the complexes (save those with Tm) for any η in the range 0° to 30°.

Our results demonstrate the dipolar origin of the proton resonance shifts in SR systems. They further show that magnetic axiality in lanthanide complexes cannot be assumed in general, and suggest that detailed structural inferences based on this assumption must be accepted with reservation.

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References and Notes

1. A. S. Mildvan and M. Cohn, *Advan. Enzymol. Relat. Areas Mol. Biol.* **33**, 1 (1970).
2. R. A. Dwek, R. E. Richards, K. G. Morallee, E. Nieboer, R. J. P. Williams, A. V. Xavier, *Eur. J. Biochem.* **21**, 204 (1971); C. D. Barry, A. C. T. North, J. A. Glasel, R. J. P. Williams, A. V. Xavier, *Nature* **232**, 236 (1971).
3. See, for example, W. DeW. Horrocks, Jr., and J. P. Sipe, III, *J. Amer. Chem. Soc.* **93**, 6800 (1971) and references therein.
4. C. C. Hinckley, *ibid.* **91**, 5160 (1969).
5. W. DeW. Horrocks, Jr., *Inorg. Chem.* **9**, 690 (1970).
6. R. J. Kurland and B. R. McGarvey, *J. Magn. Reson.* **2**, 286 (1970); B. R. McGarvey, *J. Chem. Phys.* **53**, 86 (1970).
7. W. DeW. Horrocks, Jr., and D. DeW. Hall, *Inorg. Chem.* **10**, 2368 (1971).
8. W. DeW. Horrocks, Jr., and E. S. Greenberg, *ibid.*, p. 2190.
9. The coordinates are defined as follows: r is the length of a radius vector from the metal to the nucleus in question, θ is the angle made by this vector with the molecular z axis, and Ω is the angle made by the projection of r on the x, y, plane with the x axis.
10. H. M. McConnell and R. E. Robertson, *J. Chem. Phys.* **29**, 1361 (1958); G. N. LaMar, W. DeW. Horrocks, Jr., L. C. Allen, *ibid.* **41**, 2126 (1964); J. P. Jesson, *ibid.* **47**, 579 (1967).
11. It was pointed out by J. Briggs and E. W. Randall (manuscript in preparation) and confirmed by numerical calculations of our own that, even for magnetically nonaxial complexes, if a substrate molecule is rotating freely (or with a barrier of threefold order or higher) about an axis which passes through the metal, then dipolar shifts are given by the expression: $(\Delta H^{dip}/H) = (\frac{1}{2})[-D(3\cos^2\alpha - 1) - D'(\sin^2\alpha\cos 2\beta)](3\cos^2\gamma - 1)r^{-3}$, where γ is the angle made by the radius vector to the nucleus with the axis of rotation, and α and β are the spherical polar angular coordinates of the rotational axis analogous to θ and Ω of Eq. 1, respectively. Detailed calculations of interatomic distances based on the solid-state structure of a SR adduct (*12*) suggest that, even for ligands as simple as 4-picoline, free rotation of substrate molecules is not to be expected.
12. W. DeW. Horrocks, Jr., J. P. Sipe, III, J. R. Luber, *J. Amer. Chem. Soc.* **93**, 5258 (1971).
13. The abbreviation dpm is used for $(CH_3)_3COCHCOC(CH_3)_3$.
14. A. Zalkin, D. H. Templeton, D. G. Karraker, *Inorg. Chem.* **8**, 2680 (1969).
15. J. A. Cunningham, D. E. Sands, W. F. Wagner, M. F. Richardson, *ibid.*, p. 22; E. D. Watkins, J. A. Cunningham, T. Phillips, D. E. Sands, W. F. Wagner, *ibid.*, p. 29; T. Phillips, D. E. Sands, W. F. Wagner, *ibid.* **7**, 2295 (1968); S. J. Wasson, D. E. Sands, W. F. Wagner, Abstract 014, American Crystallographic Association Summer Meeting at Ames, Iowa, August 1971.
16. R. E. Cramer and K. Seff, *Chem. Commun.* (1972), p. 400.
17. For a detailed discussion of this method see W. DeW. Horrocks, Jr., and D. DeW. Hall, *Coord. Chem. Rev.* **6**, 147 (1971).
18. The relationships between the crystal and molecular susceptibilities are:
$$\chi_a = \chi_z\cos^2\eta + \chi_x\cos^2(90° - \eta);$$
$$\chi_b = \chi_y;$$
$$\chi_c = \chi_z\cos^2(90° - \eta) + \chi_x\cos^2\eta$$
19. The assumption necessarily made here is that the solid-state structure persists in solution, retains its solid-state magnetic properties, and is the only species present. The degree of agreement found suggests that this may be true in large measure. This is further supported by the fact that $Ho(dpm)_3(4\text{-picoline})_2$ and $Eu(dpm)_3(pyridine)_2$ have virtually identical molecular structures although they crystallize in different crystal systems, orthorhombic and triclinic, respectively (*16*).
20. The NMR shifts (298°K) were obtained under conditions in which 4-picoline was present in large excess under conditions of rapid ligand exchange and have been extrapolated to those expected for a 2 : 1 complex.
21. D. F. Evans, *J. Chem. Soc.* (1959), p. 2003.
22. Supported by NSF grant GP 26148.

