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Cell Biology

Malignant transformation induced by incorporated radionuclides in BALB/3T3 mouse embryo fibroblasts

(iodine-125/tritium/DNA strand breaks/x-radiation/Auger electrons)

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ABSTRACT **The induction of lethality and malignant transformation by 5-[^{125}I]iododeoxyuridine and [^{3}H]thymidine incorporated into cellular DNA and by x-irradiation was studied *in vitro* in BALB/3T3 cells. Under these conditions, ^{125}I radiation is highly localized to small regions of the DNA at the site of each decay and produces DNA double-strand breaks with high efficiency. Incorporated ^{125}I was found to be 12–16 times as lethal per decay as incorporated ^{3}H. For the induction of malignant transformation, however, ^{125}I was approximately 25 times as effective per decay as ^{3}H. When the frequencies of transformation induced at various levels of survival by ^{125}I, ^{3}H, and x-rays were compared, lethality was found to correlate closely with transformation at doses that yielded significant cell killing. An exception occurred at low doses, where ^{125}I appeared much more efficient than x-irradiation in inducing transformation; transformation frequencies equal to those induced by 3–5 Gy of x-rays resulted from ^{125}I exposures that yielded little or no cell killing.**

Ionizing radiation is capable of inducing the malignant transformation of cells in animals and humans (1, 2). This has been demonstrated for both external radiation and internal emitters. For instance, Lisco *et al.* (3) showed that [^{3}H]thymidine injected into mice resulted in the development of a variety of tumors. There also have been a number of reports describing the carcinogenic effects of other β- and α-particle-emitting radionuclides in animals and man (2).

In vitro assays also have been used to study mammalian cell transformation by external radiation, especially x-rays, and by chemical carcinogens (4–8). However, there has been no attempt to relate the microscopic distribution of energy to the efficacy of various internal emitters in producing cell transformation, despite the repeated demonstration that the potential for cell killing strongly depends upon this distribution (9–11). This is particularly evident in comparing the effects of ^{125}I with ^{3}H; ^{125}I is much more cytotoxic than ^{3}H and produces proportionally more chromosomal aberrations (11).

The relatively high lethality of ^{125}I generally is ascribed to its release of an extremely localized burst of low-energy Auger and Coster–Kronig electrons upon decay (12). Thus, it is an efficient inducer of DNA double-strand breaks when incorporated into the DNA structure (13). The ^{125}Te nucleus of high positive charge that results from the decay of ^{125}I also may contribute to localized molecular damage (14). On the other hand, decay of ^{3}H incorporated into DNA generally has been shown to produce effects similar to low LET radiations (15).

In this report, we show that ^{125}I incorporated into DNA as 5-[^{125}I]iododeoxyuridine (^{125}IdUrd) is considerably more efficient than ^{3}H in inducing cell transformations. Moreover, when compared at equal levels of cytotoxicity with both external x-rays and ^{3}H, ^{125}I produces a greater transformation frequency at high survival fractions. This differential effect suggests that at low doses, Auger electron-emitters localized to the genome may have malignant consequences far greater than expected from their lethal properties.

MATERIALS AND METHODS

Cell Cultures. The A31-11 line of BALB/3T3 mouse embryo fibroblasts was originally obtained from T. Kakunaga at the National Cancer Institute (Bethesda, MD). Details of their maintenance and use in this laboratory have been described (16). Cells were used between passages 4 and 6 and had a cloning efficiency of 60–80%.

X-Irradiation. Cells were irradiated at room temperature on the day they reached confluence with a General Electric Maximar x-ray generator operating at 220 kV and 15 mA, yielding a dose rate of 0.8 Gy/min. Immediately thereafter they were released by trypsinization, diluted, and plated for transformation and cytotoxicity assays.

Cell Synchronization and Incorporation of Radioisotopes. For the assay of transformation, we felt that the clearest results would be obtained if the entire population of cells being treated were uniformly labeled with the radionuclides by synchronizing and labeling the cells during one S phase of growth. This would avoid problems in the interpretation of results that might arise if the cultures contained one or more subpopulations of cells with differing levels of incorporated radioactivity in one or both DNA strands. In preliminary experiments, we observed that mitotic shake off did not produce a good yield of synchronous cells. Thus, we chose to use release of cells from the density inhibition of confluent cultures. Although it was difficult to obtain a high degree of synchrony with this cell line, the procedures we used allowed each cell in the population to incorporate unifilarly an approximately equal amount of radioactivity.

