STUDY OF THE EFFECTS OF RADIATION OF
NUCLEIC ACIDS AND RELATED COMPOUNDS

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ABSTRACT

Ionizing radiation produces genetic effects in biological systems. Since genetic effects are usually the result of modifications of DNA or sometimes of RNA, interest is being centered on the chemical and physical nature of radiation-induced lesions to nucleic acids and their components. These investigations have revealed the enormous complexity of chemical events and the possible degradation of nucleic acids by strand breakage. Therefore, work in the ionization radiation of nucleic acids has proceeded along a dual course. On the one hand, molecular changes have been characterized for a number of primary radiation products. On the other hand, strand breakage has been investigated intensively as a direct primary event. Both of these aspects were emphasized in our research last year. We succeeded in improving the synthesis of 5-hydroxymethyl thymine (α -TOOH). α -TOOH was found to be much more effective than cis-5,6-dihydro-6-hydroperoxy-5-hydroxy thymine (6-TOOH) in the inactivation of transforming DNA of H. influenzae cells although α -TOOH is much less reactive chemically than 6-TOOH. 6-TOOH causes inactivation and acts as an inhibitor of DNA synthesis in mammalian cells. In addition, evidence may indicate that 6-TOOH does not induce strand breaks directly in DNA although we showed that 6-TOOH is a clastogenic compound.

SCOPE AND OBJECTIVES

Currently, it has become a national policy to pay more attention to improving and preserving the physical environment and thereby promoting the health and welfare of man. Studies on the effects of ionizing radiation exposure on man and his environments have been established as an area of interest and importance. Since it was first discovered by Müller in 1928, it has been generally acknowledged that x-rays cause mutagenic and lethal effects in biological systems. Despite these possible genetic risks, the clinical use of x-rays has been widespread because of beneficial medical results. Recently, setting up an ionizing radiation standard has become an issue with the Committee on the Biological Effects of Ionizing Radiation of the National Academy of Sciences. The main problem in establishing such standards is that there is little scientific criteria upon which they can be based.

Our understanding of the biological effects of ionizing radiation has largely been in terms of physical and biological concepts because assay of radiation damage to cells is generally based on measurements of reproductive inactivation, chromosomal aberration production, unscheduled DNA synthesis, and increased mutation rates. Although these gross changes are easily discerned, the information thus gathered provides little knowledge concerning the threshold intensity or of setting up a radiation standard. Thus, the study of radiation chemistry relevant to biological effects becomes of

crucial importance for these purposes as well as for the understanding of the biological effects of ionizing radiation on a molecular level. However, the progress in radiation chemistry of nucleic acids is rather slow for two main reasons. 1) the enormous complexities of the chemical events in these radiation reactions and 2) the immense difficulties in separating the great number of structurally similar yet highly reactive radiation products. Considering these predicaments, it is surprising that there has been continued interest and progress made in this area of research.

Therefore, I assumed this research problem as a challenge, of course, with certain apprehension. However, the approach that I proposed to use is the isolation and characterization of radiation products and has proved to be essential in the successful development of photochemistry and photobiology of nucleic acids. Especially, my laboratory has gained a great deal of experience in such an ultraviolet irradiation study.

Indeed, our results in the actual isolation and identification of radiation products of nucleic acid bases bore out my belief. The published work includes thymine glycols, cis-5,6-dihydro-6-hydroperoxy-5-hydroxy thymine (6-TOOH), 5-hydroxymethyl thymine (α -TOOH), etc. (see previous Progress Reports) and in many ways has exceeded my expectations. Consequently, our research plan was expanded to include the following areas:

1. Chemical isolation and characterization of radiation products.
2. Mutagenic study with reactive radiation products.
3. Chromosomal aberration study with reactive radiation products.
4. Radiation sensitization study with reactive radiation products.
5. Near-UV radiation study for the comparison with ionizing radiation.

