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# SEMIANNUAL PROGRESS REPORT

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# BIOLOGY DIVISION

# SEMIANNUAL PROGRESS REPORT

For Period Ending February 15, 1959

Alexander Hollaender, Director
Stanley F. Carson, Assistant Director

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## PUBLICATIONS AND LECTURES

Publications. – During the period covered by this report, 54 papers, 27 abstracts, and a symposium – "Genetic Approaches to Somatic Variation" – have been published. In press are 81 papers and 31 abstracts.

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| AUTHOR(S) | TITLE | PLACE PRESENTED |
|---|---|---|
| Adler, H. I. | Environmental Conditions That Permit Life | Biology Dept., Erskine College, Due West, S.C. |

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| Adler, H. I. | Environmental Conditions That Permit Life | Biology Dept., Wofford College, Spartanburg, S.C. |
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| Anderson, N. G. | Cell Physiology at the Molecular Level | Dept. of Biology, Spring Hill College, Mobile, Ala. |
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| | As above | Sigma Xi, University of Georgia, Athens |
| | Far-Ultraviolet Monitoring of Chromatographic Column Effluents | Am. Soc. Zool. (AAAS), Washington, D.C. |
| | Giant Molecules and Cell Function | Dept. of Biology, Loyola University, New Orleans, La. |
| | Macromolecules and Cell Function | Biology Dept., University of Puerto Rico, Rio Piedras |

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| Anderson, N. G. | Microsomes and Protein Synthesis | Dept. of Biology, Louisiana State University, Baton Rouge |
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| | Monitoring Chromatographic Column Effluents in the Far Ultraviolet | Soc. Exptl. Biol. Med., Winston-Salem, N.C. |
| | Organization of Living Cells | Biology Dept., University of Puerto Rico, Rio Piedras |
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| Arnold, W. A. | Photosynthesis | Biology Class, Oak Ridge High School, Oak Ridge, Tenn. |
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| | As above | Dept. of Zoology, University of Tennessee, Knoxville |
| Auerbach, C. | 1. Genetic Studies at the Animal Genetics Institute | Dept. of Zoology and Entomology, University of Tennessee, Knoxville |
| | 2. Report on Recent Mutation Research Carried Out in Edinburgh | |

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| | Microbial Metabolism of Carbon Compounds (3-lecture series) | E. R. Squibb Lecture Series, Institute for Microbiology, Rutgers University, New Brunswick, N.J. |
| Clayton, R. K. | Catalase from Photosynthetic Bacteria | Biophysics Dept., University of Pennsylvania, Philadelphia |
| | Induced Synthesis of Catalase in Photosynthetic Bacteria | Mellon Institute, Pittsburgh, Pa. |
| Cohn, W. E. | Minor Constituents of Ribonucleic Acids [IV Intern. Congr. Biochem., Suppl. Intern. Abstr. Biol. Sci., p 29 (1958)] | Fourth Intern. Congr. Biochemistry, Vienna, Austria |
| | An Unusual Nucleotide from Ribonucleic Acids | McCollum-Pratt Institute, Johns Hopkins University, Baltimore, Md. |

| AUTHOR(S) | TITLE | PLACE PRESENTED |
|---|---|---|
| Cohn, W. E. | An Unusual Nucleotide from Ribonucleic Acids | Biology Dept., Massachusetts Institute of Technology, Cambridge |
| | As above | Dept. of Microbiology, Harvard Medical School, Boston, Mass. |
| Congdon, C. C. | Chemical and Biological Protection Against Ionizing Radiation | Dept. of Radiology, University of North Carolina, Chapel Hill |
| | Marrow Transplantation After Whole-Body Irradiation | Medical Division, ORINS (jointly sponsored by the American Society of Clinical Pathology and ORINS), Oak Ridge, Tenn. |
| | Modification of Radiation Injury | Army Medical Research Laboratory, Fort Knox, Ky. |
| Conger, A. D. | The Fate of Metaphase Exchanges [Proc. X Intern. Congr. Genet. Vol. II, 57–58 (1958)] | Tenth Intern. Congr. Genetics, Montreal, Canada |
| Darden, E. B., Jr. | Some Effects of Ionizing Radiation on Intact and Isolated Skeletal Muscle | Depts. of Zoology, Physiology, and Entomology, Louisiana State University, Baton Rouge |
| | As above | Depts. of Chemistry and Biology, Birmingham-Southern College, Birmingham, Ala. |
| | Some Irradiation Techniques for Biological Materials | Dept. of Biology, Spring Hill College, Mobile, Ala. |

| AUTHOR(S) | TITLE | PLACE PRESENTED |
|---|---|---|
| Doherty, D. G. | Chemical Radioprotection | Symposium on Applications of Radioactivity in Pharmaceutical and Allied Industries (sponsored by the Nuclear Science and Engineering Corporation), Summit, Pa. |
| | Chemical Structure and Protection Against Ionizing Radiation in the Aminoalkylisothioureas and Related Mercaptoalkyguanidines [Abstracts of Papers, 134th Natl. Meet., Am. Chem. Soc., p 19–20 (1958)] | Am. Chem. Soc., Chicago, Ill. |
| Gaulden, M. E. | Effects of Radiation on Living Cells | Biology Dept., Alabama College, Montevallo |
| | As above | Biology Dept., Centenary College, Shreveport, La. |
| | As above | Biology Dept., Southwestern College, Memphis, Tenn. |
| Gengozian, N. | Antibody Production in Mice as a Function of Age and Antigen | Depts. of Anatomy and Pathology, University of Chicago, Chicago, Ill. |
| | As above | Dept. of Zoology, University of Wisconsin, Madison |
| | As above | Dept. of Biology, Marquette University, Milwaukee, Wis. |
| | As above | Dept. of Biology, St. Mary's College, South Bend, Ind. |

| AUTHOR(S) | TITLE | PLACE PRESENTED |
|---|---|---|
| Haber, A. H. | Physiology of Seed Germination | Botany Dept., Yale University, New Haven, Conn. |
| | As above | Tennessee Academy of Science, Oak Ridge |
| | Rendering Light-Insensitive Lettuce Seed Sensitive to Light [Plant Physiol. 33 (Suppl.), xxiii (1958)] | Am. Soc. Plant Physiol. (AIBS), Bloomington, Ind. |
| Haber, A. H., and N. E. Tolbert | Metabolism of $C^{14}$-Bicarbonate, $P^{32}$-Phosphate, or $S^{35}$-Sulfate by Lettuce Seed During Germination [Plant Physiol. 33 (Suppl.), xxiv (1958)] | Am. Soc. Plant Physiol. (AIBS), Bloomington, Ind. |
| Kennedy, S. A. | Sulfhydryl Inhibition of Melanin Formation in the Embryonic Chick | Dept. of Zoology and Entomology, University of Tennessee, Knoxville |
| Kimball, A. W., and R. F. Kimball | Estimation from Observations Having Specified but Unassigned Expectations in a Radiation Experiment with *Paramecium aurelia* | Biometric Soc., Chicago, Ill. |
| Kimball, R. F. | The Postirradiation Period and Mutation in *Paramecium* [Proc. X Intern. Congr. Genet. Vol. II, 145–146 (1958)] | Tenth Intern. Congr. Genetics, Montreal, Canada |
| Krieg, D. R. | Mutation Induced in Bacteriophage by Ultraviolet Light [Proc. X Intern. Congr. Genet. Vol. II, 151–152 (1958)] | Tenth Intern. Congr. Genetics, Montreal, Canada |
| Kroeger, H. | The Origin of Form and Shape in the Embryonic Field | Zoology Seminar, Dept. of Zoology and Entomology, University of Tennessee, Knoxville |

| AUTHOR(S) | TITLE | PLACE PRESENTED |
|---|---|---|
| Lindsley, D. L. | The Role of the Genotype in Sensitivity of Sperm to Irradiation | Depts. of Zoology and Botany, Indiana University, Bloomington |
| | As above | Dept. of Zoology, University of Chicago, Chicago, Ill. |
| | As above | Dept. of Genetics, University of Wisconsin, Madison |
| | Y-Suppressed Sex-Linked Recessive Lethals in *Drosophila* | Dept. of Genetics, University of Wisconsin, Madison |
| Lindsley, D. L., C. W. Edington, and E. S. Von Halle | Sex-Linked Recessive Lethal Changes in *Drosophila melanogaster* Suppressed by the Y Chromosome [Proc. X Intern. Congr. Genet. Vol. II, 172–173 (1958)] | Tenth Intern. Congr. Genetics, Montreal, Canada |
| Loewenberg, J. R., and N. E. Tolbert | Effects of Some Organic Compounds on Phosphorus Movement in Plants [Plant Physiol. 33 (Suppl.), xx (1958)] | Am. Soc. Plant Physiol. (AIBS), Bloomington, Ind. |
| McGrath, R. A. | Oscillation of Nuclei in the Grasshopper Neuroblast | Zoology Seminar, Dept. of Zoology and Entomology, University of Tennessee, Knoxville |
| Makinodan, T., N. Gengozian, and I. C. Shekarchi | Further Studies on Antigen-Host Relationship As Affected by X Irradiation [Abstracts of Communications, VII Intern. Congr. Microbiol., p 299 (1958)] | Seventh Intern. Congr. Microbiol., Stockholm, Sweden |
| Mota, M. | Karyokinesis Without Cytokinesis in the Grasshopper [Proc. X Intern. Congr. Genet. Vol. II, 197 (1958)] | Tenth Intern. Congr. Genetics, Montreal, Canada |

| AUTHOR(S) | TITLE | PLACE PRESENTED |
|---|---|---|
| Novelli, G. D. | Studies on Protein Synthesis | Dept. of Biochemistry, Oklahoma State University, Stillwater |
| | As above | Dept. of Biology, A. & M. College of Texas, College Station |
| | As above | Dept. of Chemistry, University of Texas, Austin |
| | As above | Dept. of Biochemistry, School of Medicine, Tulane University, New Orleans, La. |
| | As above | Depts. of Zoology, Physiology, and Entomology, Louisiana State University, Baton Rouge |
| | As above | Section of Microbiology, M. D. Anderson Hospital and Tumor Institute, University of Texas, Houston |
| | As above | Science and Math Club, Alabama State College, Montgomery |
| | The Turnover of Pantothenic Acid | Fourth Intern. Congr. Biochemistry, Vienna, Austria |
| Oakberg, E. F. | The Effect of X Rays on the Mouse Ovary [Proc. X Intern. Congr. Genet. Vol. II, 207 (1958)] | Tenth Intern. Congr. Genetics, Montreal, Canada |

| AUTHOR(S) | TITLE | PLACE PRESENTED |
|---|---|---|
| Oakberg, E. F. | Effects of Radiation on the Cell | Southeastern Regional Meeting of the National Science Teachers Association, Vanderbilt University, Nashville, Tenn. |
| | Radiation Damage to the Gonads of Mice | Radiation Laboratory, University of California, Berkeley |
| Odell, T. T., Jr. | Regulation of Platelet Production | Natl. Inst. Health Conf. on Fundamental Problems and Techniques for the Study of the Kinetics of Cellular Proliferation, Salt Lake City, Utah |
| | Tissue Transplantation in Mammals After Treatment with Radiation and Chemical Agents | Dept. of Surgery, School of Medicine, University of North Carolina, Chapel Hill |
| | As above | Dept. of Physiology, University of Tennessee, Memphis |
| | As above | Depts. of Biochemistry, Pharmacology, and Physiology, School of Medicine, University of Oklahoma, Oklahoma City |
| | As above | Medical Branch, University of Texas, Galveston |
| | As above | Dept. of Bacteriology, University of Texas, Austin |

| AUTHOR(S) | TITLE | PLACE PRESENTED |
|---|---|---|
| Owen, R. D. | Immunogenetics | Tenth Intern. Congr. Genetics, Montreal, Canada |
| Popp, R. A. | Radiation Effects on Tissue Esterases in Mice [Anat. Record 131, 590 (1958)] | Am. Soc. Zool. (AIBS), Bloomington, Ind. |
| Popp, R. A., and W. St. Amand | The Mouse Hemoglobin Locus | Am. Soc. Zool. (AAAS), Washington, D. C. |
| Russell, W. L., J. W. Bangham, and J. S. Gower | Comparison Between Mutations Induced in Spermatogonial and Postspermatogonial Stages in the Mouse [Proc. X Intern. Congr. Genet. Vol. II, 245–246 (1958)] | Tenth Intern. Congr. Genetics, Montreal, Canada |
| St. Amand, W., and L. H. Smith | A Method for the Quantitative Estimation of Rat Erythrocytes in the Mouse-Rat Chimera [Anat. Record 131, 594 (1958)] | Am. Soc. Zool. (AIBS), Bloomington, Ind. |
| Schwartz, D. | Chromosome Structure — DNA Side-Chain Model | Biology Dept., University of Georgia, Athens |
| | As Above | Dept. of Biological Sciences, Florida State University, Tallahassee |
| | As above | Dept. of Biochemistry, University of Florida, Gainesville |
| | As above | Zoology Dept., Southern Illinois University, Carbondale |
| | As above | College of Medicine, University of Chicago, Chicago, Ill. |
| | As above | Depts. of Biochemistry, Botany, and Bacteriology, University of Illinois, Urbana |

| AUTHOR(S) | TITLE | PLACE PRESENTED |
|---|---|---|
| Schwartz, D. | A New Mutable Gene in Maize [Proc. X Intern. Congr. Genet. Vol. II, 256 (1958)] | Tenth Intern. Congr. Genetics, Montreal, Canada |
| de Serres, F. J., and G. Kølmark | Quantitative Estimates of Radiation-Induced Forward-Mutation Rates at the ad-3A and ad-3B loci in Neurospora [Proc. X Intern. Congr. Genet. Vol. II, 68–69 (1958)] | Tenth Intern. Congr. Genetics, Montreal, Canada |
| Stapleton, G. E. | Effects of Ionizing Radiations on Bacteria and Viruses | Dept. of Radiology, University of North Carolina, Chapel Hill |
| | Physiological Effects of Radiations on Living Material | Inst. of Biology and Radiobiology, University of Wyoming, Laramie |
| | Protection and Recovery from the Damaging Effects of Ionizing Radiations on Biological Material | Inst. of Biology and Radiobiology, University of Wyoming, Laramie |
| Upton, A. C. | Studies on the Mechanism of Leukemogenesis by Ionizing Radiation | Tennessee Academy of Science, Oak Ridge |
| | Studies on Leukemia | Biology Class, Oak Ridge High School, Oak Ridge, Tenn. |
| | The Therapy of Chronic Radiation Damage | ANP Life Sciences Working Group, AEC Field Office, Las Vegas, Nev. |
| Volkin, E. | Ribonucleic Acid Turnover in Phage Infection | Fourth Intern. Congr. of Biochemistry, Vienna, Austria |

| AUTHOR(S) | TITLE | PLACE PRESENTED |
|---|---|---|
| Welshons, W. J. | Pseudoallelic Recessive Lethals at the *Notch* Locus of *Drosophila melanogaster* [Proc. X Intern. Congr. Genet. Vol. II, 311-312 (1958)] | Tenth Intern. Congr. Genetics, Montreal, Canada |
| Wolff, S. | Biochemical Aspects of Chromosome Breakage and Rejoining | Dept. of Biology, Massachusetts Institute of Technology, Cambridge |
| | Biochemical Nature of Chromosome Breakage and Rejoining | Dept. of Biochemistry, Tulane University, New Orleans, La. |
| | As above | Dept. of Botany, University of Florida, Gainesville |
| | As above | Dept. of Zoology, University of Georgia, Athens |
| | Protein Synthesis and Chromosome Break Rejoining [Proc. X Intern. Congr. Genet. Vol. II, 319 (1958)] | Tenth Intern. Congr. Genetics, Montreal, Canada |

Visiting Lecturers. — During the period covered by this report, scientists from various universities, laboratories, and research organizations, both from the United States and abroad, have given lectures on the Biology Seminar program.

| LECTURER | AFFILIATION | TOPIC |
|---|---|---|
| Otto Hug | International Atomic Energy Agency, Vienna, Austria | Film: Induction of Reflex Movements in Lower Animals Under the X-Ray Beam |
| Hitoshi Kihara | National Institute of Genetics, Misima, Japan | Color film: Expedition to the Hindu Kush Karakoram (Japanese Archaeological and Biological Expedition) in Search for the Origin of Wheat |

| LECTURER | AFFILIATION | TOPIC |
|---|---|---|
| F. A. Saéz | Instituto de Investigacion de Ciencias Biologicas, Montevideo, Uruguay | Recent Cytological and Cytogenetic Work at the Instituto de Investigacion de Ciencias Biologicas |
| Hans Ulrich | Zoologisches Institut der Eidgenössischen Technischen Hochschule, Zürich, Switzerland | Mutations in *Drosophila* |
| D. B. Cater | Cambridge University, Cambridge, England | The Measurement of Oxidation-Reduction Potentials, pH, and Oxygen Tension in Tumours and Normal Tissues |
| S. R. Pelc | University of London, King's College, London, England | 1. The Uptake and Retention of $H^3$-Thymidine in Bean Roots<br>2. Metabolic Activity of DNA |
| Lorentz Eldjarn | The Norwegian Radium Hospital, Oslo | Recent Studies on the Mechanism of Chemical Protection |
| Knut Mikaelsen | Agricultural College of Norway, Vollebekk | Studies on Genetic Effects of X Rays and Neutrons in Barley |
| Peter Alexander | Chester Beatty Research Institute, London, England | 1. The Primary Biochemical Lesion Produced by Radiation and Alkylating Agents<br>2. Physicochemical Changes and Energy Transfer Processes in Macromolecules Exposed to Ionizing Radiations |
| B. M. Slizynski | Institute of Animal Genetics, Edinburgh, Scotland | Translocation Sterility in the Laboratory Mouse |
| Giuseppe Sermonti | Instituto Superiore di Sanita, Rome, Italy | 1. Genetics of *Streptomyces coelicolor*<br>2. Chemical Mutagenesis and Recovery in *Penicillium* |

| LECTURER | AFFILIATION | TOPIC |
|---|---|---|
| Th. Dobzhansky | Department of Zoology, Columbia University, New York, N.Y. | The Release of Genetic Variability by Recombination |
| Otto Vos | Biology Division, Oak Ridge National Laboratory (On loan from Medical Biological Laboratory, Natl. Def. Council, T.N.O., Rijswijk, Netherlands) | Review of Bone Marrow Transplantation Studies at Rijswijk |
| Robert S. Edgar | Institut Pasteur, Paris, France | Competitive Inhibition of Genetic Transformation |
| Amoz I. Chernoff | Memorial Research Center and Hospital, University of Tennessee, Knoxville | Hemoglobin Studies — Recent Technical Advances |
| G. S. Watson | Australian National University, Canberra | Cytoplasmic Inheritance |
| G. W. Dolphin | St. Bartholomew's Hospital and Medical College, London, England, and Yale University, New Haven, Conn. | Dosimetry and Physicochemical Effects of Extremely High Intensity X-Ray and Electron Beams |
| Michael Woodruff | University of Edinburgh, Edinburgh, Scotland | Homograft Research at the University of Edinburgh |
| Frederic Ludwig | Chef du Laboratoire d'Histopathologie, Centre d'Études Nucléaires, Saclay, France | Experimental Studies About Abscopal Effects of Irradiation in Mammals |
| Øistein Strømnaes | Department of Genetics, University of California, Berkeley | The Radiobiological Study Group and the Position of Radiobiology in Norway |
| James H. McAlear | Department of Health, State of New York, Division of Laboratories and Research, Albany | The Nature and Origins of Cell Structures As Revealed by Electron Microscopy |
| Ernest H. Y. Chu | Department of Botany, Josiah Willard Gibbs Research Laboratory, New Haven, Conn. | Cytological Studies of Human Somatic Cells in Tissue Culture |

| LECTURER | AFFILIATION | TOPIC |
|---|---|---|
| Jack L. Strominger | Department of Pharmacology, Washington University School of Medicine, St. Louis, Mo. | Biosynthesis of Bacterial Cell Walls in Relation to the Mechanism of Action of Antibiotics |
| Lucien Ledoux | Centre d'Étude de l'Énergie Nucléaire, Bruxelles, Belgium | Nucleolytic Enzymes and RNA in Tumors, Bone Marrow Cells, and Other Tissues |
| Jack H. Stimpfling | Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me. | Gene Homology in Columbidae |
| T. M. Sonneborn | Department of Zoology, Indiana University, Bloomington | Kappa |
| David M. Prescott | University of California, Los Angeles | Relations Between Cell Growth and Cell Division |
| J. L. Gowans | Oxford University, Oxford, England | The Lymphocyte |
| Jerome Kaye | University of California, Los Angeles | Changes in the Fine Structure of Cells During Spermatogenesis |
| Keith R. Porter | The Rockefeller Institute for Medical Research, New York, N.Y. | Carcinogenesis in the Rat Liver |
| Peter Mazur | Princeton University, Princeton, N.J. | Effects of Subzero Temperatures on Microorganisms |

Speakers at Professional Meetings. — Thirty-seven papers on research in the Biology Division are scheduled to be given during the spring and summer of 1959. Societies and speakers are as follows.

