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NUCLEAR LESIONS PRODUCED BY ^{125}I DECAY

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ABSTRACT

^{125}I decay induced DNA damage was investigated in Chinese hamster ovary (CHO) cells which had incorporated ^{125}I-iododeoxyuridine (^{125}IUdR) into their DNA. ^{125}I decays were accumulated in frozen cells. DNA strand breaks produced by low numbers (0-1200 decays per cell) of ^{125}I decays were assayed using the alkaline unwinding technique and the alkaline filter elution technique. Approximately 300 ^{125}I decays reduced the relative damage to 50%. DNA protein cross links were not detected after accumulation of ^{125}I decay. The production of DNA double strand breaks by ^{125}I decay was assayed by the neutral filter elution technique at pH 9.6 and 7.2 after accumulating 100-5000 decays per cell. No alkali labile lesions were detected. Approximately 3000 ^{125}I decays were required to reduce the mean value of the fractions to 50%. In all the assays, DNA damage induction data was fit to a straight line by regression analysis except at very low numbers of accumulated ^{125}I decays where an initial drop in the fraction of DNA retained on the filter was observed. This drop suggests multiple chromatin fragmentation is produced by ^{125}I decay.

INTRODUCTION

^{125}I decay has been used as a tool to calibrate the neutral filter elution assay (1-3) and pulsed field gel electrophoresis (4,5). ^{125}I decay is also extremely effective in cell killing (for example, see Refs. 6-14). This is likely due to the extremely dense energy deposition of Auger electrons emitted from ^{125}I decay in a volume of 30 nm diameter (14), and because of the residual positive charge on the ^{125}Te daughter nuclide (which could produce extensive local ionizations and excitations as the charge is shared with neighboring atoms) (15) resulting in a DNA double strand break (DSB).

Recently it has been shown that cell survival changes depending on when in the cell cycle ^{125}I is incorporated into the DNA (1,13). This indicates that some lesions produced by ^{125}I decay are more lethal to the cell than other lesions produced by ^{125}I decay, *i.e.*, and not all ^{125}I decay produced DNA DSBs have the same biological effect. These data suggest that further understanding of the lesions produced by ^{125}I decay is required.

One factor that may modify the impact of energy deposition from ^{125}I decay is chromatin conformation. A computer graphics model of the energy deposition for ^{125}I decay (17) illustrates that the ^{125}I M-XY Auger electrons have a range of energy deposition that corresponds to the diameter of a solenoid fiber in chromatin structure (17). Local ionizations and excitations from ^{125}I decay could be shared with the numerous DNA strands in the solenoid fiber. Computer decay simulations (16) predict that a portion of the energy deposition from ^{125}I decay is sensitive to DNA conformation (approximately 0.17 out of the 0.82 to 1.07 DNA DSBs produced per decay of ^{125}I).

Another factor affecting DNA damage induction and cell killing could be nuclear organization. The calibration studies are based on ^{125}I damage induction studies in prokaryotes. The energy deposition event from ^{125}I decay produces 1 DNA DSB with very high probability when ^{125}I is incorporated into prokaryotic DNA (18-20). Extrapolation of the DNA DSB induction data as a result of ^{125}I decay in prokaryotes to eukaryotic cells is problematic because a phage or plasmid DNA system (a two dimensional DNA structure) is a completely different radiation target compared to the highly compacted, three dimensional mammalian cell genome. Also, a mammalian cell nucleus (a three dimensional sphere having an approximately 5 μm diameter) provides a much larger and more chemically complex "target" that could act as an "energy

sink" for the transition events from ^{125}I decay compared to a plasmid or phage DNA. Therefore, to more fully understand the role of ^{125}I decay produced lesions in mammalian cells, we initiated a study to identify the nature of lesions produced by ^{125}I decay.

MATERIALS AND METHODS

Cell Culture

Chinese hamster ovary (CHO) cells (originally obtained from Dr. R.A. Tobey) were maintained in monolayer culture using Ham's F10 medium supplemented with 5% fetal bovine serum and 5% bovine serum in a 37°C incubator continuously gassed with 5% CO_2.

