TO: Office of Grants and Contracts
National Aeronautics and Space Administration
Washington, D. C.

SUBJ: Final Progress Report of NASA Grant No. N G R 04-001-014 for the Reporting Period of September 1966 to September 1968

INTRODUCTION

This report describes the research activities of the Cellular Radiobiology Laboratory during the period of September 1966 to September 1968. The principal part of this work was supported by NASA Grant No. NGR 04-001-014.

The research efforts of the laboratory have been directed toward elucidating, in a systematic manner, the mechanism by which mammalian cells repair radiation injury. Since deoxyribonucleic acid (DNA) represents the site of radiobiological damage in the cell (and hence the site of repair), we have focused most of our attention on the behavior of this macromolecule. In general, two questions were asked: (1) Is DNA metabolism altered during repair of radiation injury? (2) Does energy deprivation (starvation or treatment with a metabolic inhibitor) effect the cellular radiation response?

In order to answer these questions, we have used radioactive precursors to follow the synthesis, and possible breakdown, of DNA. The activities of enzymes related to DNA synthesis and degradation (polymerase and nucleases) were also investigated. Because ribonucleic acid (RNA) synthesis requires structural integrity of the DNA molecule, we also studied the

behavior of this fraction. Starvation in a glucose-free salt solution and treatment with 2, 4 - dinitrophenol (DNP) served to inhibit metabolism. Cell survival kinetics were used to detect repair under these various conditions. The L-929 cell line was employed throughout these studies.

The results are presented here in terms of the questions we asked during our investigations. In summarizing, we shall propose a model which explains the repair process in terms of an enzyme that rejoins single-strand breaks in the DNA molecules.

RESULTS

A. Is DNA synthesis affected by radiation?

For these experiments, we used both pulse labeling and continuous labeling techniques to follow DNA, RNA, and ATP synthesis. Since phosphate-P^{32} labels all these fractions, it was the most frequently employed precursor. The uptake of P^{32} into the above cellular fractions after irradiation was investigated under three conditions: 1) normal growth, 2) starvation, and 3) DNP treatment.

Cells irradiated in growth medium showed no difference in their P^{32} labeling of DNA, RNA, and ATP from what was seen in unirradiated cells. The observation extended up to four hours post irradiation; and the X-ray doses ranged from 100 rad to 1000 rads.

In contrast to cells irradiated in growth media, starved or DNP-treated cells showed discrete periods of accelerated P^{32} labeling of DNA and RNA following relatively low doses. The response had three distinct features: 1) a post-irradiation delay appeared necessary; 2) the P^{32} seemed to enter and then rapidly to leave both nucleic acids; 3) the labeling of DNA closely paralleled the labeling of RNA.

This effect was most obvious at the lowest dose used (100 rads). At higher doses (250 and 500 rads), several cycles of uptake and loss of label could be seen. The response, however, appeared to become diffused at these higher doses. ATP utilization seemed to increase during the accelerated labeling of the nucleic acids. The cells also showed an accelerated labeling when tritiated adenosine and deoxyadenosine were used as labeled precursors. Thymidine and deoxycytidine, however, proved to be poor labeling agents in energy-deprived cells.

B. Is DNA degraded by radiation?

In order to answer this question, we prelabeled the cells with tritiated thymidine, a precursor specific for DNA. Twenty-four hours after irradiation, the radioactive medium was replaced with non-radioactive medium. At various times after irradiation, the cold acid-soluble fractions of the cells and the medium were examined for tritiated thymidine. The presence of radioactivity in these fractions would indicate that DNA degradation had taken place.

For these studies, the cells were irradiated under conditions of normal growth, starvation, and DNP treatment. Although the X-ray doses ranged from 100 rads to 1000 rads, we found no evidence of DNA degradation even after eight hours postirradiation.

C. Are DNA polymerase and nucleases affected by radiation?

The tests for these enzymes involve an <u>in vitro</u> assay of the amount of labeled precursor incorporated into or removed from the DNA fraction. In the case of DNA polymerase, the amount of tritiated thymidine triphosphate

incorporated into DNA served as a measure of the enzyme's activity. For the nucleases, the amount of tritiated thymidine released from prelabeled DNA indicated the activity of these enzymes in the cellular extract.

Again the cells were studied under conditions of normal growth, starvation, and DNP treatment. Under none of these conditions did radiation doses of 100 rads to 1000 rads alter polymerase or nuclease activities compared to the unirradiated control.

