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ABSTRACT

In order to understand the genetic and somatic risks associated with exposure to radon and its daughter products, it is important to characterize those lesions that lead to mutation induction and carcinogenesis. The effect of exposure to the radon daughter [212]Bi has been analyzed in 4 CHO cell lines, CHO-K1, xrs-5, AA8 and EM9. These cell lines have different radiation sensitivities and different abilities to rejoin radiation-induced DNA strand breaks. Three endpoints have been examined in these cells; cell killing, chromosome aberration induction and mutation induction. We find RBEs for AA8, EM9 and CHO-K1 of about 2.5-4 for cell killing and around 2 for G2-induced aberrations. The RBEs for xrs-5 cells are much smaller for cell killing and chromosome aberration induction, suggesting that the principle lethal lesion resulting from [212]Bi exposure is a non-repaired DNA double-strand break. (The CHO cell line xrs-5 is defective in the rejoining of DNA double-strand breaks.) The survival curves suggest that a portion of the cell killing is probably derived from the low LET beta component of the radiation. Mutation studies suggest that while the lethal and pre-mutagenic lesions might overlap, they are not exactly the same. RBEs for gene mutation induction in AA8 and EM9 cells are higher than the RBEs for killing and chromosome aberration induction. Interestingly, CHO-K1 is not more sensitive to mutation induction than killing or chromosome aberration induction by [212]Bi suggesting that this cell line differs from AA8 and EM9 in its ability to repair a premutagenic lesion induced by [212]Bi. These studies should help define the nature of the lesions induced by radon exposure and thereby help understand the carcinogenic risk from radon exposure and how it differs from low LET radiation exposure.

PROGRESS REPORT

1. Comparison of radon-daughter-induced effects in repair proficient and repair deficient CHO cell lines.

In order to understand the genetic and somatic risks associated with exposure to radon and its daughter products, it is important to characterize those DNA lesions, produced as a result of radon exposure, that lead to mutagenesis and carcinogenesis. Our approach to this problem has been to examine the effects of radon exposure at multiple levels, i.e., DNA, chromosome and cell, in a group of well-defined CHO cell lines with different radiation sensitivities and repair capabilities. In this way we hope to learn which DNA lesions are important in mutagenesis and carcinogenesis as well as how cells cope with DNA damage induced by radon and its daughters.

The source of radiation for these studies is [212]Bi. [212]Bi is a [220]Rn daughter which we elute from a [224]Ra generator. It has a 1 hour half-life and decays with the production of 1 alpha with a mean energy of 7.8 MeV. About 10% of the dose comes from beta radiation. Cells are exposed in suspension to the [212]Bi chelated to DTPA. Thus the irradiation is all external. There is no internalization of the isotope nor any attachment to the cell membrane.

We are working with 4 CHO cell lines. The first pair of cell lines include AA8 and EM9. EM9 was isolated by Dr. L. Thompson as an EMS-sensitive mutant of AA8 cells. It is very sensitive to the effects of monofunctional alkylating agents. It is also slightly sensitive to X rays (Figure 1A). We routinely find a D_0 of about 140 cGy for AA8 while EM9 has a D_0 of about 100 cGy. The defect that accounts for the sensitivity of EM9 cells to monofunctional alkylating agents and radiation is believed to be a deficiency in DNA single-strand break rejoining. So, for example, when one looks at the rejoining of DNA single-strand breaks as measured by alkaline elution after an exposure to 4 Gy X-ray, you see that most of the breaks are rejoined within 15 min in AA8 while in EM9 it takes more time (Figure 1B). By studying the molecular, cytogenetic and cellular effects of [212]Bi exposure in EM9 and AA8 cells, we hope to glean some information on the relative importance of DNA single-strand break induction in radon-induced effects.

