Nuclear Science Series
Report Number 31

Conference on Molecular and Radiation Biology

National Academy of Sciences—
National Research Council

Publication 823

Nuclear Science Series
Report Number 31

Subcommittee on
Radiobiology

Conference
on
Molecular and Radiation Biology

Gould House, December 2, 3, and 4, 1959

Ardsley-on-Hudson, New York

R. A. Deering, *Editor*

Publication 823
National Academy of Sciences—National Research Council
Washington, D. C.
1961

PREFACE

This volume was prepared from an informal conference held at Gould House, Ardsley-on-Hudson, New York, December 2, 3, 4, 1959. The purposes of the conference are adequately discussed by Dr. Ernest Pollard, conference Chairman, in his "Introductory Remarks" which lead off the text of these proceedings. Each main topic was presented in a more or less formal manner by one of the participants. Free discussion was intermixed with and followed these presentations.

The editor prepared a rough initial manuscript of the proceedings from a recording and from notes. This was not a word for word transcript but tried to achieve continuity of presentation and discussion in an intelligible way while maintaining the informal atmosphere of the conference. This rough manuscript was sent to each of the participants for modification and clarification. These edited and amended sections from each of the participants were then fitted together to give this volume. The figures in some cases are exact originals provided by the respective participants while in others they are only schematic drawings made during the course of the talks. The latter were approved by the participant and deemed sufficient to indicate the important points. The approximate nature of some of these schematic drawings should be kept in mind however. For more exact data, the reader is referred to the references listed at the end of each section.

The willing cooperation of the participants in editing and rewriting the various sections was of great assistance in the preparation of this volume. The help of Miss Carol Williams during various stages of the manuscript preparation is gratefully acknowledged.

R. A. Deering
Editor

FOREWORD

The Committee on Nuclear Science of the National Academy of Sciences-National Research Council has given special attention to those phases of nuclear studies in which two or more of the older, well-established disciplines of scientific investigation have been drawn together. To accomplish this, the Committee has organized a number of subcommittees to provide the wide range of competence needed to consider and deal with these new and rapidly developing areas of science. This is well exemplified in the work of its Subcommittee on Radiobiology, which has originated a series of conferences on the basic mechanisms of radiobiology where physicists and chemists, as well as biologists, meet to exchange information and to test hypotheses of biological actions in the light of the combined experience of the group. This has resulted in free, informal and critical discussions of information available from several specialized fields of scientific study. At the same time, deficiencies in existing data have been revealed and suggestions developed for obtaining the missing information.

The organization of these conferences, arranged to encourage frank and detailed discussion in an informal atmosphere, has been a task of some magnitude. A vital part of the conference is the publication of the proceedings, so that all interested persons may have the benefit of these deliberations. It is obvious, even to a layman in biology who may read these pages, that the vital essence of the fundamental processes are being exposed in the reasonings and arguments which have been recorded. In many instances, it is also apparent that a first tentative approach is being made to the solution of a particular problem. Therefore, it is essential to continued progress that conferences of this kind be encouraged to continue to aid in plotting a course through the myriad of complicated reaction which occur when radiation interacts with living tissues.

The Subcommittee has organized eight conferences. The first was a symposium on radiobiology held at Oberlin College, June 14-18, 1950. It consisted of a series of formal papers and formal discussions; these were published in 1952 ("Symposium on Radiobiology," J. J. Nickson, Ed., John Wiley and Sons, New York, 1952). The second was a highly informal conference held in Highland Park, Illinois, May 31-June 2, 1951, the proceedings of which were not published. The third, fourth, fifth and sixth considered physical and chemical, biochemical, cellular, and mammalian aspects of Basic Mechanisms in Radiobiology; their

proceedings have been published as Publications Nos. 305, 267, 450, and 513 of the Academy-Research Council, Nuclear Science Series. The seventh, Research in Radiology (Publication 571) sponsored jointly with the Radiation Study Section of the National Institutes of Health, attempted to bridge the gap between experimental radiobiology and chemical radiotherapy. In 1958, the Subcommittee joined with the Radiation Research Society in sponsoring the first International Congress of Radiation Research at the University of Vermont.