Biophysical Society — J. W. Hastings (coauthor, L. Astrachan), John Jagger, and M. L. Randolph (coauthor, S. Kondo)

National Academy of Sciences, National Research Council — T. T. Odell, Jr. (coauthor, B. Anderson)

Colloquium on Biological Problems of Grafting (Liége, Belgium) — R. D. Owen

American Association for Cancer Research — A. C. Upton (2 papers, one coauthored by E. P. Sniffen and F. F. Wolff)

Federation of American Societies for Experimental Biology — R. C. von Borstel, F. J. Bollum, R. K. Clayton, D. G. Doherty (coauthor, C. C. Congdon), W. Friedberg, N. Gengozian (coauthors, T. Makinodan and I. C. Shekarchi), J. W. Goodman, J. W. Hastings (coauthor, L. Astrachan), T. Iwamura, T. Makinodan (coauthors, N. Gengozian, M. G. Tolbert, and R. E. Canning), P. Rogers, Jr. (coauthor, G. D. Novelli), M. F. Stulberg, and A. C. Upton (coauthors, G. E. Cosgrove, E. E. Schwartz, C. C. Congdon, and T. Makinodan)

Association of Southeastern Biologists — N. G. Anderson (coauthor, J. A. Levinson), N. Brassington (coauthor, A. Haber), and J. Stevens (coauthor, M. E. Gaulden)

Society of American Bacteriologists — S. F. Carson (coauthor, E. F. Phares), M. S. Engel (coauthors, G. E. Stapleton and H. I. Adler), and N. P. Wood (coauthor, M. I. Dolin)

American Medical Association, Pathological and Physiological Sections — C. C. Congdon (coauthor, A. Hollaender)

Third Annual Ciba Lecture on Ageing Research (London, England) — A. C. Upton

Twenty-First International Congress of Physiological Sciences (Buenos Aires, Argentina) — C. C. Congdon. Symposium on "Radiation Protection and Recovery," organized by A. Hollaender, who will serve as chairman.

Czechoslovak Academy of Science (Prague) — T. Makinodan (2 papers)

Symposium on Immediate and Low-Level Effects of Ionizing Radiations (Venice, Italy) — J. S. Kirby-Smith, W. L. Russell, L. B. Russell, M. A. Bender, H. I. Adler, and Alexander Hollaender (chairman)

Ninth International Congress of Radiology (Munich, Germany) — L. B. Russell and W. L. Russell

Third Alligator Point Developmental Biology Conference (sponsored by Florida State University) — R. A. Popp

Twelfth Annual Biology Research Conference. — According to the custom of the past several years, the Biology Spring Conference will be held at the Mountain View Hotel in Gatlinburg, Tennessee. The title for the 1959 Symposium is "Enzyme Reaction Mechanisms." The speakers will discuss the basic enzymic mechanisms concerned with the formation and splitting of specific chemical bonds. The organizing committee for this conference is composed of S. F. Carson, W. E. Cohn, D. G. Doherty, G. D. Novelli, and E. Volkin. The program follows.

WEDNESDAY, APRIL 1

MORNING

1. Reactions Involving the P-O Bond
   Chairman and Introductory Speaker: Sir Alexander R. Todd, Cambridge University, Cambridge, England
   a. Synthesis and Structural Analysis of Polynucleotides — G. Khorana, University of British Columbia, Vancouver, B.C.
   b. Mechanisms of Enzymatic Cleavage of Organic Phosphates — Mildred Cohn, Washington University, St. Louis, Mo.

AFTERNOON

2. Reactions Involving the C-C Bond

Chairman: E. R. Stadtman, National Heart Institute, National Institutes of Health, Bethesda, Md.

a. The Participation of Acyl-CoA in Carbon Chain Biosynthesis — F. Lynen, Max-Planck-Institut für Zellchemie und Chemisches Laboratorium der Universität München, München, Germany

b. Carboxylation and Decarboxylation — M. Calvin and N. G. Pon, Lawrence Radiation Laboratory, University of California, Berkeley, Calif.

EVENING

Chairman: F. H. C. Crick, Cavendish Laboratory, Cambridge University, Cambridge, England

c. Protein Synthesis — F. Lipmann, Rockefeller Institute for Medical Research, New York, N.Y.

THURSDAY, APRIL 2

MORNING

Reactions Involving the C-C Bond (continued)

Chairman: H. G. Wood, Western Reserve University Medical School, Cleveland, Ohio

d. Aldol and Ketol Condensations — B. L. Horecker, Department of Microbiology, New York University College of Medicine, New York, N.Y.

e. Mechanisms for Enzymatic Formylation and Hydroxymethylation Reactions — F. M. Huennekens, H. R. Whiteley, and M. J. Osborn, Departments of Biochemistry and Microbiology, University of Washington, Seattle, Wash.

AFTERNOON

Mountain climbing or tour through Smoky Mountains. In case of inclement weather, the program will continue and Saturday morning will remain open for a visit to the Biology Division of the Oak Ridge National Laboratory.

FRIDAY, APRIL 3

MORNING

3. Reactions Involving C-O and C-N Bonds

Chairman: C. F. Cori, Washington University, St. Louis, Mo.

a. Reactions Involving the Carbon-Oxygen Bond of Glycosides — S. Hestrin, The Hebrew University-Hadassah Medical School, Jerusalem, Israel

Chairman: G. R. Greenberg, University of Michigan, Ann Arbor, Mich.

b. Reactions Involving the Carbon-Nitrogen Bond: Heterocyclic Compounds — J. M. Buchanan, S. C. Hartman, R. L. Herrmann, and R. A. Day, Massachusetts Institute of Technology, Cambridge, Mass.

AFTERNOON

Reactions Involving the C-N Bond (continued)

Chairman: J. S. Fruton, Yale University, New Haven, Conn.

c. Transamination and Related Reactions — E. E. Snell, University of California, Berkeley, Calif.

d. The Hydrolysis of Peptide and Ester Bonds by Proteolytic Enzymes — H. Neurath and B. S. Hartley, Department of Biochemistry, University of Washington, Seattle, Wash.

SATURDAY, APRIL 4

MORNING

4. Round Table: Active Sites

Chairman: P. Handler, Duke University, Durham, N.C.

Enzyme Function in Relation to Structure — C. B. Anfinsen, National Heart Institute, National Institutes of Health, Bethesda, Md.

The Aminoacyl Insertion Reaction — M. Brenner, Organisch-chemische Anstalt der Universität Basel, Basel, Switzerland

The Active Site of Esterases — J. A. Cohen, R. A. Oosterbaan, H. S. Jansz, and F. Berens, Medical Biological Laboratory, National Defence Research Council, T.N.O., Rijswijk Z. H., Netherlands

Enzyme Flexibility and Enzyme Action — D. E. Koshland, Jr., Biology Department, Brookhaven National Laboratory, Upton, N.Y.

ORNL Advisory Committee for Biology. — The Committee met in annual session in the Biology Division conference room on November 3, 4, and 5, 1958. On the first day of the meeting, members of all groups and sections of the Division discussed work in progress. The other days were given over to private and group consultations. Members of the Committee are Karl Sax, The Arnold Arboretum, Harvard University; Curt Stern, Department of Zoology, University of California, Berkeley; E. L. Tatum, The Rockefeller Institute for Medical Research; and E. Chargaff, College of Physicians and Surgeons, Columbia University.

Bone Marrow-AET Conferences. — The seventh in the series of informal conferences on bone marrow transplantation and chemical protection in animals and man was held in the Biology Division conference rooms on January 9 and 10, 1959. These conferences, organized by members of this Division, are gaining in popularity and usefulness. About a hundred people engaged in basic and applied research in biology and medicine were present. Proceedings of these meetings are published in Blood, The Journal of Hematology.

The next conference will be held during the Federation, April 13–17, 1959, in Atlantic City.

Education. — Several members of the Division gave short courses in various phases of radiation biology during the half year. Others have taken advantage of courses given at other laboratories.

C. C. Congdon taught a postgraduate course in internal medicine, with particular reference to bone marrow transplantation. The course was given at the University of Kansas Medical Center, Department of Postgraduate Medical Education, in Kansas City, Missouri. A. C. Upton was a lecturer at the Tenth Annual Nuclear Sciences Seminar of the ORINS Special Training Division. His subject was "Radiation Hazards to Man." Division members who have served on the ORSORT staff during the period are W. L. Russell, L. B. Russell, G. E. Stapleton, and A. C. Upton. E. F. Oakberg gave a Radiological Society of North America refresher course on "Genetic Effects of Radiation in Mammals" at the annual meeting of the Society in Chicago.

T. W. McKinley, Jr., attended a course in "The Principles of Animal Care" for supervisory personnel at Berg Institute, New York University, Bellevue Medical Center, New York City. Charlotte Auerbach, who spent several months in the Biology Division while on leave from the University of Edinburgh, spent some time at Harvard University studying Chlamydomonas work.

# CYTOLOGY AND GENETICS

## CYTOGENETIC EFFECTS OF RADIATION

R. F. Kimball
D. Davidson[1]
D. M. Green[2]
G. Y. Kikudome
H. G. Kølmark[1]
D. R. Krieg
M. Moutschen-Dahmen[1]
D. Schwartz
F. J. de Serres
P. J. Snider, Jr.[2]
O. C. Wells[2]
S. Wolff
P. A. Delker
N. T. Gaither
H. E. Luippold
K. H. McGrath
A. P. Teasley
S. M. Wilson

### Effect of Antibiotics on the Rate of Recovery from Initial Mutational Damage

R. F. Kimball N. Gaither S. M. Wilson

Introduction. – Previous work[3] on X-ray-induced recessive lethal mutations in *Paramecium aurelia* has shown that recovery from initial radiation damage occurs spontaneously up to some time during the development of the cell for division at which time the remaining damage is either converted into final mutation or lost by what will be called the terminal event. The hypothesis was advanced[4] that streptomycin and similar post-treatment agents decrease mutation by allowing more time for spontaneous recovery to occur. The evidence did not exclude, however, the possibility that such agents influence the rate of recovery as well as the time available for it.

The methods of irradiation, growing the paramecia, and detecting mutations are the same as given in many previous reports and will not be repeated here.

Results. – The main problem is to separate the effects of the post-treatment agents on the duration of the recovery period from possible effects on the rate of recovery. Two methods have been used: (1) identification of the terminal event and determination of the effect of the agents

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[1]Visiting investigator from abroad.

[2]Research Associate.

[3]R. F. Kimball, N. Gaither, and S. M. Wilson, Biol. Semiann. Prog. Rep. Aug. 15, 1958, ORNL-2593, p 19–20.

[4]R. F. Kimball, N. Gaither, and S. M. Wilson, Genetics 42, 661–69 (1957).

on it and (2) variation of the time of irradiation, and consequently of the time for recovery, while holding all other conditions constant.

The earlier work[4] suggested that the terminal event is marked, for reasons yet unknown, by a sharp drop (D) in "sensitivity" to mutation induction. Further evidence has confirmed and strengthened the association of D with the end of the period during which posttreatments are effective, and both streptomycin and chloramphenicol have now been shown to delay D. Paramecia were put into the antibiotics (3 mg/ml of streptomycin or 0.5 mg/ml of chloramphenicol) immediately after division and kept in them for from 3 to 4 hr. After removal from the antibiotic, groups were irradiated at intervals to test the "sensitivity." D occurs at about 2.5 hr in untreated log-phase paramecia;[4] at about 6 hr in streptomycin-treated ones; and at about 5 hr in chloramphenicol-treated ones. These findings support the earlier conclusion that the antibiotics delay the terminal event, at least insofar as this event can be equated with D.

Can the decrease in mutation produced by the antibiotics be accounted for by the delay in the terminal event without any change in the rate of recovery? An answer requires some measure of this rate. Earlier work[4] has shown that mutation increases as radiation is given later and later in the interdivision interval up to 2.5 hr, when D occurs. This increase has been interpreted as the result of recovery, since the later the radiation is given the less time there is for recovery before the terminal event. The rate of increase in mutation in untreated log-phase paramecia is, on this basis, a measure of the rate of recovery and is, from the earlier data, 0.14 ± 0.03 unit[5] per hour. If the rate of recovery is assumed to be unchanged by the antibiotic, the data on D in the preceding paragraph lead to the prediction that posttreatment with the antibiotics will decrease mutation by between 0.3 and 0.5 unit. The actual change is about 0.15 ± 0.03 for both antibiotics, which strongly suggests that the antibiotics slow recovery.

A quantitative measure of the rate of recovery can be obtained by varying the time of irradiation with paramecia growing at a slow rate in

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[5]The units are those of a variance-equalizing transformation (see ref 3) that gives a variable that is nearly linearly related to dose of radiation and so, presumably, to the number of mutations.

low concentrations of the antibiotics. Recently divided paramecia were put into 0.2 mg/ml of streptomycin or 0.06 mg/ml of chloramphenicol; and groups were irradiated in the antibiotic at different times after division. The paramecia were kept in the antibiotic until the day after irradiation, by which time they had undergone one to two divisions instead of the normal five. There was a small, though not significant, increase in mutation as the time of irradiation was increased. The rate of increase for the two antibiotics was nearly the same, the average being 0.045 ± 0.036 unit per hour, which is significantly lower than the rate for untreated paramecia.

Discussion. – The data suggest that the antibiotics increase the time available for recovery but also decrease its rate. The quantitative relation between these two opposing effects is such that mutation is usually decreased by the posttreatment though an increase might be anticipated under some circumstances. It seems very probable that recovery is a metabolic process, quite possibly involving protein synthesis since chloramphenicol is a well-known inhibitor of protein synthesis and our own observations show that streptomycin inhibits over-all growth in *Paramecium*. Further support for these ideas is given by the data for starved paramecia reported previously.[3] The rate of recovery under starvation conditions is only 0.011 ± 0.003 unit per hour, which is very much less than for well-fed log-phase paramecia.

Our results are in harmony with Wolff's suggestion[6] that protein synthesis is needed for restitutional reunion of chromosome breaks. They are also in harmony with the conclusion of Stapleton, Sbarra, and Hollaender[7] that recovery from potentially lethal damage in bacteria requires metabolic support, perhaps the synthesis of new enzymes. They are the opposite of Witkin's conclusion[8] that protein synthesis "stabilizes" the mutation process for ultraviolet-induced mutations in bacteria. A complete evaluation of the relation of these various findings to each other cannot be given within the confines of a short report.

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[6]S. Wolff and H. E. Luippold, Biol. Semiann. Prog. Rep. Feb. 18, 1958, ORNL-2481, p 3–4.

[7]G. E. Stapleton, A. J. Sbarra, and A. Hollaender, J. Bacteriol. 70, 7–14 (1955).

[8]E. M. Witkin, Cold Spring Harbor Symposia Quant. Biol. 21, 123–40 (1956).

## The Final Survival of Diploid and Tetraploid Clones of *Tetrahymena pyriformis* in Response to Various X-Ray Doses

O. C. Wells

Introduction. – Recently it has been demonstrated that tetraploid *Saccharomyces cerevisiae* are more radiosensitive than diploid cells of the same species.[9] Since clones of the ciliate *Tetrahymena pyriformis* lend themselves readily to cytological study, tetraploid and diploid clones of this organism have been X-irradiated in order to (1) establish survival curves for these clones and (2) combine cytological and radiation studies in an effort to gain better understanding of differences in radiosensitivity among different clones of the same species.

The clones of *T. pyriformis* used in this investigation will be designated I, II, and III in this report. The haploid chromosome number of these clones is 5. Clone II is tetraploid; the other two clones are diploid. Furthermore, clone III is an $F_1$ progeny from a cross between I and II. Organisms were maintained in an axenic peptone-tryptone medium.[10]

A series of X-ray doses ranging from 50 to 350 kr, delivered at a rate of approximately 10 kr/min, was administered to each of the three clones in the presence of catalase. Multiple single cell isolations were made in axenic medium to which 0.15% SRD antibiotic had been added. At least 100, and no more than 300, cells have been isolated for each point on the survival curves. Final survival was measured as the ability of the organisms to establish clones.

Results. – The preliminary survival data are plotted in Fig. 1. These data indicate that clone I is about three times more resistant to X radiation than clones II and III. Another difference among the clones is apparent: the survival of clone II cells is exponential, whereas survival of the two diploid clones is sigmoid. The data are not sufficient, however, to assure that the apparent difference in the form of the curves is real. Death of the cells was not immediate even at the highest doses administered. Organisms of all clones and at all doses used survive at least two days

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[9] R. K. Mortimer, Radiation Research 9, 312–26 (1958).

[10] A. M. Elliott and R. E. Hayes, Biol. Bull. 105, 269–84 (1953).