The other pair of cell lines we are studying include CHO-K1 and xrs-5 cell lines. xrs-5 was isolated by Dr. P. Jeggo from a mutagenized culture of CHO-K1 cells. It was isolated as a radiation-sensitive mutant and has therefore as its hallmark an extreme sensitivity to X-ray-induced cell killing (Figure 2A). The D_0 for xrs-5 cells is about 50 cGy while for CHO-K1 cells it is about 225 cGy. The defect underlying the radiation sensitivity in xrs-5 cells is thought to be a reduced capacity to rejoin DNA double-strand breaks. Thus on DNA neutral elution analysis of the percent DNA double-strand break damage rejoined as a function of time after exposure to 100 Gy [60]Co gamma rays, you find that by

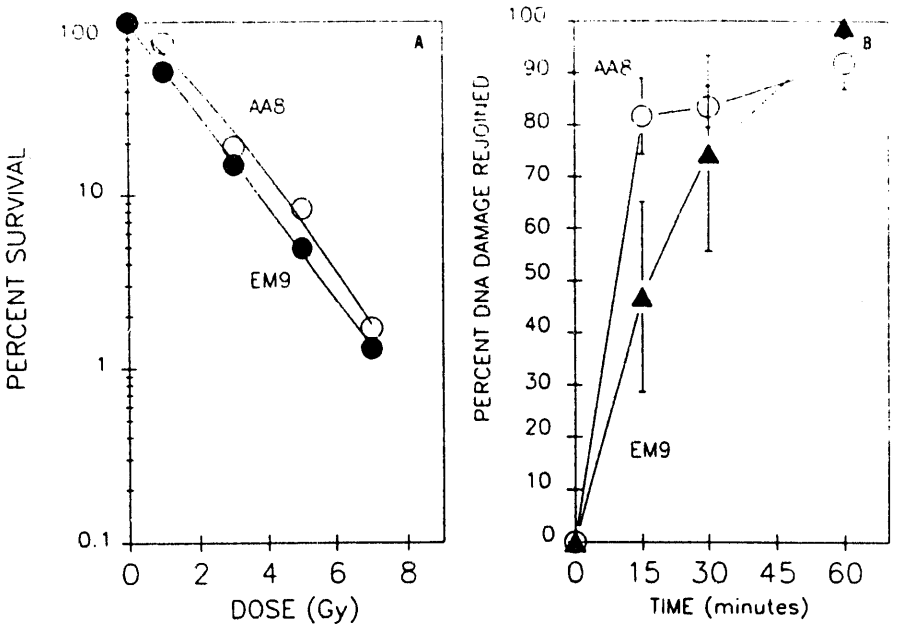

Figure 1. **Panel A:** X-ray survival curve for CHO cell lines AA8 and EM9.
Panel B: Rejoining of DNA single-strand breaks as a function of time after irradiation with 4 Gy X-ray.