The present conference on Molecular and Radiation Biology is in the tradition of its predecessors. No attempt was made to provide a comprehensive survey of the status and progress of research, but rather topics of current interest were discussed and analyzed. In planning the conference, the conference chairman and his colleagues on the subcommittee benefitted from many suggestions from their associates. Particular acknowledgement is due the National Science Foundation which provided the financial support without which neither this publication nor the conference itself would have been possible.

L. F. Curtiss, Chairman
Committee on Nuclear Science

H. J. Curtis, Chairman
Subcommittee on Radiobiology

PARTICIPANTS

Dr. Leroy G. Augenstine
United States Atomic Energy Commission
Washington 25, D. C.

Dr. Daniel Billen
M. D. Anderson Hospital
University of Texas
Houston 25, Texas

Dr. Howard Curtis
Biology Division
Brookhaven National Laboratory
Upton, L. I., N. Y.

Dr. Reginald A. Deering
Department of Biophysics
Yale University
New Haven, Conn.

Dr. Charles O. Doudney
M. D. Anderson Hospital
University of Texas
Houston 25, Texas

Dr. William Dreyer
National Heart Institute
National Institutes of Health
Bethesda 14, Maryland

Dr. Mortimer M. Elkind
National Cancer Institute
Department of Health, Education & Welfare
Bethesda 14, Maryland

Dr. Martin Gouterman
Department of Chemistry
Harvard University
Cambridge, Mass.

Dr. Franklin Hutchinson
Dept. of Biophys.,
Yale University
New Haven, Conn.

Dr. John Kirby-Smith
Biol. Div., Oak Ridge Nat'l Laboratory
Oak Ridge, Tenn.

Dr. A. Douglas McLaren
University of California
Berkeley 4, Calif.

Dr. Robert Mortimer
University of California
Berkeley 4, Calif.

Dr. Amos Norman
University of California Medical Center
Los Angeles 24, Calif.

Dr. Ernest C. Pollard, Chairman
Department of Biophysics
Yale University,
New Haven 11, Conn.

Dr. Van R. Potter
McArdle Memorial Laboratory
University of Wisconsin
Madison 6, Wis.

Dr. Henry Quastler
Brookhaven National Laboratory
Upton, L. I., N. Y.

Dr. Francis Reithel
National Science Foundation
Washington, D. C.

Dr. R. B. Roberts
Carnegie Institute of Washington
Washington 15, D. C.

Dr. John D. Spikes
Division of Biology and Medicine
United States Atomic Energy Commission
Washington 25, D. C.

Dr. Franklin W. Stahl
Institute of Molecular Biology
University of Oregon
Eugene, Oregon

Dr. J. Herbert Taylor
Department of Botany
Columbia University
New York 27, N. Y.

Dr. Gordon Whitmore
Department of Medical Biophysics
University of Toronto
Toronto 5, Canada

Dr. Thomas H. Wood
Department of Physics
University of Pennsylvania
Philadelphia 4, Pa.

Dr. Raymond Zirkle
Department of Biophysics
University of Chicago
Chicago 37, Ill.

CONTENTS

INTRODUCTORY REMARKS

E. C. Pollard

We have had great developments in the last twenty years in the subject which is loosely termed Molecular Biology, by which we often mean physical and biological studies of nucleic acid, studies of RNA and protein, and the relations of protein synthesis. All of this is linked together with the modern section electron microscopy and the growing impact of genetics on molecular structure and molecular structure on genetics.

Radiation biology is concerned with the interaction of very potent releases of energy from ionizing radiation and still potent energy releases from ultraviolet. These interactions clearly affect the molecular structure of the cell, modifying it in some way, and thus there would seem to be every theoretical reason for an interplay between molecular biology and radiation biology.

Unfortunately, many of the results of radiation biology seem mystifying; they are delayed and variable in character, and a simple pattern of explanation has not developed on its own. It is hoped that during this Conference we can see some sense in the explanation of radiation biology in terms of molecular biology and conversely, we can also hope that there will be a possibility that the findings of radiation biology can be used to give us information about the nature of molecular biology.

The objective of the Conference is therefore to try to exploit the possible relationship of the two subjects. We intend to present both molecular biology and radiation biology in an intermixed way and see whether we can develop really a usable overlap or not. Whether we develop such areas is not perhaps as significant as the fact that we find ourselves discussing important topics as an intelligent and interested group.

I would therefore like to call on Dr. Stahl to begin the proceedings.