The cells were subcultured on the day they reached confluence (when they were held in confluence for longer periods, we found that the synchronous movement of cells into the S phase was diminished). As a result, 15–25% of the cells remained in S phase at the time of reseeding (Fig. 1). For the remaining cells, there was a lag of about 10 hr before the onset of DNA synthesis. Beginning at 10 hr, a wave of cells entered the S phase, with >95% of the cells in S phase at 16 hr.

To obtain a uniformly labeled population, the cells were incubated in medium containing [^{3}H]thymidine or ^{125}IdUrd during the synchronous S phase peak from 8 to 24 hr after subculture. Cells already in S phase before this synchronous peak

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Abbreviations: ^{125}IdUrd, 5-[^{125}I]iododeoxyuridine; D_0, inverse of slope; D_{37}, dose necessary to reduce survival to 37%.

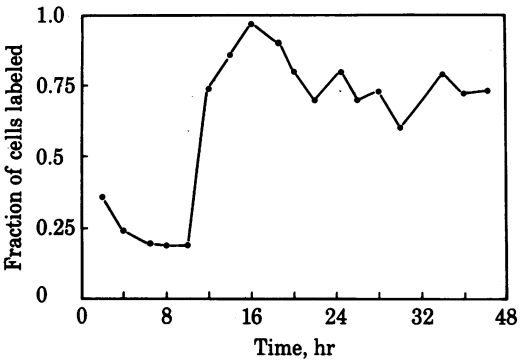

FIG. 1. Pulse-labeling index of BALB/3T3 cells after subculture from confluence at time 0. Data are from one experiment.

were killed by incubating the cultures with a "suicide" dose of [^{3}H]thymidine (5 μCi/ml; 1 Ci = 3.7×10^{10} becquerels) from 0 to 8 hr after subculture.

[*methyl*-^{3}H]Thymidine was purchased from New England Nuclear (specific activity, 40–60 Ci/mmol) and ^{125}IdUrd was purchased from New England Nuclear and from Amersham (specific activity, 2,000 Ci/mmol). For labeling of cells, [^{3}H]thymidine or ^{125}IdUrd was added to the culture medium to the desired concentration (μCi/ml); a maximum of 3×10^4 cells per ml of medium were labeled for 16 hr. For this short labeling period and at this low cell number, we found no significant depletion of incorporable radioactivity from the culture medium during the entire labeling period, even without supplying additional cold thymidine.

Radioactivity Determinations. The incorporated radioactivity was determined for each survival and transformation point in all experiments. To do this, we trypsinized a parallel plate of cells and counted the cell number with a Coulter counter. For ^{3}H assay, an aliquot of cells was loaded onto a glass fiber filter and then washed consecutively with cold 10% trichloroacetic acid and 95% ethanol; the filter was dried, and the ^{3}H cpm were determined in a cocktail of Liquifluor/toluene (New England Nuclear) in a Searle model III liquid scintillation counter. A known amount of ^{3}H standard was spotted on a filter and assayed in the same manner to determine counting efficiency. The efficiency of counting the [^{3}H]DNA cpm by this method was confirmed by scintillation counting of the cpm of isolated and degraded [^{3}H]DNA as well. For ^{125}I assay, a sample of cells was loaded on a filter and washed with cold 10% trichloroacetic acid and 95% ethanol, and the cpm were determined in a Packard Auto-Gamma Scintillation spectrometer. Counting efficiency was determined by assaying filters spotted with a known amount of ^{125}IdUrd.

To determine the average radioactivity per cell only among cells that incorporated label, we divided the average radioactivity per cell in the whole population by the percentage of cells labeled during the period of incorporation, which we determined by continuously incubating a replicate dish with [^{3}H]thymidine from 8 to 24 hr after subculture and measuring the labeling index. This correction has been applied to all of the reported dose measurements. The correction is relatively small, as >95% of the cells were labeled in every experiment.