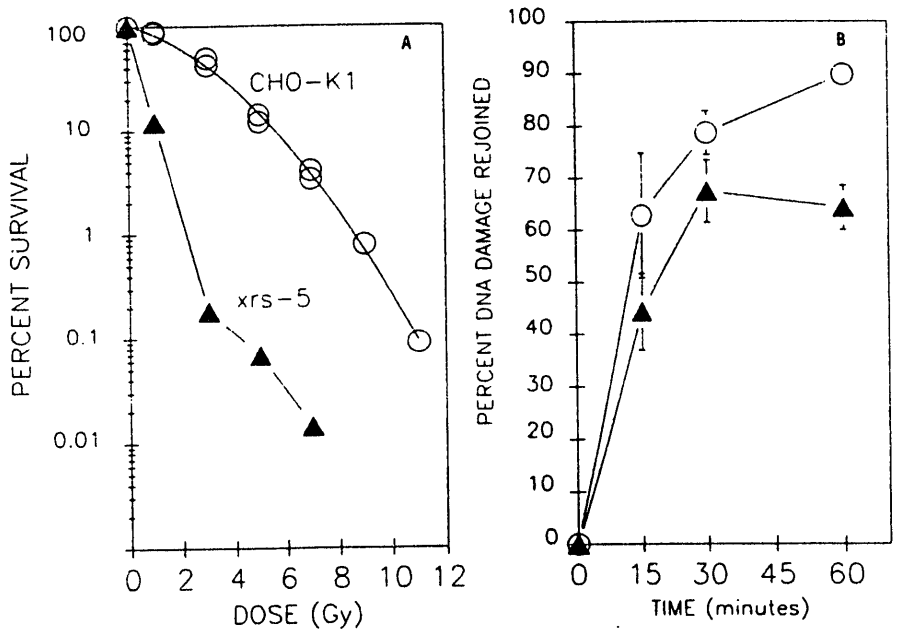

Figure 2. **Panel A:** X-ray survival curve for CHO cell lines CHO-K1 and xrs-5.
Panel B: Rejoining of DNA double-strand breaks as a function of time after irradiation with 100 Gy ^{60}Co gamma rays.

1 hour most of the breaks have rejoined in the parent CHO-K1 cell line while in xrs-5, only about 50-60% of the breaks have rejoined (Figure 2B). By comparing xrs-5 and CHO-K1 cells, we hope to learn more about the importance of DNA double-strand breaks in radon-mediated events.

To date, we have looked at 3 endpoints; cell killing, chromosome aberration induction and gene mutation induction. Previous studies by other investigators have suggested that high LET exposure, such as that produced by alpha radiation, results in a high density of DNA double-strand break damage which is not repaired by cells and it is this non-repaired DNA double-strand damage which results in cell lethality. As expected, therefore, comparison of X-ray and [212]Bi-induced cell killing in AA8 and EM9 reveals similar RBEs (Figure 3). Approximately a 3-fold increase in cell killing in both cell lines. Thus the principle lethal lesion induced by [212]Bi is probably not a DNA single-strand break or else we would expect a different RBE for EM9 cells. Interestingly, EM9 appears to be more sensitive to [212]Bi than AA8 cells. Thus there must be a component of the damage induced by [212]Bi which is repairable by the DNA single-strand break rejoining pathway that is defective in EM9 cells. We believe this to be a reflection of the low LET beta radiation which is 10% of the total [212]Bi dose. We are presently planning to test this hypothesis by looking at a pure alpha source where we would expect the survival curves for AA8 and EM9 to superimpose.

One sees a different picture with CHO-K1 and xrs-5 cells. With CHO-K1 cells, there is a slightly larger 4-fold increase in cell killing when X-rays and [212]Bi are compared (Figure 4). This probably reflects the greater inherent resistance of CHO-K1 cells to X-rays as compared to AA8 and EM9 cells. There is only a small increase in killing when these two radiations are compared in xrs-5 cells. This is what is expected if the lethal lesion induced by [212]Bi is a non-repaired DNA double-strand break. Because xrs-5 cells are defective in DNA double-strand break rejoining anyway, one would expect little increase in cell killing in [212]Bi-treated cells. The small increase that is seen, we believe to be due to the low LET component of the radiation. Again, we plan to test this hypothesis using a pure alpha source. Thus the survival studies suggest that the principle lethal lesion produced by [212]Bi is a non-repairable DNA double-strand break. However, a component of the killing curve apparently is due to the low LET radiation contaminant, and this needs to be taken into consideration when evaluating radon effects.

The second endpoint we looked at was chromosome damage. The evidence is strong that chromosome breaks result from DNA double-strand breaks. Thus one would expect the chromosome aberration studies to essentially parallel the survival studies, and they do for the most part. For example, the RBE for chromatid aberration induction by [212]Bi (1 break/cell) is close to 2 in G2-irradiated AA8 and EM9 cells (Figure 5). This is a little less than the survival curve data had suggested, but this might relect our use

