Rachmeler Martin Ph.D.
Department of Microbiology
Northwestern University
Medical School
COO-1603-3

MASTER

Effects of Tritium Decay on DNA

Terminal Summary Report

Contract AT (11-1)-1603 Mod 2

Introduction

Tritium decays within cells can ultimately lead to their death. It was the purpose of this project to (1) determine the nature of the effective agents leading to cell death, (2) to determine the nature of the defects caused by the decays and (3) to determine the extent of damage necessary before phenotypic and genotypic effects can be observed.

It appears that certainly all of the lethal effects seen in the cell can be attributed to defects in the deoxyribonucleic acid (DNA). These defects take the form of single and/or double stranded breaks in the double helix. Our study proceeded to examine (1) the effects of varying doses of tritium, incorporated into various macromolecules of the cell, on cell viability, on mutation formation and enzyme forming ability, (2) the effects of tritium decays on labeled DNA isolated and stored frozen, and (3) the effects of altered DNA repair mechanisms and/or radioprotective agents on sensitivity to tritium decays.

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

LEGAL NOTICE

This report was prepared as an account of Government sponsored work. Neither the United States, nor the Commission, nor any person acting on behalf of the Commission:

A. Makes any warranty or representation, expressed or implied, with respect to the accuracy, completeness, or usefulness of the information contained in this report, or that the use of any information, apparatus, method, or process disclosed in this report may not infringe privately owned rights; or

B. Assumes any liabilities with respect to the use of, or for damages resulting from the use of any information, apparatus, method, or process disclosed in this report.

As used in the above, "person acting on behalf of the Commission" includes any employee or contractor of the Commission, or employee of such contractor, to the extent that such employee or contractor of the Commission, or employee of such contractor prepares, disseminates, or provides access to, any information pursuant to his employment or contract with the Commission, or his employment with such contractor.

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

- 2 -

Materials and Methods

Organisms: The organisms used in the study were E. coli 15T⁻ and 15T-H⁻U⁻, E. coli K12 SH-28, a thymidine phosphorylase negative mutant (Fangman and Novick, 1966) and E. coli AB2487, a recombination deficient (rec⁻), thymine requiring mutant that is sensitive to UV irradiation (Howard-Flanders and Therit, 1966). The conditions for cultivation, storage, and the media used for various studies are in previous progress reports.

Preparation of DNA and Density Gradient Centrifugations

The phenol procedure of Thomas (1966) was used for DNA isolation. The DNA which was prepared for storage was dissolved in SSC and the DNA stored at 0°C.

CsCl gradients were used in much of this work. After collection, the densities of the various fractions were determined by refractometry.

$$\rho_{25} = a \cdot \eta_0^{25} - b \quad (\text{Thomas and Berns, 1961})$$

For sucrose gradients the sucrose was prepared either in neutral SSC or in alkaline SSC (pH 13).

Denaturation of DNA was performed either by heating at 100°C for 10' or subjecting the DNA to pH 13 buffer or alkali for 30' at 40°C and then either neutralizing for neutral sucrose gradient centrifugation or placing directly on alkaline sucrose gradient.

RESULTS AND DISCUSSION

Effects of tritium decays: Our previous data and those of others

- 3 -

leaves little doubt that the major defects caused by tritium decays are due to the reactions of the extensive ionizations produced within the cell or within a localized area close to the site of the decay. The net result is to produce breaks within the DNA chain. These breaks are undoubtedly at the phosphodiester linkage. No nucleosides or nucleic acid bases have been found in the TCA supernatant from a DNA solution having undergone extensive degradation due to tritium decays indicating that the transmutational or ionization effects produced by $^3\text{H-CH}_3$ -thymidine do not result in base loss. The implication here is that little mutagenesis can be achieved by base loss and replacement by another base. This is confirmed by our findings that $^3\text{H-CH}_3$ -thymidine or thymine is very poorly mutagenic, if at all. We were unable to find significant numbers of mutants, even after a 3-4 log loss of viability. This is apparently not true of all ^3H -bases (Person and Bockrath 1965). It appears that transmutation may play an important role in mutagenesis utilizing bases labeled in the pyrimidine ring rather than in the side chain.