DNA Damage Induced by ^{125}I Decay

CHO cells were trypsinized off of their plates and then 10^6 cells were seeded onto a tissue culture flask containing 5 ml of medium. ^{125}I-iododeoxyuridine ($^{125}IUdR$) was added to the cells to a final concentration of 0.37 to 3.7 X 10^4 Bq/ml (0.1 to 1.0 μCi/ml) (specific activity = 8.14 X 10^{10} Bq/mmol or 2.2 X 10^6 mCi/mM; ICN). After the cells had incorporated $^{125}IUdR$ during 16 h, the cells were collected by trypsinization. Before freezing the labeled cells, an aliquot of cells was removed for microscopic examination to determine cell number and the general cellular appearance. The cell radionuclide content and labeling index (L.I.) were also determined. The L.I. was greater than or equal to 97%.

The radionuclide content per cell was determined by precipitating a known number of labeled cells with approximately 10^6 unlabeled carrier cells with 10% ice-cold trichloroacetic acid (TCA) and the number of ^{125}I decays per cell per unit time was determined in a calibrated well-type crystal scintillation counter as previously described (13).

The labeled cells were suspended in cryoprotectant medium (F10 medium supplemented with 10% fetal bovine serum and 15% dimethylsulfoxide) to freeze the cells to -70°C. After the appropriate number of ^{125}I decays were accumulated, the cells were quickly thawed by immersion of the vial in a 37°C water bath. The cell samples were immediately assayed for DNA damage. The freezing and thawing procedure itself did not

introduce DNA strand breaks (also see Refs. 3,21) or change the plating efficiency from that for cells, or cell generation time, or cell cycle progression, from cells that had never been frozen.

X irradiation

CHO cells (10^6 cells/ml) were irradiated with increasing doses of X rays in medium in a 15 ml plastic centrifuge tube at 4°C. Irradiation of the cells was performed with a Phillips X ray machine operated at 250 kVp, 15 mA and the beam filtered with 3 mm Al (HVL 0.2 mm Cu). The dose rate employed was 2.5 Gy per minute as measured by a Victoreen ionization chamber.

DNA Damage Detection

The alkaline filter elution technique (22) was used to measure DNA damage induction by assaying the rate of elution of single-stranded DNA through a filter at an alkaline pH (pH 12.2). Cells (5×10^5) prelabeled with ^{14}C-TdR or ^{125}IUdR were loaded onto a polycarbonate filter (1 μm pore size) and assayed as previously described (23). Each elution profile from the ^{125}I decay irradiated cells was curvilinear and had a different Y-intercept, so did not lend itself to analysis by a strand scission factor. Instead, a mean value was calculated for the fractions collected from one column (24).

The neutral filter elution technique (25) was used to detect DNA DSBs in cells labeled with ^{125}IUdR as previously described (26). No difference in the elution profiles were observed when cells were lysed at pH 10 versus pH 9.6 versus pH 7.2. The mean value of the fractions was calculated for the neutral elution profiles (24).

The alkaline unwinding technique followed by hydroxyapatite chromatography (27) was also used to detect DNA damage produced by ^{125}I decay. Labeled cells (2×10^5) were lysed under alkaline conditions (1 M NaCl, 0.03 M NaOH) at 4°C for 1 hour in the dark. After cell lysis, the samples were neutralized and immediately sonicated (10 s) before application to hydroxyapatite chromatography at 65°C. The columns were washed with 0.012 M phosphate buffer. The single stranded (SS) fraction was eluted from the columns with 0.12 M phosphate buffer and then the double stranded (DS) fraction was eluted with 0.4 M phosphate buffer. Each eluant was collected directly into scintillation vials. Optifluor scintillation fluid was added to each

vial and the samples were counted. The percentage of double stranded DNA was calculated as described by Olive *et al.* (28).

RESULTS

DNA Damage Assayed by the Alkaline Filter Elution Technique

The induction of DNA-protein cross links was investigated after accumulation of ^{125}I decays. As shown by the data in Fig. 1, DNA protein cross links can be detected with the alkaline filter elution technique after X-irradiation. Cells irradiated with increasing doses of X rays were treated with 0.5 mg/ml proteinase K during the 45-60 min cell lysis, or no proteinase K was applied to a replicate column. The DNA from cell samples not treated with proteinase K eluted at a slower rate compared to the DNA from cell samples that were treated with proteinase K indicating DNA protein cross links were present.