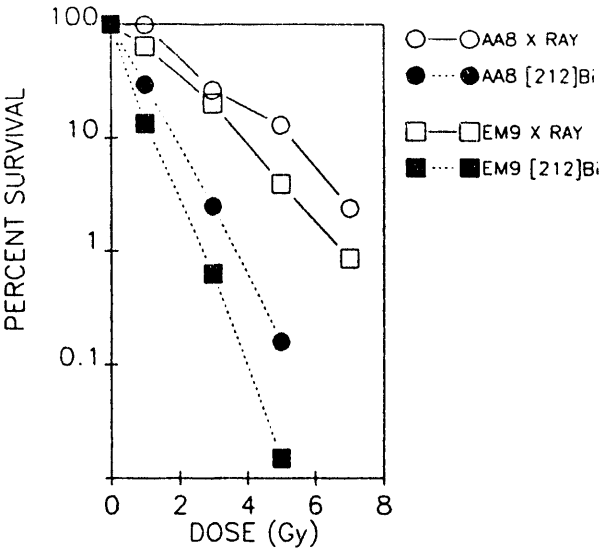

Figure 3. Comparison of X-ray- and ^{212}Bi - induced cell killing in AA8 and EM9 cells.

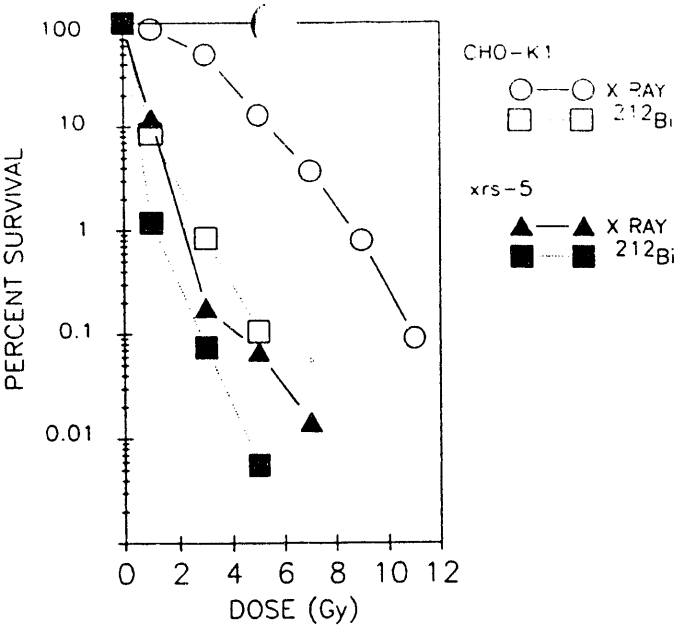

Figure 4. Comparison of x-ray-and ^{212}Bi - induced cell killing in CHO-K1 and xrs-5 cells.

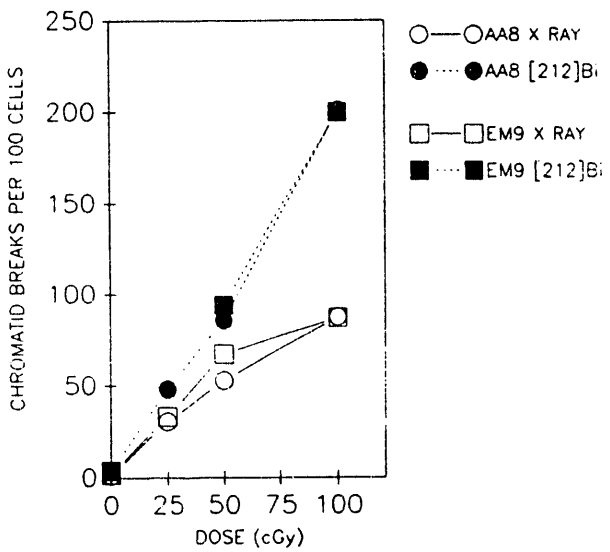

Figure 5. Induction of chromatid aberrations in G_2-irradiated AA8 and EM9 cells.

