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Annual

STATUS REPORT FOR THE NASA CONTRACT NsG-324

April 30, 1965

Period covered: April 1, 1964 to March 31, 1965

Ernest C. Pollard
Principal Investigator

The Pennsylvania State University
University Park, Pennsylvania

PHYSICS OF CELLULAR SYNTHESIS, GROWTH AND DIVISION

Introduction.

At the time of the last progress report the work on this grant was in the formative stages. Now several definitely successful lines of research have become quite clear. Among these, and admittedly rather unexpectedly, are several quite important discoveries relating to the effect of ionizing radiation on bacterial cells. These have been reported in a series of letters to the editor of Science, and two more letters are in the process of being sent off to complete the cycle. A longer article has been suggested to the editor of Science as a summary of this and other work, and he has indicated interest in it. This work has been conducted largely by Frey, Barone, Achey and Neisley.

In addition to this there has been some interest recently in the effect of pressure on the process of genetic transcription, and this also looks as though it may make a letter to the editor of Science.

Also interest in the problem of weightlessness still continues, and an article on this subject will appear in the forthcoming publication: "Hypodynamics and hypogravics - the physiology of inactivity and weightlessness" (Ed. McNally, C. C. Thomas, publisher).

The work on the effect of tritium decay on mutations has gone steadily forward as has interesting work on electron spin resonance. The whole success of this grant has been given divergent characters by the approaches taken by the various investigators in the group, and a strong

influence on a very good body of students has been exerted in the process. Separate reports from the individual members of the group are included and can speak for themselves on those areas of research.

A problem of potential interest is the question of proton tunneling as a cause of mutations. By measuring the mutation rate for two mutations in cells which are D_2O substituted and for which proton tunneling should be 1,000 times less, this phenomenon as the sole cause of mutation has been discounted (Pollard and Lemke).

The steady consolidation of work on the grant can be seen in the number of papers given at society meetings and in the number of publications which are associated with this work. The objective of trying to construct a theoretical picture of the behavior of simple cells has been steadily continued. It formed the basis of a summer term's study on the part of 15 graduate students, and some of the material they have collected is potentially suitable for publication, although the medium is not easy to find.

In addition, the principal investigator was invited to be a National Sigma Xi Lecturer during the past month. The talk was on "The Fine Structure of the Bacterial Cell and the Possibility of Its Artificial Synthesis." This will appear in the American Scientist as the National Sigma Xi Lecture and credit will be given to this group's grant for the part it played.

The ability to argue the way in which the cell fundamentally is put together and operates is probably at its sharpest peak in this group, and it does represent a level of skill which has been hard to produce and which is certainly now most interesting.

The plan of this report is as follows. There is a short discussion of the progress up to date, followed by separate reports from the people who are in charge of the various kinds of projects. At the end are lists of the personnel involved, abstracts of meeting reports and publications.

General Discussion of Progress.

In regard to our understanding of the nature of irradiation action on cells and in regard to the effect of radioactive decay in producing mutations, progress has been definitely substantial. There have also been some small achievements in the areas of the physical-chemical studies of macromolecules and in the area of understanding the nature of radiation action on double-stranded bacterial viruses.

The attempt to synthesize these findings into a firm understanding of the nature of a living cell can also be said to have progressed, but it would not be wise to say that we are more than a little closer to a good solution of this problem. More accurately, we have opened up questions, rather than produced answers.

Among the things which are suggested by our experimental studies and theoretical work is the idea that there exists a "factory" for the synthesis of DNA in which not only the polymerase action is present, but also there are enzyme systems for the formation of triphosphates necessary for the synthesis. There is also a suggestion that the cell does not produce the protein which is known to be present at specific places on its surface, such as flagellae and pili, by purely random methods, but instead that it uses a means of systematically producing messenger RNA of identical nature in a sufficient quantity to produce the necessary effects at this location. There is a third suggestion that the cell membrane not only

consists of the membrane itself, but also contains systems of enzymes designed for the twofold purpose of making the metabolite chemistry factories operate and feeding through the cell the component parts for the membrane and the cell wall. These are only suggestions and need to be followed up, but they do lead to rather an interesting picture of the possible structure of the cell.

Separate Reports on Research Progress and Current Work.