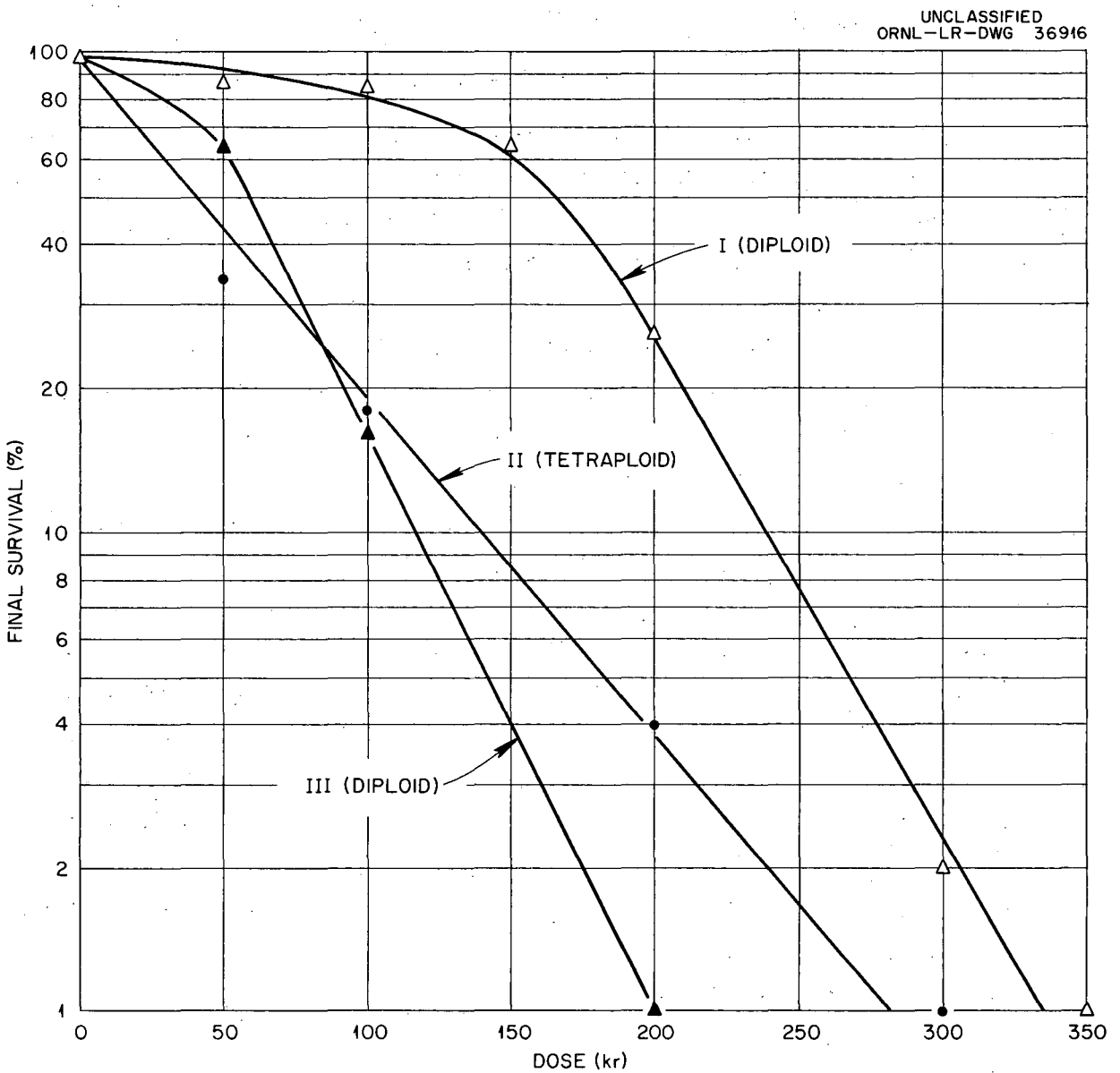


**Fig. 1. X-Ray Survival Curves for One Tetraploid and Two Diploid Strains of *Tetrahymena pyriformis*.**

after treatment. Many organisms exhibited a delayed type of death, sometimes living as long as four weeks after irradiation before dying.

Washed cells of each strain were submitted to starch gel electrophoresis to test (by the benzidene reaction) for possible differences in catalase content, which may have accounted for the observed dissimilarities in radiosensitivity. However, no difference in catalase content among the strains was detectable by this method.[11]

Discussion. – The results obtained in this investigation indicate that the ploidy of the micronucleus of *Tetrahymena* is not directly responsible for the sensitivity of the organism to X radiation. This is not a startling observation, since there is no reason to assume that the ploidy of the micronucleus determines the amount of polyploidy in the macronucleus. There has been no difference observed in the size and Feulgen staining intensity of the macronuclei of the clones in question.

The survival curves for the tetraploid (II) and for one of the diploids (III) are similar but are strikingly different from the survival curve of the other diploid (I). This is the first case of different radiosensitivities among clones of the same ciliate species that has been reported. The difference in radiosensitivity may have (1) a dominant chromosomal basis or (2) a cytoplasmic basis. The fact that the survival curve of III, an $F_1$ clone, closely resembles that of one of its parents (II) suggests a dominant chromosomal basis for the phenomenon. However, the possibility of a cytoplasmic basis for the results is not excluded. Since clone III was produced by conjugation between clones I and II, the cytoplasm of this $F_1$ clone may be directly descended, by fission, from the cytoplasm of the clone II pair member.

## Evidence for Complementation in Heterokaryons Among *ad-3B* Mutants of *Neurospora crassa*

F. J. de Serres

Early work with a series of 24 purple adenine mutants in the *ad-3* region provided evidence for complementation in heterokaryons only in A + B combinations. All A + A or B + B combinations were negative.[12] One

[11]Appreciation is expressed to R. A. Popp for the electrophoretic analysis.

[12]F. J. de Serres, Genetics 41, 668–76 (1956).

possible objection to such negative tests is that failure to complement might be simply attributable to a failure to obtain hyphal fusion and nuclear mixing with such combinations under the experimental conditions used. Later, more comprehensive experiments[13] in which the forced heterokaryon technique was used on a large series of 13 ad-3A mutants provided unequivocal evidence for the lack of complementation between these ad-3A alleles in all pairwise combinations in heterokaryons. Thus the failure to obtain complementation could not be attributed to a failure to obtain hyphal fusion and nuclear mixing. Therefore, it appeared that the proper interpretation of the negative tests with unforced heterokaryons was that the mutants involved were functionally identical. Recently, the complementation analysis in the ad-3 region has been extended further. The present experiments are concerned with such tests on a series of 160 ad-3B mutants in all pairwise combinations in heterokaryons.

Results. – Of the 25,600 pairwise combinations tested, complementation was observed only in 64. The results of these tests indicated that the 160 ad-3B mutants can be subdivided into three groups. The first group of 148 mutants do not react with any other ad-3B mutants, but the second group of 4 mutants react with all mutants in a third group of 8 mutants to give heterokaryons that grow at wild-type growth rate. The mutants in the second group of 4 differ from all the others in that they grow very slowly on minimal medium and gradually assume wild-type rate after ten or more days' growth. All mutants in this group react in the same way with the mutants in the third group. Of the 8 mutants in this group, 5 react with the 4 mutants in the second group to give heterokaryons that initiate growth after 24 hr (similar to A + B combinations) and grow at wild-type growth rate; 2 react to give heterokaryons that initiate growth after 48 hr and start off more slowly but rapidly achieve wild-type growth rate, and the remaining mutant reacts to give heterokaryons that initiate growth after 72 hr, that grow very slowly, and achieve wild-type growth rate only after 4–5 days' growth.

Discussion. – The present analysis has provided evidence for complementation among certain members of a large series of allelic ad-3B mutants

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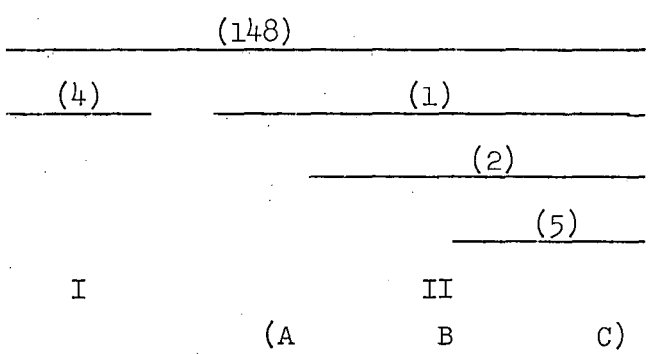
[13]F. J. de Serres, Biol. Semiann. Prog. Rep. Aug. 15, 1957, ORNL-2390, p 25–26.

that results in the adenine-independent growth of B + B heterokaryons. In this respect the present analysis is quite similar to that found with the ad-4 group in Neurospora.[14,15] If the ad-3B complementation reactions are analogous to the patterns of complementation in the ad-4 group the present data may be used to construct a tentative complementation map of the ad-3 region. To do this we must assume that the ad-3B locus consists of a number of functional regions (cistrons) arranged in a linear order along the chromosome and individual mutants have either one or more regions in the ad-3B locus nonfunctional. The ordering of mutants on such a map is based on overlapping of nonfunctional regions. Mutants with overlapping nonfunctional regions do not complement in heterokaryons, whereas mutants with nonoverlapping nonfunctional regions do complement to give adenine-independent growth. The fact that individual members of the third group of 8 mutants react differentially with the 4 members of the second group suggests (by analogy) that those combinations that react after 72-hr incubation and initially grow slowly have nonfunctional regions separated by one or a few cistrons whereas those combinations that start off rapidly, at wild-type rate, have nonfunctional regions separated by many cistrons.




Complementation Map of ad-3B Locus

The present analysis provides evidence for a minimum of two cistrons in the ad-3B locus and possibly as many as four. We have no way of knowing whether the mutants in the third group have just a single nonfunctional cistron in common or whether more are involved. If such a complementation

[14]N. J. Nelson, unpublished Ph.D. thesis, Yale University (1957).

[15]D. O. Woodward et al., Proc. Natl. Acad. Sci. U.S. 44, 1237-44 (1959).

map is a valid representation of the reactions observed, further tests of new ad-3B mutants might be expected to provide mutants with nonfunctional regions in positions II(A) or II(B) that should complement with all 4 members of the second group and only certain of the 8 mutants in the third group in a predictable pattern.

## Interaction of MEA and X Rays. Mutagenicity, Toxicity, and Protection Studied in *Neurospora*

H. G. Kølmark

Introduction. – Preliminary experiments[16] showed a marked increase in the toxic and mutagenic effects of X rays when MEA was present before and during irradiation. These results were unexpected since experiments with MEA in which other organisms were used showed a very definite protection[17,18] against the lethal effects of X rays or against mutations.[19] One main difference in the experimental technique was that, in the *Neurospora* experiments, MEA treatment was given in $H_2O$ instead of saline. The purpose of the present investigation was to determine whether the striking difference in the experimental results with *Neurospora* could possibly be attributed to such a difference in the method of MEA treatment.

Results. – The strain and general procedure were the same as in the former report.[16] Toxicity was measured by the percentage survival after treatment and the mutagenic effect as the frequency of reverse mutations per $10^6$ survivors in an ad-3A strain (38701). Pretreatment and irradiation of conidia were carried out at ice-water temperature. The saline contains 3 g of NaCl, 0.2 g of KCl, and 0.2 g of $CaCl_2$ per 1000 ml of distilled water.[20] In a pilot experiment with X rays and MEA in saline, it was found that MEA gave a definite protection, as judged by the increased

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[16] H. G. Kølmark, Biol. Semiann. Prog. Rep. Feb. 15, 1958, ORNL-2481, p 10–11.

[17] D. G. Doherty, W. T. Burnett, Jr., and R. Shapira, Radiation Research 7, 13–21 (1957).

[18] A. Hollaender and C. O. Doudney, Radiobiol. Symposium, Proc., Liège, 1954, p 112–15 (1955).

[19] A. Hollaender and G. E. Stapleton, Proc. Intern. Conf. Peaceful Uses Atomic Energy, Geneva, 1955, 11, 311–14 (1956).

[20] A. Hollaender and W. D. Claus, J. Gen. Physiol. 19, 753–65 (1936).

survival and decreased mutation rate, with a threshold of interaction between $1 \times 10^{-3}$ and $5 \times 10^{-3}$ M MEA. In the next experiment (Fig. 2a), the concentration of MEA was varied from the estimated threshold $2.5 \times 10^{-3}$ to $8 \times 10^{-2}$ M.

At all X-ray doses, essentially the same increase in survival was found when the MEA concentration was increased, until a maximum survival was reached at the concentration level 0.01 to 0.02 M MEA. Also, at these concentrations of MEA, there was a definite decrease in mutation rate. This decrease leveled off when MEA reached 0.02 M. However, at the two highest X-ray doses (96 and 120 kr), no such decrease in mutation rate was detected. In Fig. 2b is shown for comparison the preliminary experiment[16] performed in $H_2O$, in which MEA was strongly toxic. The curves in Fig. 2b show the marked decrease in survival and increase in mutation rate when MEA was present in water during X irradiation.

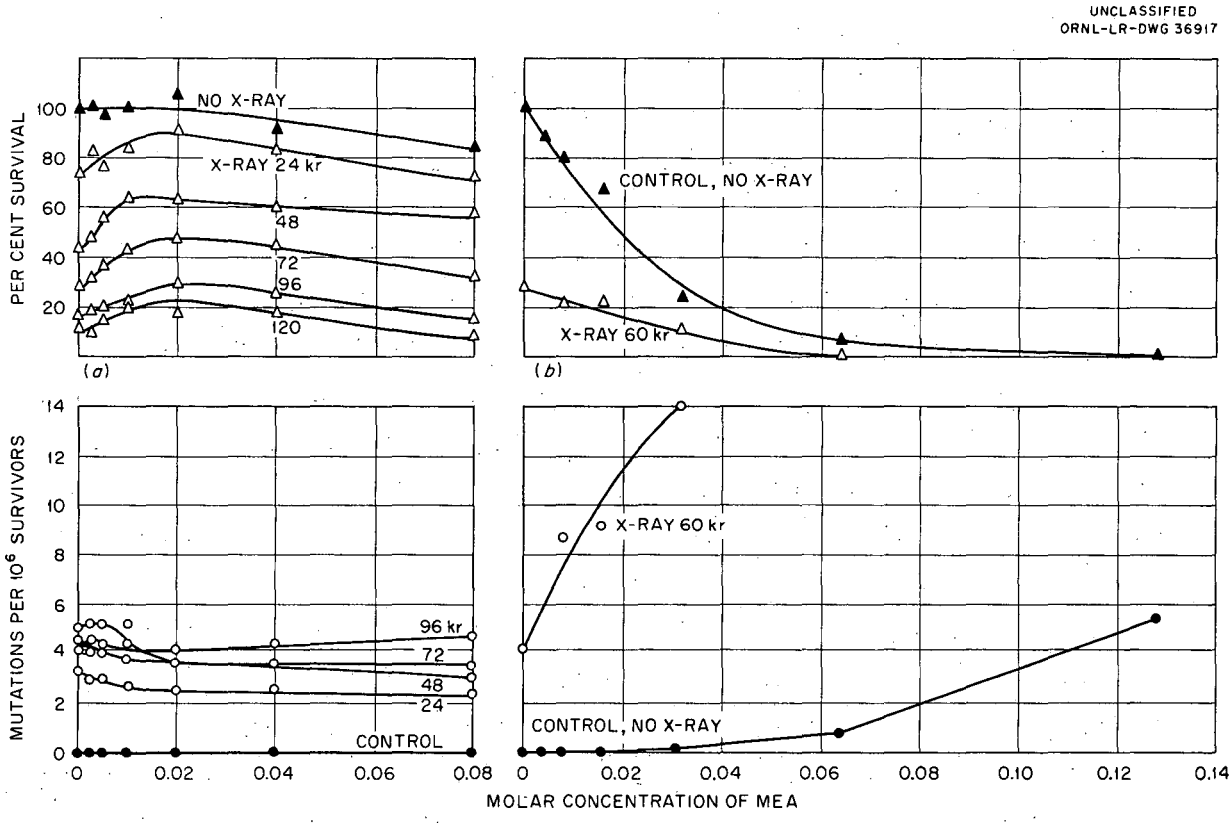


**Fig. 2. Effect of MEA on Survival and *ad-3A* (38701) Reverse Mutation Rate After X-Ray Treatment in *Neurospora*.** (*a*) Treatment in saline; $93 \times 10^6$ conidia treated at each point in the graph; (*b*) treatment in $H_2O$; $78 \times 10^6$ conidia treated at each point in the graph.

Discussion. — These experiments show that the radiation-protective ability of MEA is greatly enhanced by the presence of an environment of dissolved salts. In the *Neurospora* system, maximum protection is reached when MEA is present in the concentration level 0.01 to 0.02 *M*. This optimal concentration is the same for protection against both the lethal and the mutagenic effects of X rays. Thus there might be an interrelation of protection against the lethal and the mutagenic effects of X rays, as has also been found by Hollaender and Stapleton.[19]

At MEA concentrations of 0.02 *M* or higher, there is no further change in the rate of mutation. The decrease in survival in the X-irradiated series runs parallel with the nonirradiated series; thus X irradiation of MEA in saline solution, even very high concentrations, does not lead to the production of any new toxic or mutagenic compounds, in contrast to the experiments in water.

## Reinvestigation of Mutagen Specificity

C. Auerbach H. G. Kølmark

Introduction. — A case of apparent mutagen specificity was found when a double mutant strain (*ad-3A inos*) of *Neurospora* was given mutagenic treatment with DEB or UV. In these experiments, treatment with DEB yielded more adenine reverse mutations ($ad^+$) than inositol reverse mutations ($inos^+$), giving an $ad^+$:$inos^+$ ratio of 450:1, whereas UV treatment had the reverse effect, giving an $ad^+$:$inos^+$ ratio of 0.65. These data were interpreted as indicating that DEB produces more $ad^+$ than $inos^+$, whereas UV produces more $inos^+$ than $ad^+$.[21] The senior author thought that these results could be explained on the basis of some mutagen-specific cytoplasmic effect. With this hypothesis, it is assumed that a mutagen induces as many reverse mutations of one type as another. But, because of some mutagen-specific cytoplasmic effect, which prevents the expression of one type of reversion but not another, reversions of different mutants are not found in equal numbers after mutagenic treatment. In the previous experiments, then, treatment of the cytoplasm with DEB hindered the expression of $inos^+$, whereas treatment with UV hindered the expression of $ad^+$. This hypothesis

[21] G. Kølmark, Hereditas 39, 270–76 (1953).

was tested by using combined mutagenic treatments. If treatments were given in the order DEB + UV, one would expect a more-than-additive number of $inos^+$ and a less-than-additive number of $ad^+$. Treatment in the order UV + DEB would give an expectation of the opposite effect.

