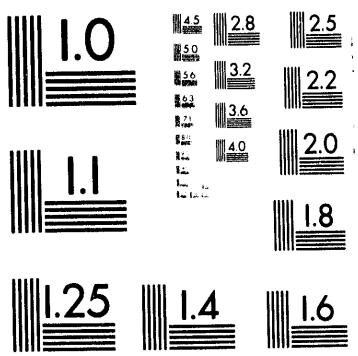
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# FINAL PROGRESS REPORT

**Project title:** Yields of Biologically Significant Damage Produced in Mammalian DNA by Irradiation Associated with Radon Decay

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**Award ID:** DE-FG03-88ER60660



**1.1. Research Objective:**
The objective of this project was to characterize the difference between damage to DNA caused by alpha particles and by low LET radiation.

**1.2. Relevance to Risk Assessment:**
Estimation of the risk posed by exposure to high LET radiation (such as that from radon) relies at present on epidemiological data, and is therefore largely empirical. This empiricism is evident from the concepts of 'quality factor' or 'RBE' that find use for describing the biological effects of high LET radiation. We argue that some effort should be made to address the mechanisms of DNA damage by high and low LET forms of radiation, and how these mechanisms might relate to the biological endpoints. This report summarizes the results of our investigations and our current understanding of these mechanisms.

**2. Method:**
Using the genome of the virus SV40, we developed a system with which to model the effect of ionizing radiation on both DNA and chromatin. The DNA from this virus can be isolated in the form of a double stranded supercoiled molecule (analogous to a plasmid), and also associated with histones arranged in 20 - 25 approximately equally spaced nucleosomes. This so called minichromosome is our model for the chromatin of mammalian cells. These DNA substrates were prepared and purified by modifications of a literature procedure. Alpha particle irradiations were performed in our laboratory using a 840 μCi $^{244}Cm$ disc source. Helium-4 ion irradiations were performed either at the Super HILAC, Lawrence Berkeley Laboratory, or at the RARAF van der Graaff accelerator, Nevis Laboratory, Columbia University.

After irradiation, the DNA substrate adopts one of three forms: 1, supercoiled or form I (undamaged); 2, open circle or form II (containing at least one single strand break [SSB]); and 3, linear or form III (containing one double strand break [DSB]). These three forms can be separated on an analytical scale using agarose gel electrophoresis. The amounts of the three forms can be estimated from the fluorescence of ethidium bound to the DNA. Fluorescence was quantified using imaging equipment constructed in this laboratory from commercially available components. The fluorescence image was aquired with a monochrome CCD

video camera, digitized by a frame grabbber board, and processed with a PC/AT compatible computer. The software needed to perform these operations was developed in this laboratory.

## 3. Results

### 3.1. General

Using the assay procedure described above, we have quantified for alpha particle (or $^4$He ion) irradiation both SSB and DSB yields in both naked SV40 DNA and the SV40 minichromosome. In order to investigate the radiation chemical processes involved in DNA damage, it was essential that these yields were determined at various scavenger concentrations and also with various particle energies (i.e. at various LETs). The results were then compared with those for low LET radiation (gamma rays), for which we have large quantities of data.

### 3.2. Alpha particle and $^4$He ion irradiations

Originally we had intended to carry out the $^4$He ion irradiations at Lawrence Berkeley Laboratory. During the course of the project, however, the accelerator became unavailable. Therefore we arranged to make use of the Radiological Research Accelerator Facility at Columbia University, courtesy of Dr. E. J. Hall. While using the RARAF accelerator, we found it necessary to redesign the irradiation chambers. In our hands, the gold plated chambers that we used at Berkeley frequently gave rise to irreproducible results. We speculate that this problem is related to the need to inject the sample into the chamber through a narrow gauge needle.

We subsequently made use of 3.5 cm diameter stainless steel rings with a mylar membrane glued to them with epoxy resin. The DNA solution is spread over the mylar by placing a 2.5 cm × 2.5 cm glass cover slip directly on top of the liquid sample. The thickness of the sample is thus determined by its volume and the surface area of the cover slip (4.8 µl having a thickness of 10 µm). These ring chambers are superior to the gold plated chambers, since they are much less expensive, trivial to assemble, and the solutions can be quickly loaded into and recovered from them. Relatively high DNA concentrations were required so that the small volumes contained sufficient DNA for our assay procedure, but this posed no serious difficulty.

