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BASIC ASPECTS OF ACTION OF RADIATION ON MICROORGANISMS

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MASTER

by

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Introduction

As an introduction we give a brief resume of our present position.

(a) DNA degradation. This area of radiation action on microorganisms has probably had our greatest thrust over the past few years. A major reason for the rather intensive study we have made of this phenomenon was the feeling that it might be the analog for chromosome breaks in cells with nuclei. Chromosome breaks must involve a secondary mechanism and it seemed possible that a local DNA degradation could be the cause. We no longer believe the two are related and, indeed, see no analogy between this process and any radiation process in human cells. This needs to be considered when our proposals for further work are read. To return to the findings in microorganisms, we believe that we have come to a point where the degradation process is reasonably well understood and where we have a chance of designating the two things that need to be established. These are first, the primary effect of radiation which elicits the action, and second, the nature of the enzymatic system which actually causes the degradation of the DNA.

One important result has recently been established by Dr. Christensen, namely that in spheroplasts the degradation of the DNA proceeds nearly as normally. A paper is being readied for RADIATION RESEARCH on this development. This result is important because it now becomes possible to introduce foreign DNA into the cell and hope to study what process of degradation takes place.

While this work was going forward a very important finding came out of Dr. Ginoza's laboratory and was made by Howard Marsden. He found that if irradiated T4 viruses are introduced into various bacterial cells then

degradation of the DNA takes place to a considerable extent, reaching up to perhaps 30%. The various strains of bacteria showed considerably parallel behavior to those in which radiation-induced degradation is within the cell. We believe that this system offers us the best opportunity to irradiate DNA outside the cell, inject it and separately study the effect of enzyme on it. Accordingly a part of our work will be shared with Marsden's and we expect this to move the project forward quite rapidly.

(b) All-or-nothing aspect of DNA degradation. An autoradiographic study of the loss of label from irradiated cells has been completed and is being forwarded to the BIOPHYSICAL JOURNAL; it is also being presented at the IV International Biophysical Congress in Moscow in August. A preprint is enclosed with this application (COO-3411-2). This work shows that the process of DNA degradation in two cells, B_{S-11} and 15JG151, occurs in an all-or-nothing manner.

(c) Inhibition by an episome. The discovery that radiation-induced degradation is inhibited in cells which carry a prophage was made some years ago in this laboratory. We have recently been studying the process further and have now established the kinetics of the development of the inhibitor, and studied the effect of rifampin on the prevention of transcription of the inhibitor. This is being completed and a draft is ready to submit to RADIATION RESEARCH. A first draft (COO-3411-3) is included with this application. Along with this study the discovery was made by Dr. Rake that colicin E2 is also inhibited by this same episomal factor. This is at present under further investigation.

(d) Transcription. The discovery was made by Sharon Davis and the writer and further confirmed by Lydersen that there are two sensitivities for the transcription process. The first, which applies to ribosomal and transfer RNA is relatively insensitive and seems to operate by the requirement that ionization

must occur in a specific DNA "target". The target is about that expected for the genetic material for these two forms of RNA. The second is more sensitive and corresponds to the production of RNA necessary for the formation of induced enzymes, of which we have studied two. This result has been reported in RADIATION RESEARCH this year (see COO-3411-4). The reason for the difference in sensitivity is now being explored.

(e) Membrane association. The idea that the sensitive fraction of transcription mentioned above could be membrane-associated has caused us to start work on the nature of the DNA-membrane association in the cells which we are studying. A very simple method which seems to be quite effective in estimating the amount of membrane binding of DNA has been worked out by Lydersen. We are actively pursuing this and while it is still in the stage of the development of technique, not yet ready for reporting of actual results, it seems very promising and we want to keep it very active as one of our projects.

