RADIATION-INDUCED CHROMOSOME ABERRATIONS

Edited by Sheldon Wolff

Radiation-Induced Chromosome Aberrations

Edited by Sheldon Wolff

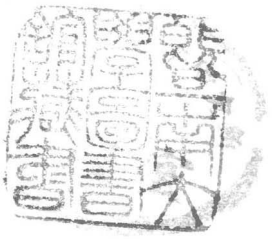
K. C. ATWOOD, C. AUERBACH, A. D. CONGER, E. H. Y. CHU,
H. J. EVANS, B. KIHLMAN, J. S. KIRBY-SMITH, D. L. LINDSLEY,
S. H. REVELL, C. P. SWANSON, J. H. TAYLOR, G. YERGANIAN

COLUMBIA UNIVERSITY PRESS
NEW YORK AND LONDON 1963

Report of A CONFERENCE ON BIOCHEMICAL AND BIOPHYSICAL MECHANISMS IN THE PRODUCTION OF RADIATION-INDUCED CHROMOSOME ABERRATIONS *held in* SAN JUAN, PUERTO RICO, NOVEMBER 16–18, 1961, *sponsored by* THE NATIONAL ACADEMY OF SCIENCES AND THE NATIONAL RESEARCH COUNCIL

Library of Congress Catalog Card Number: 63–9942
Manufactured in the United States of America

Preface



This book is a report of the ninth in a series of conferences on basic mechanisms in radiobiology that have been organized by the Subcommittee on Radiobiology, of the Committee on Nuclear Science, National Academy of Sciences-National Research Council. The funds for the support of the conference were provided by the National Institutes of Health and the Atomic Energy Commission.

The purpose of these conferences has been to bring together scientists from different disciplines so that they might consider various aspects of basic radiobiology. An attempt has always been made to make the conferences conducive to frank and detailed informal discussions. With this end in view the participants were urged to interrupt the speakers at any point. A transcript of the meetings was obtained by a stenotypist. Each participant then edited his remarks. The editing was kept to a minimum, however, to preserve both the flavor and the spontaneity of the meeting.

The conference was divided into five sessions of which Drs. Gray, Neary, Totter, Giles, and Sparrow were the chairmen. No rigid schedule was maintained, however, and on occasion a chairman found himself presiding over a paper that should have been in the previous session.

The conference committee which consisted of Sheldon Wolff, Chairman, H. Quastler, and E. C. Pollard wishes to express its profound thanks to Dr. J. Bugher, the director of the Puerto Rico Nuclear Center, and his staff who acted as local hosts during this conference. Their efforts contributed immeasurably to the success of the meeting. In particular the following members of Dr. Bugher's staff should be mentioned: Mrs. Marie Barton, Dr. H. J. Teas, and Dr. C. Garcia-Benitez.

Summer, 1962
Oak Ridge, Tennessee

SHELDON WOLFF

Committee on Nuclear Science

L. F. Curtiss, Chairman, NATIONAL BUREAU OF STANDARDS
Robley D. Evans, Vice Chairman, MASSACHUSETTS INSTITUTE OF TECHNOLOGY
J. A. DeJuren, Secretary, WESTINGHOUSE ELECTRIC CORPORATION
C. J. Borkowski, OAK RIDGE NATIONAL LABORATORY
Robert G. Cochran, PENNSYLVANIA STATE UNIVERSITY
Samuel Epstein, CALIFORNIA INSTITUTE OF TECHNOLOGY
U. Fano, NATIONAL BUREAU OF STANDARDS
Herbert Goldstein, NUCLEAR DEVELOPMENT CORPORATION OF AMERICA
J. W. Irvine, Jr., MASSACHUSETTS INSTITUTE OF TECHNOLOGY
E. D. Klema, NORTHWESTERN UNIVERSITY
W. Wayne Meinke, UNIVERSITY OF MICHIGAN
J. J. Nickson, MEMORIAL HOSPITAL
Robert Platzman, ARGONNE NATIONAL LABORATORY
D. M. Van Patter, BARTOL RESEARCH FOUNDATION

