

Protection by DMSO against Cell Death Caused by Intracellularly Localized Iodine-125, Iodine-131 and Polonium-210

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The mechanisms by which DNA-incorporated radionuclides impart lethal damage to mammalian cells were investigated by examining the capacity of dimethyl sulfoxide (DMSO) to protect against lethal damage to Chinese hamster V79 cells caused by unbound tritium (³H₂O), DNA-incorporated ¹²⁵I- and ¹³¹I-iododeoxyuridine (¹²⁵IdU, ¹³¹IdU), and cytoplasmically localized ²¹⁰Po citrate. The radionuclides ³H and ¹³¹I emit low- and medium-energy β particles, respectively, ¹²⁵I is a prolific Auger electron emitter, and ²¹⁰Po emits 5.3 MeV α particles. Cells were radiolabeled and maintained at 10.5°C for 72 h in the presence of different concentrations of DMSO (5–12.5% v/v), and the surviving fraction compared to that of unlabeled controls was determined. DMSO afforded no protection against the lethal effects of the high-LET α particles emitted by ²¹⁰Po. Protection against lethal damage caused by unbound ³H, ¹³¹IdU and ¹²⁵IdU depended on the concentration of DMSO in the culture medium. Ten percent DMSO provided maximum protection in all cases. The dose modification factors obtained at 10% DMSO for ³H₂O, ¹³¹IdU, ¹²⁵IdU and ²¹⁰Po citrate were 2.9 ± 0.01 , 2.3 ± 0.5 , 2.6 ± 0.2 and 0.95 ± 0.07 , respectively. These results indicate that the toxicity of Auger electron and β -particle emitters incorporated into the DNA of mammalian cells is largely radical-mediated and is therefore indirect in nature. This is also the case for the low-energy β particles emitted by ³H₂O. In contrast, α particles impart lethal damage largely by direct effects. Finally, calculations of cellular absorbed doses indicate that β -particle emitters are substantially more toxic when incorporated into the DNA of mammalian cells than when they are localized extracellularly. © 2000 by Radiation Research Society

INTRODUCTION

The toxicity of radionuclides that emit Auger electrons depends on their subcellular distribution. When Auger electron emitters are localized in the cytoplasm, their toxicity is akin to that of low-LET radiations (1, 2). However, when

they decay in the immediate vicinity of DNA, they are as toxic as high-LET α particles (3, 4). Thus the response of mammalian cells to DNA-incorporated Auger electron emitters has been termed “high-LET-type”. The mechanism by which DNA-incorporated Auger electron emitters elicit high-LET-type responses in biological systems has been of considerable interest and sustained debate. Because of the similarity in the dose–response curves for these radionuclides and those for high-LET α particles, and the nature of the distribution of strand breaks observed in ¹²⁵I-labeled oligonucleotides (5), it was believed at first that the mechanism was largely direct deposition of energy in the immediate vicinity of the decay site (6). This direct deposition of energy could, in principle, have two sources: (1) direct irradiation of radiosensitive targets by the low-energy Auger electrons (direct effects) or (2) localized energy deposition due to charge neutralization of the residual tellurium daughter atom (6). However, this premise was challenged when it was shown that chemical radioprotectors were able to mitigate the biological effects of Auger electron emitters *in vivo* (2). This unexpected finding provoked studies to further elucidate the mechanism of the action of irradiation by Auger electron emitters.

A variety of radioprotectors were studied by Rao and colleagues in the mouse testis model, including cysteamine (MEA) (2, 7), vitamin C (8), *S*-(2-aminoethyl)isothiouronium bromide hydrobromide (AET) (9), vitamin A and soybean oil (10), and DMSO (11). The data for DMSO are presented in ref. (11) together with a summary of the data for the studies with the other protectors. For each radioprotector, several different radiochemicals were investigated, including cytoplasmically localized *N,N,N'*-trimethyl-*N'*-(2-hydroxyl-3-methyl-5-iodobenzyl)-1,3-propanediamine (H¹²⁵IPDM), DNA-incorporated iododeoxyuridine (¹²⁵IdU), and the α -particle emitter ²¹⁰Po citrate. The hydroxyl radical scavengers DMSO and vitamin C afforded considerable protection *in vivo* against low-LET-type damage caused by cytoplasmically localized ¹²⁵I and high-LET-type damage caused by DNA-incorporated ¹²⁵IdU. However, these radical scavengers provided no protection against effects caused by the 5.3 MeV α particles emitted by ²¹⁰Po. These data, along with those for the other radio-

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protectors mentioned above, provided considerable evidence that the mechanism by which Auger electron emitters impart high-LET-type damage *in vivo* is largely radical-mediated and is therefore indirect in nature.

In contrast to the indirect mechanisms proposed by Rao and colleagues (2, 7–12), Hofer and Bao (13) reported results of experiments suggesting that direct mechanisms were dominant. They synchronized Chinese hamster ovary (CHO) cells, labeled them with ^{125}I IdU, and froze the cells at -196°C in cryoprotective medium (with or without 25 mM MEA) at different times after radiolabeling. A dose–response curve with a shoulder, characteristic of low-LET-type radiations, was observed for cells frozen 30 min after labeling. The shape of the survival curve and the capacity of MEA to afford some protection against these effects [dose modification factor (DMF) = 1.5] led the authors to conclude that these were indirect effects. In contrast, the high-LET-type response observed for cells frozen 5 h after labeling and the inability of MEA to afford protection (DMF = 1.0) suggested that direct effects were responsible. Based on these data and on the fact that MEA protects in part by scavenging free radicals, the authors concluded that direct effects are the primary mechanism for the high-LET-type lethality of Auger electron emitters incorporated into the DNA of mammalian cells (13).

In addition to the mammalian cell experiments described above, other studies have been carried out with plasmids and oligonucleotides in an effort to elucidate the mechanisms by which Auger electron emitters impart biological damage in DNA (14–18). These studies have uniformly implicated direct effects as the primary mechanism involved in the damage suffered by naked DNA.

Seemingly supporting these findings were the recent results of Howell *et al.* (19), who used cultured Chinese hamster V79 cells. It was shown that although a nontoxic concentration of DMSO (5% v/v, 0.64 M) provided substantial protection against lethal effects caused by chronic exposure (72 h) of V79 cells to unbound ^{32}P and tritiated water ($^3\text{H}_2\text{O}$) at 10.5°C (also nontoxic), no significant protection was observed against the lethal effects of chronic exposure to DNA-incorporated ^{131}I IdU, ^{125}I IdU and tritiated thymidine ($[^3\text{H}]\text{dThd}$). These results appeared to contradict those of the studies *in vivo* showing that DMSO provides substantial protection against cell killing by DNA-incorporated ^{125}I IdU and cytoplasmically localized $\text{H}^{125}\text{IPDM}$ (11). Therefore, the authors concluded that 5% DMSO was not capable of protecting mammalian cells *in vitro* against lethal damage caused by DNA-incorporated radionuclides.