In addition to the accelerator $^4$He ion beams, we also have two in house isotopic alpha particle disc sources. Both of these contain $^{244}$Cm protected by a thin gold layer. These sources have several disadvantages for quantitative work, since they can only accomodate one sample at a time; the alpha particles are uncollimated and as a consequence the dose rate and attenuation of dose with depth are uncertain; and the dose rates are relatively low, irradiation times are therefore lengthy, and as a consequence the aqueous samples tend to dry out.

### 3.3. SSB formation

The hydroxyl radical yield decreases both with increasing particle energy (i.e. increasing LET, due to an increased frequency of reactions within the track) and also with decreasing scavenger concentration (i.e. increasing lifetime). These yields are known for low LET radiation, and more recently have also been measured for $^4$He ions at various energies and lifetimes. In all cases we found that the SSB yield was proportional to the hydroxyl radical yield. This suggests that the mechanism of SSB formation is straightforward, with the SSB yield simply being a consequence of the competition for the hydroxyl radical between the DNA substrate and any scavengers that are present in solution. The nature of this competition is complicated to some extent by the phenomenon of non homogeneous kinetics (or time dependent rate constants), since one of the species involved (the DNA substrate) is a macromolecule. A fixed fraction of those hydroxyl radicals that react with the DNA then go on to form a SSB.

### 3.4. DSB formation

For $^4$He ions, we found that the DSB yield decreases with increasing scavenging capacity (as for the SSB yield). However, the DSB yield was observed to increase slightly with increasing LET (the DSB yield increases by *ca.* 2 fold as the LET increases from that of $^{137}$Cs gamma rays to that of a 5 MeV 4He ion), which is the opposite of the case for SSBs. This second observation implies that with increasing LET there is an increase in the DSB yield per hydroxyl radical. Therefore the mechanism of DSB formation is more complex than that described above for SSB formation, presumably with more than one hydroxyl radical attack being required to lead to DSB formation. Presumably the higher density of hydroxyl radicals in high LET particle tracks is responsible for the increasing efficiency of DSB formation with increasing LET.

### 3.5. Protection by histone proteins

Both SSB and DSB yields in the minichromosome were lower than in naked DNA, although the difference was smallest at the highest scavenger concentrations. We interpret these observations in terms of a physical protection of the DNA by the histones against hydroxyl radical attack. At high scavenger concentrations, a significant fraction of DNA damage is due to the direct effect, and it is not unexpected that histones are unable to protect the DNA against damage arising as a consequence of direct ionization. In this respect, alpha particle and $^4$He ions behave very similarly to gamma rays.

### 3.6. Effect of scavenging capacity

For $^4$He ion irradiation, the ratio of the SSB:DSB yield remained constant as the scavenging capacity was varied. This observation is not consistent with the decrease in this ratio with increasing LET, since both increasing LET and decreasing radical lifetime (increasing scavenger concentration) would be expected to have superficially the same effect, that being to increase the radical density within the charged particle track. Further and more sophisticated qualitative investigations may resolve this issue, since DSBs (as detected by our assay procedure) are expected to represent a variety of DNA damage structures.

### 3.7. Biological Significance

The slight increase in DSB yield with increasing LET (referred to above) is significantly smaller than the quality factors generally applied to high LET radiations. This suggests that it is not simply the increase in DSB yield that is responsible for the greater biological effectiveness of high LET radiation, but that the DSBs produced by it are more biologically active. This supports the argument for qualitative investigations of the structure of DSBs produced by alpha particles and $^4$He ions.

## 4. Resulting Publications

### 4.1. Meeting Abstracts


1. J. F. Ward. Molecular explanation of variable cell radiosensitivity. Biannual meeting of the Radiation Therapy Oncology Group, San Diego, CA (January, 1989).

2. J. F. Ward, C. L. Limoli, and P. M. Calabro-Jones. A test of the saturation of repair hypothesis for shouldered survival curves. 37th Annual Radiation Research Meeting, Seattle, WA (March, 1989).