THE DUPLICATION OF DNA

Franklin W. Stahl

A DNA molecule consists of two complimentary polynucleotide chains wound helically about a common axis and "running" in opposite directions. (For expository purposes, we represent a DNA molecule by two parallel lines and use arrow heads to indicate the polarity of the chains.)

According to an hypothesis of Watson and Crick, at the time of duplication each chain acts as a template for the synthesis of its own compliment. In its simplest form, this hypothesis predicts that after duplication the two daughter molecules each contain one chain from the parent molecule (Fig. 1). Density-label transfer experiments with E. coli, bacteriophages, Chlamydomonas, and human cells in tissue culture

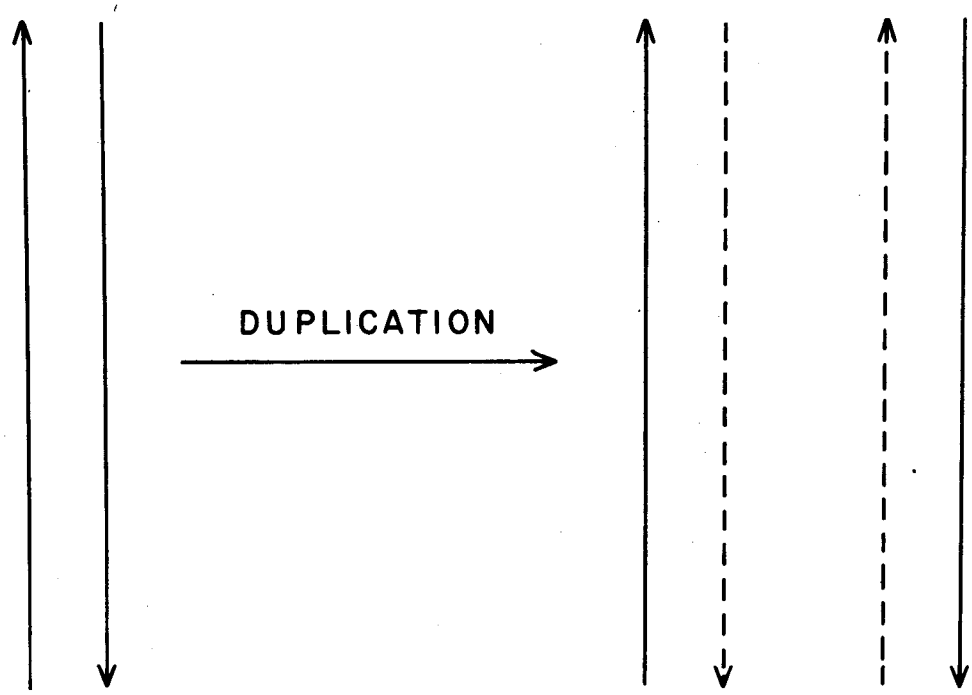

Figure 1

all support this model. This model for duplication (which we herein accept as outlined above) has been elaborated in three respects.

1. It has been proposed (Crick, unpublished musings) on grounds of simplicity that duplication proceeds in only one direction along a polynucleotide chain. This implies that the two chains of a DNA-molecule are copied beginning at opposite ends of the molecule (Fig. 2). (The duplicating molecule is held together throughout duplication by coiling and specific pairing between the "unpaired" regions of the "old" chains and the duplex regions of the presumptive daughter molecules (Fig. 3).)

2. The duplication scheme outlined in (1) has the following feature: Each level of a DNA molecule begins as a 2-chain structure, becomes next a 3-chain structure, and then a 4-chain structure. Thus, at every level of the duplicating molecule, the 4th chain to appear at that level is being formed in the presence of two chains of polarity opposite to its own. This suggests that at any level either one of these chains might act as template for the 4th chain. (The possibility that the choice of chain to act as template can be reversed repeatedly as the 4th chain is formed is attractive as an explanation for "high negative interference" (Chase and Doermann, 1958; Edgar, 1960).)