Survival and Transformation Assays. For survival and transformation assays, synchronized cells were plated in 100-mm plastic Falcon or Lux Petri dishes and allowed to incorporate [^{3}H]thymidine or ^{125}IdUrd. After the 16-hr labeling period, the radioactive medium was removed from the dishes and replaced with fresh medium containing 100 μM thymidine and 10 μM deoxycytidine as a chase. Cells then were allowed to continue growth either for the assay of transformation or on parallel plates for the assay of clonal survival. In the assay for transformation, our procedures followed those as described (16). Transformation was expressed either in terms of the frequency of type III foci per surviving cell or as the mean number of foci that developed per dish.

Autoradiography. For the determination of the pulse or continuous labeling indices, cells either were exposed for 15 min to [^{3}H]thymidine at 5 μCi/ml or were continuously labeled with [^{3}H]thymidine at 0.1 μCi/ml. Dishes then were coated with Kodak NTB-2 nuclear track emulsion, air dried, stored about 1 wk in the dark at 4°C, and developed with Kodak D-19. The cells were fixed and stained with crystal violet; at least 200 cells per dish were scored.

RESULTS

Incorporation of Radioisotopes. In order to determine the appropriate labeling period after the release of cells from the density inhibition of just confluent cultures, cells were trypsinized and resuspended at time 0, seeded into replicate dishes, and pulse-labeled every hour to determine the percentage of cells in S phase. The results of a typical experiment are shown in Fig. 1. Based on these results, the cells were incubated with [^{3}H]thymidine or ^{125}IdUrd from 8 to 24 hr after subculture. There was a linear increase in cellular incorporation (pCi per cell) with increasing concentrations of radioactivity (μCi/ml) in the medium over the entire dose range studied (Fig. 2). The slopes of the lines differ by a factor of approximately 2, indicating that under these conditions [^{3}H]thymidine was incorporated about 2-fold more effectively than was ^{125}IdUrd.

Cell Survival Studies. The dose–response relationships for cytotoxicity in 3T3 cells after incorporation of each radioisotope are shown in Fig. 3. The survival curves for both [^{3}H]thymidine and ^{125}IdUrd show a resistant tail corresponding to about 3% of the cells. The slope of the resistant component appears to be 0 because at high doses, as for [^{3}H]thymidine in Fig. 3, the curves become nearly horizontal. The fact that cultures incubated with both radioisotopes showed a similar resistant fraction implies that this effect is not related to a particular radioisotope but rather to the growth characteristics of the population of cells.

Our results indicate that the resistant tail is due to cells that escape radioisotopic labeling but go on to form surviving colonies. The following findings support this conclusion. (*i*) The

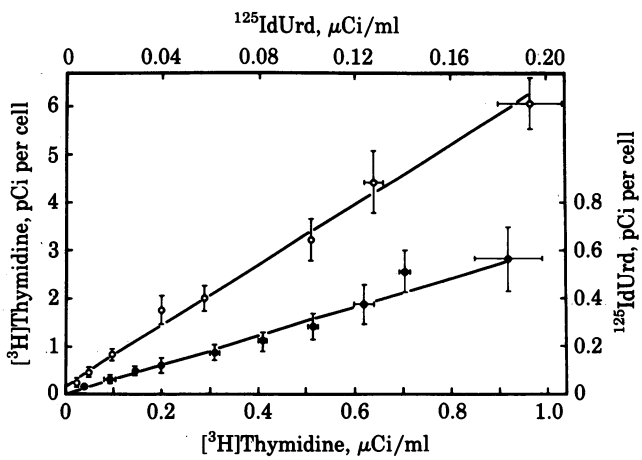

FIG. 2. Incorporation of [^{3}H]thymidine (○) or ^{125}IdUrd (●) into BALB/3T3 cells as a function of concentration in the medium. Vertical and horizontal error bars represent the SEMs; where not shown, they fall within the point. Data are from four experiments.