3. L. L. Ling and J. F. Ward. Radiosensitization by bromodeoxyuridine (BUdR): toxicity and strand break induction. 37th Annual Radiation Research Meeting, Seattle, WA (March, 1989).

4. J. F. Ward. Chemical effects of radiation associated with radon decay. 38th Annual Radiation Research Meeting Meeting, New Orleans, LA, abstract Ed-2 (April, 1990).

5. P.M. Calabro-Jones, D. T. Lai, J. R. Milligan, T. R. Nelson, and J. F. Ward. Alpha particle

irradiation of DNA. Abstract Ei-4, 38th Annual Radiation Research Society Meeting, New Orleans, LA (April, 1990).

6. J. R. Milligan, D. T. Lai, T. R. Nelson, and J. F. Ward. G value for SSB in gamma irradiated DNA. Abstract Ev-5, 38th Annual Radiation Research Society Meeting, New Orleans, LA (April, 1990).

7. J. R. Milligan, T. R. Nelson, and J. F. Ward. Estimation of G(SSB) and k2 for hydroxyl radical attack on DNA. NATO Advanced Research Workshop on "Early effects of radiation damage on DNA", San Miniato, Italy (May, 1990).

8. J. F. Ward. Mechanisms of radiation action on DNA in model systems - their relevance to cellular DNA. NATO Advanced Research Workshop on "Early effects of radiation on DNA", San Miniato, Italy, abstract (May, 1990)

9. J. F. Ward. Radiation induced DNA lesions and their repair. Workshop, 15th International Cancer Congress, Hamburg, Germany, (August, 1990).

10. J. R. Milligan, J. A. Aguilera, and J. F. Ward. Reactivity of plasmid DNA with hydroxyl radical. Abstract P-02-10, 9th International Congress for Radiation Research, Toronto, Canada (July, 1991).

11. J. F. Ward. Is there relevance of in vitro DNA damage to in vivo DNA damage. Workshop, 9th International Congress for Radiation Research, Toronto, Canada (July, 1991).

12. C. L. Limoli and J. F. Ward. production of DNA double strand breaks by a non radiolytic mechanism. Abstract P-23-10, 9th Internation Congress for Radiation Research, Toronto, Canada (July, 1991).

13. D. J. Brenner and J. F. Ward. Constraints on energy deposition and target size of multiply damaged sites associated with DNA double strand breaks. Abstract P-12-8, 40th Annual Radiation Research Meeting, Salt Lake City, UT (March, 1992).

14. G. D. D. Jones and J. F. Ward. The effect of temperature during and after irradiation on the yield of strand break formation in plasmid DNA. Abstract P-12-3, 40th Annual Radiation Research Meeting, Salt Lake City, UT (March, 1992).

15. J. R. Milligan, J. A. Aguilera, A. D. Arnold, and J. F. Ward. The effect of histone association and supercoiling on SSB yield in irradiated DNA. Symposium, 40th Annual Radiation Research Society Meeting, Salt Lake City, UT (March, 1992).

16. C. L. Limoli and J. F. Ward. Repair of photolytically induced DNA strand breaks in V79 cells. 40th Annual Radiation Research Society Meeting, Salt Lake City, UT (March, 1992).

17. J. R. Milligan, S. Kang, J. A. Aguilera, G. D. D. Jones, and J. F. Ward. Studies of complex DNA damage introduced by ionizing radiation. Abstract for meeting on "Pathways to radiation damage in DNA", Rochester, MI (June, 1992).

18. J. R. Milligan, J. A. Aguilera, A. D. Arnold, and J. F. Ward. The influence of DNA structure in its damage by ionizing radiation. Abstract for meeting on "Pathways to radiation damage in DNA", Rochester, MI (June, 1992).

19. J. F. Ward, G. D. D. Jones, and J. R. Milligan. Biological consequences of non homogeneous energy deposition by ionizing radiation. Abstract for 11th Microdosimetry Symposium, Gatlinburg,

TN (September, 1992).

20. J. F. Ward. Biologically important damage caused by ionizing radiation. 1st Australian Asian Conference on Radiation Science and Nuclear Medicine, Sydney, Australia (February, 1993).

21. J. F. Ward. DNA strand break formation by oxidizing radicals. 6th International Conference on Environmental Mutagens, Melbourne, Australia (February, 1993).