Representative elution profiles from cells damaged by ^{125}I decay and then treated, or not treated, with proteinase K can be seen in Fig. 2. No consistent difference between proteinase K treated samples and samples not treated with proteinase K was detected (Fig. 2). However, to confirm this observation a dose response curve was generated (Fig. 3). The mean values of the fractions from all the experiments that were performed can be seen in Fig. 3. Regression analysis was performed to determine the slope of the best-fit line for the data. The calculated values for the slopes were not statistically different. The slopes equaled -0.0002 for both the proteinase K treated samples and the samples not treated with proteinase K. Therefore, no ^{125}I decay induced DNA protein cross links were detected by this assay.

The data in Fig. 3 also represents the overall damage induction measured by the alkaline filter elution technique. Approximately 300 ^{125}I decays were required to reduce the mean value of the fractions to 50%. The alkaline filter elution data (Fig. 3 panel A and B) were analyzed by regression analysis ($r = -0.62$). The Y-intercept = 68.70%.

DNA Damage Assayed by the Alkaline Unwinding Assay

The alkaline unwinding assay was also performed to estimate DNA damage induced by 0-1000 ^{125}I decay in CHO cells (Fig. 4). For this assay, the DS

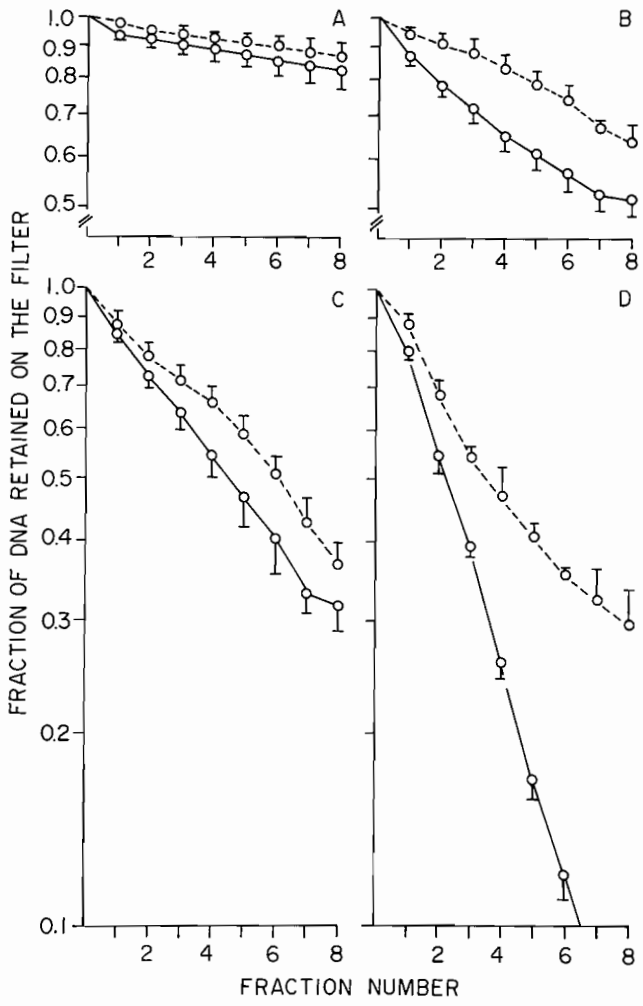

FIG. 1. DNA protein cross-links induced by X rays as determined by the alkaline filter elution technique. The lysed DNA was treated with proteinase K (solid line) or not treated with proteinase K (dashed line) after irradiation with 0 Gy (panel A), 2.5 Gy (panel B), 5.0 Gy (panel C) or 10 Gy (panel D) of X rays. The mean value for each fraction obtained from 3-5 separate experiments ± 1 standard error of the mean are plotted.

fraction is calculated as a proportion of the sum of the SS fraction plus the DS fraction. Three hundred ^{125}I decays were required to reduce the DS fraction

to 50%. The data were fit to a straight line (r = -0.92). The Y intercept = 66.49%. No constraints to unwinding (28) were apparent from the data in Fig. 4.