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Biological Damage from Intranuclear Tritium: DNA Strand Breaks and Their Repair

Abstract. *Isotopic decay in tritiated thymidine in the DNA of frozen (−196°C) Chinese hamster cells causes breaks in DNA strands to accumulate at a rate of 2.1 breaks per decay. After DNA is thawed the tritium-induced breaks repair rapidly with a half-time of 15 minutes at 37°C. In comparison to breakage by x-rays, the efficiency of DNA strand breakage by tritium is equivalent to 0.48 rad per decay. This dose per decay is close to that predicted by simple dosimetric considerations (0.38 rad per decay) for irradiation by the β particles from tritium.*

The biological consequences of radioisotopes located within tissue are of current interest in view of possible human exposure that may result from environmental contamination with industrial waste products, such as the effluent from fission power reactors (*1*). Radioisotopes, particularly tritium (^3H), which concentrate within genetic material (DNA), present several practical and theoretical problems (*1*). Tritium in the form of [^3H]water is a waste product from fission power reactors, and ^3H-labeled nucleosides, particularly [^3H]thymidine ([^3H]dT), have abundant use in studies of normal and malignant cell proliferation (*1, 2*).

The lethal effect of ^3H decays originating in DNA labeled with [^3H]dT is greater than that of decays originating elsewhere in a mammalian cell nucleus (*3*), and this is usually attributed to the various dose distributions that result from different sites of ^3H incorporation (*1, 3*). Although many experiments in bacteria (*4, 5*) and mammalian cells (*6*) are explicable in terms of energy deposited by β particles from ^3H, results that can be ascribed to transmutation processes have been detected in bacterial mutagenesis (*5*) and in inactivation of transforming DNA (*7*). In the latter, ^3H decays from [^3H]dT-labeled transforming DNA produced 0.3 DNA strand break per decay and only inactivated genetic markers on the strand in which the decay originated (*7*).