22. J. R. Milligan, S. Kang, C. L. Limoli, J. A. Aguilera, G. D. D. Jones, and J. F. Ward. Evidence that sparsely ionizing radiation causes multiply damaged sites in DNA. Abstract P-15-12, 41st Annual Radiation Research Meeting, Dallas, TX (March, 1993).

23. G. D. D. Jones, J. R. Milligan, J. F. Ward, J. A. Aguilera, and P. M. Calabro-Jones. Strand breakage as a function of LET and solution scavenging capacity for SV40 DNA and minichromosomes irradiated with 4He ions. Abstract P-15-11, 41st Annual Radiation Research Meeting, Dallas, TX (March, 1993).

24. J. F. Ward, J. R. Milligan, and G. D. D. Jones. Investigation of the protective effects of histones in a model minichromosome system. Symposium, 41st Annual Radiation Research Meeting, Dallas, TX (March, 1993).

25. C. L. Limoli and J. F. Ward. UVA exposure of BrdU substituted DNA in the presence of Hoechst dye 33258: development of a plasmid model system to characterize the damage. 41st Annual Radiation Research Meeting, Dallas, TX (March, 1993).

26. D. J. Brenner, R. K. Sachs, and J. F. Ward. Yields and spatial distributions of energy deposition clusters: from track structure to DSB induction to chromosome abberation formation. Monte Carlo Workshop, Irvine, CA (April, 1993).

27. J. R. Milligan and J. F. Ward. DNA damage by scavenger derived radicals. 18th L. H. Gray Conference (Radiation damage in DNA: physics, chemistry, and molecular biology), Bath, UK (April, 1994).

28. J. F. Ward. The complexity of DNA damage - relevance to biological consequences. 18th L. H. Gray Conference (Radiation damage in DNA: physics, chemistry, and molecular biology), Bath, UK (April, 1994).

29. G. D. D. Jones, T. V. Boswell, J. R. Milligan, J. F. Ward, M. Weinfeld, and J. Lee. DAmages produced in SV40 DNA by alpha and gamma irradiation. 18th L. H. Gray conference (Radiation damage in DNA: physics, chemistry, and molecular biology), Bath, UK (April, 1994).

30. J. R. Milligan and J. F. Ward. DNA damage by scavenger radicals. Abstract 186, 42nd Annual Radiation Research Meeting, Nashville, TN (April, 1994).

31. J. R. Milligan. Why is track structure important in radiation chemistry and radiobiology? Workshop, 42nd Annual Radiation Research Meeting, Nashville, TN (April, 1994).

32. W. C. Dewey, L. L. Thompson, M. L. Trinh, D. L. Latz, and J. F. Ward. A CCD camera image analysis system for quantifying DNA distributions in agarose gels after pulse field gel electrophoresis. 42nd Annual Radiation Research Society Meeting, Nashville, TN (April, 1994).

### 4.2. Book Chapters

1. J. F. Ward, C. F. Webb, C. L. Limoli, and J. R. Milligan. DNA lesions produced by multiple ionizing radiation: locally multiply damaged sites. In "Ionizing radiation damage to DNA: molecular aspects" (S. S. Wallace and R. B. Painter, eds.). Alan R. Liss, pp. 43-50 (1990).

2. J. F. Ward. Mechanisms of radiation action on DNA in model systems - their relevance to cellular DNA. In "The early effects of radiation on DNA" (E. M. Fielden and P. O'Neill, eds.), NATO ASI Series H: Cell Biology, **54**, 1-16 (1991).

3. J. F. Ward. DNA damage and repair. In "Physical and chemical mechanisms in molecular radiation biology" (W. Glass and M. N. Varma, eds.). Plenum press, New York, NY, pp. 403-421 (1991).

4. J. F. Ward. Summing up: a chemist's point of view. In "The early effects of radiation on DNA" (E. M. Fielden and P. O'Neill, eds.). NATO ASI Series H: Cell biology, **54**, 417-420 (1991).

5. J. F. Ward. Differential DNA damage, can we measure it, can we model it? In "Biophysical modelling of radiation effects" (K. H. Chadwick, G. Moschini, and M. N. Varma, eds.) Adam Hilger, Phildelphia, PA, pp. 323-326 (1992).