Subcommittee on Radiobiology

J. J. Nickson, Chairman, MEMORIAL HOSPITAL
Howard L. Andrews, NATIONAL INSTITUTES OF HEALTH
John Bugher, THE ROCKEFELLER FOUNDATION
H. L. Friedell, UNIVERSITY HOSPITALS OF CLEVELAND
Edwin Hart, ARGONNE NATIONAL LABORATORY
Alexander Hollaender, OAK RIDGE NATIONAL LABORATORY
Harvey M. Patt, ARGONNE NATIONAL LABORATORY
Ernest C. Pollard, YALE UNIVERSITY
Henry Quastler, BROOKHAVEN NATIONAL LABORATORY
Sheldon Wolff, OAK RIDGE NATIONAL LABORATORY

Invited Participants

Atwood, K. C., Department of Microbiology, University of Illinois, Urbana, Illinois

Auerbach, Charlotte, Institute of Animal Genetics, West Mains Road, Edinburgh 9, Scotland

Bugher, John C., Office of the Director, Puerto Rico Nuclear Center, Rio Piedras, Puerto Rico

Caldecott, Richard, Division of Biology and Medicine, U.S. Atomic Energy Commission, Washington 25, D.C.

Chu, E. H. Y., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Conger, A. D., Botany Department, University of Florida, Gainesville, Florida

Evans, H. J., MRC Radiobiological Research Unit, Atomic Energy Research Establishment, Harwell, Didcot, Berkshire, England

Garcia-Benitez, Carlos, Radiobiology Division, Puerto Rico Nuclear Center, Rio Piedras, Puerto Rico

Giles, N. H., Jr., Botany Department, Yale University, New Haven, Connecticut

Gray, L. H., BECC Research Unit in Radiobiology, Mount Vernon Hospital, Northwood, Middlesex, England

Hart, Edwin J., Argonne National Laboratory, 9700 South Cass Avenue, Argonne, Illinois

Hollaender, Alexander, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Kihlman, Bengt, Institute of Physiological Botany, University of Uppsala, Uppsala, Sweden

Kirby-Smith, J. S., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Klein, W. H., Smithsonian Institute, Washington, D.C.

Lindsley, D. L., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Moses, Montrose J., Department of Anatomy, Duke University School of Medicine, Durham, North Carolina

Neary, G. J., MRC Radiobiological Research Unit, Atomic Energy Research Establishment, Harwell, Didcot, Berkshire, England

Nebel, B. R., Division of Biological and Medical Research, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, Illinois

Nickson, James J., Memorial Hospital, 444 East 68 Street, New York 21, New York

Parker, D. R., National Science Foundation, Washington, D.C.

Pavan, C., Departamento de Biologia Geral, Universidade de São Paulo, Caixa Postal 8105, São Paulo, Brazil

Pollard, E. C., Pennsylvania State University, University Park, Pennsylvania

Revell, S. H., The Chester Beatty Research Institute, Institute for Cancer Research, Royal Cancer Hospital, Fulham Road, London, S. W. 3, England

Ris, Hans, University of Wisconsin, Madison, Wisconsin

Sparrow, A. H., Biology Department, Brookhaven National Laboratory, Upton, Long Island, New York

Steffensen, Dale, Botany Department, University of Illinois, Urbana, Illinois

Swanson, C. P., Biology Department, The Johns Hopkins University, Baltimore, Maryland

Taylor, J. H., Botany Department, Columbia University, New York, New York

Teas, Howard J., Agricultural Bio-Sciences Division, Puerto Rico Nuclear Center, Rio Piedras, Puerto Rico

Totter, John, University of Georgia, Athens, Georgia

Valencia, Juan, University of Buenos Aires

Yerganian, George, The Jimmy Foundation Building, Children's Hospital, Boston, Massachusetts

Wolff, Sheldon, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Contents

Part 1

General Survey and Interpretation of Effects

Introduction

Dr. Wolff: I would like to welcome you to this conference, which is sponsored by the National Academy of Sciences-National Research Council's Subcommittee on Radiobiology, and introduce to you the Chairman of the Subcommittee on Radiobiology, Dr. James Nickson, who would like to explain the general purpose of the Subcommittee in having conferences and the specific purpose of this conference.

Dr. J. J. Nickson: Thank you, Dr. Wolff. I would like to welcome you to the meeting on behalf of the NAS-NRC. In Dr. Bugher's absence, he has asked that I welcome you also on behalf of the Puerto Rico Nuclear Center.

