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# RADICAL-INDUCED DNA DAMAGE AND SPECIFIC IMPLICATIONS IN EVALUATING GENETIC CHANGES

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There have been many studies on the formation of radiation induced DNA strand breaks under different conditions of the buffer, however, it is not clear which of these studies can be directly correlated with important cellular phenomena such as mutations or other appropriate end points. If a certain process can be easily identified with the formation of strand breaks, one may wish to examine the correlation that may exist. Perhaps a good example of such relevance can be found in the transfection of rat embryonic cells with the plasmid, pTK2, containing the Herpes Simplex virus (HSV) thymidine kinase (TK) gene and the subsequent mutagenic effect of radiation through deletion of this particular gene. Experimental data with two different qualities of radiation are now available for the dose response curves of the mutation frequency. Since the mutation data are obtained in cells, one needs to consider the extent of validity of the *in vitro* studies on strand break cross sections with respect to cellular DNA.

Strand breaks are produced by radical mechanisms as well as by direct effects of radiation. Perhaps the results of direct effects are less dependent upon the environment and hence *in vitro* studies may be relevant to the processes in cellular DNA. However, this is not true for radical mechanisms. One needs to mimic the average $^{\cdot}OH$ migration distances for sugar and base damage in studies with aqueous solution of DNA which is between 20 A–50 A[1] in a cellular environment. Hence, one needs a very high concentration of tris as a buffer. For example, a concentration as high as 0.5 M is required to restrict the average migration distance to about 30 A. Obviously, the cross sections for the production of strand breaks are dependent upon the concentration of tris and hence these parameters must be evaluated at about 0.5 M tris concentrations. It appears from our experimental data on $D_{37}$ values for strand breaks and their comparison with theoretical calculations without considering any contribution from tris-radicals, that with such a high concentration of the buffer, the radiation chemistry of the radical attack on the DNA still remains relatively simple. Our theoretical calculations are based on a Monte Carlo technique of simulating the $^{\cdot}OH$ diffusion process as they approach the various sites on a stationary DNA molecule. These sites have been specified in a three-dimensional configuration based on X-ray diffraction data. Experimental measurements involve the use of standard gel electrophoresis technique with proper corrections for the intercalation of ethidium bromide, etc. A reasonable agreement between the experimental data and theoretical calculations for $D_{37}$ values gave us the confidence to calculate the cross sections for strand break productions. The calculations included both the direct effect[2] mechanisms and the indirect effect[3] mechanisms. Based on these calculations, we have obtained the following results on cross sections: (i) single strand breaks are produced with equal efficiencies by direct or indirect effects; (ii) double strand breaks are created with a

slightly greater efficiency by indirect effects over direct effects for low-LET (30 KeV/$\mu$M) radiation; (iii) for high-LET radiation, double strand breaks are produced through a major contribution from direct effects. These results are true only for high concentration of tris buffer. In order to calculate the frequencies for mutation of the TK gene, we have assumed that single strand breaks are mostly repaired and two double strand breaks are required for the deletion of the plasmid from the host DNA. Based on this model, and using the cross sections for the production of double strand breaks, we have been able to estimate the mutation frequencies and the dose response. Within the limit of the error bars, the experimental data are in excellent agreement with the theoretical calculations for X-ray and ion particle irradiation.

At present we do not have enough studies to arrive at a definite conclusion. However, from this solo effort it seems that the *in vitro* data with high scavenger concentration, may perhaps, be quite useful in the analysis of certain cellular phenomenon.

## *References*

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