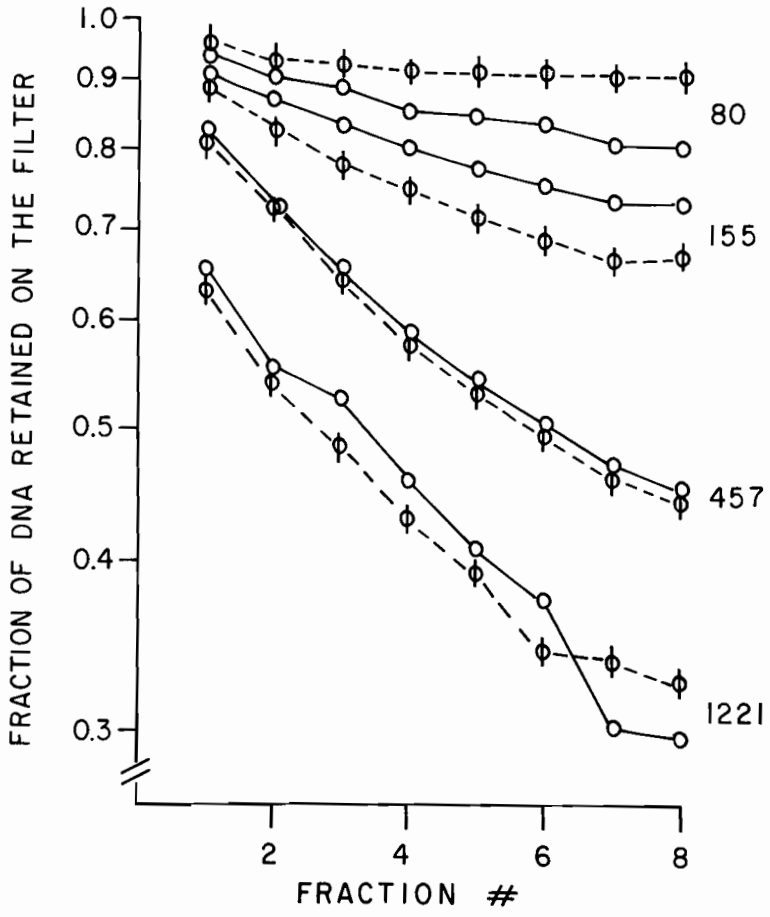

FIG. 2. DNA protein cross link induction by ^{125}I decay. DNA damage induced by ^{125}I decay was measured by the alkaline filter elution technique in samples that were treated with proteinase K (open circles) or not treated with proteinase K (dashed lines and hatched circles) in the lysis step of the elution technique. The number of ^{125}I decays accumulated are indicated to the right of the elution profiles.

DNA Damage Assayed by the Neutral Filter Elution Technique

DNA DSBs can be assayed using the neutral filter elution technique (25). Representative elution profiles for cells that had accumulated ^{125}I decays can be

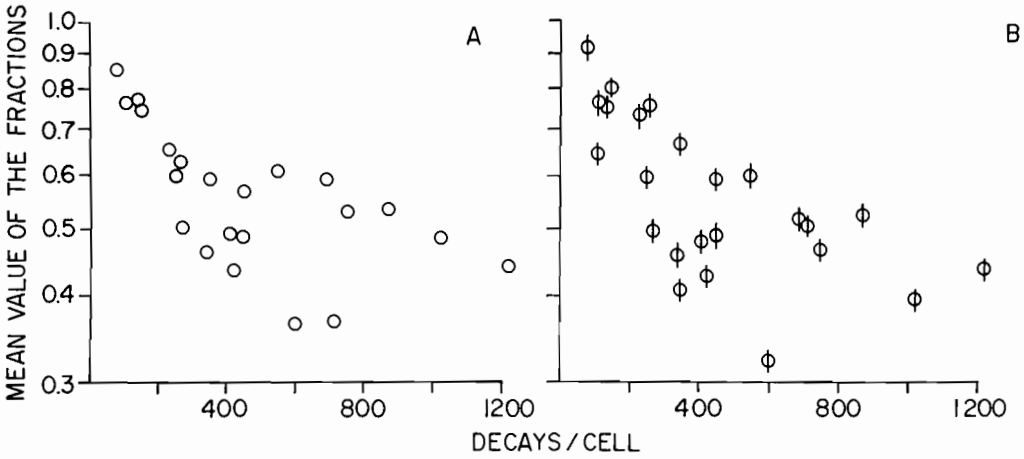

FIG. 3. Dose response curves obtained using data in Fig. 2. The mean values of the fractions for each elution profile is plotted versus the number of accumulated ^{125}I decays per cell treated with proteinase K (panel A) or not treated with proteinase K (Panel B). The slopes of the lines calculated by linear regression for the data shown in panel A and B were the same.