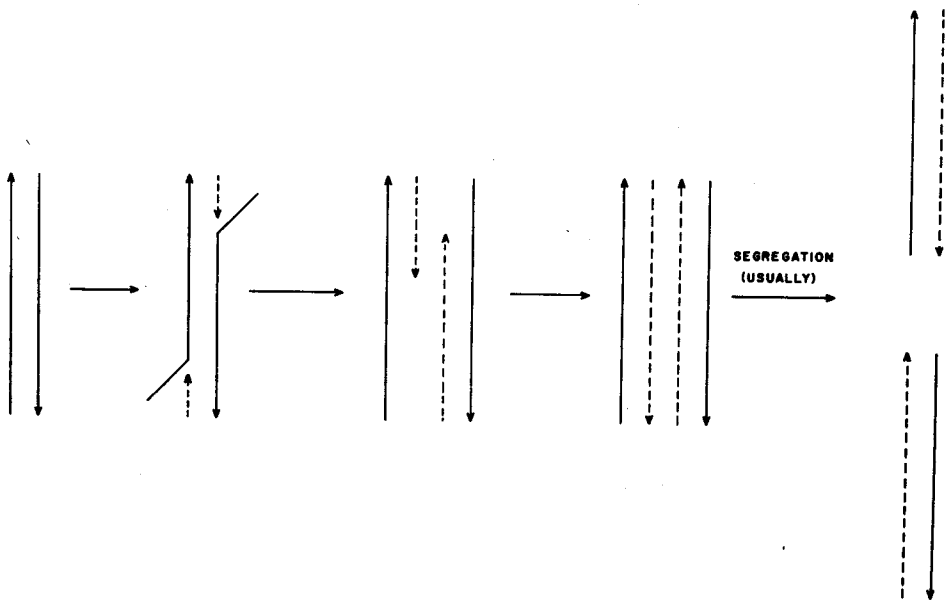

Figure 2

3. Levinthal (1959) and others have suggested schemes whereby a pair of duplicating DNA molecules can cooperate ("mate") to produce a third molecule which may be a genetic recombinant. These schemes have in common the following features: (a) The mated structures are complete DNA molecules. (b) No polynucleotide chains are broken (i.e., recombination is by "copy choice") in the process. (c) The parental DNA molecules are usually presumed to emerge intact from the mating (i.e., the duplication involved in a mating act is "conservative"). (d) At the completion of the synthetic period of the mating act, the mated structure appears as in Figure 4. (The failure of the two new chains to change templates at the same level leads to the appearance of phage heterozygotes.)

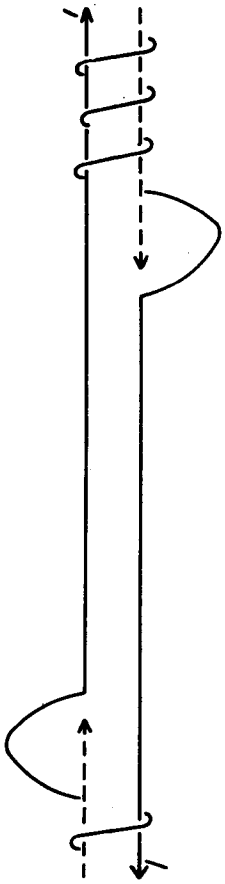

Figure 3

It is neither appropriate nor possible to consider at this time the arguments for or against these three elaborations of the Watson-Crick duplication hypothesis. We note only that despite their apparent arbitrariness, some of them, at least, may be said to constitute working hypotheses for many people in "phage genetics".

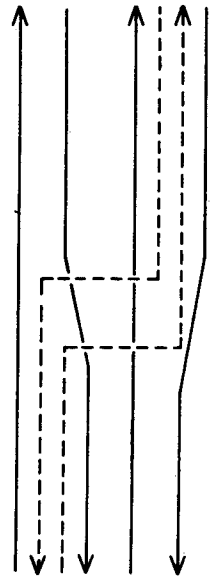

Figure 4

Pollard: The statistical mechanics of this situation might be interesting to look at. These things happen very fast, on the order of one DNA molecule produced per minute.

McLaren: How often does recombination of the above type occur?

Stahl: For the strain of virus to which this model is applied, there is about one mating per each DNA duplication when enough phage are present. There could be several points of recombination in one mating.

Hutchinson: What is the reason for assuming that genetic recombination takes place within the molecule instead of in the way in which the molecules are put together?

Stahl: It has been demonstrated that the number of places at which recombination can occur in phage far exceeds the number of DNA molecules per phage.