A. Radiation and Pressure Effects on Bacterial Cells. Pollard, Frey, Achey, Barone, Yayanos, Swez, Grady, Chapman.

In simple terms the two major discoveries of radiation action have been 1) a degradation of DNA which is due to direct radiation action on the cell and 2) a stasis or halting of the synthetic processes of the cell due to hydrogen peroxide.

The degradation has been most interesting because it shows the strange feature that a part of the DNA is seemingly harder to degrade than the rest, suggesting that the DNA is somehow in two fractions. First attempts to understand this phenomenon came with an attempt to show that one strand of the DNA was more easily degraded than the other.

Very careful work by Swez, showing that the hypochromicity of the irradiated and degraded DNA is just the same as that of normal DNA, and by Grady, showing that the density gradient sedimentation pattern of the irradiated and degraded DNA is just the same as normal DNA, has essentially disproved this hypothesis. This work is being reported at the meetings of the Radiation Research Society in Philadelphia. In view of this setback to the very simplest of explanations for the 50% less sensitive fraction, attention is being turned to the possibility that the newly synthesized DNA, which will be subsequently used in the daughter cells, is in some way so arranged that it is harder to degrade. Only one piece of evidence

supports this at the present; this is the finding by Barone and the author that the genetic transcription of cells which are irradiated in the presence of catalase, which eliminates the peroxide effect, is reduced to zero at the same time that the DNA degradation has reached 50%, This suggests that the easily degraded part is also the part which is transcribed.

It has been shown that peroxide produces no DNA degradation, but that peroxide does stop, temporarily, the transcription of the DNA and also that it destroys the function of messenger RNA once it has been made.

In the course of these studies it was discovered that the effect of irradiated medium, which seems to be the same as peroxide, is negligible upon DNA and RNA themselves, but is quite striking on some proteins in the cell. To demonstrate this effect it is necessary to study the effect of heat on the enzymatic properties of proteins. One finds that at much lower temperatures than normal heat will inactivate these proteins if the heating is done in the presence of the irradiated medium. This has been shown for beta-galactosidase and for papain. It is not universally true for all proteins, but it does suggest that possibly the effect of halting the operation of the cell is due to the action of peroxide on protein.

Recent work on the effect of pressure on bacterial cells has shown that there is a striking effect on the transcription of the DNA at pressures as low as 3000lbs/in^2. It is also probable that there is an effect on messenger RNA itself at pressures much higher, namely in the neighborhood of 15,000lbs/in^2. The lower pressures not only affect the formation of beta-galactosidase, but also stop protein synthesis in general. There is no effect on RNA synthesis as judged by the incorporation of uracil into TCA insoluble material, and only slight effect on the similar incorporation of thymine. Thus RNA and DNA synthesis are hardly affected at all.

The work in the laboratory is now aiming at an attempt to understand pressure effects, an attempt to understand the reason for the decay of messenger RNA, and an attempt to see whether all of the DNA is being transcribed at the same time. Some sort of an attempt to find the mechanism for the synthesis of DNA, which should be readily visible in the electron microscope, may be made also.

B. The Decay of Incorporated Tritium Compounds in E. coli. Person, Bockrath.

We have studied the differential mutation production by the decay of incorporated tritium compounds in E. coli (WWU) using DNA-seeking precursors (H^3-thymidine), RNA-seeking precursors (H^3-uracil and H^3-uridine) and protein-seeking precursors (H^3-histidine and H^3-proline). In particular we have determined the reversion frequency of an arginine locus. The reversion frequency is measured in units of revertants/surviving bacteria/H^3 decay. Results show that revertants are produced most effectively by H^3 decays when the label is introduced in the form of an RNA precursor. The macromolecular distribution of the label shows that 5 to 8 per cent of the H^3-uridine and H^3-uracil is incorporated into DNA.

We have also done some studies on the nature of bacterial revertants produced by some of the above decays, again in E. coli. The existence of two subclasses of revertants has been demonstrated among the revertant populations produced by H^3-thymidine, H^3-uracil and H^3-histidine: 1) the majority of the revertant population composed of dependent revertants which give rise to revertant colonies in the presence of nutrient fortification and 2) some independent revertants which form colonies without nutrient fortification. The results infer that there are at least two molecular alterations that can mediate the reversion of a bacterial mutant by H^3 decay, and that the specificity of the H^3 compounds used is sufficient to effect differentially the proportions of these two alterations.