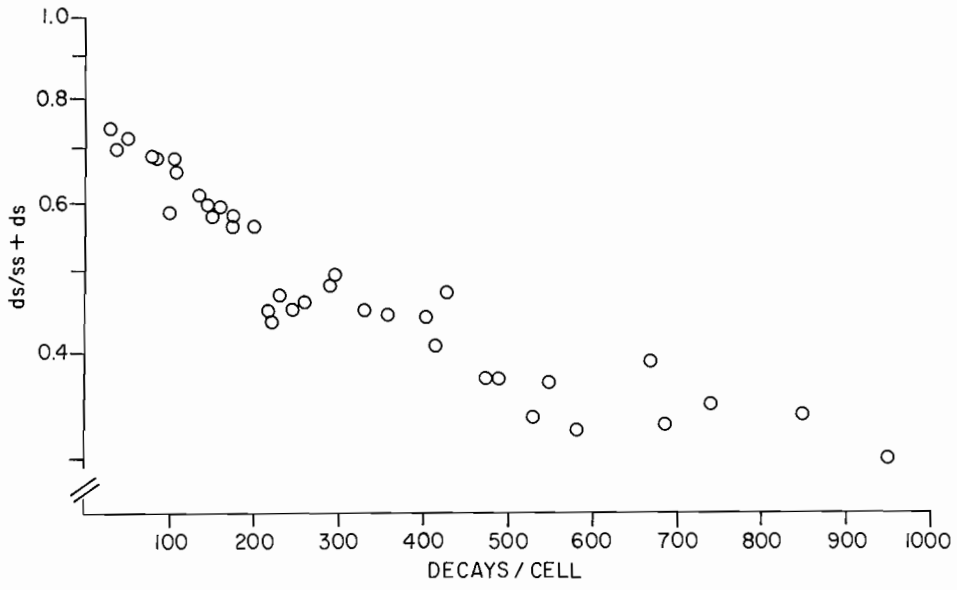

FIG. 4. DNA damage induction by ^{125}I decay as measured by the alkaline unwinding assay followed by hydroxyapatite chromatography. The double stranded (DS) fraction divided by the single stranded (SS) fraction were plotted versus the total number of accumulated ^{125}I decays per cell.

seen in Fig. 5. Figure 6 shows a dose response curve for cells that were damaged by 0 to 5,000 accumulated ^{125}I decays. As seen in Figs. 5 and 6, no difference in damage induction is observed for samples assayed at pH 7.2 versus 9.6 as also reported previously by Flick *et al.* (26). Approximately 3000 ^{125}I decays were required to reduce the mean values to 50%. The data were fit to a straight line (r = -0.94). The Y-intercept = 83.44%.