We have studied DNA breakage in mammalian cells from ^3H decays originating in DNA thymine to determine whether efficiency of breakage is similar to that in transforming DNA. Also we have determined the effective ^3H radiation dose in comparison to x-rays and the extent of strand break repair. Chinese hamster V79 cells were labeled with [methyl-^3H]dT by growth for one generation time (15 hours) in Eagle's medium plus 10 percent fetal calf serum containing $10^{-5}M$ deoxycytidine and [^3H]dT (0.1 to 1 μc/ml, 0.1 to 1 c/mmole) or [methyl-^{14}C]dT (0.3 μc/ml, 55 mc/mmole). Labeled cells suspended in growth medium with or without 5 percent dimethyl sulfoxide were frozen to −196°C at a rate of 1 deg/min and stored for various periods in liquid nitrogen. Portions containing known numbers of cells were washed in 10 percent trichloroacetic acid (TCA) at 5°C and digested in 5 percent TCA (1 hour at 90°C), and radioactivity was counted with the use of a water-miscible counting mixture (Aquasol). Counting efficiencies were determined by channel ratio methods and by the addition of internal [^3H]-toluene standards. The specific activity of the cells was calculated as disintegrations per cell per day (standard deviation, 10 percent). Cells labeled with [^{14}C]dT were irradiated with x-rays (300 kv-peak, 750 r/min) while frozen in liquid nitrogen and then stored in liquid nitrogen for later study. The dose rate was determined with the use of lithium fluoride thermoluminescent powder, irradiated at room temperature to avoid the reduction in lithium fluoride response that occurs at −196°C (*8*). At various times after freezing,

cells were thawed in a water bath at 37°C (*9*). When only a small ice fragment still remained in the thawing cell suspension, the vials were put into an ice bath. This procedure, which took less than 1 minute, was adopted to minimize strand break rejoining that might occur during thawing. Some thawed samples were diluted with 15 volumes of warmed conditioned medium and left at 37°C for various times up to 3 hours to allow cell metabolism to act on ^3H damage. Weight-average molecular weights (M_w) of DNA in thawed cells were then determined by alkaline sucrose gradient centrifugation as described, with DNA from bacteriophage T4 as a standard (*10*).

Radiation-induced strand breaks caused a progressive decrease in molecular weight of DNA with increasing doses of x-rays or ^3H decays (Fig. 1). Storage at −196°C for periods of as long as 30 days after x-irradiation produced no additional change in molecular weight. No strand breaks from ^{14}C decays in frozen cells were detected in samples in which fewer than 10^4 decays per cell had occurred. For ^3H decay and x-irradiation, there is a linear relation between dose and $1/M_w$, as reported for x-rays (*11*). The linear relation for ^3H depends, however, to a large extent on data for only the highest dose rate (9500 decays per cell per day). At low doses (less than 4×10^4 decays per cell) it appears possible that the rate of strand breakage per decay may be less than that obtained at the highest dose.

From the slope of the curves in Fig. 1, the single-strand breakage efficiency of ^3H is 2.1 ± 0.2 breaks per decay, and that for x-rays is 1 break per 115 ± 8 ev (*12*). The latter value is independent of the presence of dimethyl sulfoxide and is larger than that obtained for unfrozen cells, 1 break per 44 to 70 ev (*11*). By this comparison one ^3H decay is equivalent to 0.48 ± 0.06 rad in terms of DNA strand breaks. If the normal definition of dose (that is, energy deposited per unit volume) is applied to ^3H decays, then the expected dose for this cell would be 0.38 ± 0.04 rad per decay (*13*). These two independent estimates are very close, which suggests that the concept of a dose to the nucleus is applicable for radiation with ranges as short as that of ^3H.

Rosenthal and Fox (*7*) obtained a lower strand-break efficiency of 0.3 break per decay in frozen solutions of transforming DNA, possibly because β particle irradiation of DNA was reduced in their dilute solutions and local effects arising from ^3H transmutation effects predominated. If their value of 0.3 break per decay represents an upper limit for strand breaks resulting from local transmutation effects, then in our experiments an average of at least 1.8 breaks per decay (or 85 percent of the breakage) would be due to the β particles in the frozen state. The precise dose per decay will, however, depend on the volume of the nucleus containing [^3H]dT-labeled DNA (*14*), and experiments with other cell types might not give exactly the same dose.

Table 1. Fraction of initial strand breaks remaining after growth at 37°C. Cells were thawed from liquid nitrogen (−196°C) after (5 to 8.4) $\times 10^5$ ^3H decays have occurred. Means and standard errors were determined from the ratio of the initial molecular weight to that at various times after thawing (*15*).