This conference is one of a series which may be said to have begun with the Oberlin Conference in 1950. The Subcommittee on Radiobiology, which had been formed some time prior to that and which had a strong hand in the design and execution of the Oberlin Conference, primarily through Dr. Hymer Freidel, felt that the possibility of having symposia at fairly frequent intervals, once a year or so, in areas of radiation biology where two kinds of people who do not normally get together could be thrown together for the interchange of ideas, would be fruitful in the future.

We admit prejudice but we like to think that these series of symposia which the Subcommittee and others have sponsored and which some people have come to call the "Highland Park" Conferences, after the name of a hotel in that suburb of Chicago, which we used for the first of the series of conferences, have been worth-while.

This conference was proposed by Dr. Wolff at one of the Subcommittee meetings. He felt that it would be a good idea to consider this very rapidly expanding field with particular reference to the human aspects, to put the two kinds of people together so that they could

exchange their ideas in the hope that some of the translccations would be viable.

Dr. L. H. Gray: The Radiobiological Subcommittee of the National Research Council has given us a splendid opportunity to step out of the grooves in which we normally run and stand back and take a look at the subject as a whole, and I think I probably speak for all of you when I say that we feel this is a very great privilege enjoyed by the small group which is here; we express our sincere thanks to Dr. Nickson, Chairman of that Subcommittee, Dr. Wolff, and all those who have helped to make this meeting possible.

As Dr. Nickson said, we are not really expecting to solve a lot of problems in three days. These problems get solved in the laboratory or in the study or in the bath or any other solitary place rather than in conferences. What we can do is to help one another become aware of what the real problems are so that we may concentrate on these, and I suggest that we make this our objective. We want to see where the difficulties lie, and to see what the challenging points are. If we achieve this, the discoveries will come, after we get back to our laboratories.

Time is short and I have asked our participants to make their remarks challenging, to point to the weaknesses in the existing order, and to evaluate the experimental evidence for these weaknesses rather than to give us a tidy picture of the field as a whole. We all want to think imaginatively during these three days.

Dr. Wolff has made a good, judicious choice in selecting the specialities represented among all of the people who are here, but there are a few who could not be present. I happened to have one or two snapshots of missing colleagues, so I thought we might start by showing two or three slides.

The first is of Karl Sax who is still turning out some very interesting papers. The next is Douglas Lea who probably many of you have met. The last one is a snap I happened to take of John Read when I was out in New Zealand a couple of years ago. I think these are all people who have made such big contributions that they are bound to affect our thinking throughout this conference.

Lea's book, which was written 15 years ago, gave a very clear and logical analysis of the relation between the initial physical events

and the aberrations which we actually see. What we are trying to do is to fill in that big gap which falls between the physical events and the changes we observe in terms of biophysical and biochemical events. In attempting this I think we are at a very serious disadvantage inasmuch as we really don't know what a chromosome looks like. Is it, in fact, one or two threads coiled and recoiled, or is it 32 or 64 strands lying side by side? There are those present who, I hope, will be able to inform us on this important point, and I would urge them to speak up early in the proceedings, lest we discuss the mechanism of radiation damage in terms of a model which is utterly different from the structure of a chromosome as it exists within a living cell.

We start this morning with papers that are concerned with dose-response relations and with their formal analysis. My job is only to regulate your discussions, but I thought that there were, perhaps, two or three points which ought to be made at the beginning.

The first concerns dose relations; what we can hope to infer from a dose relation is something about the number of particles which participate in the initiation of the aberration, and not, of course, the number of targets. Thus, although two chromatids may be involved in the production of an aberration, we may infer from a linear dose relation that only one particle is involved in the initiation; or, conversely, there may be aberrations which look as if they involve only one chromatid, but for which the aberration frequency varies more rapidly than as the first power of the dose, and in such cases we must infer that more than one particle is involved in the production of this type of aberration.

When we compare the effectiveness of different radiations (e.g., gamma and alpha radiation) we must, I think, always keep in mind that, unfortunately, the change in the type of radiation changes two things simultaneously: It changes the radiation chemistry which follows after the initiating event, and it also changes the number of particles required to deliver a given amount of energy to the cell; and, hence, on geometrical grounds, the chance that a single particle having deposited energy in one critical site could go on to deliver energy in a neighboring site, relative to the chance that the same result will be produced by two particles. These two factors—the

chemical and the geometrical—have somehow to be disentangled.

