FINAL REPORT

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BASIC ASPECTS OF ACTION OF RADIATION ON MICROORGANISMS

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by

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The general purpose of this program which has lasted more than a decade has been the study of the relationship between ionizing radiation and the molecular biology of bacterial cells. During this period two review articles have been written, the first appearing in 1966 in the Encyclopedia of Medical Radiology where it forms the first article of Vol. II/2 (Springer-Verlag, Berlin), and the second appearing in 1970 in Current Topics in Radiation Research, vol.6, 51-127 (North-Holland, Amsterdam); the latter was submitted to AEC as NYO-2804-25. In addition, Addison-Wesley Publishing House has requested a unit for their series on the subject "Radiation Biology" and this can be considered as a third review article. The possibility of a more advanced review in "Current Topics" is being considered.

As we review the history of the project, we recall that the initial endeavors were aimed at the use of radiation to study the structure of the bacterial cell; among these efforts was an attempt to observe the effects of ionizing radiation on the reduction of DNA synthesis and thereby to determine the nature of the apparatus that is responsible for the synthesis of DNA. In addition, the observation of RNA and protein synthesis as affected by ionizing radiation was studied. These observations were reported on by Pollard & Vogler (1), Pollard & Macauley (2), Pollard & Barrett (3), and Kempner & Pollard (4). They were part of a presentation at the Moscow Conference on initial effects of ionizing radiation in 1960⁽¹⁷⁾. One outstanding result of historical interest is the finding (4) that the synthesis of protein required the integrity of a long and thin molecule, about 4 microns long and 20⁸ wide, which antedated the discovery of messenger RNA, clearly the molecule involved. Further, the synthesis of RNA itself required a smaller, thicker molecular structure, now identifiable with the DNA-directed RNA polymerase. The metabolism of glucose

required the integrity of a unit larger than a typical enzyme and which can probably be associated with the structural blocks of enzymes postulated for respiration by Lehninger and others.

These very promising early studies were rapidly complicated by the discovery that the response of the cell involved so many additional processes that the use of radiation to study cell structure could not be made until these responses were duly catalogued.

The first finding, reported in Science by Pollard, Ebert, Miller, Kolacz & Barone (5) was that irradiated medium sharply affects the synthetic processes in cells. The nature of this action was elucidated by Frey & Pollard (6,7). It is due ^{to} the formation of hydrogen peroxide with a G value of 0.75 to 1.50 depending on the composition of the medium. The major reason for the influence of this small concentration of peroxide is the fact that cells concentrate peroxide with a "permease" of molecular weight around 75,000. The peroxide then acts to block the respiratory chain causing a cessation of growth and synthesis until the cellular peroxidases reduce the concentration of peroxide and permit the respiratory chain to resume action. Cells which have become anaerobic are not affected by radiation-induced peroxide.

The presence of catalase in the medium during irradiation removes this action and it is routinely added in all our work.

The second program related to cellular response was the study of radiation-induced DNA degradation. We have put in years of work on this with a number of students and postdoctoral appointees helping; this work is summarized in the review article in Current Topics... (8). It is possible to summarize the present position as follows.

The products of the two genes recA and recC form a grouping of endo- and exonucleases which, together, can produce considerable DNA degradation.

The nature of the original insult that is detected by these is not clear, nor is the additional enzyme necessary to take the final product of oligonucleotides down to bases known. In spite of this it is now clear that major factors in the degradation of DNA following ionizing radiation are the products of these two genes, and it is clear that mutants which lack their function are also lacking in the normal process of DNA degradation (see Youngs & Bernstein, ref. 9).

In view of this finding two lines of work have been instituted, both of which have come to some fruition in the last year or two. The first is to look at different mutants to make sure that this conclusion, originally proposed by Youngs & Bernstein, is right and this has been done for two systems; the first (aided by Drs. Ginoza and Marsden) ^(COO-3411-7) has been on the effect of introducing irradiated T4 DNA into a variety of cells, and the second, the observation of directly produced DNA degradation in the same set of mutant cells. The conclusion is firm that the recBC genes are involved in the process, though they are probably not the only enzyme systems at work.

A second avenue follows up observations by Grady & Pollard (10) and by Pollard & Weller (11) that there is apparently an inhibitor of DNA degradation that is inducible in much the same way as a prophage may be induced. This has been the subject of considerable recent work and three papers are in preparation on the subject. A fourth is about to be completed in collaboration with Drs. Billen and Achey of the University of Florida at Gainesville. It seems possible that an inhibitor is coded for by the exr or lex gene (though this is not yet established) and that the inhibitor is not present in cells which have been cured of a known defective prophage, or which are deficient in the recA gene, known to be active in the prevention of the induction of

prophages. The character of this inducible inhibitor has been of so much interest to us that we have asked to extend the work in our laboratory specifically on the subject of the induction mechanism of prophage which is apparently related to the inducible inhibitor.

A recent finding of a great deal of interest to us is that apparently the inducible inhibitor seems to be necessary for the expression of the repair processes that are observed in the alkaline sucrose gradient type of experiments pioneered by McGrath & Williams. Work in this area is being continued as part of the new direction we are applying to the project.