Results. – Tables 1 and 2 give the results obtained when conidia of the double mutant strain were treated with DEB, UV, or both. In both experiments, the conidia were washed after the first treatment and then exposed to the second. After the combined treatments in which DEB was given first, more than 10.1 $inos^+$ and less than 16.6 $ad^+$ would be expected if the hypothesis were correct. The data do not bear out this expectation. After the combined treatment in which UV was given first, less than 4.4 $inos^+$ and more than 41.2 $ad^+$ would be expected on the basis of the hypothesis. The $inos^+$ rate is only slightly lower than the additive rate of 4.4 and is certainly not the decrease expected if DEB were hindering the expression of $inos^+$. The $ad^+$ rate from the combined treatment is lower than the additive rate of 41.2, rather than higher.

Table 1. Effect of Single Treatments with DEB and UV and Combined Treatments in the Order: DEB + UV

| Treatment | Survival (%) | $inos^+$ per $10^6$ Survival | $ad^+$ per $10^6$ Survival |
|---|---|---|---|
| Control | 100 | 0.09 | 0.03 |
| DEB 0.1 M, 34 min | 77 | 0.11 | 8.5 |
| UV 7000 ergs/mm$^2$ | 73 | 10.0 | 8.1 |
| DEB + UV | 35 | 9.5 | 34.5 |

Table 2. Effect of Single Treatments with UV and DEB and Combined Treatments in the Order: UV + DEB

| Treatment | Survival (%) | $inos^+$ per $10^6$ Survival | $ad^+$ per $10^6$ Survival |
|---|---|---|---|
| Control | 100 | 0.016 | 0.047 |
| UV 6600 ergs/mm$^2$ | 82.2 | 4.3 | 2.8 |
| DEB 0.075 M, 60 min | 82.3 | 0.1 | 38.4 |
| UV + DEB | 61.3 | 4.0 | 27.4 |

Discussion. – The present experiments provide no evidence to support the hypothesis that the phenomenon of mutagen specificity can be attributed to a mutagen-specific cytoplasmic suppression of certain types of reverse mutations. The data do not support the hypothesis that mutagens induce an equal number of reverse mutations in all mutants but rather that reverse mutation of a given mutant is a mutagen-specific reaction.

## Mutagenic Aftereffect

H. G. Kølmark C. Auerbach

Introduction. – During the study of mutagenic treatments with DEB and UV in *Neurospora* it was often found that the mutation rates obtained after combined treatments with these agents were far from being the sum of the rates of the two single treatments. Moreover, it was difficult to obtain reproducible results in repeat experiments.

These observations suggested that, in some cases, there might be a considerable mutagenic aftereffect. Production of mutations perhaps was not completely stopped by the usual procedure of washing the treated conidia twice in $H_2O$. Some experiments have now been carried out to follow in detail how the mutation rates and survival are influenced in the interval between washing and plating the treated conidia. The experiments presented here were particularly designed to follow the effect of temperature and time in this interval when conidia had been treated with DEB.

Results. – The strain used and the general procedure are as described in a previous Semiannual Report.[22] The first experiment showed a definite time- and temperature-dependent aftereffect. Conidia were treated with 0.1 M DEB at 20°C for 34 min, then washed and placed in distilled $H_2O$ at various temperatures. One group of $46 \times 10^6$ treated conidia incubated for 1 hr at 4°C yielded 100 $ad^+$, whereas a second group incubated for 4 hr at 30°C yielded 830. The number of $inos^+$ was negligible, 0 and 2, respectively. Survival decreased less than 5% at the higher temperature and longer incubation period.

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[22]H. G. Kølmark, Biol. Semiann. Prog. Rep. Feb. 15, 1958, ORNL-2481, p 10–11.

Another experiment was designed to check the possibility that the continued production of mutations after DEB treatment was caused by a trace of the chemical not removed by the washing procedure. Conidia were treated with 0.1 M DEB for 70 min at 20°C. Aliquots were then washed two and five times before incubation at 4° and 30° for periods up to 25 hr. The result is shown in Fig. 3.

It can be seen from the 30°C curves that the result was contrary to that expected if the mutagenic aftereffect had been caused by a trace of the chemical after the usual two washings. Washing five times resulted in considerable additional increase in mutation rates.

Discussion. – The marked aftereffect observed takes place under conditions usually not considered mutagenic; that is, incubation in distilled water at increased temperature and length of time. Transfer of the treated conidia to growth conditions stops the mutagenic aftereffect.

A very similar aftereffect was shown as reverse mutations induced by triethylene melamine in *Escherichia coli*.[23] The aftereffect in the present study is increased by excess washing and thus cannot be attributable to a trace of DEB. This last increase might indeed be caused by the extensive centrifugation rather than by the washing as such. An increase of chromosome aberrations in *Tradescantia* microspores and in *Vicia* seed was observed when the material was subjected to centrifugation after X irradiation.[24]

The present experiments indicate that the conditions in the period between mutagenic treatment and initiation of growth can have very marked effect on the rate of mutation. This period seems to be highly susceptible to a variety of influences usually considered as nonmutagenic.

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[23] V. N. Iyer and W. Szybalski, Proc. Natl. Acad. Sci. U.S. 44, 446–56 (1958).

[24] S. Wolff and R. C. von Borstel, Proc. Natl. Acad. Sci. U.S. 40, 1138–41 (1954).

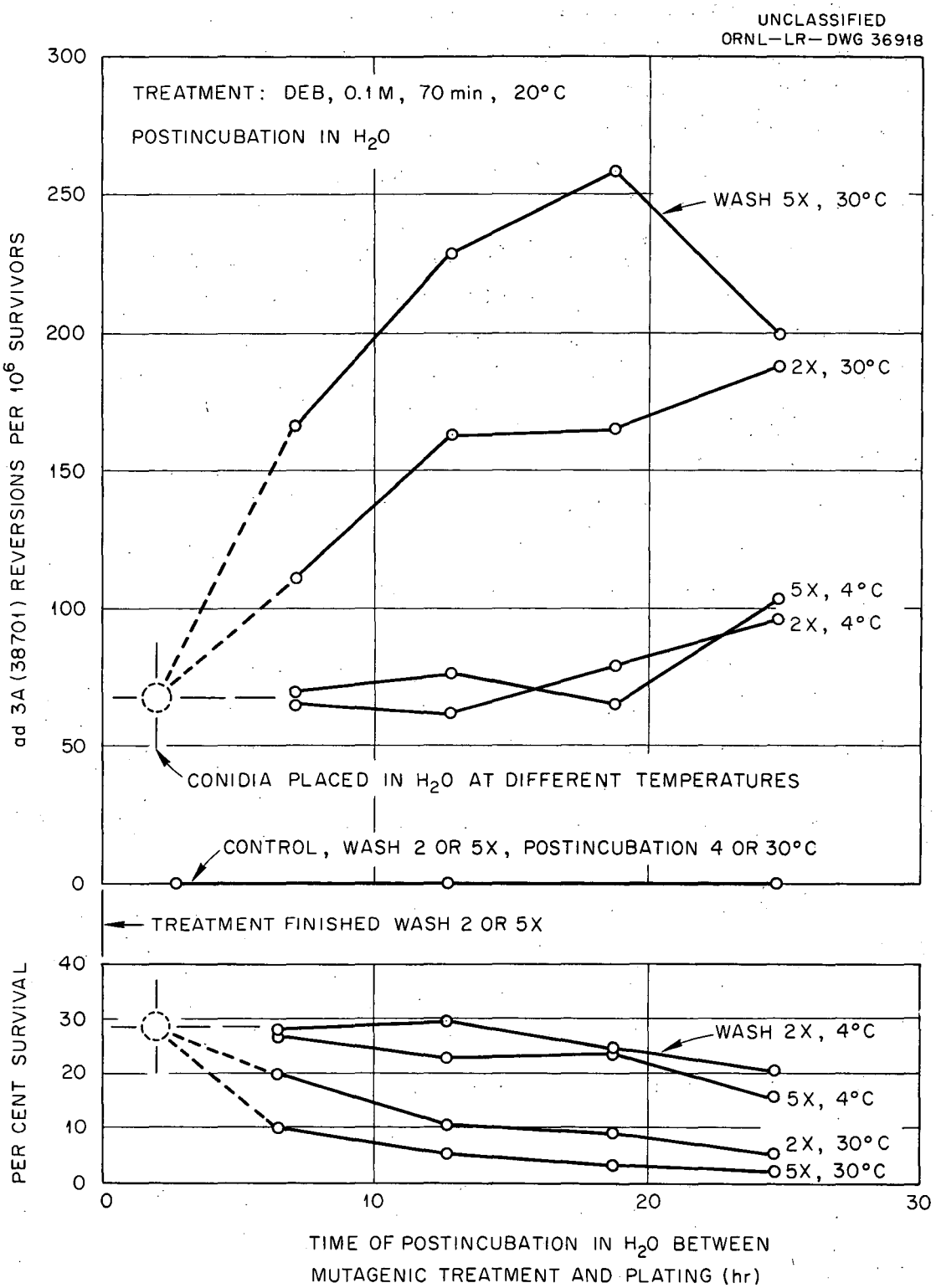


**Fig. 3. Effect of Prolonged Postincubation in Distilled $H_2O$ at 4 and 30°C on *ad-3A* (38701) Reverse Mutations and Survival in *Neurospora*.** Also, a comparison of the effect of washing two and five times and centrifugation after mutagenic treatment. Conidia were treated with 0.1 *M* DEB for 70 min at 20°C. This treatment was finished at time 0 in the graph. $78 \times 10^6$ conidia were treated at each point plotted.

Genetic Evidence Demonstrating the Occurrence of Nuclear Migration in Schizophyllum

P. J. Snider, Jr.

Introduction. – In various fungi, nuclei of one genotype can be translocated for considerable distances (0.1 to >10 cm) across a preexisting mycelium of another genotype.[25,26] Such nuclear transport could occur, in the absence of asexual spores, either by intrusive growth of new hyphae of one genotype among the preexisting hyphae of the other type or by actual intercellular migration of nuclei (after hyphal fusion) within the genetically foreign protoplasm of the preexisting hyphae. Although some of the published evidence definitely favors the actual migration of nuclei,[25,26] most of the reports do not really answer the question. The following experiment makes the conclusion absolute in Schizophyllum commune, a Basidiomycete that lacks asexual spores.

This critical experiment depends (1) upon observing translocation where intrusive growth could not possibly occur unobserved and (2) upon using meiosis as a test for what, in fact, was transported. Analysis of meiosis will distinguish between transport of whole nuclei, parts of nuclei, gene products, or cytoplasmic particles. The morphological mutant puff (p),[27] which is uniquely favorable material for deciding between migration or intrusive growth, differs from wild type in two essential characters: (1) a puff hypha looks very much like beads scattered along a string (Fig. 4a), each bead, or puff, originating from extensive branching of a lateral branch hypha, and (2) a puff mycelium can be grown with the mature hyphae so well separated from one another that single hyphae often extend for several centimeters without touching other hyphae and intrusive growth of foreign hyphae simply could not happen unnoticed.

Results. – Haploid hyphae of the genotype $A^4$ $B^4$ $u^+$ $p^+$ were placed upon a mature, haploid mycelium of the genotype $A^2$ $B^1$ u p, and translocation was observed in the puff mutant. (A and B are incompatibility loci and u is uracilless.) Progressive initiation of wild-type growth along a linear

[25] A. H. R. Buller, Researches on Fungi. IV. Longmans, Green & Co., London, 1931.

[26] P. J. Snider and J. R. Raper, Am. J. Botany 45, 538–46 (1958).

[27] J. R. Raper and P. G. Miles, Genetics 43, 530–46 (1958).

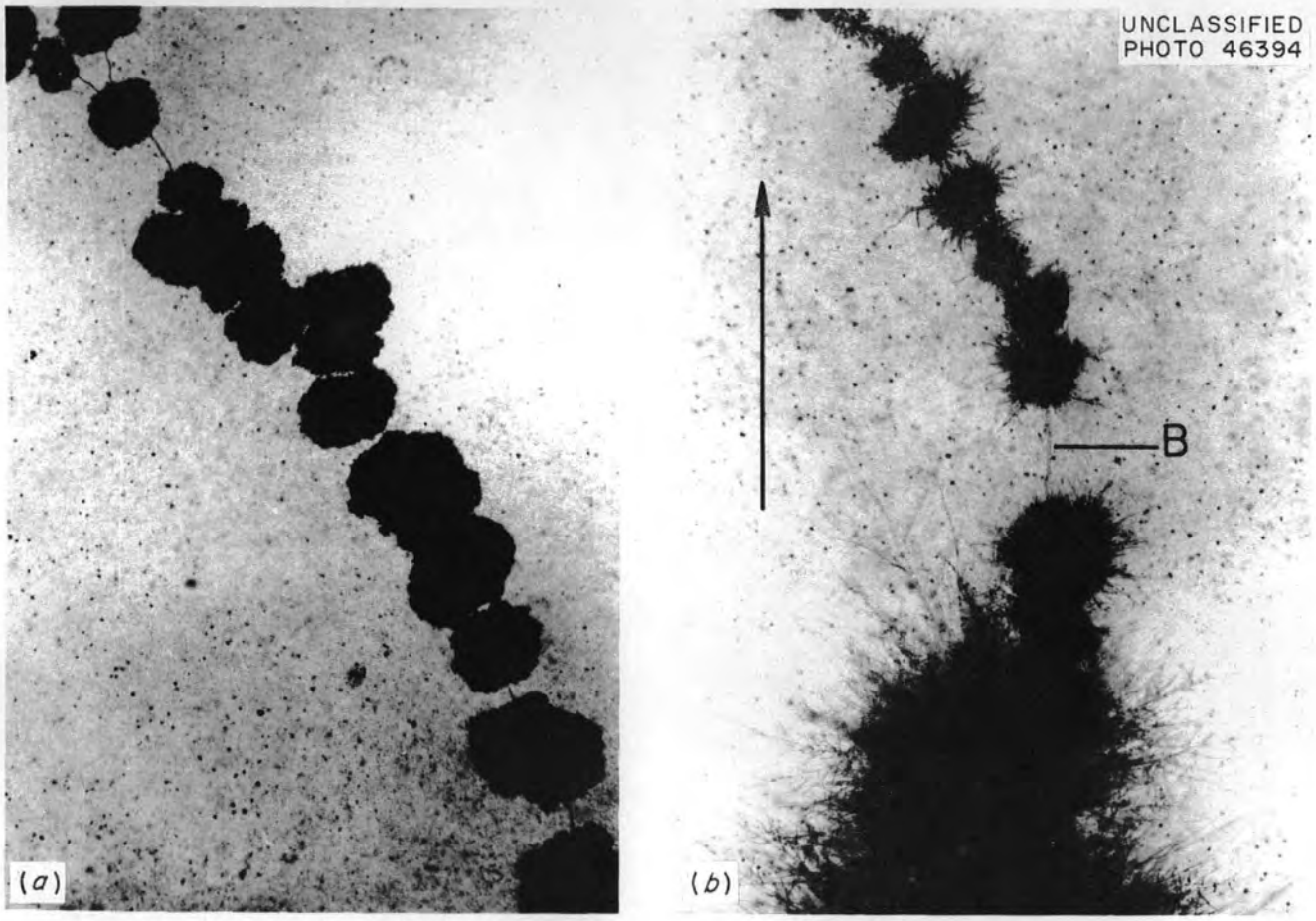


**Fig. 4. Nuclear Migration in** *Schizophyllum*. (*a*) A hypha of the morphological mutant *puff* (genotype $A^2\ B^1\ u\ p$). The single thread connecting the puffs can be seen between the well-separated puffs. 80×. (*b*) A hypha of the same *puff* mutant being induced to initiate wild-type growth by what are proved to be $A^4\ B^4\ u^+\ p^+$ nuclei which migrated through the single hyphal bridge (B) in the direction indicated (arrow). 80×.

series of puffs on a single hypha serves as a visual indicator of the translocation of $\underline{p}^+$ material. The 1–2% of the hyphae that initiated wild-type growth were easily located by scanning the cultures rapidly at 30× magnification, and then each such hypha was examined critically at 200×. In a number of instances whole strings of puffs initiated wild-type growth while still attached to the parent mycelium by unmistakably no more than the one puff hypha from which the whole string originated (Fig. 4b). Whatever the nature of the $\underline{p}^+$ material, it was certainly translocated within the preexisting hyphal bridges between the puffs. Before any new hyphal bridges became established, the most distal puff to initiate wild-type growth, on several of these critical hyphae, was isolated, cultured, and fruited, in order to obtain basidiospores, which are the direct haploid products of meiosis in Schizophyllum. During the time $\underline{p}^+$ material was translocated 30 mm, intrusive hyphal growth from the $\underline{A}^4$ $\underline{B}^4$ $\underline{u}^+$ $\underline{p}^+$ inoculum proceeded no more than 3 mm.

Previous data indicate all the genetic markers used here should behave as single gene characters and no linkage should be detected among the four loci in samples of several hundred progeny.[27] Analysis of such samples from the mushrooms known to contain translocated $\underline{p}^+$ material gave the following results (Table 3): (1) 1:1 segregation appears probable at the 1% level of significance for each of the four pairs of alleles, (2) no evidence of linkage was obtained, and (3) all 16 possible $F_1$ genotypes were recovered in about equal frequency. Meiosis appears to have occurred normally.

Conclusion. – The first part of this experiment shows conclusively that $\underline{p}^+$ material, capable of changing puff-phenotype to wild-type morphology, is translocated by intercellular migration within single, preexisting hyphae. The second part shows that translocated $\underline{p}^+$ material is genetic, can participate normally in meiosis, and remains associated with several nonlinked genetic markers. Translocated $\underline{p}^+$ material is therefore contained within whole nuclei. The experiment demonstrates unconditionally that whole nuclei migrate within preexisting hyphae of Schizophyllum for distances of centimeters. This conclusion strongly implies, in view of the literature on the translocation of nuclei, that true migration of nuclei is probably common to all major groups of filamentous fungi.