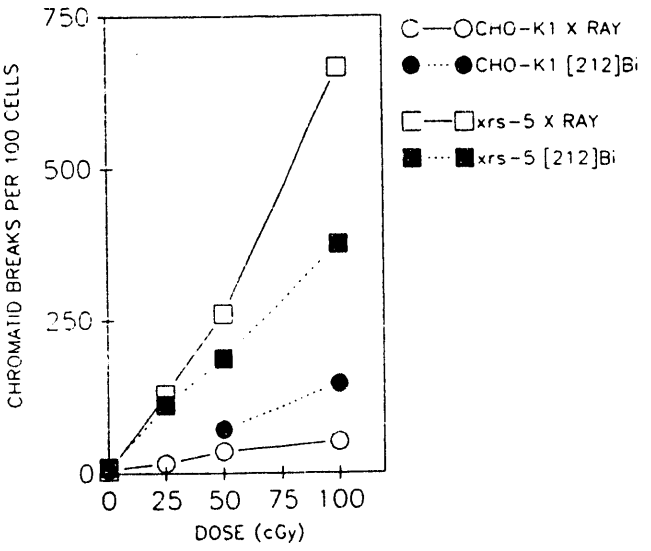

Figure 6. Inducation of chromatid aberrations in G_2-irradiated CHO-K1 and xrs-5 cells.

of G2-irradiated cells for chromosome analysis instead of asynchronous cells. We don't pick up differences between EM9 and AA8 cells in their response to X rays or [212]Bi as we did with cell killing which again might be a reflection of our use of G2 cells. Most of the aberrations seen are deletions which are thought to result from unrejoined chromosome breaks.

In CHO-K1 cells, we see similar results (Figure 6). An RBE of 2.5 for aberration induction with [212]Bi as compared to X-rays. Like X-rays survival studies, the higher RBE may reflect inherent resistance of CHO-K1 to X rays as compared to AA8 and EM9 cells. In xrs-5 cells, however, while the X-ray response is much larger than the X-ray response of CHO-K1 cells, we see fewer aberrations in [212]Bi-exposed xrs-5 cells as compared to X-rays. We believe this is a reflection of overkill. That is, in these exposed cells, the aberration frequency is so high that few cells actually make it to metaphase to be analyzed. Note the different scale used here as compared to the graph for AA8 and EM9 cells. Thus in general, the chromosome data tend to confirm the survival data suggesting that the principle lethal and clastogenic lesion resulting from [212]Bi exposure is a non-repaired DNA double-strand break.

We next turned to mutation induction in these cell lines. Here we are looking at mutations at the HGPRT locus as determined by the frequency of 6-thioguanine-resistant cells. It has been suggested that DNA single-strand breaks might be pre-mutagenic lesions. Our data for X-ray-induced mutation induction in AA8 and EM9 cells do not support this notion (Figure 7). We do not see any difference in mutation induction by X-rays in these 2 cell lines. Except for one anomalous point, so far, we do not see any difference between these 2 cell lines in mutation induction by [212]Bi either, suggesting that the pre-mutagenic lesion produced by [212]Bi-exposure is not a DNA single-strand break. Not surprisingly, we see an RBE for mutation induction by [212]Bi of close to 4, higher than that seen for cell killing. This suggests that while there may be an overlap in cytotoxic and mutagenic lesions induced by [212]Bi, they may not be exactly the same set. There may be some additional lesions produced by [212]Bi that result in mutation induction.

The mutation induction results for xrs-5 and CHO-K1 are interesting (Figure 8). For X-rays, one sees no increase in mutation induction in xrs-5 cells. At first glance, this is surprising because the chromosome aberration studies suggest that these cells are very sensitive to X-ray-induced chromosome deletion induction. 6-Thioguanine-resistance results from the loss of the HGPRT gene product either through a point mutation or a deletion mutation. The failure to see hypermutability in xrs-5 cells is probably due to an inability to recover all induced mutations. It is likely that the unrejoined DNA double-strand breaks result in large deletions. Large deletions at the HGPRT locus on the X chromosome are probably lethal as studies by others would suggest.