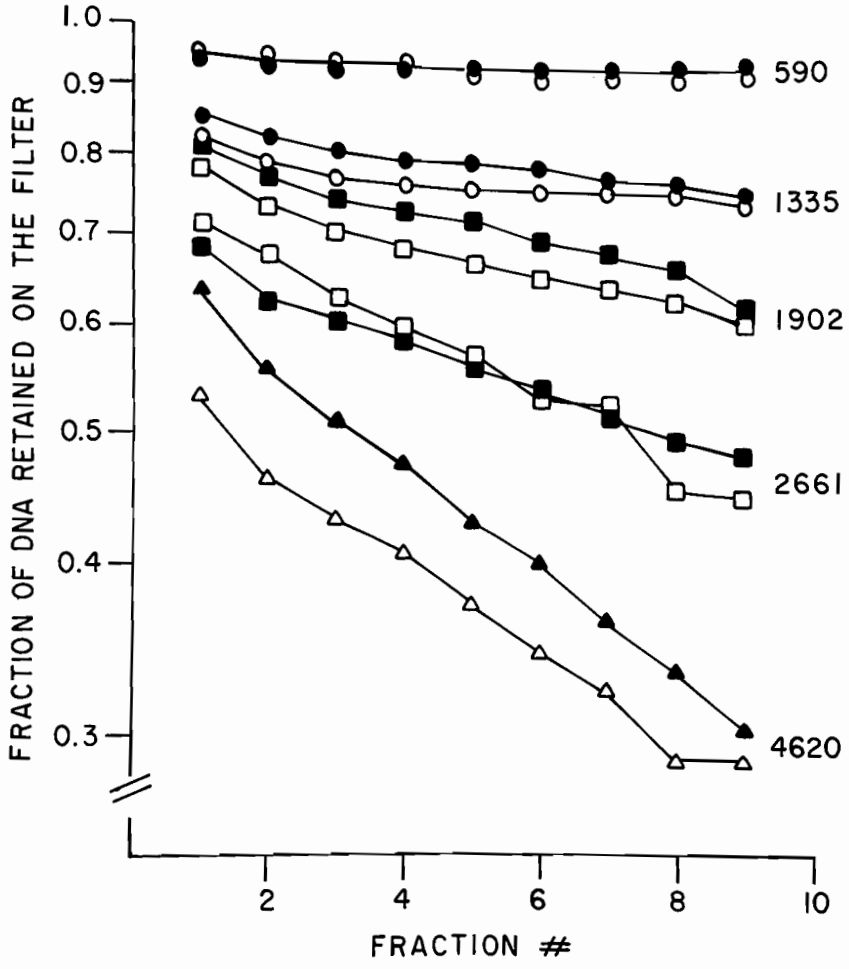

FIG. 5. Representative elution profiles of DNA damaged by ^{125}I decay and assayed by the neutral filter elution technique at pH 7.2 (open symbols) versus pH 9.6 (closed symbols). The number of accumulated ^{125}I decays is indicated to the right of each elution profile.

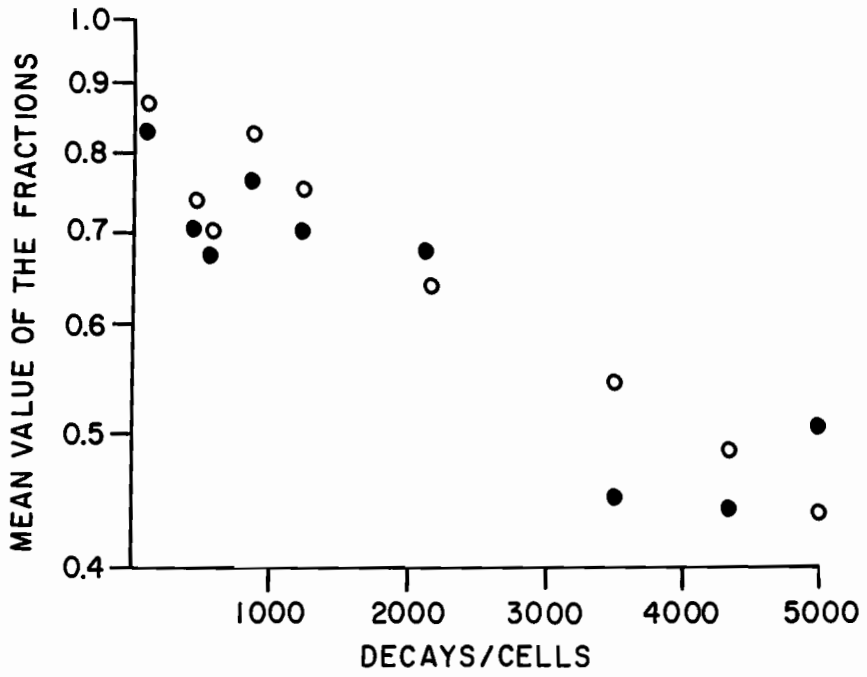

FIG. 6. A dose response curve for the neutral filter elution data. The mean value of the fractions for each elution profile was plotted versus the total number of accumulated ^{125}I decays per cell assayed at pH 7.2 (open symbols) or pH 9.6 (closed symbols). Approximately 3000 ^{125}I decays were required to reduce the mean value of the fractions to 50%. The slopes of the lines were calculated by linear regression analysis of the pH 7.2 data and the pH 9.6 data were the same.

The relative efficiency of the neutral filter elution assay compared to the alkaline filter elution assay can be calculated from the 50% values. The neutral filter elution assay was 10 times less sensitive in detecting ^{125}I decay induced DNA damage compared to the alkaline filter elution assay. As previously stated, 300 ^{125}I decays were required to reduce the fraction of retained DNA to 50% in the alkaline filter elution technique, and 300 ^{125}I decays required to reduce the double stranded fraction to 50% using the alkaline unwinding technique, whereas 3000 ^{125}I decays were required to reduce the fraction of retained DNA to 50% in the neutral filter elution technique.

DISCUSSION

The yield of DNA lesions produced by ^{125}I decay in mammalian cells has been calculated by measuring the molecular weight of DNA in alkaline (29) and both alkaline and neutral sucrose gradients (30). ^{125}I decay produces about 4-5 SSBs per decay (29,30) and about 1.4 to 2.7 DSBs per decay (30). Ward (31) discusses the following 4 temporally distinct stages for the production of chemically stable damage:

1. Physical deposition of energy (10^{-18} s).
2. Production of primary radicals on the target molecule and in molecules surrounding the target-direct and indirect effect (10^{-12} s).
3. Reaction of radicals on surrounding molecules with target molecule (10^{-8} s).
4. Reactions of unstable target radicals leading to chemically stable damage.