Table 3. Test for Normal Meiosis at Four Nonlinked Loci

Presumed Migrant Nuclei × Resident Nuclei

$\underline{A}^4\ \underline{B}^4\ \underline{u}^+\ \underline{p}^+ \times \underline{A}^2\ \underline{B}^1\ \underline{u}\ \underline{p}$

| Segregation of – | No. of Progeny | $\chi^2$ | P of 1:1 Ratio |
|---|---|---|---|
| $\underline{p}^+$:$\underline{p}$ | 165:151 | 0.62 | 0.43 |
| $\underline{u}^+$:$\underline{u}$ | 176:140 | 4.10 | 0.043 |
| $\underline{A}^4$:$\underline{A}^2$ | 139:177 | 5.06 | 0.024 |
| $\underline{B}^4$:$\underline{B}^1$ | 149:167 | 1.02 | 0.31 |
| **Linkage of –** | **Parental:Nonparental** | **$\chi^2$** | **P of 1:1 Ratio** |
| A to B | 158:158 | 0 | 1.00 |
| A to u | 164:152 | 0.456 | 0.50 |
| A to p | 157:159 | 0.012 | 0.92 |
| B to u | 161:155 | 0.114 | 0.75 |
| u to p | 156:160 | 0.050 | 0.82 |
| **No. of Progeny/Class in the 16 $F_1$ Genotypes** | | **$\chi^2$** | **P of Equal Frequency** |
| min 10, mean 20, max 28 | | 18.65 | 0.25 |

## Genetic Studies on Enzymes in Maize Endosperm

D. Schwartz
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K. H. McGrath
A. N. Best

The technique of starch gel electrophoresis is being used in a study of the enzymes involved in starch synthesis and degradation. The experiments are designed to compare the enzymes produced by a series of mutants that show abnormal starch synthesis in a study of gene enzyme relationships. Starch gel electrophoresis is well suited for study of these enzymes since the starch is also the substrate upon which these enzymes act. The migration of the enzymes in the gel is dependent both on their charge

and their affinity for the substrate. The enzymes that show substrate affinity are separated from all other soluble proteins in the extract, whose mobility on the gel is dependent solely on their charge. The other proteins move out rapidly, and the enzymes involved in starch synthesis are left behind and are found as bands closest to the point of origin. A second advantage of this technique is that it is possible to recognize these enzymes also by their enzymatic activity in cleavage of the starch molecules of the gel. The gel is made up of both amylose and amylopectin and turns blue when stained with iodine. The protein bands that are enzymically active in cleaving the starch molecules can be recognized as such by the change in the reaction with iodine, appearing as red bands on the blue background of the gel. Of the five slowest moving protein bands (as determined by protein staining with Buffalo Black) four act on the starch and appear as red bands. One of these enzymes has been tentatively identified as β-amylase. It shows only weak activity by the iodine test but has such a pronounced effect on the structure of the gel that the position of the band can easily be seen even in unstained material. In extracts with a high concentration of this enzyme the migration of the slower moving bands is affected because of the modification in gel structure. Fortunately, it was found that this enzyme is not salted out by 55% ammonium sulfate, a concentration which does precipitate the other four enzymes we are studying. It is therefore quite easy to fractionate the crude extracts from young endosperms and eliminate the disturbing effect of this enzyme. The 55% ammonium sulfate fractions from young endosperms of the following genotypes were analyzed: Wx/Wx/Wx Su/Su/Su (KYS inbred); Wx/Wx/wx Su/Su/Su; wx/wx/wx Su/Su/Su (waxy); and Wx/Wx/Wx su/su/su (sugary). Pending identification of the enzymes, the bands will be numbered 1 to 4 starting from the point of origin. The banding patterns of the first two genotypes, homozygous and heterozygous Wx, are essentially identical, differing only in the concentrations of the enzymes in the bands. These will be referred to as standard. The only noticeable difference in the banding pattern of the waxy mutant was a change in the position of band No. 2, which moved slower than standard. In the sugary mutant the position of band No. 4 was identical to standard but the other three bands showed considerable lagging. The same was the case for the β-amylase-like enzyme, which was precipitated above 55% ammonium sulfate and run separately on the

gel. The differential mobility of these bands can reflect differences in either charge or affinity. The sugary mutant contains a much higher concentration of the enzyme that bands out in the No. 4 position than do the other genotypes; however, it shows much reduced activity in cleavage of the starch molecules as determined by the starch test, suggesting enzymatic modification.

The sugary mutant has four bands in positions different from those in standard. However, when mixtures are made of the sugary and standard extracts only five bands are formed instead of the expected nine. These bands are in positions intermediate between those found in sugary and in standard. If the two extracts are run side by side on the same gel so that opposing gradients are formed across the gel, a continuous, sharply defined band connects each of the fast moving bands of standard with each of the corresponding slower moving bands of sugary. Since band No. 4 shows the same rate of migration in both types it forms a straight line across the gel. These experiments suggest interactions of the enzymes, but explanation of this phenomenon must await more complete fractionations so that each enzyme can be run separately on the gel. Either the slower moving enzymes in the sugary mutant are different from those in standard or their migration is influenced differentially by other proteins which moved ahead. A large number of different genes that affect starch synthesis in the maize endosperm are under investigation as well as a comparison of the same enzymes produced by different tissues of the same plant.

## Further Studies of Extracellular Ultraviolet-Induced Mutation in Phage T4

D. R. Krieg

Introduction. — Irradiation of T4r bacteriophage with ultraviolet light prior to infection of unirradiated bacteria induces $r^+$ reversions. Almost all the previous experiments of this kind were done with a particular allele, $r_{59}$, but more recent work shows that the induced reversion reflects a more general process of induced mutation. Additional work has investigated the dependence of induced mutation on multiplicity reactivation, and a preliminary experiment indicates the feasibility of a direct test to decide whether induced mutation can occur without any such reactivation.

Results and Discussion. — The phenomenon of induced reversion is not unique to T4 $r_{59}$. Parallel treatment of this and two other $r_{II}$ mutants of spontaneous origin gave similar results. T4 $r_{71}$, with about the same spontaneous reversion rate, yielded about 40% as many induced revertants as T4 $r_{59}$; T4 $r_{65}$, with at least ten times higher spontaneous reversion, produced not more than twice as many induced revertants.

The same treatment which induced reversions of T4 $r_{II}$ mutants to T4 $r^+$ can also induce genetic changes in the opposite direction. This was shown by an experiment on wild-type T4 phage, with and without extracellular irradiation. Significantly (about tenfold) more $r$ mutants were found in the progeny of the irradiated than the unirradiated phage. This could not be attributed to any selective advantage conferred by the $r$ character; a parallel reconstruction experiment with a mixture of T4 $r_{59}$ and excess T4 $r^+$ showed no appreciable changes in the proportions of $r$ and $r^+$ progeny. The question of whether genetic change can be induced in either direction at a single genetic locus is important in testing the interpretation of induced mutation. Alternative interpretations for radiation-induced genetic changes in viruses (e.g., replacement of a phage gene by a distinguishable bacterial homologue) have been considered,[28] but these could account only for changes in one direction.

Progeny phage have been produced in these experiments by multiplicity reactivation, a phenomenon in which two or more inactivated phage adsorbed to the same bacterium interact to produce viable progeny. Experiments of various designs have investigated the influence of this reactivation phenomenon on the induction of mutations. If it were found that the frequency of induced mutants at a given dose were independent of the conditions or amount of multiplicity reactivation, it would suggest that mutations were induced independently of lethal or other damages and the reactivation process simply facilitated their detection. Reducing the multiplicity of phage per bacterium strongly reduces the amount of reactivation; some experiments have indicated more induced revertants (up to fourfold) with lower multiple infection. When progeny are harvested at various times during phage reproduction, earlier lysates would tend to sample relatively undamaged or easily

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[28] D. Fraser, Virology 3, 527–53 (1957).

reactivated phage. Some experiments indicated that fewer induced revertants (as low as half as many) are found in the earliest lysates. However, the conclusions are still uncertain; in both cases other experiments showed no differences. The extremes are cited to show the direction and possible range of the results.

The interpretation should be simpler, however, if we could compare induced mutation among survivors whose reproduction does not involve reactivation, and among phage produced by multiplicity reactivation of killed phage at the same dose. Technical improvements and recent data indicate that this approach may now be practicable. In a preliminary experiment, wild-type T4 was irradiated with a low dose and progeny were obtained with multiple infection (mostly by reactivation) and with predominantly single infection (mostly from survivors). The numbers of r mutants observed were low, but significantly more were found with multiple infection than with either single infection or unirradiated controls. Additional work should reveal how many, if any, induced mutants can be recovered from survivors.

## The Induction of r Mutants in T4r$^+$ Bacteriophage by Ethyl Methane Sulfonate

D. M. Green

Introduction. — The exposure of extracellular bacteriophage to ethyl methane sulfonate leads to a substantial production of induced mutants. When T4r$^+$ phage are exposed to 0.4 M ethyl methane sulfonate, diluted more than $10^6$-fold, and plated on *Escherichia coli* strain B, about 1% of the plaques are recognizably r or mottled (partially r), as contrasted to 0.05% in controls. Less than 25% of the phage are inactivated by such treatment. These results confirm a report by Loveless[29] of the mutagenicity of ethyl methane sulfonate in phage. He observed that this agent induced r and host range mutants in a related phage, T2.

Results. — Thirty-four plaques scored as r or mottled were picked and the contents sampled to determine what proportion of the phage in each were mutant. No purely mutant plaque was found; the fraction of mutants varied from 0.96 to 0.008; and, in most cases, the mutant type was in the minority.

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[29]A. Loveless, Nature 181, 1212–13 (1958).

These results suggested that even upon single infection of bacteria by treated phage the induced mutants are produced in mixed bursts, and raised the possibility that bursts with too small a proportion of mutant types to produce recognizable mottling would not be scored as mutant. Data on the numbers and kinds of phage produced in the first interbacterial cycle of growth of the treated phage particles could more accurately test this inference and clarify its implications as to how the induced mutants are produced.

A preliminary "single-burst" experiment has been performed. Bacteria infected with treated phage at a mean multiplicity of 0.28 phage per bacterium were randomly distributed to tubes before lysis. Three series were made with means of 1, 2, and 4 infected bacteria per tube. Tubes were incubated to allow lysis and the contents of each tube plated and scored for r and mottled plaques. A total of 72 plates were observed to have one or more r or mottled plaques, and on each of these plates $r^+$ plaques were also observed. The numbers of r and mottled plaques in a burst ranged from 1 to 171. The mean total burst size of phage in this experiment was 180. The discrimination of mutants by the single-burst technique was decidely improved over the scoring of mottled plaques; the former showed 5% of the total bursts had mutant r types, whereas in the same experiment only 0.8% of the plaques produced by direct plating of the infected bacteria were scored as r or mottled plaques.

If it is assumed that these bursts of mutants arose by clonal growth with a normal rate of division once it had begun, the data may be analyzed as to the number of generations required to produce each "clone" of mutants (Table 4). Chi-square test of the numbers of clones in each size range from 1 to 127 shows no significant variation from the mean. These observations are consistent with a constant probability of a mutant clone beginning in any phage generation, except possibly the earliest. This distribution is distinctly different from that found by Luria[30] for spontaneous mutations in phage T2. Since this experiment did not reveal the total size of each burst in which a mutant clone appeared, it is not possible to draw inferences as to the fraction of mutant phage in a burst.

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[30]S. E. Luria, Cold Spring Harbor Symposia Quant. Biol. 16, 463–70 (1951).

Table 4. Size Distribution of Mutant Clones

| Generation | Clone Size | Number of Clones |
|---|---|---|
| 0 | 1 | 15 |
| 1 | 2–3 | 10 |
| 2 | 4–7 | 7 |
| 3 | 8–15 | 11 |
| 4 | 16–31 | 13 |
| 5 | 32–63 | 8 |
| 6 | 64–127 | 7 |
| 7 | 128–256 | 1 |

Discussion. – The implications of these results on the mechanism of induced mutation may now be considered. It is useful to conceive of two stages or events in the mutation process: the first interaction of the mutagen with the genetic material of the phage and "stabilization" of the mutated gene so that all its descendants will be mutant. If both events occurred during the extracellular treatment and phage were "haploid," no mixed bursts would be found, and this is not the case. Both events may occur before the reproduction of the phage, and the mixed bursts may be attributed to an effectively diploid phage (e.g., because of the two-stranded nature of DNA). Unless reproduction of the mutant gene is impeded relative to its nonmutated allele, the bursts would tend to be half mutant, and this is not the case. The stabilization may not occur until after the onset of viral reproduction within the bacterium. The first interaction might not itself "mutate" the gene (except to place it in a mutable state) but the gene so affected might have a constant chance per generation of initiating a pure mutant clone. The first event need not occur outside the cell; the mutagen may be associated with the phage in a genetically nonspecific way that merely conveys it to the infected cell. The mutagen may then have a constant chance of mutating one of the reproducing virus genomes in each generation. Experiments to distinguish between these possible alternatives are in progress.

The nature and locations of the $r_{II}$ mutants generated through the action of ethyl methane sulfonate are being studied by means of segment mapping tests.

## Protein Synthesis Inhibitors and the Rejoining of Chromosome Breaks

S. Wolff H. E. Luippold

Introduction. – It is now well established that the rejoining of radiation-induced chromosome breaks is not a passive process but is somehow dependent upon cellular metabolism and the production of ATP.[31] The unknown link by which the breaks actually rejoin has been called "the rejoining system."[32] Since chromosomes consist of nucleoprotein it seemed possible that this rejoining system could be the protein synthesis system of the cell or nucleus. This possibility was encouraged by the discovery[31] that chloramphenicol, a known protein-synthesis inhibitor, prevented the rejoining of radiation-induced chromosome breaks in *Vicia faba* seed. However, chloramphenicol has not been shown to be a specific inhibitor in the bean seed at the concentrations required to affect rejoining of chromosome breaks. The present experiments were performed to test the hypothesis that protein synthesis is necessary for breaks to rejoin.

Results. – Biological Experiments. – Aureomycin, another compound that is known to inhibit protein synthesis in certain organisms, was applied to *Vicia* seeds between two doses of radiation. It was found that the breaks from the first dose would then remain open and rejoin quantitatively with breaks from the second dose, that is, the numbers of dicentric and ring chromosomes induced were proportional to the square of the total dose. If no Aureomycin was used, the breaks did not remain open and the aberration yield was simply the sum of the yields of the two doses. Another antibiotic, one that does not inhibit protein synthesis, penicillin, was found not to affect rejoining.

Biochemical Experiments. – To test whether or not chloramphenicol actually did inhibit protein synthesis in *Vicia*, soaked seeds were incubated for 90 min in the presence of 2-$C^{14}$-glycine. The soluble proteins were then

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[31] S. Wolff, in *Proc. First Intern. Congr. Radiation Research*, p 453–62, Radiation Research, Suppl. 1 (1959).

[32] S. Wolff and H. E. Luippold, in *Progress in Radiobiology* (ed. by J. S. Mitchell, B. E. Holmes, and C. L. Smith), p 217–21, Oliver and Boyd, Edinburgh, 1956.

extracted and checked for the incorporation of labeled carbon. It was found that chloramphenicol partially inhibited this incorporation.

Since it had not been established that chloramphenicol was a specific protein-synthesis inhibitor in beans, experiments were performed to check the incorporation of $P^{32}$ into the nucleic acids of Vicia. It was found that 600 μg/ml of chloramphenicol did not inhibit this incorporation. On the contrary, those beans treated with this concentration of chloramphenicol incorporated as much $P^{32}$ in their nucleic acids as did those not treated with the inhibitor.

Discussion. – These experiments indicate that, of the antibiotics tested, only those that are known to affect protein synthesis can prevent the rejoining of radiation-induced breaks. Furthermore, chloramphenicol has been found to inhibit the incorporation of labeled carbon from glycine into the proteins of Vicia. A similar inhibition has not been found on the incorporation of labeled phosphorus into the nucleic acids. These results support the hypothesis that protein synthesis is implicated in the rejoining of radiation-induced chromosome breaks.

## Studies on Chromosome Breakage

D. Davidson

Introduction. – Primary roots of Vicia faba were X-rayed and the survival of cells with aberrant chromosomes was studied. Previously, a single reciprocal translocation had been found, in sections of each of three primary roots of Vicia fixed three weeks after irradiation;[33] six types of aberrant chromosomes were found in squashes of lateral roots of Vicia derived from irradiated primary roots.[34]

The effect of azaserine on roots of Vicia faba was studied.

Results. – Aberrant chromosomes were found in dividing cells 9, 11, and 21 days after irradiation. Translocations were the most frequent type of aberration, but some deficiencies and some dicentric chromosomes were also present. The number of different chromosome changes (several often occurred in the same root) and the percentage of cells with changed complements varied (Table 5). Often several cells in a root showed a similar

[33] R. T. Brumfield, Am. J. Botany 30, 101 (1943).
[34] D. Davidson, J. Exptl. Botany (in press).

Table 5. Effect of 600 r of X Rays on *Vicia faba* Primary Roots

| Root* | Cells | | No. of Chromosome Changes | Per Cent Changed Cells | Total Cells |
|---|---|---|---|---|---|
| | Normal | Damaged | | | |
| | | 9 Days After X-Raying | | | |
| 15 | 158 | 46 | 11 | 22.33 | 204 |
| 12 | 171 | 47 | 10 | 21.55 | 218 |
| 3 | 125 | 13 | 6 | 10.42 | 138 |
| 8 | 42 | 11 | 7 | 20.7 | 53 |
| 4 | 42 | | | | 42 |
| 14 | 25 | 2 | 1 | 10.7 | 27 |
| 6-1** | 41 | 3 | 1 | 6.81 | 44 |
| 6-2** | 71 | 10 | 4 | 12.34 | 81 |
| 18-1** | 131 | 55 | 5 | 29.56 | 186 |
| 18-2** | 75 | 27 | 1 | 26.47 | 102 |
| | | 11 Days After X-Raying | | | |
| 11-1** | 76 | 9 | 4 | 10.5 | 85 |
| 11-2** | 74 | 7 | 2 | 8.64 | 81 |
| 1 | 106 | 13 | 4 | 10.92 | 119 |
| 5 | 97 | 21 | 5 | 17.79 | 118 |
| 10-1** | 72 | 6 | 3 | 7.69 | 78 |
| 10-2** | 62 | 17 | 3 | 21.51 | 79 |
| 9 | 125 | 38 | 8 | 23.31 | 163 |
| 2 | 139 | 63 | 10 | 31.18 | 202 |
| 13 | 70 | 11 | 5 | 13.58 | 81 |
| 11 | 68 | 14 | 2 | 17.07 | 82 |

*Roots 7 and 16 were without divisions.
**Roots with a split apex.

aberration. It is assumed that all cells with a particular change, or, more rarely, with a particular double change, are descended from one damaged cell. On this basis, the minimum number of divisions that occurred between irradiation and fixation has been estimated.[35] The study of roots

[35]D. Davidson, Brit. J. Radiol. (in press).

which are cytological chimeras is revealing how the apical meristem changes and is reorganized following irradiation.

Chromosome breakage was seen in roots grown in solutions of azaserine. Breaks were not distributed at random. They occurred at a few points which appear to coincide with regions known to be heterochromatin. On the basis of comparative lengths, breaks are disproportionately frequent in the non-nucleolar (S) chromosomes. Most broken ends were involved in reunions and there were few simple terminal deletions; B" was almost invariably followed by SR, in both centric and acentric fragments, and B' by R', giving chromatid exchanges; R" was not found.

Azaserine also inhibits cell division. In concentrations which do not suppress mitosis completely the entry of cells into mitosis may be upset and there is evidence that cells in late interphase may be retarded more than cells at earlier stages.

