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

TO

DIVISION OF BIOMEDICAL AND ENVIRONMENTAL
RESEARCH

U. S. ATOMIC ENERGY COMMISSION

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Introduction

It is generally accepted that a genetic effect is usually the result of modifications of DNA or, sometimes, of RNA. The nucleic acids are important targets for radiation effects in biological systems. Consequently, a great deal of interest is being centered on the physical and chemical natures of radiation-induced damage to nucleic acids and their components under various conditions. The results of these investigations have shown that among other changes the chemical changes induced in purine and pyrimidine moieties of nucleic acids, may be of decisive importance in the functioning of multicomponent cells. However, the exact nature and absolute chemical yields of many radiation products are not yet completely understood. Due to the necessity of a thorough knowledge of these radiation products before understanding their radiobiological effects, a systematic chemical study is being carried out under conditions ranging from the simple aqueous to the in vivo.

Isolation and Identification of Radiation

Products (with B.S. Hahn)

Subsequent to our preliminary study, we first investigated the radiolysis of thymine in aerated aqueous solutions. The progress of this study surpassed our expectations and the results are far more important and exciting than would have been expected of a

project that has been undertaken in other laboratories for almost half a century. We were then anxious to begin another project: the irradiation of cytosine in an aerated aqueous solution, which is presently being performed concurrently with the study of thymine. After irradiation, evaporation, extraction, thin layer chromatography, etc., the mixture of the radiation products was separated into at least 17 different products, the major one of which was further separated into two structural isomers. In addition to the UV, IR, NMR and mass spectra, X-ray diffraction analysis was also carried out in order to establish the molecular structures of the two products as 1-carbamylimidazolidone glycols.[J. Am. Chem. Soc. 95, 2711 (1973)]. This is also a surprising finding since earlier notions had led to the assignment of other structures.

Synthesis of trans 1-carbamylimidazolidone-4,5
glycol (with B.S. Hahn)

This synthesis provides further evidence for the identification of the major radiation product from cytosine, and may provide compounds for the testing of antiviral activity. It is interesting to note that recently the riboside of an analog of the above compound was shown to have broad-spectrum antiviral activity[Science 177, 705 (1972)]. Condensation of glyoxal and biuret results in a fair yield of the trans glycol identical to that obtained from the irradiation of cytosine. The synthesis of its riboside is now in progress.

Chromosomal Aberrations by Thymine Hydroperoxide, TOO_H.

[with J.E.T. Kelley and T. Merz]

In our last report to DBER, we mentioned that TOO_H reacts with transforming DNA, chromosomes, etc., causing inactivation and possibly aberrations. We believe that further studies may add a great deal to the understanding of the molecular basis of radiation biology. We were happy to learn that R. M. Herriott has shown TOO_H to be a mutagen.

Preliminary experiments with Kelley and Merz have indicated that TOO_H has radiomimic properties for human cells. The cell system being studied is the human lymphocyte in short term culture. The most obvious effects observed have been the production of chromosomal aberrations and a marked mitotic delay. All of the aberrations are of the chromatid type and are mostly achromatic gaps, although some deletions were also observed. The treatment time was generally a two hour exposure at the initiation of the culture which places the cell at the G₀ - G₁ interface. This implies that the effect of TOO_H is possibly sub-chromosomal and/or involves DNA replications. An interesting aspect is that TOO_H seems to be reasonably potent. Treatment at 10⁻⁵ M concentration produces achromatic gaps in 18 percent of the metaphases scored at 72-75 hr of culture. In contrast, the peroxide most often studied was hydrogen peroxide and, with the exception of catalase deficient mutants, deleterious effects were observed only at super-biological

doses. Thus, previous investigations of the role of peroxide in radiation biology have not been fruitful. However, there is little doubt that ionizing radiation produces peroxides within biological systems. While catalase-sensitive peroxides are probably not significant participants in the pathways leading to the biological effects of ionizing radiation, TOOH is apparently catalase-resistant and would conceivably be formed from thymine within or adjacent to the most radiosensitive cellular organelle. Investigations of the actions and effects of TOOH as well as its related nucleosides and nucleotides are fundamental to our understanding of the early radiation chemical events following exposure of biological systems to ionizing radiation.

The effect of TOOH on nucleic acid components

(with L. Parkhill)

In understanding the molecular basis of the deleterious, mutagenic and other effects produced by TOOH in biological systems, a logical approach was the study of the chemical reactions of TOOH with various nucleic acid components. These compounds are quite reactive toward TOOH as indicated by UV spectral changes and by the detection of product formation by ¹⁴C-labeled compounds. Based on these data, the reactivity of the compounds with TOOH may be shown in the following decreasing order: guanine > adenine > cytidine > cytosine > uracil > thymidine > thymine. It was observed that many

different products are formed as a result of each reaction. This was expected since these heterocyclic compounds have many reactive sites. The surprising finding is that TOO_H reacts differently with various bases. This was indicated by the differences in the end products of TOO_H after each reaction. Thus, biological effects resulting from TOO_H largely depend upon its environment. TOO_H not only changes the compounds it attacks, but it also undergoes changes itself which vary according to the compounds with which it reacts. From this preliminary study it is evident that a thorough investigation of these reactions would be interesting and significant.