At about the same time, Walicka *et al.* (20, 21) demonstrated that higher concentrations of DMSO (10%, 1.28 M) provide marked protection against DNA double-strand breaks and death of V79 cells caused by ^{125}I IdU at 0.3°C . Under their experimental conditions, where ^{125}I decays were accumulated over a period of 6 to 48 h, the temperature of 0.3°C was cryotoxic, although the use of 10% DMSO mitigated the cryotoxicity to some extent. The observed tox-

icity was factored out by using similarly treated controls to assess the effects of the ^{125}I decays. The high DMF of 5.4 for the survival end point led the authors to conclude that indirect effects were principally responsible for the high-LET-type effects of DNA-incorporated ^{125}I . To explain their findings, Walicka *et al.* (21) proposed a model consisting of densely packed nucleosomes that would facilitate cross irradiation by the low-energy Auger electrons whereby indirect effects may be expected to predominate. Notably, Terrissol's (22) track structure calculations in a nucleosome model suggest that 75% of double-strand breaks produced by DNA-incorporated ^{125}I are due to indirect interactions.

In an attempt to sort out the inconsistencies between the various experimental data sets, the present work examines the concentration dependence of the protective action of DMSO against lethal damage caused by incorporated radionuclides. In addition, studies have been carried out to determine the maximum protection provided against several different radiochemicals. As in our previous communication (19), cultured Chinese hamster V79 cells are used as the experimental model with cell survival serving as the biological end point. The radiochemicals studied include IdU labeled with either the prolific Auger electron emitter ^{125}I or the β -particle emitter ^{131}I , $^3\text{H}_2\text{O}$ or ^{210}Po citrate.

MATERIALS AND METHODS

Radiochemicals

Tritiated water ($^3\text{H}_2\text{O}$, 0.37 GBq/ml), Na^{131}I in 0.1 N NaOH (36 GBq/ml), and Na^{125}I in 0.1 N NaOH (13.6 GBq/ml) were obtained from NEN Life Science Products (Boston, MA). The radionuclide ^{210}Po (3700 kBq/ml) was obtained as PoCl_4 in 2 M HCl from Isotope Products Laboratories (Burbank, CA). Radiolabeled iododeoxyuridine (^{131}I IdU and ^{125}I IdU) was synthesized and purified by HPLC in our laboratory according to procedures reported previously (10). Polonium-210 citrate was prepared by mixing the stock ^{210}Po solution with 1 M sodium citrate (pH 7.0) in the ratio of 1:9 as described previously (4). All radiochemical solutions were sterile and carrier-free. The radiochemicals ^{131}I IdU, ^{125}I IdU and ^{210}Po citrate are incorporated into the V79 cells, whereas $^3\text{H}_2\text{O}$ diffuses freely in the cells (19, 23).

Quantification of Radioactivity

The ^3H and ^{210}Po activities were determined with a Beckman LS3800 automatic liquid scintillation counter (Fullerton, CA) by transferring aliquots of radioactive culture medium into 6 ml of Aquasol[®] liquid scintillation cocktail (NEN Research Products, Boston, MA). The detection efficiencies for the ^3H 5.7 keV β particles and ^{210}Po 5.3 MeV α particles were 0.65 and 1.0, respectively. The ^{131}I activity was quantified with a Canberra HpGe (Meriden, CT) well detector (364.5 keV photopeak, efficiency = 0.175, yield = 0.812). The ^{125}I activity was quantified with a Beckman 5500 automatic γ -ray counter equipped with a 3-inch sodium iodide crystal (K-shell X rays + 35.5 keV γ rays, efficiency = 0.48, yield = 1.47).

Cell Culture and Rationale for Protocol Design

Chinese hamster V79 lung fibroblasts (kindly provided by A. I. Kassis, Harvard Medical School, Boston, MA) were used in these studies with clonogenic survival as the biological end point. Culturing conditions, culture media (MEMA, MEMB and wash MEMA), and the rationale for the

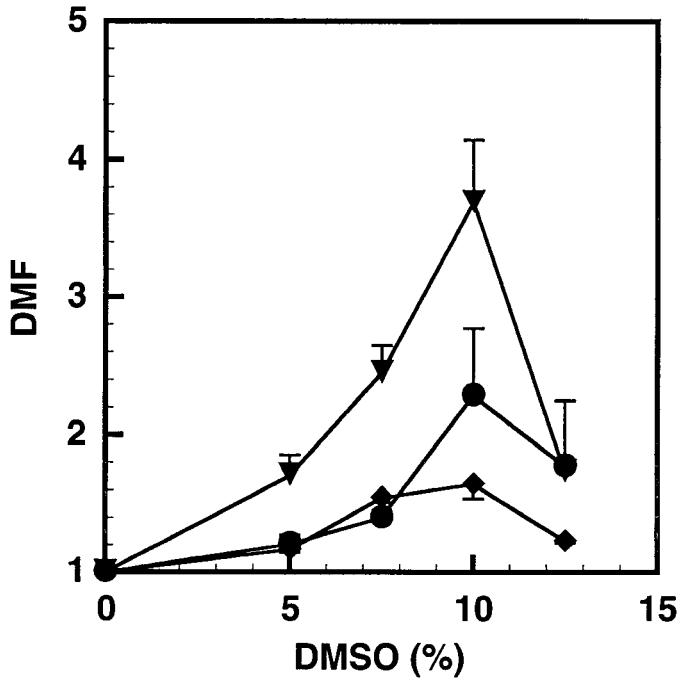


FIG. 1. Dose modification factor (DMF) as a function of DMSO concentration. V79 cells were exposed to $^3\text{H}_2\text{O}$ (\blacktriangledown), $^{131}\text{IIdU}$ (\blacklozenge) or $^{125}\text{IIdU}$ (\bullet) at 37°C for 12 h and subsequently treated with different concentrations of DMSO at 10.5°C for 72 h. Each point indicates the mean \pm SD for two individual experiments.

$$\text{SF}(^3\text{H}_2\text{O}) = \exp(-C/C_0); \quad (4)$$

$$\text{SF}(^{125}\text{IIdU}, ^{131}\text{IIdU}) = \exp(-A/A_0). \quad (5)$$

Since the absorbed dose received by the cells is directly proportional to the concentration of $^3\text{H}_2\text{O}$ in the culture medium or the mean cellular uptake of $^{131}\text{IIdU}$ or $^{125}\text{IIdU}$, the DMF, or the degree of protection provided by DMSO against the lethal effects of radionuclides, is given by the ratio of the C_0 's or A_0 's in the presence and absence of DMSO:

$$\text{DMF}(^3\text{H}_2\text{O}) = \frac{C_0(\text{with DMSO})}{C_0(0\% \text{ DMSO})},$$

$$\text{DMF}(^{125}\text{IIdU}, ^{131}\text{IIdU}) = \frac{A_0(\text{with DMSO})}{A_0(0\% \text{ DMSO})}. \quad (6)$$

The results in Fig. 1 illustrate that DMSO protects V79 cells from damage caused by DNA-bound $^{131}\text{IIdU}$ and $^{125}\text{IIdU}$ and unbound $^3\text{H}_2\text{O}$ in a concentration-dependent manner. Little protection was afforded by 5% DMSO against damage caused by DNA-bound $^{131}\text{IIdU}$ or $^{125}\text{IIdU}$; however, more substantial protection was observed in the case of $^3\text{H}_2\text{O}$. DMSO-mediated protection was optimal at 10% for all three radiochemicals. A marked decline in the DMF was observed for all three radiochemicals at 12.5% DMSO.

