ESR STUDY IN RADIATION
DAMAGE IN PYRIMIDINES

3-Year Comprehensive Progress Report

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1. Main research accomplishments with reference to the originally stated objectives

The objectives of this work were stated in general terms, so they have evolved only slightly during the course of the experiments. We proposed to look for general mechanisms of radiation damage to biomolecules using substituted pyrimidines as our model system. We chose to put particular emphasis on barbituric acid derivatives because we could either obtain or synthesize a variety of closely related derivatives, some of which could be crystallized into more than one crystalline structure. With this system we expected to be able to separately determine the effects of molecular structure and crystalline environment on the radiation damage processes. The uracil derivatives selected for study are closely related to the barbituric acid derivatives, and are associated with potential biological damage.

Our work has shown that radicals in barbituric acid derivatives are formed by selective abstraction of one of the two groups on C(5) of the pyrimidine ring. The mechanism for ethyl group abstraction was found in the several derivatives studied to involve a prior hydrogen abstraction from the ethyl group, followed by the transfer of a hydrogen within that group at the time of dissociation. A very similar mechanism prevails for the abstraction of an isoamyl group in amobarbital. In contrast, our dose response studies of various methylated derivatives show that methyl abstraction is associated with a hydrogen addition process.

Initial crystal structure effects on radical formation processes include a significant dose response effect in barbital, and a difference of radical structure in the case of 5-ethylbarbituric acid.

We have also developed a general mechanism for iminoxy radical formation in crystalline nitropyrimidines. Recent work by others has indicated that the

same radical structure is formed by a different mechanism in acidic low temperature glasses, apparently another environmental effect on a radical formation process.

In the process of this work we have synthesized a number of barbituric acid and uracil derivatives, some of which are selectively deuterated at specific sites, for use in further studies in the development of our mechanism.

2. Plans for continuation of the present and/or modified objectives.

As stated above, the major objectives of this work were stated in sufficiently general terms that they remain current. The first year of this contract provided funds only for the purchase of a CO-60 irradiation source. In the second and third years, moderate amounts were provided for summer salary of the principal investigator and a graduate assistant, and supplies. In the third year, we also purchased a digital signal averager and processor, an item that was used only briefly until a component failure caused it to become inoperative until replaced by the manufacturer this week. Although the contracts have provided only 40 per cent of the amount requested, we have still been able to make significant progress toward the program objectives. The rate, however, has been limited.

We expect to continue on our present course, but hopefully with additional cryogenic capabilities. In the accompanying continuation proposal we support the need to go to lower temperatures in order to trap early precursors in the radiation damage process. The key to our work continues to be the selection and development of barbituric acid and uracil derivatives as a model system with the appropriate characteristics for investigation of the various aspects of radiation damage in a separable and systematic fashion.

3. Graduate Students trained:

Julianne Russ	(current degree candidate)
Patrick Lorenz	(contributors to this work but not paid under this contract)
Theodore Krupa	(summer research assistant, degree candidate at Penn State)

As part of their undergraduate research projects, Charles Montague and Neil Sudol participated in the research of this contract.

4. Bibliography

Publications:

"Free Radicals in Pyrimidines: ESR Study of Selective Abstraction of C(5) Substituents by X-Irradiation" Radiat. Res. 58, 141 (1974).

"Nitro-Centered Free Radicals in α -irradiated 5-Nitro-6-Methyluracil at 77°K" Radiat. Res. 60, 405 (1974).

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Meeting Abstracts:

"Free Radicals in 5-Substituted Pyrimidines: An ESR Study" 5th Internat. Cong. Radiat. Res. D-35-6 (1974).

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"Free Radicals in Gamma Irradiated Pyrimidines: An ESR Study" Radiat. Res. Abstracts BC-6 (1975).

"Free Radicals in Pyrimidines: ESR of α -Irradiated 5-Isobutyl-5-Allylbarbituric Acid" Radiat. Res. (June 1976 meeting).

5. Present state of knowledge; significance in fields of biology and medicine; need for future investigations.

I will comment on the specific area of radiation chemical physics. Some years ago it was realized that ESR techniques were superior to most alternatives in identifying specific molecular alterations due to ionizing radiation. This has led to the identification of the predominant free radicals produced at room temperature in a variety of biological molecules and their close analogs. In almost all cases, it is not yet clear how these free radical structures directly relate to the important biological effect of cell death and mutagenesis. The main exception is the case of EUDR sensitization where the proposed mechanism is reasonable. From a large variety of biological work using protective agents, sensitizing agents, and radical scavengers, it is clear that free radicals are the major type of molecular damage relevant to the biological effects of radiation. The fact remains also that a complete understanding of the biological effects of radiation will require a detailed knowledge of the mechanisms of radiation damage to the molecular constituents of cells which obtain under conditions similar to the highly structured interior of cells.

The problem has been more easily stated than solved, and the solutions have proved to be much less obvious than anticipated from initial experiments. Major emphasis is now being placed on mechanisms of damage, and the sequence of products starting from the earliest damage trapped at 4.2°K are being followed. Variations of local environments are being found to be significant to the damage process.

There has been a distinct leveling, or perhaps even a reduction, in the amount of research in this area. Some good workers have left the field, while others are devoting a smaller fraction of their effort to this work. I believe that the problem is still unsolved, the basic techniques, with progressive

refinements in cryogenics and ENDOR methods, are still providing good results. Alternative techniques for following molecular damage have not been forthcoming, and continued support of radiation chemistry will yield mechanisms for the biological effects of radiation.

6. Division of Federal Support

There are no other federal sources of support.