## INSECT CYTOLOGY AND GENETICS

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M. L. P. Rekemeyer[38]
P. A. Smith
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### Effects of X Rays on Individual Phases of Mitosis

M. E. Gaulden B. J. Stevens

Introduction. — The effects of radiation on mitosis have been studied by a large number of investigators on a wide variety of cells, with mitotic ratio as the main tool for analysis. With all these data, however, it is not possible at the present time to delineate in radiobiological terms the cause and effect sequence of events in the cell that lead to mitotic inhibition. Interpretation of this type of radiation damage is undoubtedly made difficult by the highly complicated nature of the mitotic process. Mitosis involves to some extent all the numerous cell components; many of these vary

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[37]Visiting investigator from abroad.
[38]Graduate student, University of Tennessee.

greatly in state and function, and most probably in radiation sensitivity, with the mitotic phase. There are not many cell types that lend themselves to a detailed analysis of the response of the entire mitotic cycle, including interphase, to radiation. The neuroblast of the grasshopper embryo is one of the more favorable cells for such an analysis. Mitosis as observed in living neuroblasts can be subdivided, on the basis of morphological characteristics of cell structures, into 14 distinct phases and can, therefore, be studied in considerable detail. The present work was performed to determine the response of each of these phases to three doses of X rays.

Methods. – Culture preparations of grasshopper embryos were made according to the method of Gaulden, Nix, and Moshman,[39] with Shaw's culture medium.[40] Isotonic preparations were examined at room temperature, and cells in known mitotic phases were located and recorded. The preparation was then treated with X rays or remained untreated to serve as a control.

Embryo cultures were treated with 8, 32, or 64 r of X rays (32 r/min). A General Electric Maxitron 250-kvp unit was used for the experiments, operated at 150 kvp and 15 ma, with a target distance of approximately 62 cm.

Twenty-one minutes after treatment, the preparations were placed in a 38°C constant temperature box and observed at intervals. The cells were observed until they reached the next metaphase or for a maximum time of 12 hr. Metaphase was chosen as the end point because it is an easily identifiable stage and because cells reaching this stage usually complete division.

For each dose of radiation, data were collected for at least ten cells irradiated in each of the 14 mitotic phases: interphase, very early prophase, early prophase, middle prophase with three subdivisions, late prophase with two subdivisions, very late prophase, prometaphase and metaphase, anaphase, early telophase, middle telophase, and late telophase. A total of 700 cells were observed; for each mitotic phase the median value of division time following each dose was compared to the control median value.

Since 38°C appears to be detrimental to neuroblasts after 12 hr, several culture preparations were treated in the above manner but were observed

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[39] M. E. Gaulden, M. Nix, and J. Moshman, J. Cellular Comp. Physiol. 41, 451–70 (1953).

[40] E. I. Shaw, Exptl. Cell Research 11, 580–86 (1956).

at room temperature over a period of 4–5 days in order to determine whether those cells not reaching metaphase in 12 hr at 38°C were in fact able to divide eventually.

Results and Discussion. – The irradiated cells could be placed in one of two categories: those that divided, that is, reached the next metaphase, and those that did not divide in the 12-hr period of observation.

Even after 8 r a few cells irradiated in prometaphase-metaphase, anaphase, and middle telophase did not divide. As the dose was increased the number of cells not dividing within the 12 hr increased; the number of phases thus affected also increased to include all the mitotic cycle except very late prophase. The phase showing the greatest number of nondividing cells was early telophase (Fig. 5). That this stage should be so drastically affected is perhaps not surprising in view of the facts that the chromosomes are undergoing rapid and extensive changes in physical state and that the cell is not known to be synthesizing any materials at this point in the cycle. Results of cursory experiments carried out at room temperature indicate that all cells, irrespective of phase at time of treatment, eventually divide.

The cells that divided within 12 hr of irradiation could be classified as showing or not showing mitotic delay. The only phase that showed absolutely no delay, even after 64 r, was very late prophase. In the remaining phases the extent of mitotic delay varied with the phase and the dose. Cells irradiated at any of the phases from prometaphase through middle prophase showed a relatively wide and often evenly spread range of division times from a point of no delay (when compared with unirradiated cells) to considerable delay (note data for metaphase in Fig. 5). The cells irradiated in late prophase with all doses and in middle prophase with 64 r fell into two distinctly separated groups: those showing no delay and those with much delay (Fig. 5).

Most of our previous knowledge of the effects of these doses of radiation on mitosis in the neuroblast has been gained by periodic counts of mid-mitotic cells in living embryos for 6 to 8 hr after irradiation. This end point of observation was necessitated by the limited growth of cells in the culture medium used; it appeared justifiable because the mitotic

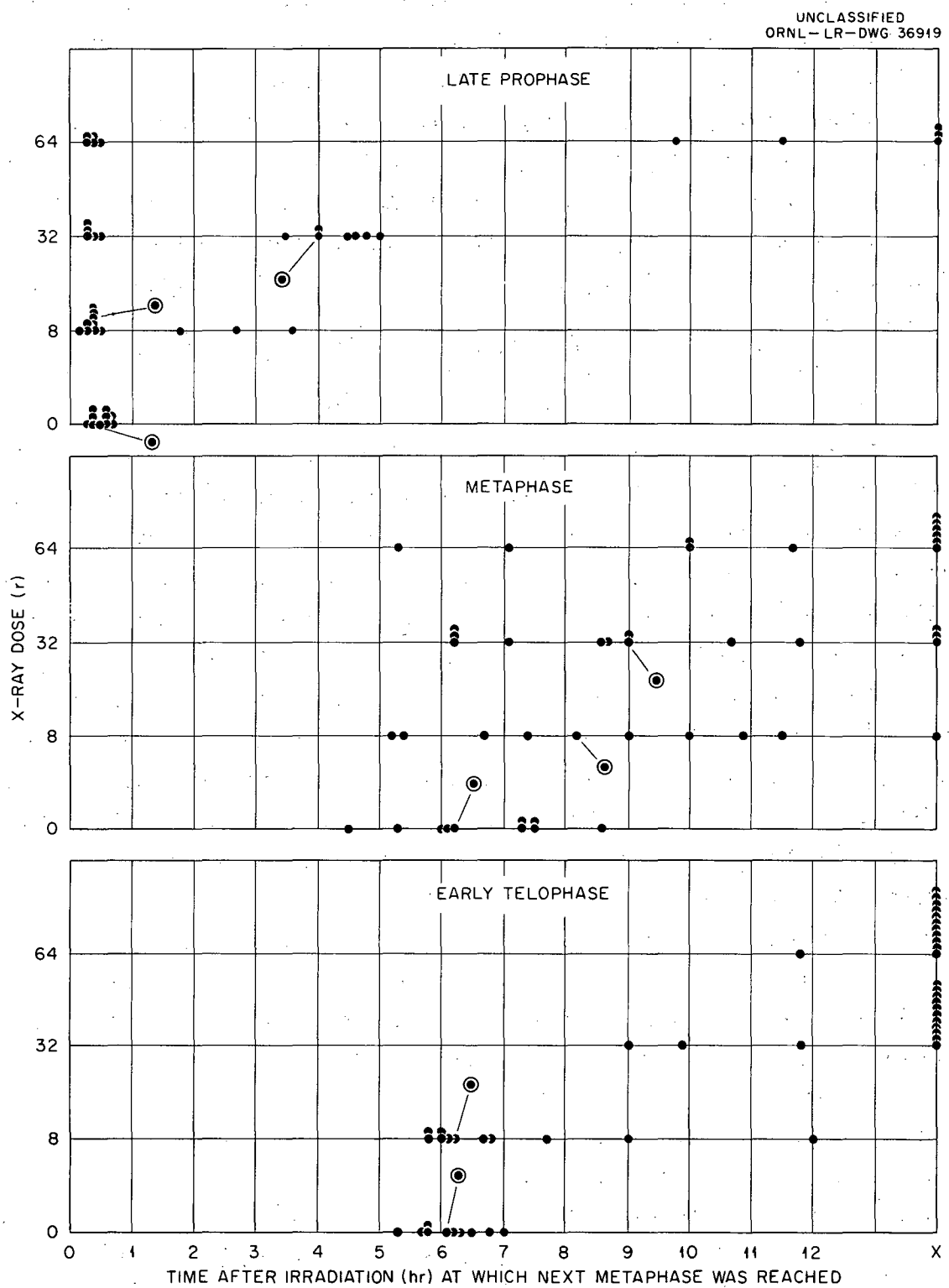


**Fig. 5. The Effects of Three Doses of X Rays on Late Prophase, Metaphase, and Early Telophase.** Each dot represents one cell. The encircled points represent median values.

ratio returned to 1.0 by this time. It is obvious from the present experiments that not only did the mitotic ratio suggest a premature termination of observations, even after a dose as low as 8 r, but also that the information gained was extremely limited. The present data show a varied and great range in sensitivities for the different mitotic phases. Data such as these may make possible an analysis of radiation-induced mitotic inhibition in terms of specific targets and reactions in the cell, a goal that must be realized if we are to better understand this phenomenon.

## X-Ray-Induced Prophase Reversion in *Vicia faba* Root Tips

M. E. Gaulden H. Gaskins[41]

Introduction. — Analysis of X-radiation damage to mitosis in living neuroblasts of grasshopper embryos has revealed that one of the prominent effects involves what appears to be a "reversion" of prophase chromatin to the interphase state. This cellular response assumes considerable significance as a radiobiological phenomenon in view of the fact that it can be induced in neuroblasts by only a few roentgens of X rays. Since prophase reversion has also been demonstrated following irradiation of several other cell types,[42-46] it may be of more common occurrence than has previously been recognized. The present experiments were initiated to determine whether this effect of radiation occurs in the mitotic cells of the root tip of *Vicia faba*, a plant that has been widely used to study radiation damage to cell structures.

The best method for detecting prophase reversion is direct observation of living cells. In the absence of good culture methods for root tip cells, however, it is necessary to resort to analysis of the number of cells in the various stages of mitosis in tips fixed at frequent intervals after irradiation.

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[41]Summer employee, 1958.

[42]J. G. Carlson, J. Morphol. 71, 449–62 (1942).

[43]C. D. Darlington and L. F. La Cour, J. Genet. 46, 180–267 (1945).

[44]M. E. Gaulden, M. Nix, and J. Moshman, J. Cellular Comp. Physiol. 41, 451–70 (1953).

[45]A. V. Beatty and J. W. Beatty, J. Tenn. Acad. Sci. 29, 175 (1954).

[46]A. V. Beatty and J. W. Beatty, Am. J. Botany 41, 242–50 (1954).

Methods. – Three groups of Vicia seedlings were given 250, 500, and 1000 r of X rays. At 30-min intervals up to 6 hr after irradiation, four lateral root tips from each group of seedlings and from an unirradiated group were fixed in Carnoy's solution (6 parts absolute alcohol, 3 parts chloroform, 1 part acetic acid). Prior to fixation all seedlings were kept in the dark at 26°C. The root tips were stained with Feulgen reagent and squashed one to a slide in a temporary mounting mixture of 3 parts aceto-carmine, 3 parts water, and 2 parts Karo syrup. The total number of fields in a squash, as seen with a 10× ocular and a 10× objective, was determined. One-fourth of these fields, chosen at random, were used for mitotic counts as follows: for each field the 44× objective was switched into position and the mitotic stage was determined for each cell within or touching the 16 central squares of a 36-square ocular grid. Cells were scored as being in one of seven mitotic phases: interphase, early, middle, or late pho-phase, prometaphase-metaphase, anaphase, or telophase.

Results and Discussion. – Prophase reversion represents in essence a blockage of cells in one part of the mitotic cycle – the cells are stopped at prophase, revert to interphase, and pile up at that point. Evidence of prophase reversion, therefore, in a series of fixed and stained cells is most convincing if soon after irradiation there is complete disappearance of cells in mid-mitosis, followed by a gradual decrease in the number of prophase cells and a concurrent increase in the number in interphase.

The series of Vicia root tips given 1000 r of X rays satisfied these criteria – the number of cells in mid-mitosis dropped to zero 2 hr after irradiation and remained at that level through the 6-hr interval. The number of cells in prophase began decreasing rapidly prior to this time and reached the zero point at about 5 hr after irradiation. The data obtained after 250 and 500 r suggest that prophase reversion also occurs at the lower doses. It should be noted that in unirradiated root tips of Vicia an unusually high proportion, 94%, of the cells are in interphase at any given time. This possibly contributes to the difficulties encountered in demonstrating reversion at the lower doses.

Thus it appears that prophase reversion does indeed occur in Vicia faba but cannot be established with confidence except at relatively high doses.

Investigations in Intersexual Gynandromorphs on the Genetic Control of Sex Expression in *Drosophila*

H. Kroeger

Introduction. – The genital imaginal disc of *Drosophila* consists, at the end of the last instar, of a mosaic of independent morphogenetic fields, which are, however, in themselves capable of full regulation.[47] By the use of an unstable ring-X-chromosome ($w^{vc}$) and a chromosome-3 mutation ix (intersex), compound external genitalia were produced that were composed of varying amounts of male and female or male and intersexual tissue, respectively. The analysis of these compound sets of genitalia provided information on the means by which the genotype exerts its control over the build-up and expression of the mosaic fields in the genital imaginal disc.

Results. – Six hundred gynandromorphs with compound external genitalia were analyzed. The mutual replacement of parts of the male and female set of genitalia showed that the typical apparatus of the two sexes is brought about by a differential response of the different genotypes to the same prepattern.[48,49] However, in most cases the border line between XX and XO tissue, when crossing the genital disc, caused the production of two of these prepatterns, one in the XX, one in the XO area of the mosaic; the symmetry axis of both of these sets stands always in an angle vertical to the border line, regardless of the orientation of the border line to the body axis. In a number of cases a morphogenetic field of one set can fuse with the corresponding one from the other, thereby producing areas that are, although typically male or female in structure, shared by both sets. The way in which stages of progressive reduction of the compound sets can be arranged to form a gapless sequence indicates that the elements of this sequence represent subsequent stages of segregation of symmetric elements from one single unit.[50,51] In cases where the border line between XX and

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[47] E. Hadorn, G. Bertani, and J. Gallera, Wilhelm Roux' Arch. Entwicklungsmech. Organ. 144, 31–70 (1950).

[48] C. Stern, Science in Progress, Ninth Series (ed. by G. A. Baitsell), Yale Univ. Press, New Haven, Conn.

[49] C. Stern, Cold Spring Harbor Symposia Quant. Biol. 21, 375–82 (1956).

[50] H. Kroeger, Wilhelm Roux' Arch. Entwicklungsmech. Organ. 150, 401–24 (1958).

[51] H. Kroeger, ibid. (in press).

XO tissue lies tangential to the normally structured male set, a supernumerary female set is produced, thereby mimicking the condition in females homozygous for ix. The distribution of these supernumerary sets reveals a gradient from maleness toward femaleness from the tip of the abdomen to the ventral side.

Discussion. – It can be concluded that the threshold in the reaction to this gradient is lowered in the intersexes, which then results in the production of one incomplete male and one incomplete female set of genitalia. From the sequence of reductional stages of the compound sets in combination with the independent capability of regulation as exhibited by the fusing of fields it can be concluded that the mosaic of independent fields on the genital disc is produced by a segregation of symmetric elements from a centrally located origin in much the same way as this has been hypothesized from the production of symmetric structures inside a morphogenetic field.[50,51]

## The Nature of Primary Radiation Death in *Habrobracon* Gametes

R. C. von Borstel

Introduction. – When *Habrobracon* males or females are treated with X radiation or nitrogen mustard, lethal changes that are genetically dominant are induced in the mature gametes; that is, the lethality will be expressed when combined with a normal unirradiated gamete. Induced dominant lethality can be expressed in the embryo in several ways.[52] The most predominant type of death is that occurring in the first few nuclear divisions with subsequent production of Feulgen-negative nuclei that enlarge tremendously.[53] This will be referred to here as primary radiation death. It has not been possible to mimic this type of dominant lethality with genetically contrived chromosome loss or loss of chromosome parts, although the other types of dominant lethality can be mimicked by chromosome imbalance phenomena.[54]

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[52]K. C. Atwood, R. C. von Borstel, and A. R. Whiting, Genetics 41, 804–13 (1956).

[53]R. C. von Borstel, Nature 175, 342 (1955).

[54]R. C. von Borstel and M. L. Rekemeyer, Genetics (in press).

Results. — By a fortunate circumstance, a recessive mutation in Habrobracon was discovered that mimicked the primary radiation death. When a female is homozygous for the mutation, her offspring die under conditions in which Feulgen-negative nuclei are produced that eventually enlarge. The mutation is associated with a chromosomal translocation and was discovered in a routine test for translocations.

The mimicry of primary radiation death by this mutation is not absolute. During normal oogenesis, meiosis is inhibited at the first meiotic metaphase until the egg is laid, when the nucleus is displaced from the egg center to the periphery and meiotic division proceeds. After irradiation of normal eggs or sperm, the rate of meiosis is unimpaired but the subsequent normally rapid nuclear divisions (mitosis every 10 min) are drastically slowed and then inhibited completely after two or three divisions. Production of Feulgen-negative nuclei begins a few hours later. Meiosis in eggs from the mutant is inhibited. After several hours, the meiotic nucleus abortively divides and production of Feulgen-negative nuclei begins. Also, in unirradiated or irradiated normal, newly laid eggs, the meiotic nucleus is adjacent to the periphery on the convex surface. In eggs from the mutant, the meiotic nucleus maintains the same position it does in the uterine sacs before laying. These facts indicate that, in the mutant, the process of egg activation is disrupted.

Since unfertilized eggs of Habrobracon become normal males and fertilized eggs become females, it has long been assumed that activation of the Habrobracon egg must be associated with the laying process rather than with the usual combined fertilization-activation that takes place during zygote formation in most animal species. During the laying process the egg is squeezed and distorted markedly as it passes down the ovipositor. At this time the meiotic nucleus shifts its position and the nucleus is activated to divide.

Since activation was suspected as being associated with oviposition, the act of laying eggs by the mutants was carefully observed. It was found that eggs do not pass down the ovipositor when laid by the females that carry the radiation-mimicking mutation. They come out just anterior to the ovipositor and are not distorted during the laying process. Therefore it appears likely that the passage down the ovipositor is necessary for egg

activation; it follows that the inhibition of egg activation accounts for the mimicry of the primary radiation syndrome.

Discussion.— We believe that the primary radiation death is caused by drastically reduced rates of nuclear synthesis and division, since an actual inhibition of nuclear division by means other than radiation elicits the same pattern of death. The production of Feulgen-negative nuclei appears to be a syndrome associated with a block of nuclear division during very early development rather than any specific effect induced by radiation. It seems likely that when the egg is laid, differentiation of egg cytoplasm begins, and if nuclear multiplication fails to keep pace, then at some later age of the eggs, specific moribund effects are observable.

There is good reason to believe that the phenomenon of cell death from interruption of division with radiation is a common one, but the associated syndromes in each organism studied thus far are remarkably diverse. In *Drosophila* embryos, nuclear division ceases but Feulgen-negative nuclei are not produced; in yeast, death occurs when a bud starts to protrude from a cell; in human and mammalian tissue cultures, giant cells form which do not divide; in bacteria, growth occurs but division does not, with the result that long filamentous "snake" forms develop. Cell death from radiation has been ascribed to such unlike phenomena as chromosome breakage or inhibition of DNA synthesis. The death is the consequence of unbalanced growth with either explanation.[55,56] In the case of the *Habrobracon* egg, chromosome breakage is known to cause death at some other stage than the stage discussed here, and cessation of DNA synthesis is not a necessary prerequisite for unbalanced growth, although such cessation is the eventual outcome of an unbalanced nucleocytoplasmic relation.