However, the budget support for many years remained approximately the same level and this year became only sufficient to cover ~50% of the salary of one Associate. Therefore, research areas 4 and 5 had to be suspended. Research areas 2 and 3 were continued without support. Despite these financial stringencies, satisfactory progress has been made in these two areas as well as Area 1. We can not maintain this level of research activity unless DBER decides to give a substantial increase in the funding level. Since I became aware that DBER may decrease its support for extra-mural research, I must voice my concern that this nation's ability to continue to conduct high-quality scientific research at university campuses may be hampered. This project, in my opinion, seems to deserve special consideration because the rather "unique" approach and some "breakthrough" findings.

SIGNIFICANT RESULTS

The most significant result of the last year or, some believe, for the last half of the century since Müller discovered radiation mutagenesis, is that we are able to explain this phenomenon on a molecular level. In addition, 6-TOOH was evidence to be clastogenic, i.e. producing chromosomal aberrations and the synthesis of α -TOOH has been improved so that sufficient quantities can be obtained for our intended biological studies.

1. "Biological" Study:

Because a large number of structurally similar products are formed even in a simple radiation reaction, it is impossible to specify the action of a particular radiation product or products in a biological system. Therefore, radiobiological effects have been described most frequently in terms of physical and biological changes. In order to correlate radiobiological effects with radiation chemical modifications, 6-TOOH and α -TOOH have been used to investigate the chemical mechanism causing some of the biological effects. This approach seems to be effective.

Mutagenic Study with 6-TOOH and α -TOOH

Our first publication concerning a thymine hydroperoxide as a mediator in ionizing radiation mutagenesis appeared in Nature, [259, 341 (1976), copy of reprint enclosed] recently. Based on our results, a mechanism for in vivo radiation mutagenesis was proposed. Apparently, ionizing radiation produces 6-TOOH in

the DNA or metabolic pool of cells. It reacts with transition metal ions, in particular Cu^{++} , forming free radicals that react with other bases of the cell's genome. Some of these modified bases cause base mispairing during replication leading to what are generally regarded as mutations.

α -TOOH

6-TOOH

In addition, we are studying α -TOOH, which is also a radiation product found in DNA but in lesser quantity than 6-TOOH, in a similar manner. The most interesting preliminary finding is that α -TOOH is 10^3 -fold more effective than 6-TOOH. This is unexpected because α -TOOH is 10^3 -fold less reactive chemically than 6-TOOH and should be much less effective. This evidence suggests that the mutagenic actions of these two compounds must occur via different mechanisms despite their similar hydroperoxy functional groups.

Chromosomal Aberration Study with 6-TOOH

Mr. Jonathan E.T. Kelly (a graduate student of Dr. Timothy Merz) will complete his Ph.D. research work shortly with the submission of his thesis entitled "The Evaluation of cis-5,6-Dihydro-6-hydroperoxy-5-hydroxythymine as a Radiomimetic Compound" [June 1976]. The data collected constitute evidence that shows that 6-TOOH is:

- a. an inhibitor of DNA synthesis
- b. lethal
- c. clastogenic but does not induce strand breaks in DNA directly.

This study has shown that chromosomal aberrations may be produced chemically by a radiation product. Further investigation should lead to an understanding of a chemical mechanism for clastogenic actions.

2. Chemical Study:

Our main effort has been the preparation of various compounds for the "biological" study. However, a communication on the "Synthesis and Characterization of 5-Hydroxymethyluracil" appeared in J. Org. Chem. [41, 567 (1976), copy of reprint enclosed]. Progress also made in the preparations of nucleosides of thymine glycols, thymine hydroperoxides, and 5-hydroxymethylthymine. Despite several reports in the literature, the actual isolation of various isomers of these nucleosides remains to be accomplished. Apparently, the presence of the ribose moiety has resulted in some complications. Dr. Jean Cadet, who has considerable experience in this problem, is currently a Visiting Associate in my laboratory until the end of August. Satisfactory data should be forthcoming.