The third point I wish to make here is that unfortunately most of the experiments that we shall be talking about concern the induction of chromosome aberrations in cells which form part of an organized tissue. This is the situation which exists in the *Tradescantia* anther, the *Vicia* root tip, and the *Drosophila* testis. We now know that in all these situations we are liable to be dealing with a population of cells whose sensitivities are being influenced to varying degrees by the fact that the oxygen tension is not uniform throughout the irradiated structure. This is very beautifully illustrated in some recent observations made by Dr. Alvin Beatty and Dr. Jeanne Beatty on the frequency with which chromosome aberrations are induced at different sites within the *Tradescantia* anther.

I am greatly indebted to Dr. Beatty for sending me the data from which it is possible to compute the yield of aberrations per 100 cells in three different zones in each of the two locules of the anther. The aberrations were scored separately in three concentric zones in each locule—an inner, an intermediate, and an outer zone. When the anther is situated in oxygen, the aberration frequency is approximately the same in all three zones. When the anther is irradiated in helium, and therefore completely devoid of oxygen, the aberration frequencies are again the same in all three zones. If, however, the anther is irradiated in a gas phase which contains 5 percent oxygen, the aberration frequencies are very different in the three zones, being greatest in the outer zone and least in the inner zone. Even when the gas surrounding the anther is air, very marked differences in aberration frequency are observed in the three zones. We may infer, therefore, that when an anther is situated in air, or in gas less rich in oxygen than air, the population of cells which is being irradiated is heterogeneous with respect to oxygen tension. Since the gradients in oxygen tension which are demonstrated by these observations arise on account of cellular respiration, we have also to reckon with the fact that a change in any factor, such as temperature, which changes the magnitude of cellular respiration will change this oxygen gradient, and likewise the heterogeneity of the radiosensitivity of the cell population. Furthermore, we have also to remember that the Q_{O_2} of cells almost certainly varies

throughout the mitotic cycle, and when synchrony exists, as it does within the anther, the oxygen gradient will also undergo cyclic variation. Thus, the heterogeneity of the microspores in early interphase, when chromosome aberrations are induced, might be different from that at later interphase, when chromatid aberrations are induced. Miss Gillian Douglas and I have measured the total oxygen consumed by anthers at different degrees of development as a function of the oxygen tension in the gas phase surrounding the anther. The relation is temperature-dependent, and markedly different for early-interphase and late-anaphase anthers.

We will now proceed to the papers. I think speakers will have different preferences as to the manner in which you raise your questions. I think Dr. Evans will be very happy to have you interject your questions at any time in the course of his talk.

Chromosome Aberrations and Target Theory

Dr. H. J. Evans: If I understand my assignment correctly, I have to try to give some sort of general background on chromosome aberrations and target theory in 10 minutes or a quarter of an hour and, at the same time, try to get you to stand up and argue with me or, preferably, argue with others around the table. I think the best thing for me to do is to very briefly describe some of the findings made in experiments using *Tradescantia,* because the target hypothesis and its application is perhaps more widely or better understood in terms of chromosome aberrations in plant microspores than in *Drosophila;* but this does not mean we are not to discuss *Drosophila.* At the same time I want to raise a number of questions relating to the scoring of the aberrations, the nature of the target, and, finally, if time permits, to discuss the importance of the modifying effects of certain physiological factors as exemplified by some recent fractionation experiments such as those carried out by Elkind and Sutton (1960) and Davies and Wall (1961).

I should start by saying that the first critical cytological evaluation of radiation-induced chromosome aberrations was made by Sax (1938, 1939, 1940, 1941) using *Tradescantia* microspores, and I would like to begin by enumerating some of the basic findings which resulted from Sax's experiments.

First, it was shown that simple terminal deletions and isochromatid aberrations increased *approximately* linearly with increasing dose. I would stress the word approximately, for later data indicate that this statement is not quite true.

The yield of these aberrations was apparently unaffected by altering the dose rate or by splitting the radiation treatment into two equal doses separated by varying time intervals (fractionation).