Survival after Exposure to $^3\text{H}_2\text{O}$, $^{131}\text{IIdU}$, $^{125}\text{IIdU}$ and ^{210}Po in the Presence of 10% DMSO

The radioprotective efficacy of 10% DMSO was investigated further by exposing V79 cells to different concen-

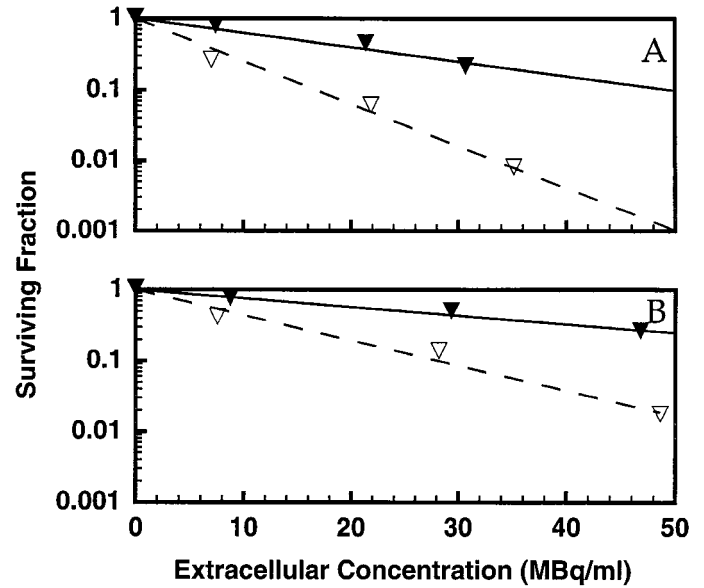


FIG. 2. Survival of V79 cells after a 12-h exposure at 37°C followed by a 72-h exposure at 10.5°C to various concentrations of $^3\text{H}_2\text{O}$ in the culture medium in the absence (∇) and presence (\blacktriangledown) of 10% DMSO. The data for two independent experiments are shown in panels A and B. Least-squares fits of the data to a monoexponential function are shown. Standard deviations for individual data points are of the order of the dimensions of the symbols.

trations of unbound $^3\text{H}_2\text{O}$ and different cellular uptakes of DNA-incorporated $^{131}\text{IIdU}$ and $^{125}\text{IIdU}$. In addition, the capacity of 10% DMSO to protect against the direct effects of ^{210}Po α particles was investigated. Figure 2 shows the survival of V79 cells after a 72-h exposure at 10.5°C to various concentrations (0–46.3 MBq/ml) of $^3\text{H}_2\text{O}$ in the absence and presence of 10% DMSO. The data were fitted by least squares to Eq. (4) to obtain the mean lethal concentration, C_0 , for $^3\text{H}_2\text{O}$. The DMF values for each experiment as presented in Table 1 were calculated using Eq. (6). The mean DMF for the two experiments was 2.9 ± 0.01 .

Figures 3–5 show the survival of V79 cells after treatment with $^{131}\text{IIdU}$, $^{125}\text{IIdU}$ or ^{210}Po . The surviving fraction as a function of mean activity per cell was plotted for each experiment, and the data were fitted to the relationship

$$\text{SF} = 1 - [1 - \exp(-A/A_0)]^n, \quad (7)$$

where A is the mean activity per cell at the end of the uptake period and A_0 and n are analogous to the D_0 and n in the single-hit multi-target model (24), respectively. The DMF is given by the ratio of the values of A_0 in the presence and absence of DMSO. A value of $n = 1$ was used for the fit to the data for $^{125}\text{IIdU}$ and ^{210}Po . As given in Table 1, the mean DMFs for protection by 10% DMSO against the lethal effects of $^{131}\text{IIdU}$, $^{125}\text{IIdU}$ and ^{210}Po are 2.3 ± 0.5 , 2.6 ± 0.2 and 0.95 ± 0.07 , respectively.

TABLE 1
Dose Modification Factors in the Presence of 10% DMSO

Radiochemical	Experiment no.	10% DMSO		0% DMSO		DMF
		<i>n</i>	<i>C</i> ₀ (MBq/ml)	<i>n</i>	<i>C</i> ₀ (MBq/ml)	
³ H ₂ O	1	1	21.6	1	7.26	2.96
	2	1	36.0	1	12.2	2.94
						2.9 ± 0.01
Radiochemical	Experiment no.	10% DMSO		0% DMSO		DMF
		<i>n</i>	<i>A</i> ₀ (mBq/cell)	<i>n</i>	<i>A</i> ₀ (mBq/cell)	
²¹⁰ Po citrate	1	1	0.0218	1	0.0243	0.90
	2	1	0.0193	1	0.0191	1.01
						0.95 ± 0.07
¹³¹ IdU	1	2.10	7.85	3.25	4.03	1.94
	2	1.78	8.40	3.87	3.13	2.68
						2.3 ± 0.5
¹²⁵ IdU	1	1	0.383	1	0.141	2.72
	2	1	0.382	1	0.153	2.50
						2.6 ± 0.2

DISCUSSION

Mean Lethal Number of Decays, Mean Lethal Dose, and Relative Biological Effectiveness

Cellular dosimetry is used to calculate the mean lethal number of intracellular decays and mean absorbed dose to the cell nucleus for each of the intracellularly incorporated radiochemicals examined in the present study. Using the methods outlined in the Appendix, the mean lethal number

of decays, N_{37} , that are required to achieve 37% survival in the absence of DMSO is 7.1, 2240 and 49 for ²¹⁰Po citrate, ¹³¹IdU and ¹²⁵IdU, respectively (Table A1). The values for ²¹⁰Po citrate and ¹²⁵IdU are in good agreement but are slightly lower than the values of 9 and 66 obtained in our earlier work carried out at 37°C (4). The mean lethal absorbed dose, D_{37} , to the cell nucleus (calculated as described in the Appendix) in the absence of DMSO is 0.68, 2.4 and 0.33 Gy, respectively. The 0.68 Gy obtained for ²¹⁰Po citrate