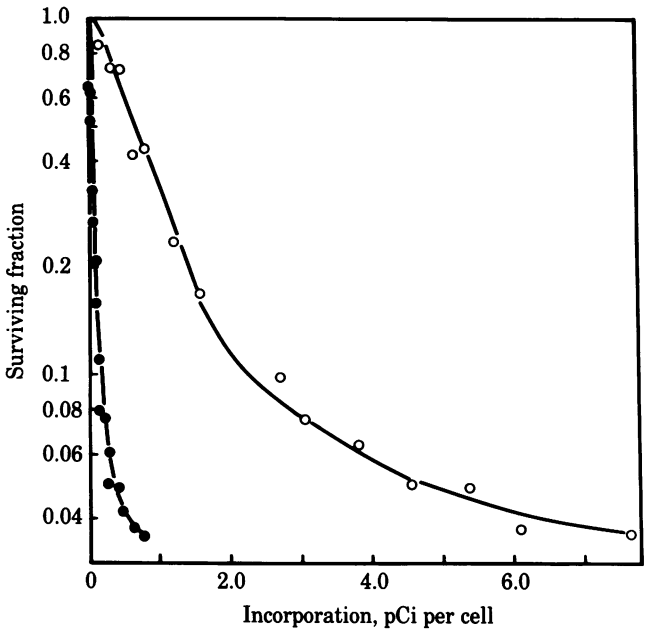

FIG. 3. Survival of BALB/3T3 cells after incorporation of [^{3}H]-thymidine (○) or ^{125}IdUrd (●). Data are from two experiments.

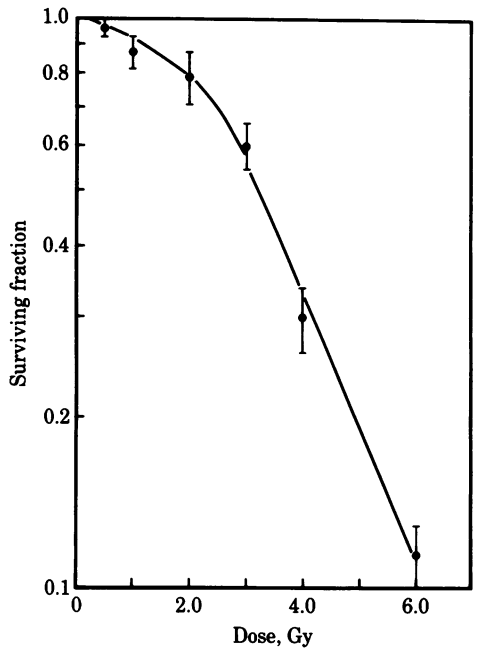

FIG. 4. Survival of BALB/3T3 cells after x-irradiation. Vertical error bars represent the SEMs. Data are from four experiments.

percentage of unlabeled cells in any given experiment generally correlated with the percentage of the cell population forming the resistant fraction based on extrapolation from the survival curves. For example, the survival curves shown in Fig. 3 have a resistant fraction of about 3%, whereas the continuous labeling index after 24 hr in this experiment was 97%. (*ii*) Autoradiographic studies carried out several days after incubation with a high dose of [^{3}H]thymidine (while the colonies were still small) showed the surviving colonies to be completely unlabeled while surrounding dying cells were heavily labeled. These surviving colonies were not resistant to incorporation of label because they were labeled readily after subsequent incubation with [^{3}H]thymidine. (*iii*) When the high thymidine chase was not applied after labeling, the resistant tail of the survival curve acquired a steeper negative slope. This apparently was due to low-level incorporation of residual label from the medium after the initial labeling period. This interpretation was confirmed by the autoradiographic observation of small numbers of grains over the nuclei of surviving cells in dishes receiving no thymidine chase after a high dose of [^{3}H]thymidine but not over cells receiving a thymidine chase.

To compare the D_0s (inverse of the slope) of the initial components of the ^{3}H and ^{125}I survival curves, we first subtracted out the small contribution from the resistant fraction. As calculated from the curves in Fig. 3 (pooled data from two experiments), the D_0 for [^{3}H]thymidine was 0.80 pCi per cell and for ^{125}IdUrd was 0.064 pCi per cell. Thus, by a comparison of D_0s, incorporated ^{125}IdUrd is 12.5 times more toxic per decay than incorporated [^{3}H]thymidine. The D_{37} (dose necessary to reduce survival to 37%) value for ^{3}H is 1.0 pCi per cell and for ^{125}I is 0.068 pCi per cell, so a comparison of D_{37} values shows ^{125}IdUrd to be 15.6 times as lethal per decay as [^{3}H]thymidine. The extrapolation numbers for ^{3}H and ^{125}I were 1.1 and 1.0, respectively. The survival of 3T3 cells after x-irradiation is shown in Fig. 4. The survival curve exhibits a D_0 of 1.84 Gy and an extrapolation number of 2.9.