We turn now to several radiation experiments with bacteriophage. The results of these experiments appear compatible with the elaborated model for duplication outlined above; there is even the possibility that they add restrictions to the model.

Most, at least, of the UV "hits" on a phage result in small, discrete damages to the genetic map as we know it from crosses (see below). These damages are probably not duplicated (for arguments, see Doermann, Chase and Stahl, 1955; and Tomizawa, 1958). These considerations lead us to formulate the following model for the UV-inactivation of the ability of a phage (or DNA-molecule) to multiply. (For the purposes of this discussion we assume that the UV dose has not disturbed the environment in which the DNA is to be duplicated, i.e., we assume that all the machinery for DNA duplication is intact but that the templates to be copied are damaged.)

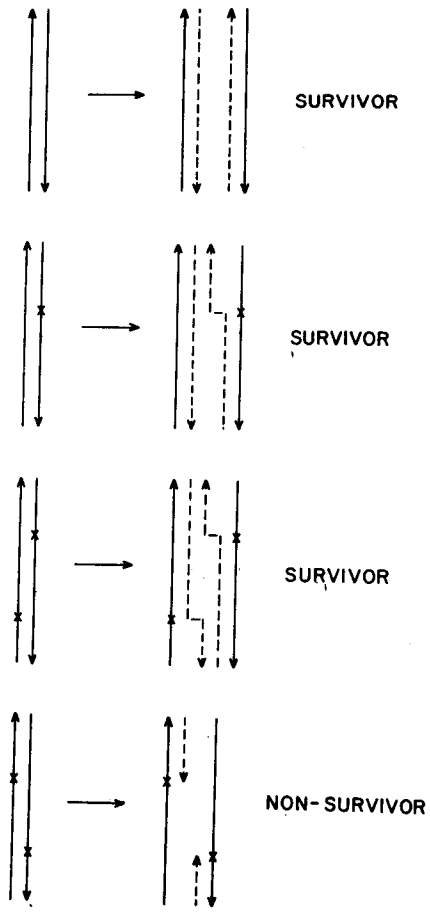

Figure 5

It follows then from our elaborated model for DNA duplication (points (1) and (2)) that a UV-damaged DNA molecule will survive if all the damages on one chain are "below" all the damages on the other chain. Under this condition the two newly forming chains will be able to reach each other and then to copy each other, thus circumventing the UV-damages (Fig. 5).

It is easy to show (Stahl, unpublished; Epstein and Steinberg, unpublished) that the survival curve for such a system is given by

$$S/S_0 = (1 + h)\, e^{-h}$$

where h is proportional to dose and has the units "damages per single polynucleotide chain". In Figure 6

the theoretical survival curve is compared with a classical published UV-survival curve for T2.

We turn now to a series of experiments involving mixed infection of bacteria with a single UV'd phage particle and a few non-UV'd particles. The first of these experiments is a study of the inactivation by UV of genic function.

Mutants of T4, called rII, cannot grow in E. coli strain K. Wild-type T4 grows well in K. The rII mutants fall into two cistrons called A and B. The A cistron is about 5 map units long and the B cistron is about 3 map units long. We may define the function of the wild-type state of an rII cistron as its ability in mixed infection to promote the growth of mutant particles in K. Krieg (1959) has studied the UV-inactivation of this genic function. He found exponential (one hit) survival curves for both cistrons. The function of the A-cistron was inactivated 0.1 as fast as whole phage, the B-cistron 0.05 as fast as whole phage. A conclusion we wish to point out here is that many damages lethal to the phage in single infection do not impair the functioning of these cistrons.

The experiments to be discussed indicate that those damages which occur within the cistron inactivate the function of the cistron while most, at least, of those outside the cistron have no effect.