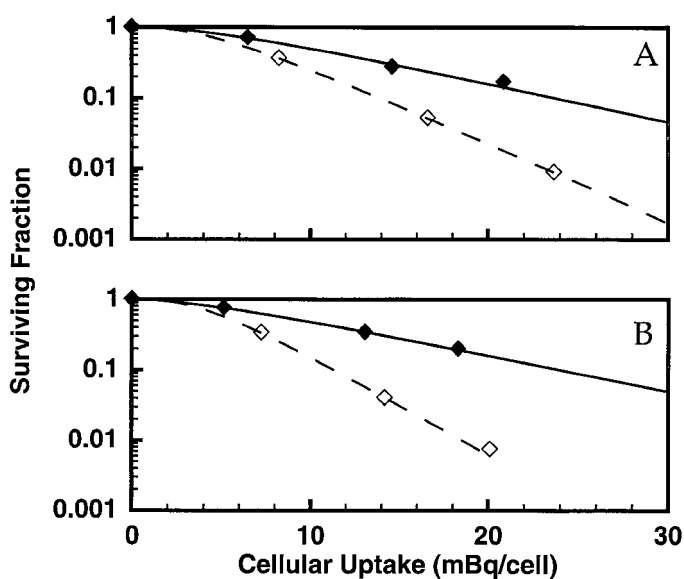


FIG. 3. Survival of V79 cells as a function of intracellular activity of ¹³¹IdU in the absence (◇) and presence (◆) of 10% DMSO in the culture medium. The data for two independent experiments are shown in panels A and B along with the corresponding least-squares fits to the single-hit multi-target model. Standard deviations for individual data points are of the order of the dimensions of the symbols.

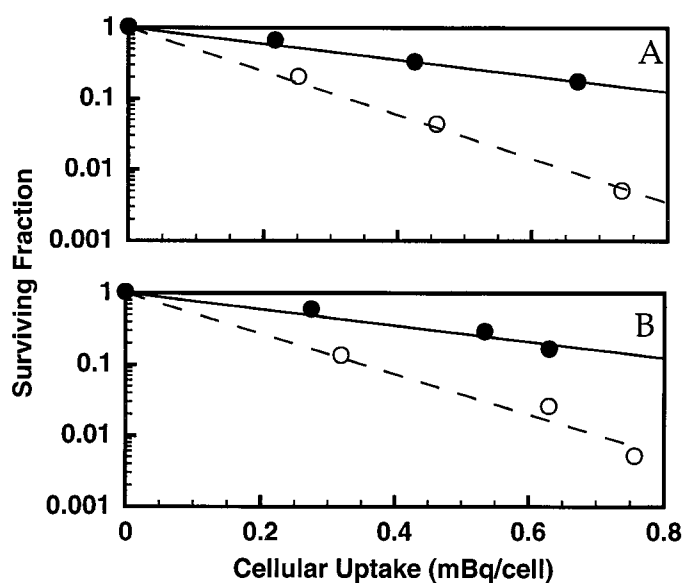


FIG. 4. Survival of V79 cells as a function of intracellular activity of ¹²⁵IdU in the absence (○) and presence (●) of 10% DMSO in the culture medium. The data for two independent experiments are shown in panels A and B. Least-squares fits of the data to a monoexponential function are shown. Standard deviations for individual data points are of the order of the dimensions of the symbols.

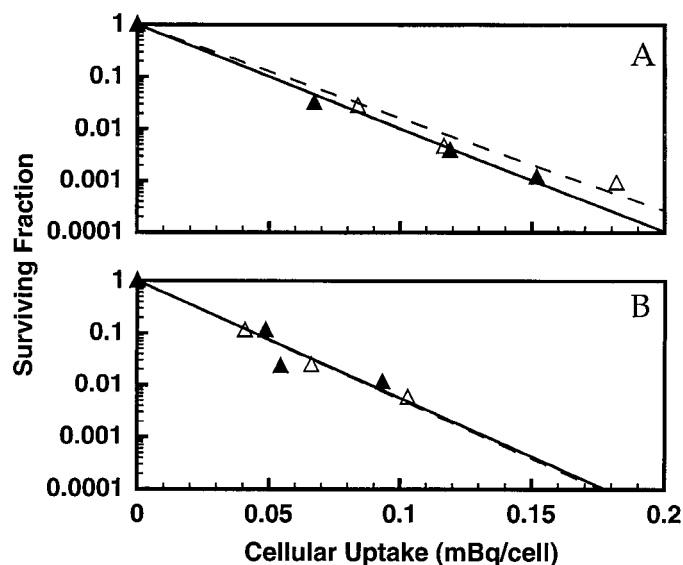


FIG. 5. Survival of V79 cells as a function of intracellular activity of ^{210}Po in the absence (Δ) and presence (\blacktriangle) of 10% DMSO. The data for two independent experiments are shown in panels A and B. Least-squares fits of the data to a monoexponential function are shown. Standard deviations for individual data points are of the order of the dimensions of the symbols.

corresponds to about two complete α -particle traversals across the cell nucleus (4). Also calculated in the Appendix is the D_{37} for $^3\text{H}_2\text{O}$. This radiochemical yields a D_{37} of 2.7 Gy. These data are presented in Table 2 and are compared with the D_{37} of 7.1 Gy for unbound ^{32}P , an emitter of energetic β particles (see ref. 19 and Appendix). This radionuclide emits energetic β particles which are clearly low-LET in nature and are the same type of particle emitted by ^3H , ^{125}I and ^{131}I (i.e. electron); it also delivers the exposure in a chronic fashion. Thus, in keeping with the definition of RBE (24), it serves as a good reference radiation. In view of the historical use of external γ rays as the reference radiation, it would also be useful to study the effects of acute ^{137}Cs or chronic $^{99\text{m}}\text{Tc}$ γ rays. However, the geometry of the present experiment precluded their use. The RBE of $^3\text{H}_2\text{O}$, ^{210}Po citrate, ^{131}IdU and ^{125}IdU compared to extracellular ^{32}P (D_{37} of 7.1 Gy) at the D_{37} are 2.6, 10, 3.0 and 22, respectively (Table 2). The high RBE values for ^{210}Po citrate and ^{125}IdU are expected and are similar to those obtained by Howell *et al.* (4) when chronic $^{99\text{m}}\text{Tc}$ γ rays were used as the reference radiation and the V79 cells were maintained at 37°C . While the RBE of 2.6 for $^3\text{H}_2\text{O}$ is not surprising in view of the LET (3.3 keV/ μm) of the 5.7 keV β particles emitted by ^3H (25), it also falls within the range of RBE values observed for $^3\text{H}_2\text{O}$ for a variety of end points (23). The RBE of 3.0 for ^{131}IdU is perhaps surprising given the low-LET nature of the β particles (mean energy = 191 keV) emitted by ^{131}I . Possible explanations for this relatively high RBE are discussed at the end of the Discussion section.

TABLE 2
Relative Biological Effectiveness

Radiochemical	D_{37} (Gy)	RBE
Unbound		
^{32}P	7.1 ± 0.7	1.0
$^3\text{H}_2\text{O}$	2.7 ± 1.0	2.6 ± 1.0
Intracellular		
^{210}Po citrate	0.68 ± 0.12	10 ± 2
^{131}IdU	2.4 ± 0.4	3.0 ± 0.6
^{125}IdU	0.33 ± 0.02	22 ± 2

DMSO as a Radioprotector

Ionizing radiation is generally believed to impart damage to biological tissue by two primary mechanisms: direct and indirect. Direct effects are the result of energy deposited directly in critical molecular structures within the cell. Indirect effects are due to interactions between a variety of free radicals (OH^\cdot , H^\cdot , etc.) produced in the radiolysis of water with the critical molecules (26). The biological damage caused by high-LET radiations such as α particles has been attributed primarily to direct effects (27), whereas the biological effects of low-LET radiations (e.g. β particles, X rays) are caused primarily by indirect effects (26).