Step 1 is the first event involved in DNA damage induction by ^{125}I decay. The time required for the vacancy cascade associated with ^{125}I decay is less than 10^{-15} seconds (32). Steps 2-4 are likely swamped out by the range of energy deposition from ^{125}I decay (approximately 30 nm) (14) relative to the range of migration of OH· radicals (only about 6.8 nm) (33). In fact, indirect DNA damage produced by ^{125}I decay may be biologically inconsequential. LeMotte and Little (21) measure approximately a 5 fold increase in DNA DSBs produced by ^{125}I decay after decays were accumulated at 0°C in medium compared to when accumulation occurred at -90°C in medium containing 10% glycerol (radical scavenger). However, no change in cell survival is detected when decays are accumulated with or without the scavenger present (21). The chemically stable DNA lesion mentioned in step #4 above, should be fundamentally different for those lesions produced by ^{125}I decay compared to X or γ rays. This conclusion is an extrapolation of our simple microdosimetric calculations (17). The above conclusion is in contrast to conclusions by Iliakis *et al.* (1). They compared repair kinetics after ^{125}I decay and X irradiation and find that the rates are the same. Based on these results, the authors suggest that the DNA lesions produced by these two radiations are identical. However, the authors (1) do not state whether equitoxic levels of radiations were used or whether DNA damage induction levels were the same for the two radiations used. Indeed, the authors do not state the number of decays of ^{125}I that were used for their experiments. Much more work needs to be performed before such conclusions can be made.

DNA damage induction by ^{125}I decay was investigated by the alkaline (Figs. 2 and 3) and neutral filter elution (Figs. 5 and 6) assays and the alkaline unwinding assay (Fig. 4). In this investigation, no DNA protein cross links (Figs. 2 and 3) are detected. Also, no pH 9.6 labile lesions (Figs. 5 and 6) (see also Ref. 26) are detected suggesting that these assays are measuring frank DNA DSBs produced by ^{125}I decay. It is found that 300 ^{125}I decays per cell are required to reach the 50% damage level for both the alkaline unwinding (Fig. 4) and the alkaline filter elution assays (Fig. 3). Approximately 10 times greater number of accumulated ^{125}I decays are required to reach the 50% damage level for the neutral filter elution technique (Fig. 6).

The three different methods of measuring DNA damage (alkaline and neutral filter elution and alkaline unwinding) all indicate that damage induction relative to the number of accumulated ^{125}I decays can be fit to a straight line (no shoulder) except at very low numbers of ^{125}I decays where there is an initial increase in damage induction rate, *i.e.*, Y-intercept is significantly less than 100% (also see Ref. 21). Linear ^{125}I decay induced DNA damage curves are also found by Peak *et al.* (2), Radford and Hodgson (3), and LeMotte and Little (21).

All of the alkaline and neutral elution profiles show a marked drop in the fraction of retained DNA in the first fraction collected (Figs. 2 and 5). This large drop is attributed to ^{125}I decay induced clusters of lesions or multiply fragmented DNA released from genomic DNA under the elution conditions. The formation of multiple chromatin breaks or clusters of lesions in DNA has previously been suggested in connection with the strand breaking effectiveness of high-LET radiations (34). Martin and Haseltine (35) detected multiple chromatin breaks produced by ^{125}I decay in a small region of DNA in a synthetic linear piece of DNA when using DNA sequencing methodology. Turner *et al.* (36) also measured fragmentation of chromatin by ^{125}I decay into much smaller pieces of DNA than by ^{3}H decay using neutral sucrose gradient methods. It is predicted that conventional assays that measure DNA damage provide an underestimate of the numbers of strand breaks produced by ^{125}I decay. This prediction is supported by: 1) data presented here using the filter elution techniques and alkaline unwinding where an initial drop is observed in the damage induction curves (Figs. 3, 4 and 6), and 2) physical calculations (38). Calculations indicate that ^{125}I decay deposits a mean energy of 1.6 keV in a spherical volume of 10 nm (38) and this could lead to energy deposition that either has no impact on DNA (energy misses target and is "wasted") or causes

the induction of more than 1 DNA DSB (energy "hits" target) (30,37). In comparison, 600 eV from external γ irradiation can produce a DNA DSB (30,37).

