig. 2. Gas-liquid chromatograms of *N*-trifluoroacetyl-D-2-butyl esters and *O*-acetyl-*N*-trifluoroacetyl-D-2-butyl esters of amino acids in hydrolyzate from sediment core 3B (34.5 to 34.8) from Saanich Inlet. Abbreviations for amino acids are listed in Table 1. Peak corresponding to derivative of D-alloisoleucine indicated. Columns: 0.5 mm by 46 m, Carbowax 20M, and 0.5 mm by 46 m, XE-60. Conditions same as Fig. 1 except that the temperature was programmed from 110° to 155°C at 1°C/min and 110° to 175°C at 1°C/min, respectively.

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

KEITH A. KVENVOLDEN
ETTA PETERSON
*Exobiology Division,*
*NASA–Ames Research Center,*
*Moffett Field, California 94035*
FREDERICK S. BROWN
*Biochemistry and Electrochemistry*
*Department, Systems Group of TRW,*
*Inc., Redondo Beach, California 90278*

### References and Notes

1. E. T. Degens, A. Prashnowsky, K. O. Emery, J. Pimenta, *Neues. Jahrb. Geol. Palaeont. Abh. B* (1961), p. 413; E. T. Degens, K. O. Emery, J. H. Reuter, *ibid.* (1963), p. 231; R. H. Clarke, *Nature* **213**, 1003 (1967).
2. P. E. Hare and P. H. Abelson, *Carnegie Inst. Wash. Year B.* **66**, 526 (1968); P. E. Hare and R. M. Mitterer, *ibid.* **65**, 362 (1967).
3. S. M. Gueluer and M. G. Gross, *Limnol. Oceanogr.* **9**, 359 (1964); R. W. Buddemeier, Ph.D. thesis, Univ. of Washington, Seattle (1969).
4. F. S. Brown, M. J. Baedecker, A. Nissenbaum, I. R. Kaplan, in preparation.
5. E. Gil-Av, R. Charles, G. Fischer, *J. Chromatogr.* **17**, 408 (1965); G. E. Pollock, V. I. Oyama, R. D. Johnson, *J. Gas Chromatogr.* **3**, 174 (1965); G. E. Pollock and V. I. Oyama, *ibid.* **4**, 126 (1966).
6. G. E. Pollock and A. H. Kawauchi, *Anal. Chem.* **40**, 1356 (1968).
7. J. M. Manning and S. Moore, *J. Biol. Chem.* **243**, 5591 (1968); G. H. Wiltshire, *Biochem. J.* **55**, 46 (1953); A. Neuberger, *Advan. Protein Chem.* **4**, 297 (1948).
8. G. E. Pollock and L. H. Frommhagen, *Anal. Biochem.* **24**, 18 (1968).
9. K. A. Kvenvolden, E. Peterson, G. E. Pollock, *Nature* **221**, 141 (1969).
10. K. A. Kvenvolden and E. Peterson, *Geol. Soc. Amer., Abstr.* **7**, 132 (1969).
11. P. H. Abelson and P. E Hare, *Carnegie Inst. Wash. Year B.* **67**, 208 (1969).
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.

16 March 1970; revised 11 June 1970

## 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