Transformation. Comparisons of malignant transformation induced by [^{3}H]thymidine, ^{125}IdUrd, and x-rays are shown in Figs. 5–8. The dose–response relationship for the induction of transformation by ^{3}H and ^{125}I is plotted in two ways. In Fig. 5, the transformation frequency is expressed as the total number of transformed foci observed at each dose point divided by the total number of surviving cells at that dose. However, it has been reported previously with these cells (16) that the x-ray transformation frequency per survivor induced by a given radiation dose appears to be inversely proportional to the number of cells initially seeded and thus, to the number of survivors on the dish after the initial treatment. Therefore, in order to establish a dose–response relationship for a given agent, it is necessary to have approximately the same number of surviving cells present in each dish at each dose level studied. Therefore, we adjusted the number of cells seeded in order to achieve as nearly as possible the range of 4,000–7,000 surviving cells per dish as has been described for x-ray transformation (16). Because of the unpredictable fluctuation from experiment to experiment in the cloning efficiency and in the uptake and killing efficiency of the

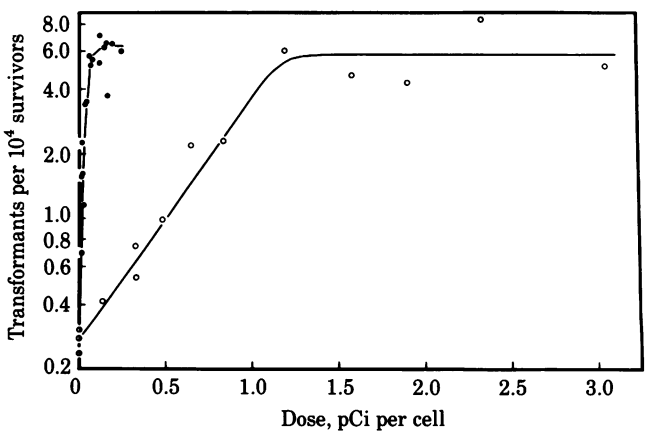

FIG. 5. Transformation of BALB/3T3 cells induced by [^{3}H]-thymidine (○) or ^{125}IdUrd (●). Transformation is expressed as transformants per 10^4 survivors vs. mean incorporated radioactivity per cell for labeled cells. Individual data points are shown from five experiments, but only those points are included for which the initial number of survivors per dish was in the range of 4,000–7,000 cells, as discussed in the text.

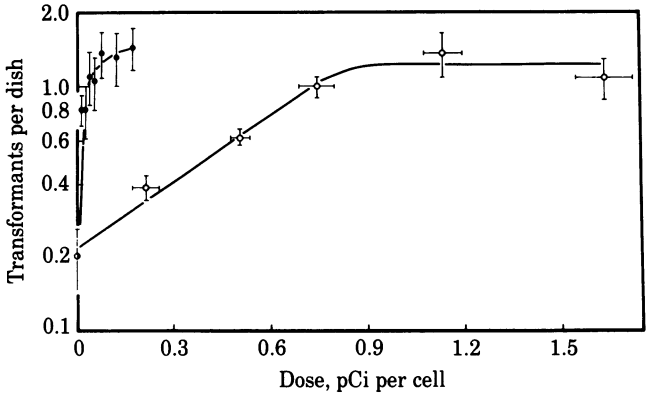

FIG. 6. Transformation of BALB/3T3 cells induced by [^{3}H]-thymidine (○) or ^{125}IdUrd (●). Transformation is expressed as transformants per dish vs. mean incorporated radioactivity per cell for labeled cells. All data points are included, regardless of the initial number of survivors per dish. Vertical and horizontal error bars represent the SEMs for data from five separate experiments. Where horizontal error bars are not shown, they fall within the point.

treatments, this narrow range of survival was not infrequently missed. Therefore, only those points that fell within this range of survivors were plotted in Fig. 5 as transformants per 10^4 survivors vs. dose (pCi per cell).