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[55] M. S. Cave and S. W. Brown, Am. J. Botany 44, 1–8 (1957).

[56] H. D. Barner and S. S. Cohen, J. Bacteriol. 71, 149–57 (1956).

D. L. Lindsley
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## Evidence for a Chromosomal Target for Induced Gamete Inactivation and a Nonchromosomal Target for Induced Dominant Lethality in the Mature Sperm of *Drosophila melanogaster*

D. L. Lindsley

A model has been constructed that attempts to reconcile the apparently contradictory observations that $Y^S X \cdot Y^L/O$ males show a much greater shift in sex ratio of their progeny with increasing doses of radiation than do $Y^S X \cdot Y^L/Y$ males whereas the rates of induced zygote mortality in the two genotypes are not different (see Fig. 6).

A population of spermatozoa from $Y^S X \cdot Y^L/O$ males is composed of two genotypes XY A and A in the ratio of x:1 - x. Fertilization of an XA ovum by the first sperm type will lead to the production of a female and by the second sperm type to a male. Consequently the sex ratio in an unirradiated cross is

$$SR_O = \frac{x}{1 - x} .$$

Furthermore, the probability that an irradiated XY A sperm will survive is

$$S_{XYA} = e^{-n_1} ,$$

when $n_1$ is simply the average number of induced events that prevent a sperm from producing an adult fly; this event may interfere at any point between the time of irradiation of the gamete and eclosion of the imago. Similarly

$$S_A = e^{-n_2} .$$

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[57]Research Associate.

[58]Visiting investigator from abroad.

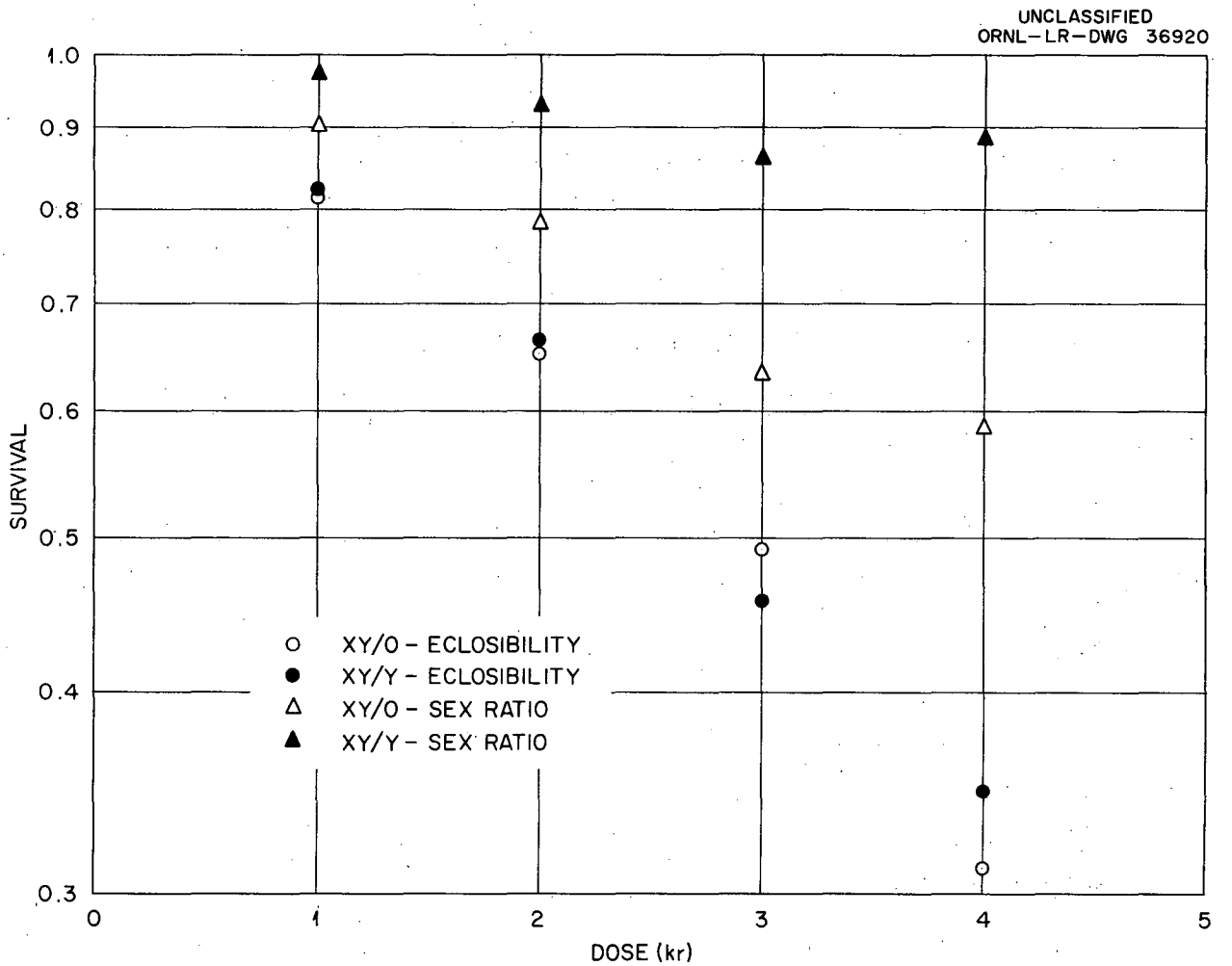


**Fig. 6. Comparison of Survival of Sperm of XY/Y and XY/O Males As Measured by Sex Ratio and Eclosibility.**

It follows that the sex ratio after some dose of irradiation is

$$SR_O = \frac{x\ e^{-n_1}}{(1 - x)\ e^{-n_2}} = SR_O\ e^{-(n_1 - n_2)} = SR_O\ e^{-\Delta n} \quad .$$

The events represented by $n_1$ may be subdivided into those that are sex linked (XY) and those that are not (A), also into prefertilization or gametic and postfertilization or zygotic times of action:

$$n_1 = XY_g + XY_z + A_g + A_z \quad ;$$

similarly,

$$n_2 = A_g + A_z \quad .$$

Assuming non-sex-linked damage to be identical in the two genotypes,

$$\Delta n = XY_g + XY_z \quad .$$

That is to say, sex ratio provides a measure of sperm survival attributable to the XY chromosome. By the same argument in the case of XY/Y males $\Delta n = XY_g + XY_z - Y_g - Y_z$, and from a comparison of $\Delta n$ for XY/O and XY/Y males we can estimate $Y_g + Y_z$. The observations indicate that $Y_g + Y_z$ is a linear function of dose and equals 0.108D. From the eclosion data we can estimate that $Y_z = 0$ and consequently that $Y_g = 0.108D$, and further that $XY_z = 0$ and $XY_g = 0.142D$.

From this we have concluded that the measured shift in sex ratio represents elimination of gametes from the fertilizing pool and that the target for this phenomenon is chromosomal. On the other hand, the reduction in eclosibility of eggs is the consequence of irradiation of a nonchromosomal target.

## Preliminary Approaches to the Genetic Basis of Antibody Production

D. L. Lindsley M. S. Moore

The observation that a mammal, once it has been stimulated to produce antibody to a particular antigen, retains for extended periods of time greatly enhanced ability to produce the same antibody upon additional stimulation, strongly suggests that the information required for the synthesis of the particular antibody protein is stored somewhere in the cellular machinery of the body. The observations of Coons[59] on the localization of labeled antigen indicate that scattered lymphoid cells respond to primary injection by the production antibody and that the secondary response is effected by the proliferation of the primary antibody-producing cells into clones. A corollary of this interpretation is that the information required for the synthesis of a given antibody molecule, whatever its physical basis, is passed to both products of a cell division and may in fact be self-replicating.

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[59]A. H. Coons, J. Exptl. Med. 102, 49 (1955).

Recently the cellular selection theory of antibody formation has been developed by Burnet,[60] Talmage,[61] and Lederberg.[62] It postulates the preexistence of cells producing an immense variety of globulin molecules specified by an array of randomized information. The lymphoid system, consequently, is potentially able to produce $\gamma$-globulin molecules complementary to virtually any antigen that might be administered. The role of antigen is visualized as that of mobilizing those cells whose globulin is complementary into proliferation and increased globulin synthesis.

Investigation of the possible genetic basis of these cellular events requires observation of the clonal development of a cell that can produce an identifiable antibody molecule. To this end we have attempted to produce *in vivo* clones of lymphoid cells using Ford's observation[63] that reversion to mouse type of radiation-produced rat-mouse chimeras is often accomplished from a small number of surviving cells. Essentially the idea has been to protect supralethally irradiated mice with a massive dose of rat bone marrow cells and to administer, in addition, a small inoculum of lymphoid cells that are hyperimmune to bacteriophage and select cases in which the rat marrow graft is eventually sloughed for an investigation of antiphage-producing capabilities of the repopulating cells. The results of these attempts are summarized in Table 6. It can be seen that with the possible exception of the last line, which is the most recent experiment, it has not been possible to induce appreciable regression of the rat graft with small inocula of unirradiated isologous spleen or marrow cells.

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[60] F. M. Burnet, Australian J. Sci. 20, 67–69 (1957).

[61] D. W. Talmage, Ann. Rev. Med. 8, 239 (1957).

[62] J. Lederburg, J. Cellular Comp. Physiol. 52 (Suppl. 1), 383–401 (1958).

[63] C. E. Ford, P. L. T. Ilbery, and J. F. Loutit, J. Cellular Comp. Physiol. 50 (Suppl. 1), 109–38 (1957).

Table 6. Summary of Attempts To Clone Isologous Cells in Mouse-Rat Chimeras from Small Inocula

| X-Ray Dose (r) | Time of Administration of $1.4 \times 10^8$ RBM (hr after X ray) | Isologous Cell Treatment | | | No. of Animals | No. Surviving on – | | No. of Stable Reversions to Mouse RBC After Showing 100% Rat RBC |
|---|---|---|---|---|---|---|---|---|
| | | Cell Type | No. | Time of Administration (days after X ray) | | Day 30 | Day 90 | |
| 1050 | 2–4 | Spleen | $10^2$–$10^3$ | 0 | 224 | 133 | 59 | 1 |
| 1050 | 2–4 | Bone marrow | $10^3$ | 0 | 54 | 26 | 9 | 1 |
| 1050 | 16–20 | Spleen or bone marrow | 500 | 0 | 98 | 42 | 17 | 0 |
| 1050 | 2–4 | Spleen | $10^2$ | 3–4 | 109 | 47 | 20 | 0 |
| | | | $10^4$ | 3–4 | 23 | 20 | 9 | 2 |
| | | | $10^5$ | 3–4 | 24 | 4 | 0 | |
| 1050 | 2–4 | Bone marrow | $10^3$ | 3–4 | 57 | 29 | 7 | 1 |
| | | | $10^4$ | 3–4 | 23 | 21 | 1 | 0 |
| | | | $10^5$ | 3–4 | 20 | 14 | 4 | 4 |
| 0* | None | Bone marrow: spleen = 1:1 | $10^4$ | – | 31 | 23 | 17 | 2 |
| 300* | None | Bone marrow | $10^4$ | 0 | 13 | 8 | | 4 |
| 300* | None | None | | | 13 | 6 | | 0 |

*Hosts are survivors of previous experiments which showed 100% rat RBC.

Reciprocal Translocations Between the X and Y Chromosomes in *Drosophila melanogaster*

B. Nicoletti D. L. Lindsley

The genetic and cytological study of X;Y translocations seems to be particularly interesting and promising since they can be used for the analysis of many general problems. In order to obtain translocations between the X and Y chromosomes the following method has been used.

Crosses of homozygous yellow females carrying an extra Y chromosome marked with $y^+$ ($y/y/sc^8 \cdot Y$) with males carrying a Y chromosome marked with the gene B ($Y \cdot B^S$) yield sons that carry both the marked Y chromosomes ($y/sc^8 \cdot Y/Y \cdot B^S$). Such males produce $y/sc^8 \cdot Y$ and $y/Y \cdot B^S$ sperm in which translocation between the X and Y can be induced. Immediately after irradiation these XYY males are mated in mass culture with females homozygous for a balancer chromosome $\left[Y^S X \cdot Y^L, \text{Ins}(1)\text{dl-49}, \text{EN}, \text{y v f car} = \text{Bal}\right]$. The $y/\text{Bal}/Y \cdot B^S$ and $y/\text{Bal}/sc^8 \cdot Y$ daughters are selected and mated individually with Bal/marked Y males. The Y chromosome of the male carries the marker different from that of the female to allow classification of the nondisjunctional progeny. The translocations are scored by looking for the vials lacking aneuploid classes.

From 573 $y/sc^8 \cdot Y$ sperms, 4 carried the desired translocations (0.69%), as did 7 (0.72%) from 974 $y/Y \cdot B^S$ sperms. Of these 11 translocations, 5 were male lethal, 3 were male sterile, and 3 were male fertile. The male sterility persists in the presence of an extra normal Y. The X chromosome break point of 5 of these translocations has been determined by salivary chromosome analysis (see Table 7). The break points of the other translocations are now being studied.

Furthermore, these experiments produce X chromosomes broken at different points along their length; the duplications and deficiencies derived from them provide valuable tools for other studies.

Table 7. Summary of Available Information on T(X;Y)'s

| No. | Lethal | Sterile | Sterile | X Break | Y Break |
|---|---|---|---|---|---|
| 1 | | x | | 15 F - 18* | $Y^L$ |
| 7 | x | | | 14 A | $Y^S$ |
| 12 | | x | | 15 F - 18* | $Y^L$ |
| 118 | | | x | 15 F - 18* | $Y^L$ |
| 120 | x | | | 15 F - 18* | $Y^S$ |
| 140 | | | x | 3 C4 | $Y^L$ |
| 150 | | x | | 3 F5 | $Y^S$ |
| 240 | | | x | 14 A | $Y^L$ |
| 290 | x | | | 1 A | ** |

*Determined genetically.
**The break point has not yet been determined.

## Complementation Studies with the Y Chromosome in *Drosophila*

G. E. Brosseau, Jr.

Introduction. — In a previous report,[64] data on the number and genetic organization of the male fertility factors on the Y chromosome were given. At that time the data were consistent with seven functional units on the long arm of the Y as defined by the ability of the sterile Y chromosomes to complement each other. Additional evidence now indicates that there are actually five units in this complex. It was found that some combinations of two sterile Y's with overlapping deficiencies were capable of producing some fertility in the males bearing them. This has been termed partial complementation. Additional data gathered on partial complementation will be reported here.

Results. — Experiments were carried out to determine the frequency of fertile males among males carrying various combinations of sterile Y's and to determine whether temperature affected this frequency. The data from these experiments are given in Table 8. In other experiments, it became apparent that males carrying certain sterile Y's produced a few progeny.

[64]G. E. Brosseau, Jr., Biol. Semiann. Prog. Rep. Aug. 15, 1958, ORNL-2593, p 37–38.

Table 8. The Fertility of *Drosophila* Males Carrying Sterile Y Chromosomes Singly and in Combination

| Cross No. | Sterile Y No. | No. Fertile Males / No. Males Tested | | Per Cent Fertile | | Average No. Progeny Mating | Factors Deficient |
|---|---|---|---|---|---|---|---|
| | | 25°C | 18°C | 25°C | 18°C | 25°C | |
| 1 | L41 | 14/2948 | | 0.47 | | 10.93 | $k_3$ |
| 2 | L19 | 9/2765 | | 0.32 | | 1.56 | $k_{3-5}$ |
| 3 | L34 | 68/8881 | | 0.76 | | 11.31 | $k_5$ |
| 4 | L3 | 67/5334 | | 1.26 | | 17.30 | $k_5$ |
| 5 | L28 | 20/2802 | | 0.71 | | 5.60 | $k_5$ |
| 6 | L36 | 27/3001 | | 0.90 | | 4.89 | $k_{4-5}$ |
| 7 | L9/L9 | 0/275 | | 0 | | | $k_{2-5}/k_{2-5}$ |
| 8 | L36/L9 | 4/308 | | 1.30 | | 1.5 | $k_{4-5}/k_{2-5}$ |
| 9 | L9/L36 | 0/316 | | 0 | | | $k_{2-5}/k_{5-5}$ |
| 10 | L36/L36 | 0/314 | | 0 | | | $k_{4-5}/k_{4-5}$ |
| 11 | L34/L9 | 11/269 | 2/320 | 4.09 | 0.63 | 2.27 | $k_5/k_{2-5}$ |
| 12 | L1/L34 | 1/201 | 5/332 | 0.50 | 1.51 | | $k_{2-5}/k_5$ |
| 13 | L9/L1 | 0/406 | 0/414 | 0 | 0 | | $k_{2-5}/k_{2-5}$ |
| 14 | L34/- | 1/547 | 0/412 | 0.18 | 0 | | $k_5$/- |
| 15 | -/L34 | 3/1047 | 0/1050 | 0.29 | 0 | 1.00 | -/$k_5$ |
| 16 | L34/- | 2/1869 | 1/855 | 0.11 | 0.12 | 1.00 | $k_5$/- |
| 17 | L34/L34 | 2/261 | 0/345 | 0.77 | 0 | 1.50 | $k_5/k_5$ |
| 18 | L34/L9 | 10/540 | 0/564 | 1.85 | 0 | 2.00 | $k_5/k_{2-5}$ |
| 19 | L9/L34 | 9/1050 | 0/1002 | 0.86 | 0 | 1.30 | $k_{2-5}/k_5$ |

Reexamination of the crosses that showed partial complementation showed that all these combinations included one of these "leaky" Y's. Data on the fertility of males with a leaky Y are given at the top of Table 8. In one combination at least, L34/L9, the frequency of fertility is increased over that which can be attributed to L34 alone. Some of the other combinations probably also increase the frequency of fertile males, but larger numbers are needed to establish this observation. Males grown at 18°C (but tested at 25°C) are generally less fertile than those grown at 25°C. This relationship is clearly so for the L34/L9 combination and probably so for some of the others. The difference in the fertility observed with the same genotypes tested at different times may be caused by a decrease in fertility with the age of the male, because the males showing the highest fertility were generally younger when tested than those with lower fertility (e.g., Table 8, cross 3 vs crosses 14 and 16).