Dimethyl sulfoxide is a radioprotector whose protective action is due principally to scavenging of hydroxyl radicals (28–30). The capacity of DMSO to provide substantial protection against biological effects caused by low-LET radiations has been demonstrated using a variety of experimental end points (11, 28, 31–34). As a radical scavenger, the ability of DMSO to protect against the direct action of high-LET α particles is minimal (11, 34). Therefore, it is well suited for the study and comparison of the mechanisms of the action of different radiations emitted by tissue-incorporated radionuclides. However, typical cell survival experiments *in vitro* using DMSO as a radioprotector involve acute radiation exposures in the presence of DMSO concentrations as high as 15–20% (v/v), after which the cells are immediately washed free of the radioprotector to minimize the toxic effects of DMSO (31). When cells are irradiated by radionuclides, the dose is delivered chronically, and therefore the chemical agent should be present during the extended irradiation time. To minimize the toxicity associated with exposure of cultured cells to radioprotective concentrations of DMSO at 37°C , we have used our techniques reported previously (19) and have maintained the cells at 10.5°C throughout most of the exposure period. Under these conditions, the V79 cells were not proliferating, no cryotoxicity was observed, and only minimal toxicity resulted even at the highest DMSO concentration used (12.5%). This is in contrast to the studies of Walicka *et al.* (20), where a high degree of cryotoxicity was observed when the V79 cells were maintained at 0.3°C in the absence of DMSO. This is expected based on our previous studies (19) and therefore suggests that 10.5°C may be better suited for the present studies.

DMF as a Function of DMSO Concentration

The results presented in Fig. 1 show a marked dependence of the DMF on DMSO concentration in the culture medium. As indicated in the Materials and Methods section, these data were obtained using procedures that emphasized obtaining relative DMF values. The DMFs for the various radiochemicals rise rapidly as the DMSO concentration increases from 5% to 10% and then fall precipitously at 12.5% DMSO. This is in keeping with data reported by Vos and Kaalen (31), who found that when cells were acutely irradiated with 250 kVp X rays in the absence and presence of DMSO, the DMF increased linearly with DMSO concentration up to 30% v/v and then could not be measured due to extreme chemotoxicity. Therefore, for both chronic and acute irradiation conditions, there appears to be a window for optimum radioprotection with minimal undesired chemotoxic effects on the V79 cells. Similar observations were made by Roberts *et al.* (35) for the radioprotector MEA.

Contribution of Indirect Effects to Lethality of DNA-Incorporated Radionuclides

Figures 2–5 and Table 1 directly compare the radioprotection provided by 10% DMSO against lethal damage to V79 cells caused by $^3\text{H}_2\text{O}$, ^{131}IdU , ^{125}IdU and ^{210}Po citrate. The highest DMF was obtained for $^3\text{H}_2\text{O}$: 2.9 ± 0.01 . This radiochemical freely diffuses into the cells and does not bind to DNA. Similar DMF values were obtained for DNA-incorporated ^{131}IdU (2.3 ± 0.5) and ^{125}IdU (2.6 ± 0.2) despite their disparate radiation emissions and RBE values. Each decay of ^{131}I emits low-LET β particles with a yield close to unity (25), whereas ^{125}I emits about 25 low-energy Auger electrons per decay (36). In contrast to these results, a DMF of 0.95 ± 0.07 was obtained for intracellular ^{210}Po , which emits a single 5.3 MeV α particle per decay. Therefore, 10% DMSO offers no protection against high-LET α particles, which are known to impart biological damage largely by direct effects. Substantial protection is provided against low-energy β particles emitted by unbound ^3H , which cause biological damage primarily by indirect effects. Given the high degree of protection afforded by 10% DMSO in the case of ^{125}IdU and ^{131}IdU , these data suggest that DNA-incorporated ^{131}I and ^{125}I impart lethal damage by a similar mechanism (e.g. indirect). This conclusion is in agreement with that of Rao and colleagues, who showed that DNA-incorporated Auger electron emitters cause lethal damage to spermatogonial cells in mouse testes principally through indirect effects (2, 7–12). This series of *in vivo* experiments culminated with the work of Goddu *et al.* (11), which showed that DMSO provides substantial protection against lethal damage from ^{125}IdU (DMF = 4.4 ± 1.0) and cytoplasmically localized ^{125}I (DMF = 3.1 ± 1.0) and offers no protection against lethal damage caused by 5.3 MeV α particles emitted by ^{210}Po citrate (DMF = 1.1 ± 0.2).

Walicka *et al.* (20) also found that 10% DMSO provided

ample protection against the lethal effects of ^{125}IdU in V79 cells (DMF = 5.4 ± 0.6) and reached the same conclusions as Rao and colleagues (2, 7–12) regarding the indirect mechanism of action of DNA-incorporated Auger electron emitters in mammalian cells. Walicka *et al.* (20) labeled V79 cells with ^{125}IdU for 18 h at 37°C and then stored them on ice for 6 to 48 h for accumulation of decays prior to plating for colony formation. Decays of ^{125}I occur in the cell during the 18-h uptake period, the time during which the cells were stored on ice, and during the colony-forming period. The effect of decays that occurred during the 18-h uptake period and colony-forming period was factored out by using appropriate controls. The resulting surviving fraction, corrected for cryotoxicity and the effects of decays that occurred during the uptake and colony-forming periods, was plotted as a function of intracellular decays that occurred during the storage time on ice. If one examines the number of decays that occur during each period, the correction is substantial. Consider, for example, a cellular uptake of 0.1 mBq/cell after the 18-h uptake period. The intracellular cumulated activity during the uptake period would be 3.2 Bq s (1 Bq s = 1 decay). If the intracellular activity during the colony-forming period decreases with an effective half-time of 12 h, the cumulated activity during this period would be 6.2 decays. Only 2.2 decays would occur in the cells during the period on ice if they were maintained there for 6 h. Therefore, only 20% of the decays occurred in the presence of DMSO, and the effects of the remaining decays had to be factored out by using controls. Hence the surviving fractions were extracted from data that contain high “background noise”. This problem is alleviated somewhat for the later times. When the cells were kept on ice for 24 and 48 h, 48 and 65% of the decays, respectively, occurred in the presence of DMSO. These large corrections may explain in part why Walicka *et al.* (20) obtained a much higher DMF for ^{125}IdU than for acute ^{137}Cs γ rays. The chronic and acute irradiation protocols for these two radiation sources may also be a factor.