Although the number of transformed foci per survivor decreased as the number of surviving cells per dish increased, the number of transformed foci per dish appeared to remain fairly constant over a wide range of initial cell densities. This phenomenon has been described by Little (16) in these 3T3 cells and by Kennedy *et al.* (17) in the similar 10T½ mouse fibroblast line. Because of this relationship, the results were plotted (Fig. 6) in terms of the mean number of transformants per dish. We have included in Fig. 6 all of our data rather than only those points that fell within a narrow range of survivors per dish as in Fig. 5. Because the actual levels of radioactivity incorporated per cell varied somewhat among experiments, the data for similar levels were pooled, and this variability was expressed as the horizontal error bars in Figs. 6 and 8.

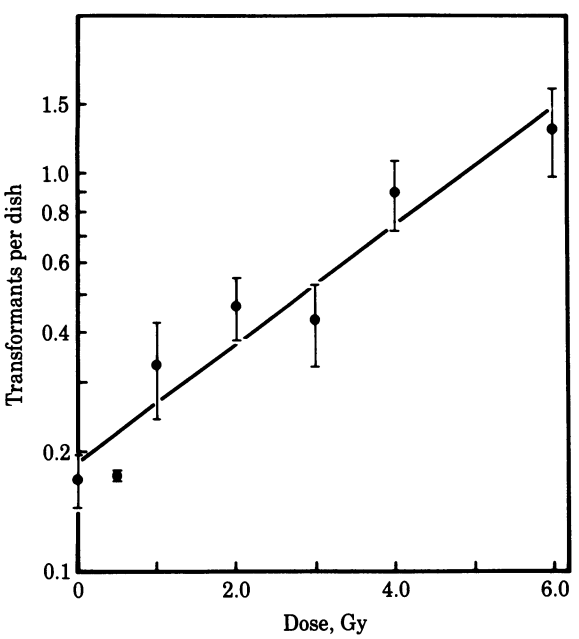

FIG. 7. Transformation of BALB/3T3 cells induced by x-rays. Error bars represent the SEMs of data from five experiments.

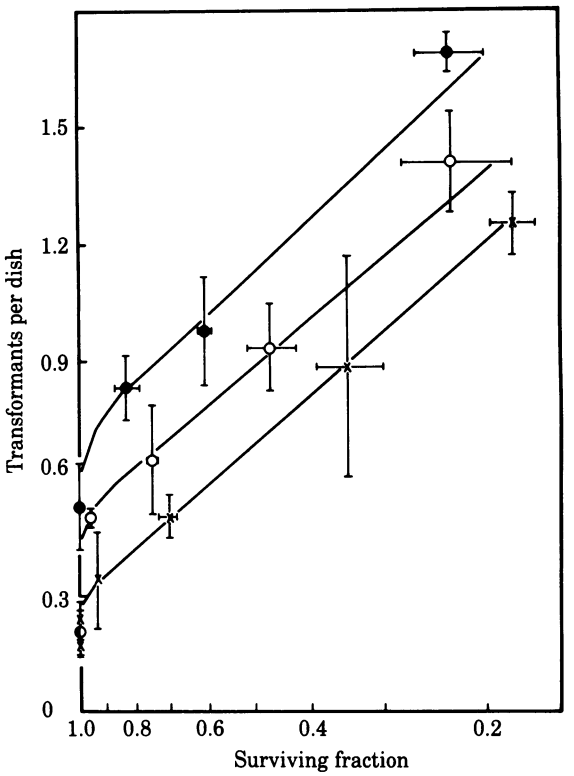

FIG. 8. Transformants per dish induced by [^{3}H]thymidine (○), ^{125}IdUrd (●), or x-rays (×) plotted as a function of the surviving fraction. Vertical and horizontal error bars represent the SEMs. Where horizontal error bars are not shown, they fall within the point. Data are from five experiments. The mean background level of transformation for [^{3}H]thymidine and ^{125}IdUrd experiments was 0.2 transformants per dish. For x-ray experiments, it was 0.17 transformants per dish.