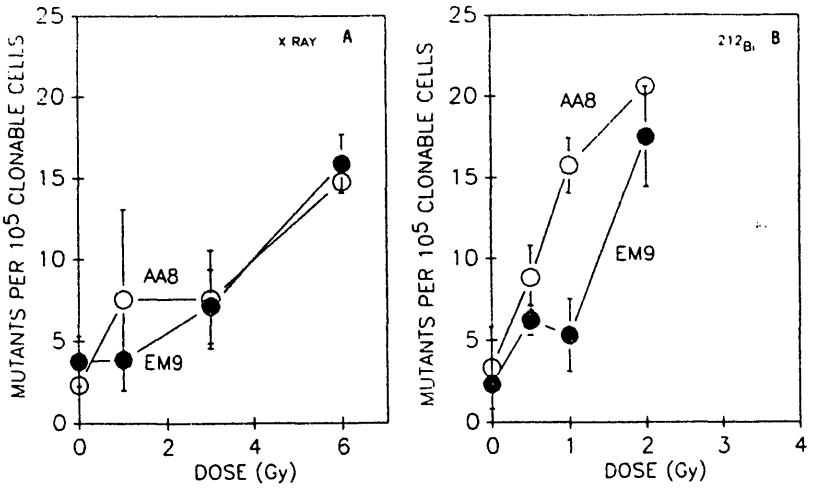

Figure 7. Induction of 6-thioguanine-resistant mutants in AA8 and EM9 cells. Panel A. X-ray-oxposed. Panel B. ^{212}Bi-exposed.

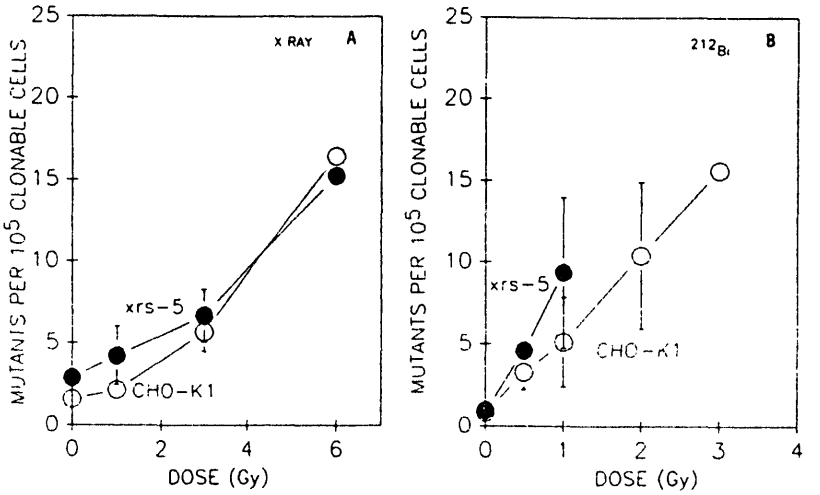

Figure 8. Induction of 6-thioguanine-resistant mutants in CHO-K1 and xrs-5 cells. Panel A. X-ray-exposed. Panel B. ^{212}Bi exposed.

The RBE for mutation induction by [212]Bi in CHO-K1 cells is about 2, lower than that for AA8 and EM9 cells and lower than the RBE for cell killing and chromosome aberration induction. These preliminary studies suggest that AA8 and EM9 might be defective in repair of some [212]Bi-induced pre-mutagenic lesions that are handled by CHO-K1 cells. All 3 cell lines respond similarly to X-rays. For xrs-5, the RBE is about 4, similar to AA8 and EM9 cells, suggesting that defective DNA double-strand break rejoining might sensitize cells to the mutagenic effects of [212]Bi exposure, and that the premutagenic lesions produced by [212]Bi are different from those produced by X-rays.

To summarize our data then (Table 1), we find RBEs for AA8, EM9 and CHO-K1 of about 2.5-4 for cell killing and around 2 for G2-induced aberrations. The RBEs for xrs-5 cells are much smaller for cell killing and chromosome aberration induction, suggesting that the principle lethal lesion resulting from [212]Bi exposure is a non-repaired DNA double-strand break. The survival curves suggest that a portion of the cell killing is probably derived from the low LET beta component of the radiation. Mutation studies suggest that while the lethal and pre-mutagenic lesions might overlap, they are not exactly the same. RBEs for gene mutation induction in AA8 and EM9 cells are higher than the RBEs for killing and chromosome aberration induction. Interestingly, CHO-K1 cells are not more sensitive to mutation induction than killing or chromosome aberration induction by [212]Bi suggesting that these cells differ from AA8 and EM9 in their ability to repair a premutagenic lesion induced by [212]Bi.