Despite the different experimental models and procedures used to obtain the various data sets on radioprotection against the lethal effects of DNA-incorporated ^{125}I in mammalian cells with radical scavengers and other radioprotectors, most of the results are in relatively good agreement. The early work of Rao and colleagues with mouse testis (11), the recent studies of Walicka *et al.* (20), and the present studies all strongly support the hypothesis of Rao *et al.* (7) that indirect mechanisms predominate in the lethal effects of DNA-incorporated Auger electron emitters. Our data for ^{131}IdU indicate that the same can be concluded for DNA-incorporated β -particle emitters. This is expected based on conventional wisdom.

Given that DNA-incorporated Auger electron emitters appear to impart biological damage largely through indirect effects, how is it that they can produce high-LET-type survival curves? Linz and Stöcklin (37) used a supercoiled plasmid DNA model to show that ^{125}I decays cause DSBs

hundreds of base pairs from the decay site and that formation of these particular DSBs is strongly influenced by the presence of radical scavengers. In contrast, the presence of radical scavengers had no impact on DSBs formed in the immediate vicinity of the decay site. Furthermore, they found that the ^{125}I decays caused one to three DSBs per decay. Although Linz and Stöcklin argued that the distant DSBs were unimportant compared with the DSBs formed in the immediate vicinity of the decay site, their data show that significant indirect damage occurs at locations distant from the decay site. This latter finding is supported by the theoretical calculations of Terrissol (22), who showed that the convoluted structure of chromatin in the cell nucleus provides an environment whereby radicals resulting from the dense shower of low-energy Auger electrons can cause DSBs hundreds of base pairs distant from the actual site of decay. In fact, Terrissol's calculations predict that 75% of all DSBs caused by DNA-incorporated ^{125}I are due to indirect effects. Similar to Linz and Stöcklin (37), Walicka *et al.* (21) have found that DSBs produced by ^{125}IdU in V79 cells can also be protected in part by 10% DMSO and that more than one DSB is produced per decay. Like Terrissol, they invoke a nucleosome model of chromatin structure and suggest that one DSB is due to local effects whereas the additional DSBs are due to interaction of SSBs at distances far from the decay site. This is a plausible hypothesis for explaining the capacity of radioprotectors to mitigate the high-LET-type effects of DNA-incorporated ^{125}I . Interestingly, in 1979, Rao *et al.*² hypothesized that the nucleosome was the relevant biological target for DNA-incorporated Auger electron emitters.

Contribution of Direct Effects to Lethality of DNA-Incorporated Radionuclides

Despite the apparent importance of indirect effects in the lethal action of DNA-incorporated Auger electron and β -particle emitters, our data also provide insights into the contribution of direct effects to their lethality. It is interesting to note that the DMFs shown in Table 1 for 10% DMSO are somewhat higher for unbound $^3\text{H}_2\text{O}$ (2.9 ± 0.01) compared to DNA-incorporated ^{125}IdU (2.6 ± 0.2) and ^{131}IdU (2.3 ± 0.5). In addition, our previous communication showed that 5% DMSO provided ample protection against lethal effects caused by unbound $^3\text{H}_2\text{O}$ ($\text{DMF} = 2.3 \pm 0.3$) as well as unbound ^{32}P ($\text{DMF} = 2.6 \pm 0.5$), which emits energetic β particles (19). However, little or no protection was afforded by 5% DMSO in the case of DNA-incorporated [^3H]dThd, ^{125}IdU and ^{131}IdU (19). Therefore, although indirect effects appear to be principally responsible for the lethal damage caused by [^3H]dThd, ^{125}IdU and ^{131}IdU , other

mechanisms are involved in the damage imparted by DNA-incorporated radionuclides. However, the damage caused by other mechanisms appears to constitute a smaller fraction of the overall damage responsible for cell death.

The portion of the lethal damage to mammalian cells that is imparted by DNA-incorporated radionuclides that is not due to indirect effects can, in principle, be due to direct effects, charge-induced molecular fragmentation (^{125}I) (38, 39), chemical transmutation (40), recoil, and other mechanisms such as long-range energy transfer (41). Consider first the case of the Auger electron emitter ^{125}I . Charge-induced molecular fragmentation as a consequence of the ^{125}I Auger electron cascade is not supported by the data of Hofer *et al.* (42) or Goddu *et al.* (11), nor is it supported by the present data. While chemical transmutation cannot be ruled out by the present data, the arguments of Makrigiorgos *et al.* (43), who compared the effects of $^{77}\text{BrdU}$, ^{123}IdU and ^{125}IdU , strongly indicate that transmutation is not a significant factor. Recoil obviously is not a significant factor in the case of Auger electron emitters. While long-range energy transfer may be important in the case of ^{125}I (44), it appears that direct effects are the most likely secondary mechanism (11). The presence of a direct component is supported by the data of Kandaiya *et al.* (15) and Lobachevsky and Martin (16), who found that in the case of ^{125}I -labeled oligonucleotides *in vitro*, where no cross irradiation from nearby labeled oligonucleotides is present, DMSO does not provide protection against strand breaks within 9 bp of the decay site and provides only limited protection against breaks that occur at more distant locations. Similarly, Adelstein and Kassis (14) found that DMSO protected against damage to plasmid DNA caused by unbound ^{125}I in the solution; however, no protection was observed against damage caused by ^{125}I -labeled Hoechst 33342, which binds to the minor groove of DNA in A-T rich regions. These data suggest that direct effects from ^{125}I decays in the DNA may be responsible for DNA damage within a few base pairs of the decay site.

The importance of direct effects is also supported by the data of Hofer and Bao (13), who examined the capacity of MEA to protect against lethality and induction of micronuclei in CHO cells maintained at -196°C . A low-LET-type response was observed when cells were synchronized at the G_1/S -phase boundary, allowed to progress synchronously into S phase for 30 min, pulse-labeled with ^{125}IdU for 10 min, and then incubated for 30 min in nonradioactive medium prior to freezing. In contrast, a high-LET-type response was observed when the cells were incubated 5 h prior to freezing. Cysteamine provided protection against the effects of ^{125}I during the initial phase of effects characteristic of low-LET radiation, but no protection was seen during the phase of effects characteristic of high-LET radiation. Through comparison with parallel studies with external X rays, Hofer and Bao concluded that the latter phase was indeed of a high-LET nature and suggested that the ^{125}I must be contained within higher-order structures in the

² D. V. Rao, G. F. Govelitz, K. S. R. Sastry, J. W. Bowers and J. T. Mallams, Radiotoxicity of I-125 deoxyuridine *in vivo* and microdosimetry of Auger and Coster Kronig electrons. Presented at the Fifth International Conference on Medical Physics, Medical Physics Department, Beilinson Medical Center, Petah Tikva, Israel, 1979.

cell nucleus to observe this phenomenon. It is difficult to reconcile the absence of protection against their high-LET-type effects with the protection observed for mammalian cells in a liquid environment. One clue to this difference may lie in the lack of protection provided by MEA against the high-LET-type effects. MEA has been shown to provide protection against high-LET radiation (45), so it is curious that it was unable to provide any protection against the high-LET-type effects in the studies of Hofer and Bao. It is possible that 25 mM MEA was sufficient to protect against low-LET-type effects while higher concentrations may be required to protect against high-LET-type effects. Perhaps the frozen conditions may also contribute to the differences observed between the various studies. Livesey has indicated that indirect effects are diminished or eliminated in dry or frozen biological preparations, while hydrogen donation is maintained (46). Accordingly, these conditions have been used by investigators to eliminate radical scavenging to study the hydrogen donation mechanism of radioprotection by thiols such as cysteamine (46). If Hofer and Bao have effectively eliminated radical scavenging and retained hydrogen donation, then the protective mechanisms operative in the liquid environment will be very different from those in the frozen state and therefore will be difficult to compare. If this is so, then perhaps the low-LET-type response observed by Hofer and Bao is modified by hydrogen donation whereas the high-LET-type response is not. The implication for the Auger electron effect is unclear.