Time at 37°C (minutes)	Fraction of initial strand breaks
0	1.00
15	0.49 ± 0.04
40	0.31 ± 0.03
60	0.26 ± 0.04
105	0.19 ± 0.05
165	0.14 ± 0.02

The strand breaks caused by ^3H decays are rejoined rapidly, with a half-time of about 15 minutes after cells are thawed (Table 1). This is very similar in rate and extent to the rejoining seen at 37°C for cells irradiated with x-rays (*15*) and indicates a rapid return to apparently normal metabolism during recovery from the frozen state. Therefore, our data indicate that damage resulting from decays in [^3H]dT in DNA is no more than that predicted for irradiation by the β particles, and that the kinetics of repair damage are similar

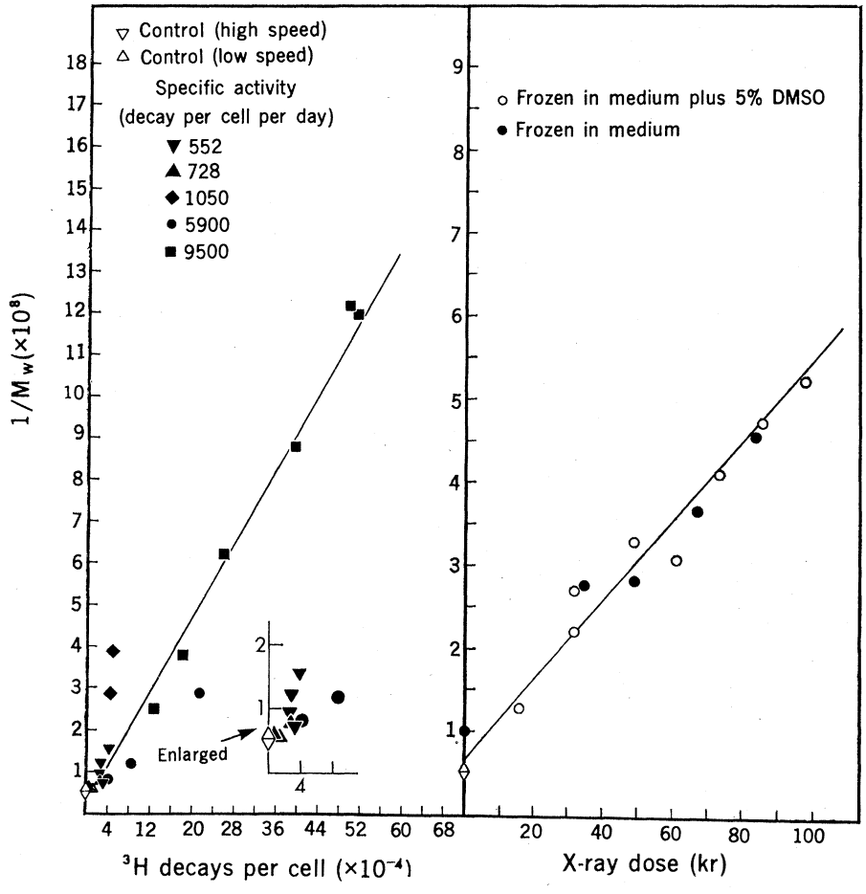

Fig. 1. Reciprocal weight-average molecular weight as a function of ^3H decays or x-ray dose. All cells were frozen in 5 percent dimethyl sulfoxide (DMSO) except where indicated. The controls were cells labeled with [^{14}C]dT and centrifuged at 11,500 or 25,000 rev/min; each control point is the mean of three determinations. All other samples were centrifuged at speeds between 11,500 and 25,000 rev/min, with no dependence of data on centrifugation speed. The standard deviation of each specific activity is ±10 percent. Cells were x-irradiated in liquid nitrogen

to those for external ionizing radiation. Thus, the decays of ^3H in DNA do not appear to cause amounts of breakage or irreparable breaks greater than those expected for β particle irradiation.