D. What are the survival properties of cells under conditions of normal growth and energy deprivation?

The single cell survival technique was used to determine repair of radiation injury. The survival studies showed that cells treated with DNP had a significantly higher survival than cells in growth medium. This increased radioresistance in DNP appeared for both single and paired-irradiations. Starved cells had a slightly higher survival than cells in growth medium, but the significance of the increase remains doubtful. The doses for the survival studies ranged from 100 rads to 800 rads. In the paired-irradiation experiment, the cells usually received two 400-rad doses.

CONCLUSION

The results of our studies indicate the following:

(1) Under conditions of energy deprivation, L cells show an accelerated P^{32} labeling of both DNA and RNA at relatively low doses. This response does not appear for cells irradiated under normal growth conditions.

(2) There is no evidence for a radiation-induced DNA breakdown under any of the conditions we used. This conclusion is based on the results from the

prelabeling experiments and on the measurements of nuclease activity. (3) Energy deprivation reduces cellular radiosensitivity. In the case of DNP-treated cells, the survival may increase threefold over the growth medium controls at higher doses.

The two positive results of these studies are the accelerated P^{32} labeling of the nucleic acids and the increased cellular survival, both of which result from energy deprivation. How can these two phenomena be reconciled? A possible explanation comes from the recent description of enzymes which join single-strand breaks in the DNA molecule. These enzymes have been termed DNA ligase, DNA sealase, and polynucleotide-joining enzyme. They appear to act on all single-strand breaks regardless of how the breaks are produced. Since breakage of the phosphodiester backbone is a known action of ionizing radiation, any enzyme which rejoins the ends of a single-strand break would be of prime importance in recovery from radiation injury.

The accepted mechanism for the DNA rejoining enzyme involves the addition of adenylate to one terminus of the broken strand. This condition activates the DNA strand for rejoining, which results in the release of the adenylate moiety. If the adenylate should be labeled with P^{32}, the label would enter and then leave the DNA fraction; this is precisely what we observed during the accelerated labeling. Thus, we can explain our accelerated labeling of DNA and consequently, the increased repair during energy deprivation, in terms of an enzyme which rejoins single-strand breaks in the DNA molecule.

Unfortunately, the reason for an accelerated RNA labeling remains unclear. Possibly, future studies will reveal its role, if any, in the

repair of radiation injury. The above model, however, does explain nicely why we observed no DNA degradation or net DNA synthesis. None would be required according to the rejoining enzyme mechanism. The question arises as to why we see the accelerated labeling in energy-deprived cells but not in growing cells. Probably, the accelerated labeling does occur in growing cells, but it is overshadowed by the cell's normal metabolism.

Publications Sponsored by the Grant:

a. Dalrymple, G. V., Sanders, J. L., and Baker, M. L., : Dinitrophenol Decreases the Radiation Sensitivity of L Cells, Nature, 216: 708, 1967.

b. Tyson, J. W., Meade, J. A., Dalrymple, G. V., and Marvin, H. N.: Some Biomedical Applications of a Non-linear Curve Fit Method, J Nuc Med., 8:558-569, 1967.

c. Dalrymple, G. V., Sanders, J. L., and Baker, Max L., : Do Cultured Mammalian Cells Repair Radiation Damaged DNA by the "Cut-and-Patch" Mechanism? J Theoretical Biology, (In Press)

d. Dalrymple, G. V., Sanders, J. L., Baker, Max L., and Wilkinson, K. P.: The Effect of 2,4-Dinitrophenol on the Repair of Radiation Injury by L Cells, Radiat Res. (In Press)

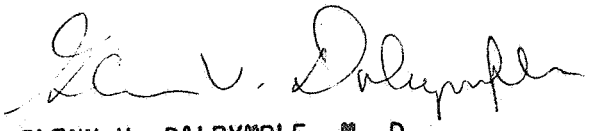
e. Tyson, J. W. and Dalrymple, G. V.: The Effect of Radiation on the DF ^{32}P RBC Survival Curne on the Mouse, Proc Soc Exp Biol and Med, (In Press).

f. Sanders, J. L., Dalrymple, G. V., Baker, Max L., and Wilkinson, K. P.: Evidence for an Accelerated ^{32}P Labeling of DNA and RNA In Irradiated L Cells. Radiat Res (submitted).

Presentations

a. "A Radiation-Induced Nucleic Acid Synthesis in L Cells Under Energy Deprivation", Sanders, J. L., Dalrymple, G. V., Baker, M. L., and Wilkinson, K. P. To be presented at 16th Annual Meeting, The Radiation Research Society, Houston, Texas, April 21-25, 1968.

b. "Radiation Response of L Cells treated with 2,4-Dinitrophenol," Sanders, J. L., Dalrymple, G. V., Baker, M. L., and Wilkinson, K. P. To be presented at 13th Annual Meeting, Health Physics Society, Denver, Colorado, June 16, 1968.



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