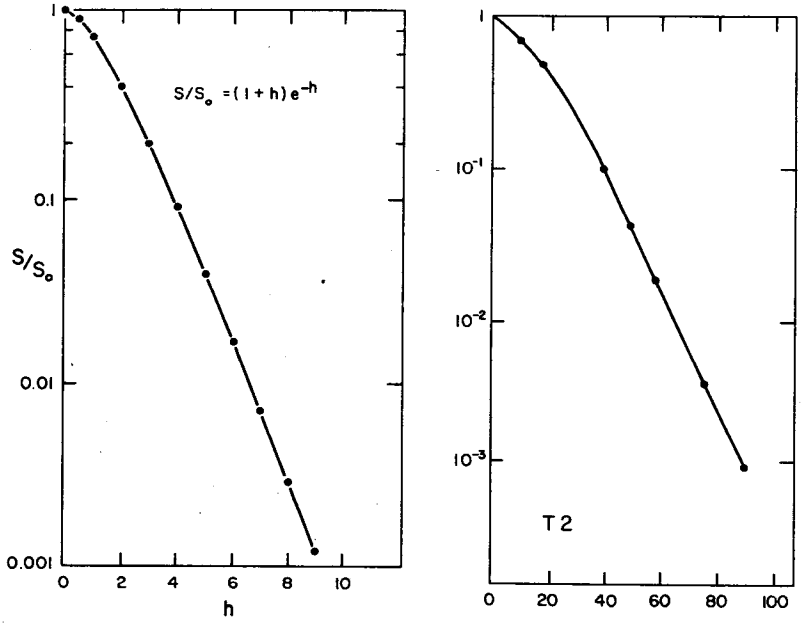

Figure 6. The T2 survival curve is reproduced from Viruses 1950 by permission of the Biology Division, California Institute of Technology.

Pollard: This was for UV. Till and I found no such clear-cut relationship between marker size and radiation sensitivity for ionizing radiation.

Stahl: UV is probably more delicate.

The next experiments employ the same genetic markers of T4 as did the previous one. However, the experiments are designed to study not the inactivation of genic function but, instead, the "rescue of a marker", i.e., the ability of irradiated phage to contribute at least one copy of a particular genetic marker to the viable offspring produced following mixed infection of a bacterium by one irradiated phage and several un-irradiated ones. This experiment has been performed in two different ways; in both cases wild-type T4 is irradiated and its ability to transmit the wild-type marker in a mixed infection with an rII mutant is measured. In the first case, E. coli strain B is employed as host (Doermann and Chase, unpublished); in the second case, strain K is used as host (Krieg, 1959). In both cases the measurement made as a function of dose is the fraction of phage-yielding bacteria which yield at least one wild-type particle. At low doses, the two experiments give about the same result; marker "knock-out" is roughly exponential and the rate is 1/17 that of whole-phage inactivation. At higher doses, the two curves diverge. For the experiment performed in K, the curve levels off at a fractional survival of about 0.14; for the experiment performed in B, the curve continues falling, although its slope decreases by about a factor of 10 as compared to the initial slope. These observations are qualitatively summarized in Figure 7. What is the essential difference between the two experiments? In the experiment performed in B, the function of the rII region is not required for phage production (since rII mutants by themselves grow perfectly well in B). In K, however, the wild-type phage which contribute their r + marker to the progeny are selected as having an intact r + function. (We are plotting the fraction of phage-yielding cells which yield at least one r + phage.) The consequence is that among particles so selected, the r + marker is contributed to the progeny with a high probability which is independent of dose at high dose. This sequence of experiments, and others by Doermann and Chase (unpublished) not reported here (see Stahl, 1959), are explained very simply. The function of a region of DNA is destroyed if a UV-damage occurs within that region. A marker in a UV'd phage particle appears among the viable progeny if in mating(s) with the non-irradiated phage the genetic recombinations necessary to produce a non-damaged phage carrying the marker occur. If the probabilities of such recombinational events are like those for ordinary recombination in phage, they will depend on the distance between the genetic marker and the neighboring UV-damage(s). Thus, when phage are selected for being undamaged in an rII cistron (by using K as host), the probabilities for the necessary recombinational events for an r + marker within the cistron are higher than is the case for the unselected population (i.e., when B is the host).