In conclusion, these data indicate no DNA protein cross links or pH 9.6 labile lesions are produced by ^{125}I decay. Frank breaks are assayed by the neutral filter elution assay. Clusters of lesions or multiple chromatin breaks are detected after ^{125}I decay. Such multiple fragmentation of DNA may be modified by local chromatin compaction and in some cases may represent a devastating lesion with little or no chance for "repair". Based on these data and previous work with ^{125}I decay, we conclude that the consequences of ^{125}I decay in DNA and chromatin are not yet fully understood and, therefore, care should be exercised in the use of ^{125}I decay as a tool in calibrating assays to measure DNA damage in mammalian cells.

ACKNOWLEDGMENTS

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DISCUSSION

Schneiderman, M. If you get a number of breaks clustered around the ^{125}I decay, then on pulsed field gel electrophoresis you should have a large number of small DNA pieces. Have you used PFGE?

Yasui, L. No, I have not used PFGE. We are currently thinking of pushing the alkaline unwinding technique with lower concentrations of phosphate buffer to pick up the fragments produced by ^{125}I decay at the clustered lesion site.

Martin, R. 1) Linda, the 30 nm range figure you quoted seems very high to me, but there are other people in the audience who can comment on that better than I. In short, I am trying to draw a comment from the microdosimetry people.

Humm, J. L. 2) If you look at the DNA doughnut model of Charlton, presented at the last Auger Symposium, you will find that the dose deposition in adjacent strands in supercoiled DNA is between one tenth and one hundredth of the dose to the strands within which the ^{125}I is located.

Yasui, L. S. 1) The 30 nm range was taken from our studies using older Auger spectra. I am aware of newer data that also lists N-shell Auger electrons from ^{125}I decay - these have much shorter ranges. However, the main point I wanted to make with our model was that the range of energy deposition in DNA is much larger for ^{125}I decay than for X ray energy deposition. 2) If ^{125}I decay deposits 1.6 keV in a 10 nm target size (Tisljar-Lentulis *et al., Radiat. Environ. Biophys.* **26**: 189-195, 1987), then adjacent fibers could receive 16 to 160 eV. Assuming 100 eV is sufficient energy to produce a single strand break, there could certainly be enough energy to cause a single strand break in adjacent DNA. In addition, the residual positive charge on the ^{125}Te daughter nuclide could produce extensive local ionizations and excitations as the charge is shared with neighboring atoms producing DNA single strand and double strand breaks in adjacent DNA molecules.

Goodhead, D. You suggested looking specifically for clustered damage extending over about 30 nm, based on the estimated average size of the dense-ionization region around the Auger decay. Because of the large statistical variations in individual decays in the tracks of the emitted electrons, would it not be more reasonable to expect a wide spectrum of different clustered damages?

Yasui, L. I think it is reasonable to expect a wide spectrum of different clustered damages produced by ^{125}I decay in the DNA. One possible explanation for the differential cell survival data from ^{125}I decay is that local

chromatin or nuclear conformation modifies the DNA damage induction pattern and these different lesions are handled differently in the cells.

Rao, D. V. Linda, are you suggesting that when ^{125}I decay occurs on the coiled or folded DNA, there is more than one double strand break, but they all show up as one double strand break in your measurement?

Yasui, L. Yes, the model we propose would have highly localized multiple fragmentation of DNA. By the assays used in our investigation this cluster of lesions would fall through the filters and not be bound by hydroxyapatite. Thus, the assay measures these clusters of lesions as frank DNA double strand breaks.

Nagasawa, H. N. What are the different cell killing mechanisms for α particle and ^{125}IUdR irradiation of CHO K-1 and XRS-5? I found that the D_0 doses were nearly 1 for Il-treated, G_1-phase, CHO K-1 and XRS-5 after α particle irradiation.

Yasui, L. There does appear to be a different mechanisms of killing by ^{125}I decay and α particle irradiation. The RBE calculated for the K-1 and XRS-5 cells relative to ^{137}Cs γ radiation at 10% survival is about 8 and 10.3, respectively. The RBE values after α particle irradiation (depending upon the lab where studies were performed) have a reversed or equivalent relationship. Perhaps the spectrum of lesions produced by these radiations are handled differently by cells.