C. Regional Energy Deposits in Localized Tritium: Calculated in Non-Spherical Models. Bockrath.

H^3-labeled compounds may be differentially incorporated into specific molecular regions of a biological system. A computer program was designed to record the energy transferred to specific regions from tritium localized in either identical or non-identical regions. The biological system was approximated by a cylindrical model with hemispherical ends and adjustable length. The results obtained, which were reported at the ninth annual Biophysical Society meetings, predict significant variations in dose as a function of target and source (tritium) positions. In the case of T4 the energy deposited in the DNA target from tritium in the DNA region is 2.6 times that deposited on the same target from tritium in the protein region (per decay).

D. The Mechanism of Lethality by Tritium Decay in T4 Coliphage. Person, Funk, Bockrath.

The decay of incorporated H^3 compounds in phage may produce biological damage by two types of phenomena: those which occur at the site of radioactive decay and those which occur randomly throughout the phage, e.g. ionizations along the path of the beta particle. Experimental data have been obtained in this lab for the effect of H^3 decays originating in phage DNA and protein. Assuming that plaque forming ability is an assay for DNA and protein is unimportant, that the dimensions of phage DNA and protein are known, that a phage population is uniform in its DNA and protein content and that the rate of energy loss in phage DNA is the same for H^3 decays originating in DNA as it is for decays originating in protein, one can calculate the ratio of energy deposited in the DNA by H^3 decays from phage DNA and from phage protein. The results led to the conclusion that the loss of plaque forming ability by H^3 decay in T4 may be entirely accounted for by radiation (beta particle) damage associated with the decay.

E. Polyelectrolytic Solutions. (Started in September, 1963) Taylor.

In order to understand the basic mechanisms of cell function, it is important to understand the nature of interactions between macromolecules and small molecules. Present theories of the behavior of simple model polyelectrolytes are in a poor state owing to the dearth of experimental data available.

One aspect of the problem is the effect of the distribution of charged sites on the configuration and ion-binding properties of polyelectrolytes. A series of copolymers of vinyl acetate and acrylic acid have been prepared by a free radical mechanism in vacuum at 60°C to about 20% conversion; the resulting polymers were purified by repeated precipitation and hydrolysed to make them water soluble. The polymers prepared contain approximately 10%, 20%, 30%, 40% and 50% of acrylic acid as determined by potentiometric titrations. Transference studies using a stable voltage supply, accurate milliameter and coulombmeter, have been carried out on a solution of one of these polymers in sodium chloride solution containing trace amounts of Na^{24}. Self diffusion studies have also been made of the same polymer solution. These measurements are now being made on the other members of the series.

The effect of added salt and degree of ionization on the configuration of these polymers is being measured using light scattering techniques. The molecular weights of the polymers are sufficiently high for the radii of gyration to be measured.

The final measurements necessary to adequately test currently available theories are of the activities of the counter ions. Apparatus is being set up to do this using ion-selective membranes and electrodes.

F. Effect of Ionizing Radiation on the Configuration of DNA. Taylor

Recent studies of the effect of ionizing radiation on cells have shown a progressive degradation with time after irradiation to a limit which depends to some extent on the conditions of the experiment. Several physical methods (density-gradient, hyperchromicity, T_m) failed to show significant differences from the unirradiated control. A method was needed to investigate small changes in configuration and flexibility of the DNA and a 'Zimm,' low-shear viscometer was constructed for this purpose. A viscometer rotor was designed to operate at $3 \times 10^{-2} sec^{-1}$ shear rate which is in the small region of shear independence near zero shear. The effect of salt concentration on the shape of the reduced viscosity number $\left(\frac{n - n_o}{n_o c}\right)$ vs concentration curves, for irradiated and control samples of salmon sperm and calf thymus DNA, has been studied. In addition the effect of the presence of small amounts of bound protein is being examined. Extraction procedures have been set up for the 'in vivo' studies on E. coli DNA and these measurements are in progress.