TABLE 1. SUMMARY OF RBEs FOR [212]Bi-INDUCED EFFECTS

CELL	KILLING (D_{10})	ABERRATION (1/CELL)	MUTATION (10 INDUCED)
AA8	2.50	1.85	3.91
EM9	3.25	1.72	3.93
CHO-K1	3.93	2.67	2.14
xrs-5	1.50	0.78	4.27

2. The Molecular Spectrum of [212]Bi-Induced Mutations.

The studies mentioned above suggest that while the DNA double-strand break is probably the major lethal and clastogenic lesion induced by [212]Bi, it is not the only premutagenic lesion. The RBEs for the different CHO cell lines suggest that there might be another DNA lesion, induced by [212]Bi, that results in mutation. To begin to investigate what this lesion could be, we have begun studies to analyze the molecular spectrum of mutations induced by [212]Bi (as compared to X rays). The molecular spectrum of mutations induced by [212]Bi is being determined in a chromosomally integrated shuttle vector plasmid. The system being used is the T5 system developed by our co-investigator Dr. C. Ashman. It is a CHO-K1 cell that contains a chromosomally-integrated gpt vector. Initial studies were designed to develop dose response curves for

the induction of gpt deletions. Preliminary results of this study are shown on figure 9. As expected, T5 is much more sensitive to the induction of gpt mutations by either X rays or [212]Bi than CHO-K1 is to HGPRT mutations. This is probably due to the fact that many of the X ray- and [212]Bi-induced mutations are deletions and (as mentioned above) large deletions at the HGPRT locus on the X chromosome are most likely lethal and nonrecoverable. The gpt gene is probably located on an autosome in a position where large deletions can be recovered. We plan localize the gpt chromosomally using in situ hybridization studies. We have now begun to isolate mutant T5 clones for analysis of the molecular lesion underlying the loss of gpt activity.

3. Comparison of Different Radon Sources.

In order to better evaluate radon studies from different laboratories, we have begun to compare radon/alpha radiation sources at the University of Chicago (this study), Case Western Reserve University (Helen Evans' laboratory), Argonne National Laboratory (Tom Seed's laboratory), and Pacific Northwest Laboratory (Rick Jostes' laboratory). While we use a radon daughter ([212]Bi) chelated to prevent entry and attachment to the cell, studies at Pacific Northwest laboratories and at Case Western Reserve expose cells to radon gas directly in a closed vessel. At Argonne, Tom seed has designed a system where cells are externally exposed to radon daughters through a mylar window. In an attempt to better compare results from one laboratory to another, we are measuring cell killing in a common cell line (C-18) using the different exposure systems.

Survival curves have been generated at each institution using the CHO cell line (C-18). Preliminary results for our source and that of Tom Seed's are shown in figure 10. The results from each laboratory compare well with each other. Any differences in killing can be accounted for by the measurable attachment of radon daughters to the cell. Compare, for example, the effects of [212]Bi chelated to DTPA, which does not attach or enter the cell with non-chelated DTPA. The excess killing with the non-chelated is due to the attachment of the isotope to the cell. Approximtely 10% of the total activity added is found attached to the cell in the unchelated experiments. The data from studies with direct exposure to radon gas find survival curves similar to the unchelated [212]Bi. Radon daughter attachment does not occur in Tom Seed's irradiation chamber and as expected, his survival curve falls closely on our [212]Bi-DTPA survival curve line. Thus, given the caveat of radon daughter attachment to cells, use of these 4 different exposure systems should yield similar dose-response curves.