Let us now turn our attention to the secondary mechanisms involved in the case of ^{131}I IdU. Our data indicate that DNA-incorporated ^{131}I is about three times more lethal than external β particles (e.g. ^{32}P). Furthermore, the differences in protection afforded by both 5 and 10% DMSO against the lethal effects of $^3\text{H}_2\text{O}$ and ^{131}I IdU suggest that a significant fraction of the damage imparted by this radiochemical is through mechanisms other than indirect. These findings are in accord with the data of Whaley and Little (47) for ^{131}I IdU as well as extensive data on DNA-incorporated ^3H and ^{32}P that have been reviewed by Halpern and Stöcklin (41). Halpern and Stöcklin have discussed the many possible mechanisms including direct effects, chemical transmutation, recoil, and other mechanisms such as long-range energy transfer. Whaley and Little have ruled out recoil as a mechanism for the enhanced toxicity of DNA-incorporated ^{131}I due to the very low nuclear recoil energy of the xenon daughter. However, the remaining mechanisms could explain the observed effects. Elucidation of the exact mechanism will require further study.

CONCLUSIONS

The present data support the hypothesis that indirect effects are largely responsible for lethal damage to mammalian cells from DNA-incorporated Auger electron emitters both *in vivo* (2, 7–12) and *in vitro* (19–21). Only a small

portion of the lethal damage is likely to be due to direct effects. In addition, β -particle emitters are substantially more toxic when incorporated into the DNA of mammalian cells than when they are localized extracellularly.

APPENDIX

Absorbed Dose From $^3\text{H}_2\text{O}$

The cells were exposed to $^3\text{H}_2\text{O}$ in 17×100 -mm Falcon sterile polypropylene culture tubes placed on a rocker-roller. The rocker-roller maintained the cells as a uniform suspension in 2 ml of culture medium. Given that $^3\text{H}_2\text{O}$ diffuses freely into the cells, the mean absorbed dose to the cells is equal to that to the culture medium. The mean absorbed dose to the culture medium per unit cumulated activity of ^3H in the culture medium (S) is given by (48):

$$S = \Delta\phi/m = 4.54 \times 10^{-13} \text{ Gy/Bq s}, \quad (\text{A1})$$

where Δ is the mean energy emitter per transition, ϕ is the absorbed fraction of energy emitted by ^3H that is absorbed in the culture medium, and m is the mass of the culture medium ($m = 0.002 \text{ kg}$). For ^3H , $\Delta = 9.08 \times 10^{-16} \text{ Gy kg Bq}^{-1} \text{ s}^{-1}$ (25); $\phi = 1$ due to the short range of the tritium β particles (mean energy = 5.67 keV). Therefore, $S = 4.54 \times 10^{-13} \text{ Gy/Bq s}$ for $^3\text{H}_2\text{O}$. The cumulated activity, \tilde{A} , in the tube is given by

$$\tilde{A} = A(t=0) \int_0^3 \exp(-0.693t/T_p) dt, \quad (\text{A2})$$

where $A(t=0)$ is the initial activity in the tube, is the total exposure time, and T_p is the physical half-life. For ^3H , is the initial 12-h period at 37°C followed by 72 h at 10.5°C for a total of 84 h. The physical half-life, T_p , is 12.33 years (25). In the absence of DMSO, the mean C_0 for the two experiments is $9.7 \pm 3.5 \text{ MBq/ml}$ (Table 1), therefore, $A(t=0) = 19.5 \text{ MBq}$. Since T_p is very long compared to 84 h, the cumulated activity corresponding to C_0 is given by

$$\tilde{A} = A(t=0) \cdot 84 \text{ h} = 19.5 \text{ MBq} (84 \text{ h}) = 5.89 \times 10^{12} \text{ Bq s}.$$

The mean absorbed dose is given by (49)

$$D = \tilde{A} S. \quad (\text{A3})$$

Therefore, the mean absorbed dose delivered to the cells in the absence of DMSO at a $^3\text{H}_2\text{O}$ concentration corresponding to C_0 is $(5.89 \times 10^{12} \text{ Bq s}) (4.54 \times 10^{-13} \text{ Gy/Bq s}) = 2.7 \pm 1.0 \text{ Gy}$. Because the dose–response relationship is exponential, this is equal to the dose required to achieve the D_{37} .

Mean Lethal Absorbed Dose and Number of Intracellular Decays from Intracellular ^{131}I IdU, ^{125}I IdU and ^{210}Po Citrate

The V79 cells were exposed to low concentrations of extracellular ^{131}I IdU, ^{125}I IdU or ^{210}Po citrate for a short period, washed free of extracellular activity, maintained at 10.5°C for a 72 h, plated for colony formation, and incubated for 7 days, during which the activity per cell decreased as the cells divided to form colonies (4). Following the general formalism for cellular dosimetry given by Eq. 7 of ref. (49), the mean absorbed dose to the cell nucleus, D_N , is given by

$$D_N = \tilde{A}[f_N S(N \leftarrow N) + f_{Cy} S(N \leftarrow Cy)]; \quad (\text{A4})$$

$$\tilde{A} = \tilde{A}_I + \tilde{A}_M + \tilde{A}_{CF}; \quad (\text{A5})$$

where \tilde{A}_I , \tilde{A}_M and \tilde{A}_{CF} are the cellular cumulated activities during the periods of incubation for cellular uptake of radioactivity, maintenance at 10.5°C for 72 h, and colony formation, respectively. The quantities f_N and f_{Cy} are the fraction of cellular activity in the nucleus and cytoplasm, respectively, where $f_N + f_{Cy} = 1$. The values of f_N are 0.28, 1.0 and 1.0 for ^{210}Po citrate, ^{131}I IdU and ^{125}I IdU, respectively (4). The quantities $S(N \leftarrow N)$ and $S(N \leftarrow Cy)$ are the cellular S values (absorbed dose per unit cumulated