G. ESR of an Irradiated Single Crystal of Thymidine. Snipes, et al.

This work was begun with other investigators at Duke University, but a portion of it was finished at The Pennsylvania State University and was supported by this grant. A paper will be coming out in May in the Proceedings of the National Academy of Science. A gamma-irradiated single crystal of thymidine was prepared and studied to gain more specific knowledge about the radical giving the thymidine-like electron-spin resonance in irradiated DNA. From ESR measurement of these single crystals, the principal, long-lived free radical produced by the irradiation is shown to be a secondary one formed by a hydrogen atom addition reaction on the $C_{(6)}$ of the thymidine ring. The unpaired electron is in a π orbital of the ring

with 0.70 of its spin density on $C_{(5)}$. A similar free radical gives rise to the thymidine-like resonance observed in DNA.

Work is currently being carried on in this laboratory on the radiation damage to pyrimidine derivatives.

H. Microspectrophotometry. Strother.

Development of a simplified microspectrophotometer was reported by Strother in Science in 1959. During the past semester the limits of instrumentation of the microspectrophotometer have been evaluated. The errors of instrumentation were observed as a function of the power of magnification. Results of this study are presently being evaluated.

Meeting Abstracts

Several papers were given at the ninth annual meeting of the Biophysical Society in San Francisco, February 24-26, 1965:

Regional Energy Deposits From Localized Tritium: Calculated in Nonspherical Models. R. Bockrath.

Features of the Mechanism of Halting Genetic Transcription by Ionizing Radiation. H. E. Frey, T. F. Barone and E. C. Pollard.

The Mechanism of Lethality by Tritium Decay in T4 Coliphage. S. R. Person, F. D. Funk and R. C. Bockrath, Jr.

Mutation Rates in Bacteria Grown in D_2O. E. C. Pollard and M. Lemke.

Development of Progeny From Single E. coli B/r Cells. W. G. Yeisley and E. C. Pollard.

A paper to be presented at the Washington meeting of the American Physics Society was also partially supported by this grant:

Electron Spin Resonance of an Irradiated Single Crystal of Thymidine. B. Pruden*, W. Snipes and W. Gordy.*

Three papers will be presented at the Radiation Research Society meeting in Philadelphia, May 24-25, 1965:

The Influence of Post-Irradiation Conditions on the Degradation of DNA of E. coli 15 T^-L^- Induced by Gamma-Irradiation. P. M. Achey and E. C. Pollard.

DNA Degradation by Tritium Decay in Escherichia coli. M. Sclair and S. Person.

Physical Characteristics of DNA from Escherichia coli 15 T^-L^- Following Whole Cell Irradiation by Co^{60} Gamma Rays. E. C. Pollard, J. Swez and L. Grady.

Publications

1. Bockrath, R. C., Jr. and S. Person. 1964 The Nature of Bacterial Revertants Produced by the Decay of Incorporated Tritium Compounds in Escherichia coli. Mut. Res., 1 373-380.

2. Lehman, R. C. and E. C. Pollard. 1965 Diffusion Rates in Disrupted Bacterial Cells. Biophys. J., 5 109-119.

3. Person, S. and R. C. Bockrath, Jr. 1964 Differential Mutation Production by the Decay of Incorporated Tritium Compounds in Escherichia coli. Biophys. J., 4 355-365.

4. Pollard, E. C. 1963 Collision Kinetics Applied to Phage Synthesis, Messenger RNA and Glucose Metabolism. J. Theoret. Biol., 4 98-112.

5. _____ 1964 Ionizing Radiation: Effect on Genetic Transcription. Science, 146 927-929.

6. _____ 1965 Theoretical Studies on Living Systems in the Absence of Mechanical Stress. J. Theoret. Biol., 8 113-123.

7. Pollard, E. C., M. J. Ebert, C. Miller, K. Kolacz and T. F. Barone. 1965 Ionizing Radiation: Effect of Irradiated Medium on Synthetic Processes. Science, 147 1045-1047.

8. Pollard, E. C. and M. Lemke. 1965 Rate of Mutation to Phage Resistance in ^2H_2O Medium. Mutation Res., in press.

9. Pruden, B., W. Snipes and W. Gordy. 1965 Electron Spin Resonance of an Irradiated Single Crystal of Thymidine. Proc. Natl. Acad. Sci., in press.

10. Yeisley, W. C. and E. C. Pollard. 1964 An Analog Computer Study of Differential Equations Concerned With Bacterial Cell Synthesis. J. Theoret. Biol., 7 485-503.