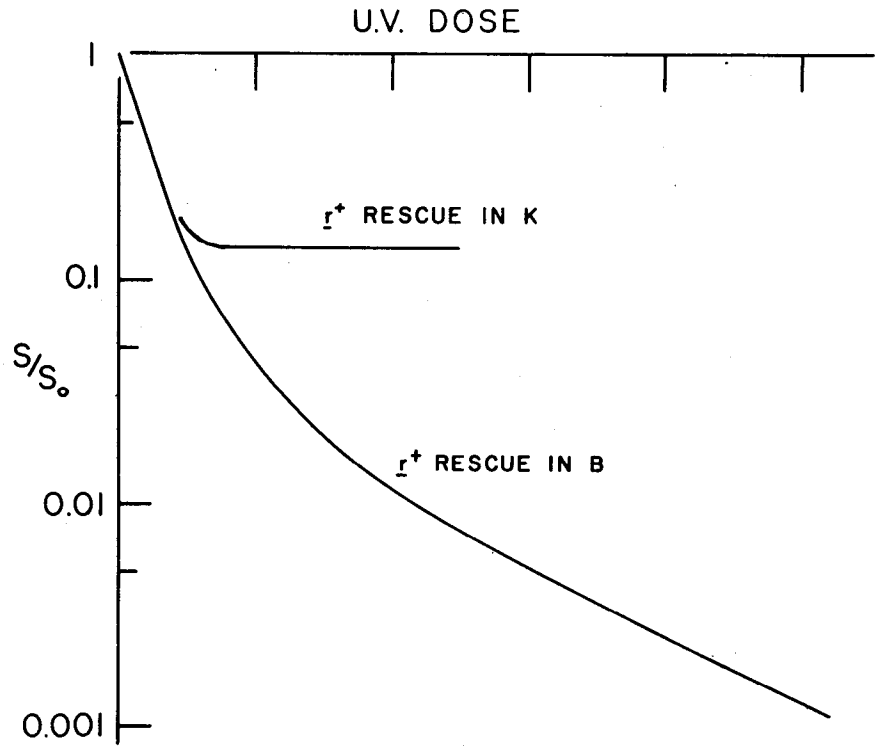

Figure 7

Wood: Could you call the UV lesions "mutations"?

Stahl: The lesions are defined operationally by the experiments described.

Potter: Could this lesion be the deamination of cytosine?

Stahl: I don't know.

Norman: I think there is no case known of mutations in DNA or RNA irradiated alone, i. e., produced directly by UV without interactions of some sort with other metabolic processes.

Stahl: Kreig has experiments which show a high frequency of mutants among the recombinants of UV-inactivated phage, but has not found mutants among the offspring of survivors. This mutation production may be a function of genetic recombination among the irradiated phages.

Pollard: Maybe the mutants were deletions. I also don't think Dr. Norman's last statement should be quite so general.

Stahl: In Krieg's experiments, the mutations were reversible; thus, it would seem unlikely that they were deletions.

McLaren: Could the mutations be caused by the effect of the host on the virus DNA?

Stahl: They are probably not the consequence of recombination with host genetic material.

McLaren: In relation to Norman's comments, it's not clear to me whether the UV produces a mutation directly or whether the UV alters the DNA such that the cell can then act on the molecule to give a mutation.

Stahl: There is no way of telling in this system since the phage won't reproduce in the absence of the host.

Let us examine now in detail a curve for marker KO. Since rescue of the marker depends on recombination which in turn depends on distance, the survival curve for a marker should be a function of $\frac{1}{h}$ where h is the number of hits per phage "chromosome". If we let C be the number of recombinational events per damaged chromosome, then the probability of getting a recombination required for marker "rescue" is $C/(C + h)$ (as long as either C or h is large) (Stahl and Meselson, unpublished).

We may now ask how many recombinational events (each of which has a probability $f(\frac{1}{h})$) are required for reactivation? This question is pertinent if we are to inquire into the possible molecular basis of "marker rescue". Goodgal and Rupert (pers. com.) have pointed out that this question is conveniently answered by inspection of a plot of log S versus log dose for large values of dose. The survival equation under these conditions reduces to $S/S_o = kh^{-n}$ where n is the number of recombinational events required. Thus, log S/S_o versus log dose will be linear and have a slope whose absolute value gives the number of required recombinational events. For the case of the phage experiments described here and for analgous experiments with transforming principle of Haemophilus influenzae (Goodgal and Rupert, pers. com.), n is equal to 2.

We may now attempt to explain marker rescue in terms of the elaborated models for DNA duplication. The model pictured in Figure 8 is simultaneously (reasonably) harmonious with our elaborated models for DNA duplication and with the results of the UV-experiments. At high doses, duplication of the UV'd chromosome is prevented; i.e., it can proceed only to the first damage on each chain. Recombination then depends on duplication along a normal chromosome which is "mating" with the UV'd chromosome. One of the new chains abandons the undamaged