6. J. F. Ward. The intracellular molecular damage which is dependent on radiation energy deposition patterns at the nanometer level. Genes, cancer, and radiation protection, NCRP Proceedings No. 13, pp. 38-48 (1992).

7. D. Brenner, J. F. Ward, and R. Sachs. Track structure, chromosome geometry and chromosome abberations. In "Radiobiological research" (A. Chatterjee and M. Varma, eds.). Plenum press (1993 in press).

### 4.3. Peer Reviewed Journals

1. J. F. Ward. DNA damage in mammalian cells. *Free Rad. Res. Commun.*, **6**, 179-180 (1989).

2. L. L. Ling, and J. F. Ward. Radiosensitization of chinese hamster cells by bromodeoxyuridine substitution of thymine: enhancement of radiation induced toxicity and DNA strand break production by monofilar and bifilar substitution. *Radiat. Res.*, **121**, 76-83 (1990).

3. J. F. Ward. The yield of DNA double strand breaks produced by ionizing radiation. *Int. J. Radiat. Biol.*, **57**, 1141-1150 (1990).

4. J. F. Ward. Response to commentary by D. Billen. *Radiat. Res.*, **124**, 385-387 (1991).

5. J. F. Ward, C. L. Limoli, and P. M. Calabro-Jones. An examination of the repair saturation hypothesis for describing shouldered survival curves. *Radiat. Res.*, **127**, 90-96 (1991).

6. D. J. Brenner and J. F. Ward. Constraints on energy deposition and target size of multiply damaged sites associated with DNA double strand breaks. *Int. J. Radiat. Biol.*, **61**, 737-748 (1992).

7. J. R. Milligan, A. D. Arnold, and J. F. Ward. The effect of superhelical density on single strand break yield for gamma irradiated plasmid DNA. *Radiat. Res.*, **132**, 69-73 (1992).

8. G. D. Jones, J. R. Milligan, J. F. Ward, P. M. Calabro-Jones, and J. A. Aguilera. Yield of strand breaks as a function of scavenger concentration and LET for SV40 irradiated with $^4$He ions. *Radiat. Res.*, **136**, 190-196 (1993).

9. J. R. Milligan, J. A. Aguilera, and J. F. Ward. Variation of single strand break yield with scavenger concentration for the SV40 minichromosome irradiated in aqueous solution. *Radiat. Res.*, **133**, 158-162 (1993).

10. J. R. Milligan, J. A. Aguilera, and J. F. Ward. Variation of single strand break yield with scavenger concentration for plasmid DNA irradiated in aqueous solution. *Radiat. Res.*, **133**, 151-157 (1993).

11. C. L. Limoli and J. F. Ward. A new method for introducing double strand breaks into cellular DNA. *Radiat. Res.*, **134**, 160-169 (1993).

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13. N. Arnhem, J. Boice, R. Cox, M. Gould, E. Hall, A. Knudson, H. Mohrenweiser, W. Sinclair, E. Stanbridge, R. Ullrich, J. Ward, H. Weinstein, A. Karaoglu, D. Galas, D. Smith, M. Varma, and R. Wood. Report on workshop to examine methods to arrive at risk estimates for radiation induced cancer in the human based on laboratory data. *Radiat. Res.*, **135**, 434-437 (1993).

14. J. F. Ward, J. R. Milligan, and G. D. D. Jones. Biological consequences of non homogeneous energy depositions by ionizing radiation. *Radait. Prot. Dosim.*, **52** (1994, in press).

15. J. F. Ward. DNA damage as the cause of radiation induced gene inactivation. *Radiat. Res.*, **137S** (1994, in press).

16. J. F. Ward. The hazard of hydroxyl radicals: response to comments of W. H. Koppenol and Z. Maskos. *Free Rad. Biol. Med.*, **16** (1994).

17. J. R. Milligan and J. F. Ward. Yield of single strand breaks due to attack on DNA by scavenger derived radicals. *Radiat. Res.*, **137**, 295-299 (1994).

18. G. D. D. Jones, T. V. Boswell, and J. F. Ward. Effects of post irradiation temperature on the yields of radiaiton induced single and double strand breaks in SV40 DNA. *Radiat. Res.* (1994, in press).

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