TABLE A1
Calculation of D_{37} to Cell Nucleus from Radiochemicals

Radiochemical	A_0 (mBq/cell)	\tilde{A}_I (Bq s)	\tilde{A}_M (Bq s)	\tilde{A}_{CF} (Bq s)	\tilde{A}_0 (Bq s) ^a	D_0 (Gy)	D_{37} (Gy)	N_{37} (decays) ^a
²¹⁰ Po citrate	0.0217 ± 0.0037	1.95×10^{-2}	5.57×10^0	1.53×10^0	7.1 ± 1.3	0.68 ± 0.12	0.68 ± 0.12	7.1 ± 1.3
¹³¹ IIdU	3.58 ± 0.64	7.73×10^1	8.15×10^2	1.71×10^2	1060 ± 190	1.14 ± 0.20	2.4 ± 0.4	2240 ± 370
¹²⁵ IIdU	0.147 ± 0.0085	3.18×10^0	3.74×10^1	8.83×10^0	49.0 ± 3.0	0.33 ± 0.02	0.33 ± 0.02	49.0 ± 3.0

^a The cumulated activity, \tilde{A} , is equivalent to the number of decays. Hence the number of decays required to achieve 37% survival, N_{37} , is equivalent to \tilde{A}_{37} .

activity) for the radionuclide localized in the nucleus and cytoplasm, respectively. The values for $S(N \leftarrow N)$ are 0.155, 0.00107 and 0.00667 Gy Bq⁻¹ s⁻¹ for ²¹⁰Po, ¹³¹I and ¹²⁵I when localized in cells with a radius of 5 μm and a nuclear radius of 4 μm (50). These dimensions correspond to those of V79 cells (4). The value for $S(N \leftarrow Cy)$ for ²¹⁰Po is 0.0712 Gy Bq⁻¹ s⁻¹ (50).

Since these radiopharmaceuticals are taken up by the cells linearly with time (4), the cellular cumulated activity during the uptake period \tilde{A}_I is given by

$$\tilde{A}_I = 0.5 t_I A_p \quad (A6)$$

where A_I is the average cellular activity (Bq/cell) at the end of the uptake period and t_I is the incubation time during which the radioactivity is taken up by the cells [t_I (²¹⁰Po) = 0.5 h, t_I (¹²⁵IIdU/¹³¹IIdU) = 12 h]. During a maintenance period, t_M of 72 h, the radioactivity undergoes physical decay only. Therefore, the cumulated activity during this time is given by

$$\tilde{A}_M = A_I \int_0^{t_M} \exp(-0.693t/T_p) dt. \quad (A7)$$

The T_p of ²¹⁰Po, ¹³¹I and ¹²⁵I is 138, 8 and 60 days, respectively (25). Finally, the cumulated activity during the colony-forming period, t_{CF} is given by

$$\tilde{A}_{CF} = \exp(-0.693t_M/T_p) A_I \int_0^{t_{CF}} \exp(-0.693t/T_{e,CF}) dt, \quad (A8)$$

where $T_{e,CF}$ is the effective half-time of the radioactivity in the cells during this period. These values are 13.8, 12 and 12 h for ²¹⁰Po citrate, ¹³¹IIdU and ¹²⁵IIdU (4).

Column 2 of Table A1 lists the mean A_0 based on the values given for two experiments in Table 1. In addition, the cellular cumulated activities during each period assuming a cellular uptake equal to A_0 are provided in columns 3–5 of Table A1. The total cumulated activity assuming a cellular uptake of A_0 is given by \tilde{A}_0 in column 6 of Table A1. The D_0 is calculated according to Eq. (A4): $D_0 = \tilde{A}_0 [f_N S(N \leftarrow N) + f_{Cy} S(N \leftarrow Cy)]$ and is given in column 7. Since the dose D is directly proportional to the intracellular activity A , the surviving fraction can also be expressed as

$$SF = 1 - [1 - \exp(-D/D_0)]^n. \quad (A9)$$

Therefore, the mean absorbed dose to the cell nucleus required to achieve the D_{37} can be obtained by setting the SF as 0.37 and solving Eq. (A9) for $D = D_{37}$.

$$D_{37} = -D_0 \ln(1 - 0.63^{1/n}). \quad (A10)$$

The mean value of n from two independent experiments is taken from Table 1. The D_{37} values are given in column 8 of Table A1. Finally, the corresponding number of decays required to achieve 37% survival is equal to \tilde{A}_{37} and is given by N_{37} in the last column of Table A1.

Absorbed Dose from Extracellular ³²P

In our earlier publication, we used the same experimental techniques to irradiate the suspended V79 cells with β particles emitted by extracellular ³²P (19). This is a good reference radiation for use in calculating RBE values for chronic irradiation by incorporated radionuclides. However, no dosimetry calculations were presented in our earlier work. There-

fore, the dosimetry calculations are outlined below for the purpose of determining the D_{37} for β particles emitted by extracellular ³²P. As in the present study, the cells were exposed to the radiochemical in 17 × 100-mm Falcon sterile polypropylene culture tubes placed on a rocker-roller. The rocker-roller maintained the cells as a uniform suspension in 2 ml of culture medium. The absorbed fraction, ϕ , or the fraction of energy emitted by ³²P that is absorbed in the culture medium, depended on the geometry of the solution in the tube. The absorbed fraction is maximum when the culture tube is oriented vertically and minimum in the horizontal position. When placed on the rocker-roller, the geometry oscillates between ones that approximate these two configurations. The average of the absorbed fractions in these two configurations may be used to calculate the mean absorbed dose to the culture medium. Accordingly, the EGS4 Monte Carlo track structure code (50–52) was used to simulate 100,000 ³²P β-particle histories for each geometry (vertical and horizontal). For the vertical geometry, the 2 ml of culture medium in the tube was taken to have a cylindrical geometry of 1.13 cm in length and a radius of 0.75 cm. The horizontal geometry was taken as a slice along the long axis of the cylindrical tube with dimensions of length 8 cm and a radius of 0.75 cm. This geometry resembles a shallow trough. When the full ³²P β-particle spectrum (53) was used in these calculations, the mean absorbed dose per unit cumulated activity (S) was calculated to be 4.64×10^{-11} and 3.37×10^{-11} Gy/Bq s for the vertical and horizontal geometry, respectively. Thus the mean S for the culture medium is 4.00×10^{-11} Gy/Bq s. The cumulated activity, \tilde{A} , in the tubes is given by Eq. (A2). For the ³²P experiments (19), $t = 72$ h and $T_p = 14.2$ days (25). Thus Eq. (A2) can be integrated to give $\tilde{A} = A(t=0) \cdot 66.9$ h. Finally, the mean absorbed dose delivered to the cultured cells by the ³²P β particles is given by

$$D = \tilde{A} S = 9.63 \times 10^{-6} A(t=0) \text{ Gy}, \quad (A11)$$

where $A(t=0)$ is in Bq. Thus, with the mean value of $A_0(t=0) = 2$ ml (0.21 ± 0.028 MBq/ml) = 0.42 ± 0.056 MBq from ref. (19), the mean D_0 value is 4.0 Gy in the absence of DMSO. Finally, with $n = 2.5$ (19), Eq. (A10) can be used to calculate the mean $D_{37} = 7.1 \pm 0.7$ Gy.

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