(f) Repair of breaks in strain B_{s-1}. A question which has not yet had a full answer concerns the possibility of the repair of single-strand breaks in sensitive strains such as B_{s-1}. Some preliminary work by Horan indicated that possibly such a repair process can take place and that it is masked by the extensive DNA degradation that goes on in this cell. Asli Tolun has been studying this cell and its response, using a number of doses, and varying the conditions of irradiation. The study is not complete but it would seem as though there is conclusive evidence that in this cell there is no apparent repair of the broken DNA, but rather this broken DNA is further broken down into small fragments we associate with DNA degradation.

(g) Kinetics of repair. A preliminary study, again by Horan, enables us to make some measurement of the kinetics of repair of one of the types of breaks in DNA. We have been furthering this work, and now have a series of measurements that indicate the rapidity with which these breaks are repaired and it is intended to continue study over a range of temperature. This is being done by Dr. Christensen.

(h) Single-cell division studies. These were started with Dr. Billen. They follow the pattern described in Pollard and Tilberg's recent paper (COO-3411-5) except that a greatly simplified method is used. The study involved a comparison between P3478 cells, which lack one Kornberg polymerase, and W3110, which have this enzyme. For the mutant it was found that cells which survive divide normally while for W3110 there is a pattern of delay. Since the precise difference between these cells is not known the work has been suspended until more knowledge is available. It will be completed in the next few months.

(i) T4 attachment. An interesting small project, which may be useful in future interpretation of the membrane was carried out by an undergraduate, Gerry Sterling. He showed that the target size expressed as a "radiation molecular weight" for the attachment process is 500,000 and that there is a conceivable multiplicity.

Personnel on the Project: (including those paid on other budgets)

Co-principal investigators: E. C. Pollard, 60%
W. D. Taylor, 10%

Faculty Associate: A. V. Rake, 5%

Postdoctoral Fellows: R. Christensen, NIH trainee, 100%

Research Aide: E. Randall, 100%

Graduate Assistants: B. K. Lydersen, 50%
A. Tolun, 50%

Part time: K. Keller
K. Kraus

Publications

E. C. Pollard & A. Tilberg. Action of Ionizing Radiation on Sensitive Strains of Escherichia coli B. Biophys. J. 12:133-156 (1972) COO-3411-5 (reprints enclosed) *Removed*

E. C. Pollard & B. K. Lydersen. The Action of Ionizing Radiation on the Synthesis of RNA and Two Inducible Enzymes in Escherichia coli. Radiat. Res. 50:293-300 (1972). COO-3411-4 (reprints enclosed) *Removed*

A. V. Rake & E. C. Pollard. The Nature of Residual Escherichia coli DNA after Degradation Induced by Ionizing Radiation. Radiat. Res. 50:334-338 (1972). COO-3411-6 (reprints enclosed) *Removed*

H. Marsden, W. Ginoza & E. C. Pollard. Effects of Ionizing Radiation on the capacity of Escherichia coli to support bacteriophage T4. J. Virol. 9:1004-1016 (1972). COO-3411-7.

Accepted for publication:

P. K. Horan, K. Hird & E. C. Pollard. A strain of Escherichia coli with minimum postirradiation degradation properties. Radiat. Res.

Cyr, W. H. & E. C. Pollard. Ionizing Radiation-Induced Degradation of DNA in Germination Spores. Radiat. Res.

Submitted:

E. C. Pollard & K. Kraus. All-or-nothing character of DNA degradation in bacteria following ionizing radiation. Biophys. J. COO-3411-2 (preprint enclosed).

In preparation:

E. Pollard & E. Randall. Further studies on the inducible inhibitor of radiation-induced DNA degradation in E. coli. COO-3411-3 (draft enclosed) *Lab. Res. Abstracts. Removed*

Abstracts:

E.C. Pollard. The all-or-nothing character of radiation-induced DNA degradation of E. coli. IV Intern. Biophys. Congr., Moscow, August, 1972. COO-3411-8 (copies enclosed) *Abstracts. Removed*

E. C. Pollard & B. K. Lydersen. The action of ionizing radiation on bacterial RNA synthesis. 16th Ann. Biophys. Soc. Mtg., Toronto, Feb. 1972. COO-3411-9 (copies enclosed) *Abstracts. Removed*