A Progress Report

to

Division of Biology and Medicine
U. S. Atomic Energy Commission
Washington, D. C.

BIOLOGICAL AND CHEMICAL STUDIES ON PHYSICAL MUTAGENS

Contract No. AT(11-1)-3419

Period Covered: August 1, 1972 -- July 31, 1973

Submitted by

Stanley Person
Principal Investigator

Biophysics Department
College of Science
The Pennsylvania State University
University Park, Pennsylvania 16802

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or 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.

MASTER

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

101

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.

Progress Report

Our progress during the past year has been mainly in the area of transmutation effects of tritium decays from nucleic acid precursors. In particular we noted that single-strand breaks in bacterial DNA stored in vitro are four times more frequent per decay when decay occurs as cytosine-6-³H as opposed to cytosine-5-³H. The values obtained for single-strand breaks per decay are 0.37 for cytosine-6-³H and 0.09 for cytosine-5-³H. When our data are taken together with those of Rosenthal and Fox (1970) it would seem that the transmutation effect occurs for thymidine-6-³H as well. Also Fred Funk reports that thymidine-methyl-³H causes single-strand breaks in Φ X174 RF DNA with an efficiency of 0.075 (personal communication). Our data are presented in Appendix I which is the page proof of an article accepted for publication in the International Journal of Radiation Biology. In the immediate future we want to extend these studies to decays occurring from the 2- and 8-positions of purines.

Although the 6-position of pyrimidines is important in producing single-strand breaks, this is not detectable as an increased killing efficiency for bacteria accumulating decays from DNA-incorporated thymidine-6-³H and thymidine-methyl-³H (see Fig. 1). For this study we used the isogenic strains W3110 thy⁻pol⁺A1 and P3478 thy⁻pol⁻A1. The polymerase mutation was included because the mutant is five times as sensitive as the parent strain to ionizing radiation, and we thought we might notice the transmutation effect for 6-position ³H-labeled pyrimidines in the mutant cell. Reference to Fig. 1 shows that there is no difference in killing efficiency for decays originating from the 5- or 6-position of thymidine in these two strains. We conclude that the single-strand breaks specifically associated with decays from 6-position pyrimidines are efficiently repaired in bacteria. If an assay were specific for single-stranded

SURVIVING FRACTION OF THE INITIAL POPULATION

Figure 1. Plot of the survival of bacteria as a function of accumulated tritium decays. Cells were labeled with the indicated tritium-labeled precursor, frozen at -196°C and stored at -60°C to accumulate decays. Periodically samples were thawed, diluted and plated on tryptone agar to determine the survival value. Other samples were combusted ^{prior} to determining the amount of incorporated radioactivity. The killing efficiency (probability of lethal events per decay) is 0.0030 for W3110 and 0.0142 for P3478. The mutant strain is 4.7 times more sensitive to decays than is the pol⁺ strain. This number may be compared to 5.2 for external ionizing radiation (Paterson, Boyle and Setlow, 1971). The open squares represent data points for thymidine-methyl-³H decays and the closed circles represent data points for thymidine-6-³H decays.

---

nucleic acid one would expect to see a difference in decays originating from the two labeled positions. The data of Rosenthal and Fox (1970) for Pneumococcus transforming DNA, and for ΦX174 survival (Funk and Person, 1969) indicate the possibility of such a local effect.

Frank Krasin, a graduate student in our department, has also investigated the chemical product of tritium decays from 5-position labeled cytosine. Previously we had shown that decays originating from this position effectively cause mutations and that the genetic coding change associated with these mutations is specific with C now coding as T. Krasin used cytosine-5-³H with a ¹⁴C label in the 2-position as well. Although it was difficult to determine a non-radiation associated product when aqueous cytosine-5-³H-2-¹⁴C was stored to accumulate decays, a transmutation product was discernible when the cytosine-5-³H-2-¹⁴C was incorporated into the DNA of bacteria and purified

DNA stored at -60°C to accumulate decays. The stored DNA was hydrolyzed, lyophilized and subjected to two-dimensional thin-layer chromatography, employing a different developer for each dimension. Sheets of Kodak X-ray film were exposed to the chromatogram and were subsequently developed to reveal a series of spots, including of course cytosine. Several unknown spots appeared, but only one accumulated significant quantities of ^{14}C radioactivity as a function of storage time. For the two solvent systems employed this spot occupied the same location as uracil which was co-chromatographed with the products of tritium decay. A second evidence that the spot was uracil is that the amount of ^{14}C radioactivity did not go through the origin at zero storage time, but instead extrapolated to an amount of radioactivity that can be attributed to deamination of cytosine to uracil by the acid hydrolysis conditions used. Data are shown in Figs. 2 and 3; the conclusion from the slope of Fig. 3 is that 0.28 μmoles of uracil are produced per μmole of tritium decay. Fig. 2 shows the fraction of radioactivity applied to the chromatograms that resides in the spot that co-chromatographs with uracil as a function of accumulated tritium decays. In Fig. 3 the zero value is subtracted and the data are expressed in terms of molecules of product per tritium decay. Data that will not be included here indicate that we expect on the basis of genetic experiments 0.33 molecules of product formed that code as thymine. Chemically we find uracil or a uracil-like product formed with an efficiency of 0.28 per tritium decay. We are still giving thought as well as doing experiments that should give us a clue as to the molecular rearrangements of cytosine following a tritium decay at the 5-position.

Figure 2

^{14}C -product formed
(pico moles)

Tritium decays from cytosine - 5- ^3H -2- ^{14}C (pico moles)

Figure 3

Last year we attempted to develop mutational systems in mammalian cells in order to determine if decays from cytosine-5-³H caused a transmutation effect in these cells as previously shown for bacterial viruses and bacteria. We were totally unsuccessful in these endeavors and eventually turned our attention to an animal virus, Herpes simplex type I, instead. Since Alec Keith, who is concerned with physical properties of membranes, was coming to our department, Wallace Snipes and I decided to focus our research on membrane functions. Dr. Snipes decided to study membrane interactions in the enveloped bacteriophage, PM2 and its host, BAL-31. I have decided to study the mechanism of virus-induced cell fusion caused by mutants of Herpes Simplex Virus. Some of our proposed studies are outlined in the accompanying renewal proposal.

Personnel on the project:

S. Person, Co-principal Investigator, Professor, 25%,	[REDACTED]
W. Snipes, " " " , Assoc. Prof., 24%,	[REDACTED]
*J. Cupp, Graduate Assistant, 50% (5 mos.)	[REDACTED]
*S. Read, " " 50% (3 mos.)	[REDACTED]
*T. Holland, " " 50% (3 mos.)	[REDACTED]

Part-time help

References Cited:

Funk, F. and S. Person, 1969, Science 166:1629.

Paterson, M. C., J. M. Boyle and R. B. Setlow, 1971, J. Bacteriol. 107:61.

Rosenthal, P. N. and M. S. Fox, 1970, J. Mol. Biol. 54:441.

---

* As of March 31, 1973