At the same time that we have studied DNA degradation we have also carried on work on the effect of radiation on transcription (12-15). Apart from the finding that hydrogen peroxide, which, as has been said, blocks the respiratory system, also causes a temporary cessation of transcription, several new findings have been made.

- o The first is that the transcription of ribosomal RNA or transfer RNA is relatively insensitive (14,15).
- o The second is that the transcription of messenger RNA has a variable sensitivity, heavily dependent on the strain and in general suggesting a target region in the cell some three to four times greater than that for ribosomal RNA.
- o The third is that the transcription of a gene near a prophage can actually be increased by ionizing radiation (14).
- o The fourth is that there is a polar effect in the lac operon, less transacetylase than beta-galactosidase being transcribed after radiation (14).
- o The fifth is that messenger half-life is increased in irradiated cells.

All these processes have a dose-reduction factor for O_2/N_2 of 2 to 3.

If we attempt to analyze by target theory these effects of radiation on transcription, then it is possible to explain what occurs by supposing that a single-strand unrepaired break occurs in a stretch of DNA approximately the right length to code for the ribosomal RNA. If we accept the premise of Hagen et al. (16) that a single break stops the process of transcription then this explains the findings. On the other hand, the stretch in which a single-strand break is sufficient to eliminate the formation of the messenger RNA, for example for beta-galactosidase, is some four times greater than one would expect. There is thus some indication that damage to the DNA outside the precise region of the operon itself may be important in affecting transcription.

We have made an attempt to see whether this last sensitive form of transcription is in some way related to the membrane relationships believed to exist between the cell and the DNA. We have not been able to follow this up to any satisfactory conclusion.

In order to understand the relationship of this work to radiation action in general we can say the following. First of all, since molecular biological processes presumably underlie cell behavior, they must be examined with regard to their sensitivity to ionizing radiation or it is possible that important mechanisms may be missed. For this reason, time was put in on the phenomenon of DNA degradation, which seemed to be a possible model for chromosome damage in higher cells. It does seem to be unlikely that it will play much part, although quite recent work has demonstrated that there is indeed some DNA degradation in mammalian cells. Therefore the background of understanding this phenomenon may prove useful in considering all radiation effects, though perhaps not of detailed use in understanding the effect on human cells.

On the other hand, observations with regard to transcription may very well suggest a process which is closely related to cellular sensitivity. The sensitivity of cells which have a cell cycle is high in the period preceding the S stage and also preceding mitosis, both of which involve considerable transcription of new material. It is quite possible that the basis for this sensitivity will be found to lie in the damage to the DNA, which is necessary to be transcribed at this time, and it may bear a relationship to the observations on bacteria, outlined below.

As a result of our own work and also based on discussions with other laboratories (notably Dr. Alper's, Dr. Billen's, The Oak Ridge Group, Dr. Hutchinson's and Dr. Smith's) the following factors to consider in assessing radiation damage to microorganisms appear. These can exert influence to different degrees and, of course, the list may not be inclusive. We think that a proper assessment of the relative importance of these factors is a key to understanding why cells lose their colony-forming ability when exposed to ionizing radiation.

Factors affecting cell sensitivity to ionizing radiation

A. Mechanisms of cell death

1. Gross alteration in the DNA synthetic machinery, including the presence of considerable unscheduled synthesis.
- *2. Alteration of the transcription machinery so that needed units for division are not made.
- *3. Damage to the DNA which cannot be repaired or bypassed (includes rapid DNA degradation after irradiation).
- *4. Induction of a prophage which lyses the cell before division.
5. Damage to the membrane and respiratory* functions so that the cell is choked.
- *6. Introduction of excessive division delay so that the cell may not be able to reproduce a normal cell (exemplified by snake formation),

B. Mechanisms of cellular recovery from damage

1. Enzymatic repair of radiation-induced damage.
2. Recombination to provide an undamaged genome in the presence of damage.
- *3. Transcription of the inducible inhibitor of enzymatic DNA degradation,

C. Mechanisms of cellular protection from damage

- *1. The presence of multiple genomes to provide a chance that an undamaged genome can produce a normal cell.
- 2. The presence of radiation modifiers such as glutathione within the cell.

Of these factors, those marked with an asterisk have been studied on the project. It is planned to attempt two follow-ups. The first involves a small informal workshop similar to the recently held one (May 7-8, 1973 at U. Florida, Gainesville) on "Cellular and Molecular Effects of Solar Ultraviolet Radiation" in which those concerned with the factors listed above would participate for two or three days. The second is an attempt to bring the whole of the ideas together to give a theoretical picture of sensitivity involving strain difference, medium effects and so on. This will be attempted this summer (1973).

Of really considerable interest is the phenomenon of the defective prophage and the inducible inhibitor. It is possible to study both these phenomena by observation of either the inhibition of DNA degradation, or the partial lysis of the cell wall. Both are actively under study at the present time and both seem to suggest that studies of the effect of ionizing radiation on this induction process may very easily be the ^{recent} clue to the induction of cancer by ionizing radiation which is described in the Academy Report as the most serious factor standing in the way of the use of ionizing radiation for the purposes of humanity.

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