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References and Notes

1. *Biological Effects of Transmutation and Decay of Incorporated Radioisotopes* (International Atomic Energy Agency, Vienna, 1968); E. P. Cronkite *et al.* (Committee 24 of the National Council on Radiation Protection and Measurements), *Radioactive Nucleic Acids and Precursors*, in preparation.
2. L. E. Feinendegen, *Tritium-Labeled Molecules in Biology and Medicine* (Academic Press, New York, 1967); J. E. Cleaver, *Thymidine Metabolism and Cell Kinetics* (North-Holland, Amsterdam, 1967).
3. G. Marin and M. A. Bender, *Int. J. Radiat. Biol.* **7**, 235 (1963); *ibid.*, p. 221; H. J. Burki and S. Okada, *Biophys. J.* **8**, 445 (1968).
4. S. Apelgot and M. Duquesne, *Int. J. Radiat. Biol.* **7**, 65 (1963); S. Apelgot, *ibid.* **10**, 495 (1966); R. Bockrath, S. Person, F. Funk, *Biophys. J.* **8**, 1027 (1968).
5. S. Person and H. L. Lewis, *Biophys. J.* **2**, 451 (1962); S. Person, in *Biological Effects of Transmutation and Decay of Incorporated Radioisotopes* (International Atomic Energy Agency, Vienna, 1968), p. 29.
6. D. E. Wimber, *Proc. Nat. Acad. Sci. U.S.A.* **45**, 839 (1959); J. G. Brewen and G. Olivieri, *Radiat. Res.* **28**, 779 (1966); W. C. Dewey, R. M. Humphrey, B. A. Jones, *ibid.* **24**, 214 (1965).
7. P. M. Rosenthal and M. S. Fox, *J. Mol. Biol.* **54**, 441 (1970).
8. D. R. Hall, J. D. Lewis, C. D. Town, P. J. Fish, P. J. Lindop, *Int. J. Radiat. Biol.* **16**, 43 (1969).
9. The survival curve of V79 cells irradiated by ^3H decays in DNA at −196°C is given by an extrapolation number of 5 and a D_0 dose of 1100 decays per cell (H. J. Burki, R. Roots, L. E. Feinendegen, in preparation).
10. J. E. Cleaver, G. H. Thomas, J. E. Trosko, J. T. Lett, *Exp. Cell. Res.*, in press.
11. J. T. Lett, I. Caldwell, C. J. Dean, P. Alexander, *Nature* **214**, 790 (1967); C. J. Dean, M. G. Ormerod, R. W. Serianni, P. Alexander, *ibid.* **224**, 1042 (1969); A. R. Lehmann and M. G. Ormerod, *Biochim. Biophys. Acta* **204**, 128 (1970); J. T. Lett and C. Sun, *Radiat. Res.* **44**, 771 (1970).
12. For these estimates we assume that M_w is equal to twice the number-average molecular weight for random distributions (*11*), the average DNA content of V79 cells in exponential growth is 8×10^{-12} g, and the nuclear volume is 190 ± 20 μm^3 (D. W. Ross and H. C. Mel, *Biophys. J.*, in press). Errors for breakage efficiency and dose per decay are derived from the slope of regression lines.
13. If the energy from ^3H decays originating in DNA thymine is uniformly distributed in the cell nucleus (*12*), a ^3H decay with average energy of 5.7 kev deposits 0.38 rad in a nucleus of density 1.0 if allowance is made for an edge effect of 20 percent (*2*). The actual volume of frozen cells may be slightly less than 190 μm^3 because of shrinkage during freezing, and the dose of 0.38 rad per decay is thus a minimum estimate. No allowance is made here for the relative biological effectiveness of β particles from ^3H, but current estimates suggest that it is close to 1 in relation to 250 kv-peak x-rays (*2*).
14. B. S. Strauss, *Radiat. Res.* **8**, 234 (1958).
15. M. G. Ormerod and U. Stevens, *Biochim. Biophys. Acta* **232**, 72 (1971).
16. Supported by the Atomic Energy Commission.