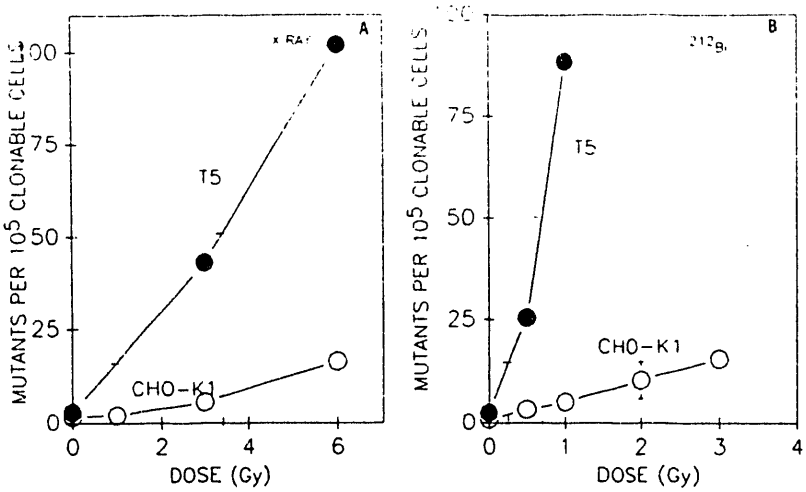

Figure 9. Comparison of mutation induction in CHO-K1 (HGPRT locus) and T5 (gpt locus). Panel A. X-ray. Panel B. ^{212}Bi.

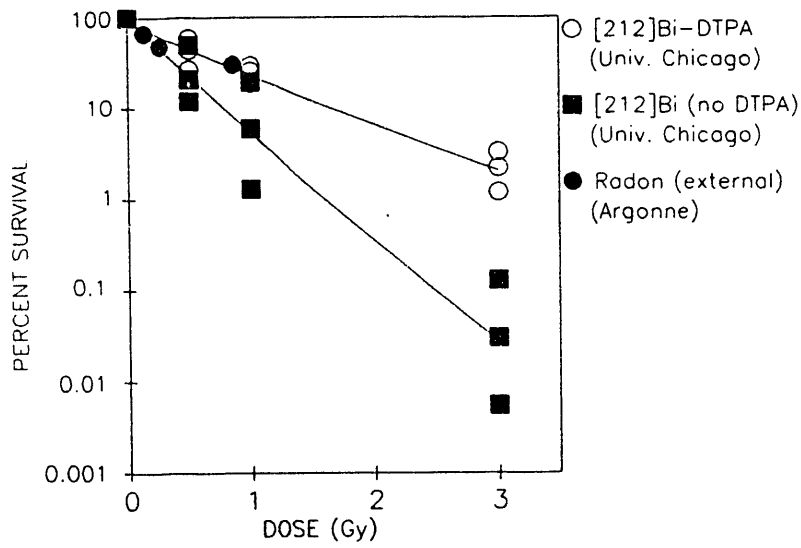

Figure 10. Comparison of radon sources. Cell survival curves of CHO C-18 exposed to $^{[212]}$Bi-chelated to DTPA, unchelated $^{[212]}$Bi, and radon daughters (through a mylar film).

RESULTING PUBLICATIONS:

Shadley, J.D., Atcher, R.W., Toohill, M., Rotmensch, J. and Schwartz, J.L., Cytotoxic, clastogenic and mutagenic effects of Bismith-212, a radon daughter, (Abstract), Thirty-Seventh Annual Meeting of the Radiation Research Society, 1989.

Shadley, J.D., Toohill, M., Whitlock, J., Rotmensch, J. and Schwartz, J.L., XRS-5 cells are not hypersensitive to X-ray-induced mutation, (Abstract), FIfth International Conference on Environmental Mutagens, 1989.

Schwartz, J.L., Shadley, J.D., Jaffe, D.J., Whitlock, J., Rotmensch, J., Cowan, J.M., Gordon, D.J., and Vaughan, A.T.M., Association between radiation sensitivity, DNA repair and chromosome organization in the Chinese hamster ovary cell line xrs-5, Fifth International Conference on Environmental Mutagens. M.L. Mendelson, Editor, A.R. Liss, Inc. New York, (in press).

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