The level of transformation plotted at zero dosage in Figs. 5 and 6 represents the spontaneous transformation frequency plus a slight increase (about 22%) as a result of the [^{3}H]thymidine suicide treatment at time 0 to 8 hr. Cultures exposed to nonradiolabeled IdUrd at the same concentrations (about 0.1 nM) as were used for ^{125}IdUrd showed no additional transformation above the background level, nor did such cultures show an enhanced x-ray-induced transformation frequency due to the incorporated nonradioactive IdUrd. In both Figs. 5 and 6, the curves show an increase in transformation frequency with increasing dose followed by a plateau. At still higher doses, the transformation frequency began to drop (data not shown). The resistant fraction of cells that escaped labeling were the cause of the eventual drop in transformation frequency. At sufficiently high doses, the unlabeled cells became a significant proportion of the total surviving cells. Because they did not receive radiation damage, they did not become transformed, and so the net transformation frequency dropped.

A comparison of the relative transforming potential of ^{3}H and ^{125}I can be made by examining the slope of the initial straight line components on each dose–response curve. The initial slope of the ^{125}I curve in both Figs. 5 and 6 is about 25-fold greater than that of the ^{3}H curve. The dose–response curve for x-ray-induced transformation measured in parallel experiments is shown in Fig. 7. The transformation frequency increased as a function of dose up to 6.0 Gy, the highest dose examined.

In order to compare the efficiency of transformation of ^{3}H, ^{125}I and x-rays at doses yielding similar levels of lethality, the number of transformants per dish was plotted as a function of the surviving fraction (Fig. 8). At least-squares linear regression

line was drawn for each agent by using all points below 100% survival. A t test showed no significant difference in their slopes. This would imply that the ability of these agents to induce transformation is correlated over this range with their ability to induce lethality. On the other hand, a t test shows the y intercept of these lines to be significantly different from the spontaneous level of transformation (for ^{125}I, $P < 0.0005$; for ^{3}H, $P < 0.005$). This implies that at high-survival levels, before much killing was induced, there was a rapid rise in transformation particularly for ^{125}I and to a lesser extent for ^{3}H.

DISCUSSION

We assessed the potential of incorporated radioisotopes to induce malignant transformation of BALB/3T3 mouse fibroblasts *in vitro*. ^{3}H emits a single low-energy β-particle upon decay. ^{125}I decays by electron capture and emits cascades of low-energy Auger electrons of ultrashort range; an average of 21 electrons are emitted per decay (18). In addition, the ^{125}I decay results in a daughter nucleus, ^{125}Te, with a high positive charge. It has recently been calculated (K. Sastry, personal communication) that the dose within a 10-Å sphere around the decay of an ^{125}I atom in the condensed phase is about 300 eV or 10^{7} Gy. Thus, ^{125}I incorporated into cellular DNA provides a tool for inducing highly localized damage in the DNA.

In a number of studies, ^{125}I has been found to be highly lethal to cells when incorporated into their DNA as ^{125}IdUrd (9–11). We also found incorporated ^{125}I to be 12–16 times more lethal per decay than incorporated ^{3}H. Molecular studies of DNA damage in phage and in bacteria have shown that ^{125}I is an efficient inducer of double-strand DNA breaks. For example, Krisch and Sauri (19) have calculated that one double-strand DNA break is produced per ^{125}I decay in bacteriophage under frozen conditions. In a parallel study using neutral elution techniques (unpublished data), we found a similarly high yield of DNA double-strand breaks in mammalian cells exposed to ^{125}I under conditions similar to those in the present experiments. Martin and Haseltine (13) have recently studied in detail the molecular damage produced in DNA *in vitro* from decay of incorporated ^{125}I. They found that an ^{125}I decay breaks the DNA within a region approximately one to five bases adjacent to the iodine site. Thus, it appears that the particularly high toxicity of ^{125}I may be a result of its highly localized energy deposition and the resultant efficient induction of DNA double-strand breaks.

Malignant transformation in mammalian cells has been shown to be a progressive process (20) that may be initiated, at least in some cases, by damage to the cellular DNA (21, 22). We have used ^{125}I as a probe specifically to assess the importance of highly localized DNA damage in transformation; the results clearly show that ^{125}I is an efficient inducer of transformation. Indeed, the transformation rates were increased by 25-fold while lethality was increased 12- to 16-fold in comparison with ^{3}H. When the dose–response relationships for transformation induced by ^{125}I, ^{3}H, or x-rays are normalized for survival (Fig. 8), the incremental increase in transformation with dose produced by each agent was similar for doses yielding greater than about 20% cell killing. The results in Fig. 8 indicate, however, that there are significant differences in the low-dose region, including the extrapolated intercepts of the transformation–survival curves (Fig. 8). These differences imply that the densely ionizing radiation produced by ^{125}I can lead to considerable transformation (equivalent to that caused by about 4.0 Gy of x-irradiation) before there is any significant cell killing. We have no clear understanding of the cause of this difference among the initial shoulders on the curves shown in Fig. 8. Perhaps it is related to the relative repairability of the DNA damage induced by each agent.