Viability studies: There are differences in sensitivity to tritium decays between strains. Although it is not possible at this time to explain all these differences, in one instance, that of the AB2487 strain, the increased sensitivity appears attributable to the deficient DNA repair mechanism of the cell. It seems fairly certain now that the loss of viability in cells is due to chromosome breakage. In view of the postulated role of DNA repair mechanisms, apparently many of the breaks, especially single strand breaks, caused by the effects of tritium decays are repaired. The first order kinetics of survival

- 4 -

suggest a single hit is necessary for loss of viability in a cell. The efficiencies ranging from 300 to 25 indicate that many events take place in a cell on the average before a lethal effect is recorded. The DNA repair mechanism undoubtedly repairs many of the breaks caused by the decays. It is possible, and further analysis of some of our data may show, that a double stranded break is what is required for the lethal effect to occur.

Enzyme studies: Using K12 strains we have found that ^3H -decays do not appreciably affect the enzyme forming ability of the cells by assaying three inducible enzymes: B-galactosidase, serine deaminase and tryptophanase. This is at variance with a previously published report (Rachmeler and Pardee 1963) but we have not been able to resolve the difference.

DNA studies: (In vivo and in vitro). Cells were labeled with the appropriate ^3H -labeled precursor and then stored at -70°C . At 0 time, DNA was prepared from a large aliquot and stored at 0°C (in vitro DNA). At various intervals aliquots of cells were removed and DNA prepared (in vivo DNA). The DNA, both in vivo and in vitro, was analyzed by either CsCl or sucrose density gradient centrifugation. In some instances the DNA was x-linked by mitomycin (Iyer and Szybalski 1964) or NaNO_2 (Becker et al, 1964) prior to centrifugation. In all cases samples were centrifuged before and after denaturation. The data suggests that the differences in sensitivities of cells to ^3H -decays is apparent with the DNA isolated from stored cells; i.e.

- 5 -

more breakage with more sensitive cells, but this is not readily apparent with DNA stored in vitro, i.e. all in vitro DNA seems equally sensitive to decays. Thus the sensitivities are not inherent in the DNA molecules themselves but in the environment in which they are placed. This again is strongly suggestive that more damage, in the way of DNA strand breaks, occurs than is seen because much of it is repaired in the cells. In the "in vitro" DNA more overall damage due to decays is seen but the exact correlation between decays and breaks has not yet been analyzed.

Both cysteine (1mM) and 2.8% AET tend to diminish the effects of ^3H decays on in vitro DNA. Since these agents are radioprotective we conclude that the ^3H decay effects on in vitro DNA are due to the ionizations produced.

These two agents have a pronounced protective effect on the viability of cells but we have not yet been able to correlate this with any decrease in breaks in in vivo DNA. This analysis is currently underway.

SUMMARY CONCLUSION: The major product of ^3H decays in the cell are ion pairs. These in turn react with the DNA, and other macromolecules of lesser importance for the effects noted, to cause strand breaks, very likely the phosphodiester bond. Cell death is probably caused by one of these breaks or a double strand break produced as a result of a single decay. It appears that not all decays lead to DNA strand breakage. DNA repair mechanisms tend to decrease the efficiency of ^3H decays in causing cell death. The decays of $^3\text{H-CH}_3^-$ thymidine are poorly mutagenic and cause no rupture of bonds between base and

- 6 -

sugar or sugar and phosphate. In addition there appears to be no long range effects along the length of the chromosome as determined by the lack of effect of randomly occurring decays on the transcriptional ability of three independent cistrons not closely linked. The quantitative relationship between ³H-decays and strand breakage is now being determined.

REFERENCES

- Becker, E. F. Jr., Zimmerman B. K. and Geiduschek, E. P. 1964. J. Mol. Biol. 8, 377.
- Fangman, W. L. and Novick, A. 1966. J. Bacteriol. 91, 2390.
- Howard-Flanders, P. and Theriot, L. 1966. Genetics 53, 1137.
- Iyer, V. N. and Szybalski, W. 1964. Science 145, 55.
- Person, S. and Bockrath, R. C. Jr. 1965. J. Mol. Biol. 13, 600.
- Rachmeller, M. and Pardee, A. B. 1963. Biochim. Biophys. Acta 69, 62.
- Thomas, C. A. Jr. and Berns, K. I. 1961. J. Mol. Biol. 3, 277.
- Thomas, C. A. Jr., Berns, K. I. and Kelly, T. J. Jr. 1966. in Procedures in Nucleic acid Research G. L. Cantoni and D. R. Davies eds. Harper and Row, 535.

GRADUATE STUDENTS AND POSTDOCTORALS ON THIS GRANT:

None.

PUBLICATIONS:

None (manuscript in preparation).