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Control of Carcinogenesis: A Possible Role for the Activated Macrophage

Abstract. *Cytotoxic activity of activated mouse macrophages against mouse embryo fibroblasts was tested before and after spontaneous transformation of the fibroblasts in vitro. Activated macrophages caused little or no destruction of untransformed fibroblasts but were markedly cytotoxic to the same fibroblasts after spontaneous transformation. The efferent limb of this cytotoxic reaction appears to be nonimmunologic and to be related to abnormal growth properties rather than to the antigenic composition of target cells.*

When normal mammalian cells are serially cultivated they retain their normal characteristics for a limited period; during this time they are termed a cell strain (*1*). Cell strains are either lost after a variable number of subcultivations (*2*), or they spontaneously develop altered morphology, karyotype, and growth properties (which include unlimited capacity for multiplication) characteristic of cell lines (*3*, *4*). The ease of spontaneous establishment of a cell line appears to be species-dependent; spontaneous establishment of a cell line from a human cell strain rarely if ever occurs (*2*), while in practically all cases murine cell strains develop spontaneously into cell lines within 3 months of culture (*3*, *4*). When cultivated under conditions of extensive cell-to-cell contact, spontaneously established lines of mouse cells usually have, in addition to abnormal in vitro growth properties, in vivo malignant potential (*4–6*). We have reported that activated peritoneal macrophages from mice, unlike normal macrophages, appear to cause in vitro the selective destruction of cells with abnormal growth properties—that is, tumor cells and a cell line—by a nonimmunologic mechanism (*7–9*). We have suggested that the activated macrophage may have a homeostatic role in destroying cells that develop abnormal growth properties in vivo (*9*, *10*).

In the experiments reported here we used mouse fibroblasts as target cells, before and after spontaneous transformation, and showed an altered in vitro reactivity of the activated macrophages to newly established lines of mouse fibroblasts. We report that the cytotoxocity of activated C3H/HeJ macrophages for fibroblast target cells appeared to be related to the acquisition of abnormal growth properties by the fibroblasts, which include loss of contact inhibition of cell division, rather than to antigenic differences between the activated macrophages and target cells. Activated C3H/HeJ macrophages did not destroy allogeneic fibroblast cell strains that have cell surfaces of high immunogenic potential, but were markedly cytotoxic to both syngeneic and allogeneic cell lines. We propose that the cytotoxic effect of activated macrophages for fibroblast cell lines that have developed under subcultivation conditions of extensive cell-to-cell contact may reflect a fundamental host reaction to abnormal cell growth.

Primary cultures of mouse fibroblasts were prepared from 17- to 19-day embryos by the method of Reinhold (*11*). Cells were cultured in Eagle's minimal medium with Earle's salts, streptomycin (100 μg/ml), penicillin (100 unit/ml), and 10 percent fetal calf serum (Gibco, Berkeley, California). All cultures were maintained on a rigid transfer schedule in 950-ml prescription bottles, subcultures at a 1 : 2 ratio being made every 7 days. Medium was changed on day 3. Cells were detached for transfer by adding 0.25 percent trypsin to the monolayers and incubating for 15 minutes at 37°C. Action of trypsin was stopped by adding 4 ml of medium containing serum. When spontaneous transformation to a cell line occurred, changes in morphology and growth characteristics included loss of fusiform shape and parallel orientation of the fibroblasts, cell overgrowth, increased saturation density, and ability to grow at low inoculation density. By these criteria, cell lines were present by subculture 16 in the case of C57BL/6J fibroblasts, by subculture 17 for BALB/c fibroblasts, and by subculture 20 for C3H/HeJ fibroblasts.

To activate macrophages, female C3H/HeJ mice were chronically infected with *Toxoplasma gondii* (*7*) or treated with complete Freund's adjuvant