The high efficiency of transformation induced by ^{125}I is further emphasized by the fact that incorporation of ^{125}I at levels yielding only about 30 decays per cell produces transformation at a frequency of approximately 10^{-4} per surviving cell (Fig. 5). This frequency is proportionately higher at lower densities of survivors per dish. Although DNA damage is induced at sites other than the decay site of ^{125}I, the major damage is nevertheless near the decay site (13). Thus, if DNA damage is the initial event leading to transformation, only a small number of sites of damage are apparently necessary to yield a significant number of transformants.

In summary, we have shown that ^{125}I efficiently induces malignant transformation as well as lethality in mammalian cells. In comparing the efficiency of ^{125}I with ^{3}H, the former is 12–16 times more lethal and 25 times more efficient in producing transformation. Moreover, transformation–survival curves demonstrate that considerable transformation can occur before significant cell killing is produced by ^{125}I. In the light of this observation, we suggest that the possible use of Auger electron-emitting radionuclides *in vivo* be carefully considered when they are to be distributed within the cell nucleus.

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1. Walburg, H. E. (1973) *Adv. Radiat. Biol.* **10**, 209–254.
2. United Nations Scientific Committee on the Effects of Atomic Radiation (1977) *Sources and Effects of Ionizing Radiation*, (United Nations, New York).
3. Lisco, H., Baserga, R. & Kisieleski, W. E. (1961) *Nature (London)* **192**, 571–572.
4. Borek, C. & Sachs, L. (1966) *Nature (London)* **210**, 276–278.
5. Terzaghi, M. & Little, J. B. (1976) *Cancer Res.* **36**, 1367–1374.
6. Reznikoff, C. A., Bertram, J. S., Brankow, D. W. & Heidelberger, C. (1973) *Cancer Res.* **33**, 3239–3249.
7. Kakunaga, T. (1973) *Int. J. Cancer* **12**, 463–473.
8. Pienta, R. J., Poiley, J. A. & Lebherz, W. B. (1977) *Int. J. Cancer* **19**, 642–655.
9. Burki, H. J., Roots, R., Feinendegen, L. E. & Bond, V. P. (1973) *Int. J. Radiat. Biol.* **24**, 363–375.
10. Hofer, K. G., Harris, C. R. & Smith, J. M. (1975) *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* **28**, 225–241.
11. Chan, P. C., Lisco, E., Lisco, H. & Adelstein, S. J. (1976) *Radiat. Res.* **67**, 332–343.
12. Ertl, H. H., Feinendegen, L. E. & Heiniger, H. J. (1970) *Phys. Med. Biol.* **15**, 447–456.
13. Martin, R. F. & Haseltine, W. A. (1981) *Science* **213**, 896–898.
14. Stocklin, G. (1979) in *Proceedings of the Sixth International Congress of Radiation Research*, eds. Okada, S., Imamura, M., Terashima, T. & Yamaguchi, H. (Toppan Printing, Tokyo), pp. 382–391.
15. Bond, V. P. & Feinendegen, L. E. (1966) *Health Phys.* **12**, 1007–1020.
16. Little, J. B. (1979) *Cancer Res.* **39**, 1474–1480.
17. Kennedy, A. R., Fox, M., Murphy, G. & Little, J. B. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 7262–7266.
18. Charlton, D. E. & Booz, J. (1981) *Radiat. Res.* **87**, 10–13.
19. Krisch, R. E. & Sauri, C. J. (1975) *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* **27**, 553–560.
20. Barrett, J. C. & Ts'o, P. O. P. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3761–3765.
21. Little, J. B. (1977) in *Origins of Human Cancer*, eds. Hiatt, H. H., Watson, J. D. & Winsten, J. A. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 923–939.
22. Barrett, J. C., Tsutsui, T. & Ts'o, P. O. P. (1978) *Nature (London)* **274**, 229–232.