Discussion. — Although the basis of Y chromosome-associated male sterility is not known, the phenotype for all the sterile Y chromosomes appears to be the same and similar to the phenotype when the entire Y chromosome is absent. Thus all the functional units seem to produce the same effect although they are functionally distinct as demonstrated by the complementation between the various deficiencies. Why some overlapping deficiencies partially complement each other is not clear. One possibility that is in accord with the available data is that some of the deficiencies are actually the result of a restricted break within the functional complex with the inactivation of adjacent loci because of position effect. The leakiness would be a manifestation of variegation for fertility. The case of L19 is difficult to explain on any other basis. The overlapping deficiencies that complement would be those that have their breaks fairly far apart. The temperature effect would also be in accord with this interpretation. In the case of the Y chromosome it is difficult to establish the presence of intrachromosomal rearrangement (interchromosomal rearrangements have been eliminated), one of the criteria for position effect. As the affected system, the Y chromosome, is itself a strong modifier of variegation, the effects of added heterochromatin are also difficult to study. However, it should be possible to establish that some of the normal alleles are present, but inactive, in a multiple site deficiency by obtaining a

normal Y chromosome as a result of crossing over between two sterile Y's carrying overlapping deficiencies.

## Studies of the lysine and red cell Mutants of Drosophila melanogaster

E. H. Grell

Introduction. – A biochemical description of gene action is available for relatively few mutants of Drosophila. In the past, biochemically defined phenotypes were ones in which a block in the synthesis of eye pigments occurred.[65-67] Recently a mutant that seems to be unable to degrade the amino acid, lysine, has been described.[68] Lysine cannot be degraded and tends to accumulate in the mutant flies when they are living on a diet containing an excess of lysine.

The mutation, lys, causes effects other than the accumulation of lysine. Two peptides present in normal pupae are absent in lys pupae, and lys acts as an enhancer of the red cell phenotype. The red cell phenotype is characterized by the presence of pigment in certain large fat cells located most numerously under the cuticle of the thorax.[69] In the original strain two mutant loci, lys and rc (red cell), had to be homozygous for the expression of the red cell phenotype. A second strain of flies ($rc^2$) was discovered that exhibits a red cell phenotype if grown at a low temperature (15°C) but not at a higher temperature (25°). The $rc^2$ strain does not accumulate lysine.

Studies have been made to determine the genetic basis of the red cell phenotype of the $rc^2$ strain and its relationship to the original red cell strain. Attempts have been made to induce new alleles of lys and rc by X irradiation.

Results. – The results listed in Table 9 are consistent with the following interpretation: (1) lys, rc and $rc^2$ are recessive genes; (2) lys

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[65]A. Butenandt, W. Weidel, and E. Becker, Naturwissenschaften 28, 63 (1940).

[66]A. Butenandt, W. Weidel, and H. Schlossberger, Z. Naturforsch. 46, 242 (1949).

[67]H. S. Forrest, E. Glassman, and H. K. Mitchell, Science 124, 725 (1956).

[68]E. H. Grell, Proc. X Intern. Congr. Genet., p 104, 1958.

[69]J. C. Jones and E. B. Lewis, Biol. Bull. 112, 220 (1957).

Table 9. The Phenotypes of Combinations of lys, rc, and $rc^2$

| Genotype | Phenotype |
|---|---|
| lys rc/lys rc | Red cell and lysine |
| lys rc/+ + | Wild type |
| lys rc/+ rc | Wild type |
| lys rc/lys + | Lysine |
| + rc/+ rc | Wild type |
| lys +/lys + | Lysine |
| + $rc^2$/ + $rc^2$ | Red cell at 15°, wild type at 25° |
| + $rc^2$/+ + | Wild type |
| lys $rc^2$/+ $rc^2$ | Red cell at 15°, wild type at 25° |
| lys $rc^2$/lys $rc^2$ | Red cell and lysine |
| lys rc/lys $rc^2$ | Red cell and lysine |
| lys rc/+ $rc^2$ | Wild type |
| + $rc^2$/+ rc | Wild type |

is an enhancer of rc, rc produces a red cell phenotype only in combination with lys, $rc^2$ produces a red cell phenotype at 25°C when combined with lys; (3) rc and $rc^2$ are alleles, $rc^2$ is a stronger allele than rc and produces a red cell phenotype without the enhancer if grown at low temperatures, rc is dominant to $rc^2$ because the heterozygote is wild type even at 15°.

Attempts have been made to produce more alleles of lys and rc by X irradiation. Of approximately 15,000 gametes from males exposed to 4000 r no mutations of $rc^+$ were detected. From 35,000 gametes from males exposed to 4000 r one mutation of $lys^+$ has been obtained. The new allele ($lys^2$) accumulates lysine and enhances rc. It differs from lys in that it is semilethal and the wings of the homozygote are abnormal. The wings have a heavier texture than wild type and are curved downward. The abnormal wing may be an effect of $lys^2$ but the possibility cannot be eliminated that it is caused by a mutation at another locus.

## Studies on Synaptic Affinities Between Nonhomologues

R. F. Grell

Introduction. – A marked nonrandom assortment between two nonhomologous chromosomes in *D. melanogaster* has been described. The greatest amount of nonrandom assortment was observed between a normal Y and a normal fourth chromosome; the frequency with which the two nonhomologues segregate to opposite poles was found to be approximately 92%. These segregation results were interpreted as the consequence of an association between the two nonhomologues at meiosis, and the association was thought to be induced by the absence of available pairing homologues for both the Y and the fourth chromosome.[70] It was now proposed to test whether the fourth chromosome could compete successfully with the sex chromosomes for an association with one of them.

Results. – Sturtevant and Beadle[71] showed that when the homology between the X's is partially destroyed by the introduction of the dl-49 inversion into one of them, the frequency of secondary exceptions is increased from 4 to 45% whereas the frequency of primary exceptions is not altered. Presumably, the Y chromosome is now able to pair with the X's more successfully than the X's pair with each other. The introduction into this situation of a free fourth chromosome that lacks a pairing homologue provides a test of the ability of a nonhomologue to interfere with the pairing between or among homologues. If the synaptic affinity between the homologues is much greater than that between the nonhomologues, the presence of the fourth chromosome would be expected to have little effect on the pairing and disjunction of the two X's and the Y and the percentage of secondary exceptions would remain approximately the same. Conversely, if the synaptic affinity between the four and any of the sex chromosomes is appreciable, the percentage of secondary exceptions is expected to be altered. A nonhomologous association might occur between the Y and the fourth chromosome or between one of the X's and the four. The effect of either type of association would be expected to decrease the frequency of XXY trivalent formation, the pairing model that best accounts for the high frequency of secondary nondisjunction

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[70] R. Grell (in press).

[71] A. H. Sturtevant and G. W. Beadle, Genetics 21, 554 (1936).

observed in this situation, and to decrease the frequency of exceptional progeny.

Accordingly, XXY females were constructed that were heterozygous for In(1)dl-49, homozygous for T(3;4)86D and that carried a free fourth chromosome marked with $ci^D$. Females of identical genotype that lacked a free four served as a control. The percentage of exceptional progeny was 28.1 when the free four was present and 51.1 in the control.

Discussion. – These data show that the small fourth chromosome can compete successfully with the sex chromosomes for an association with one of them. Although direct genetic evidence has established that the Y and fourth chromosome are capable of segregating from each other with a very high frequency, no such evidence exists for the X and the fourth chromosome. A test is now under way to elucidate this point. The results of this test may discriminate between the alternatives of predominately Y-4 or X-4 pairing as the cause of the decrease. These data suggest that nonhomologues may possess affinities of a very high order for each other since the percentage of exceptional progeny is reduced approximately 45% when the free fourth chromosome is present. Ultimately, it is hoped that numerical values may be assigned to such "quasihomologues" that describe the degree of synaptic affinity between them.

## MAMMALIAN GENETICS AND DEVELOPMENT

### GENETIC AND DEVELOPMENTAL EFFECTS OF RADIATION IN MICE

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#### Dependence of Mutation Frequency on Radiation Dose Rate in Female Mice

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Results from specific locus mutation rate studies in females are summarized in Table 10. The task of obtaining a reliable rate from acutely irradiated females is beset by the practical difficulty that the number of

[1]Consultant.
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[3]Visiting investigator from abroad.

Table 10. Mutations at Specific Loci in Mouse Oocytes

| Radiation | Dose (r) | Intensity (r/min) | No. of Offspring | No. of Mutations at Locus | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | a | b | c | d | p | s | se | d se |
| None* | 0 | | 5,845 | - | - | - | - | - | - | - | - |
| X* | 400 | 96 | 1,729 | - | - | - | - | - | 1 | - | - |
| X | 400 | 92 | 6,130 | - | 2 | - | - | 1 | 2 | - | 1 |
| None | 0 | | 40,918 | - | - | - | - | - | - | - | - |
| Gamma ($Cs^{137}$) | 258 | 0.0085 | 26,468 | - | 1 | - | - | - | - | - | 1 |

*1950-51 experiment.

offspring that can be obtained before sterility ensues is very small. Nevertheless, as is shown below and in the succeeding report, such data were urgently needed to make proper interpretation of other results.

The results show the specific locus mutation rate in females to be significantly higher following acute than following chronic irradiation. The respective values are $31.8 \times 10^{-8}$ per roentgen per locus (95% confidence interval: 12.8 to $65.5 \times 10^{-8}$) and $4.2 \times 10^{-8}$ per roentgen per locus (95% confidence interval: 0.51 to $15.1 \times 10^{-8}$). Since conditions in the adult mouse ovary are such that complications from secondary factors are unlikely, it can be concluded that in oocytes there is a dose-rate effect on the mutation process itself. The influence of dose rate on the frequency of mutations following irradiation of spermatogonia (see the succeeding report), which could have been due to secondary factors, is now, in the light of the oocyte results, also considered to be due to a dose-rate effect on the mutation process.

The most significant features of the results, from the point of view of human hazards, are, first, that there is, at present, no evidence that females are less sensitive than males, and, second, that the finding of a dependence of mutation frequency on radiation dose rate, now extended from males to females, applies to those germ cell stages that are important in human hazards.

## Radiation Dose Rate and Mutation Frequency

W. L. Russell
L. B. Russell
E. M. Kelly
J. W. Bangham
M. B. Cupp
M. C. Bahner

New data have clearly confirmed the earlier finding[4,5] that specific locus mutation rates obtained with chronic gamma irradiation of spermatogonia (Table 11) are lower than those obtained with acute X rays (Table 12, Fig. 7). Since this result is in contrast to classical findings for *Drosophila* spermatozoa, and apparently contradicts one of the basic tenets of radiation genetics, it was important to determine what factors were responsible for it.

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[4]W. L. Russell and E. M. Kelly, Science 127, 1062 (1958).

[5]W. L. Russell and L. B. Russell, Proc. Second Intern. Conf. Peaceful Uses Atomic Energy, Geneva, 1958 (in press).

Table 11. Mutations at Specific Loci Induced in Spermatogonia of Mice by Chronic Gamma Irradiation

| Dose (r) | Intensity (r/week) | No. of Offspring | No. of Mutations at 7 Loci | Mean No. of Mutations per Locus, per Gamete, $\times 10^5$ |
|---|---|---|---|---|
| 0 | | 105,403 | 8 | 1.08 |
| 86 | 10 | 48,500 | 6 | 1.77 |
| 516 | 90 | 20,752 | 4 | 2.75 |
| 861 | 90 | 20,993 | 9 | 6.12 |

Table 12. Mutations at Specific Loci Induced in Spermatogonia of Mice by Acute X Irradiation

| Dose (r) | No. of Offspring | No. of Mutations at 7 Loci | Mean No. of Mutations per Locus, per Gamete, $\times 10^5$ |
|---|---|---|---|
| 0 | 42,833 | 1 | 0.33 |
| 300 | 40,408 | 25 | 8.85 |
| 0 | 106,408 | 6 | 0.81 |
| 600 | 119,326 | 111 | 13.29 |
| 0 | 33,972 | 2 | 0.84 |
| 1000 | 31,815 | 23 | 10.33 |

Experiments undertaken for this purpose reveal the following: (1) the lower mutation frequency is due mainly to difference in dose rate of radiation, rather than quality; (2) a dose-rate effect is not obtained in experiments with mouse spermatozoa, confirming classical findings for spermatozoa, and indicating that the explanation for intensity dependence in spermatogonia resides in some characteristic of gametogenic stage; and (3) a dose-rate effect is found not only in spermatogonia, but also in oocytes (see the preceding report), where cell selection is improbable,

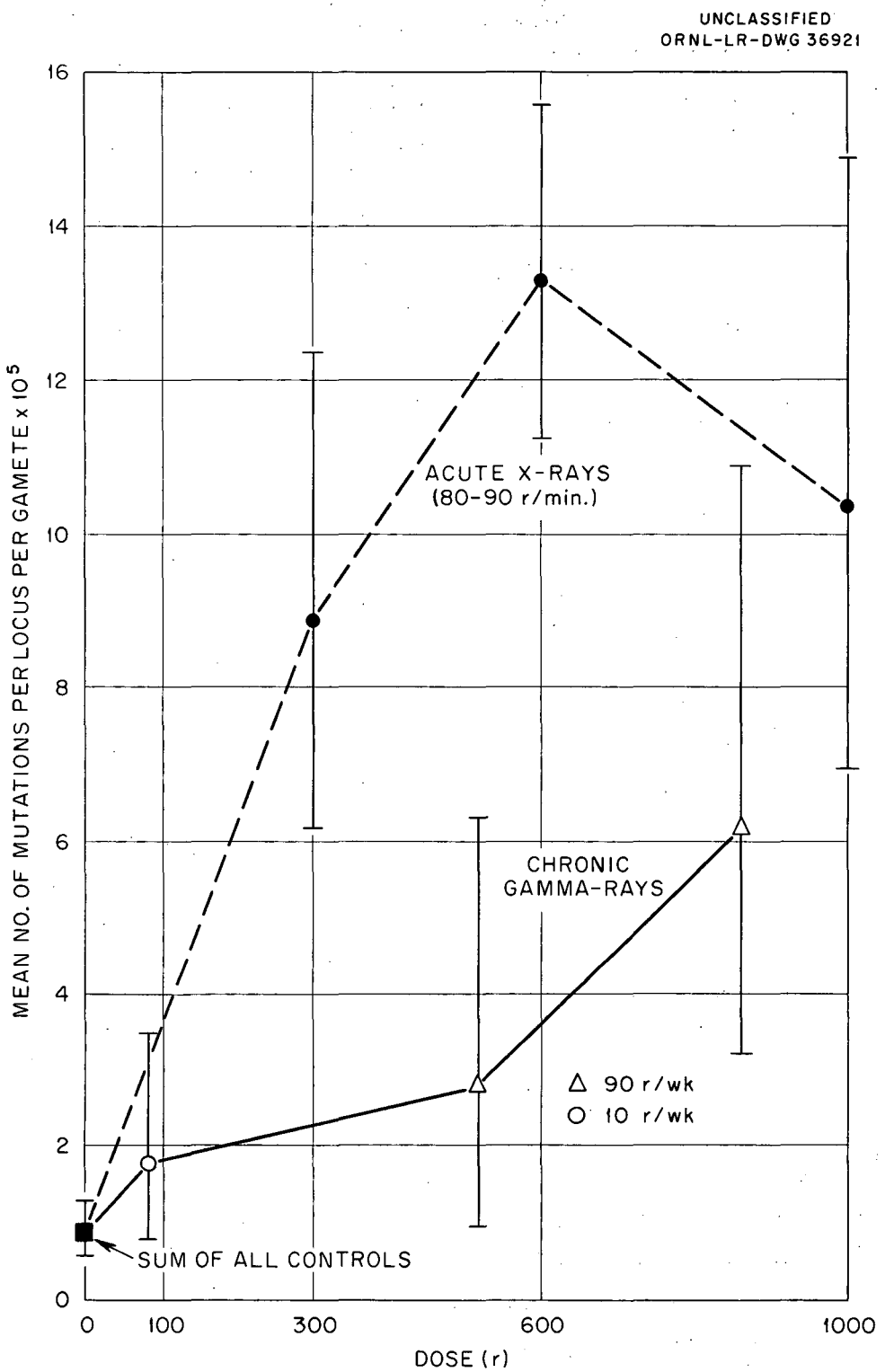


**Fig. 7. Mutation Rates at Seven Specific Loci in the Mouse, with 90% Confidence Intervals.**

indicating that the radiation intensity effect is on the mutation process itself.

A threshold response for all mutations in spermatogonia and oocytes is not a necessary consequence of the findings. Plausible hypotheses consistent with the present results can lead to other predictions.

From a practical point of view, the results indicate that the genetic hazards, at least under some radiation conditions, may not be as great as those estimated from the mutation rates obtained with acute irradiation. However, it should not be forgotten that even the lower mutation rates obtained with the present intensity levels are still appreciable.

## Exceptional Inheritance of a Sex-Linked Gene in the Mouse Explained on the Basis that the XO Sex-Chromosome Constitution Is Female

W. L. Russell L. B. Russell J. S. Gower

A sex-linked recessive gene, *scurfy*, that kills male mice before they reproduce, occasionally shows exceptional inheritance in producing the *scurfy* phenotype in females. The genetics of such females, which also die before reproductive age, was analyzed by transplanting their ovaries and obtaining offspring from them. Several possible explanations for these females could be ruled out by the results of the progeny tests. Of the remaining possibilities, the one that now appears most likely is that the *scurfy* females have the sex-chromosome constitution $X^{sf}/O$. That $X/O$ is female in the mouse is proved by independent data in the accompanying report by Welshons and Russell.[6] In the *scurfy* stock, the frequency of primary occurrence of $X/O$ is 0.9% or possibly greater. The $X^{+}/O$ daughters of $X^{sf}/O$ ovaries are probably of somewhat reduced viability, but those that survive to weaning age are fertile. Their $Y/O$ progeny probably die prenatally.

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[6]Also W. J. Welshons and L. B. Russell, Proc. Natl. Acad. Sci. U.S. 45 (1959) (in press).

## The Male Determining Action of the Y Chromosome in the Mouse

W. J. Welshons L. B. Russell

In the accompanying report by Russell, Russell, and Gower,[7] some of several possible explanations for the unexpected inheritance of a sex-linked gene were eliminated by genetic tests, some of which required ovarian transplantation. One of the remaining ones rested on the hypothesis that exceptional females were of the X/O constitution.

In order to test this X/O hypothesis, a series of crosses with other sex-linked markers were performed which produced additional exceptional females. These females were fertile and could, therefore, be analyzed genetically without ovarian transplantation. The analysis was successful in eliminating other possible hypotheses and indicated that the exceptional females behaved in a manner consistent with the hypothesis that they were hemizygous for an X chromosome. If these females have an X/O constitution, they should possess only 39 chromosomes. Bone marrow preparations indicated that this was the case. Thus, the cytological observations indicate that the exceptional females lack a chromosome, and the genetic evidence indicates that the missing element is an X chromosome.

Since a fertile female can be of the X/X or X/O constitution, it follows that the Y chromosome of the mouse is male determining. This finding may apply to other mammals. Thus, the mechanism of sex determination in the mouse is unlike that in *Drosophila*.[8]

## Effects of Chronic Gamma Irradiation on the Ovary

E. F. Oakberg L. B. Blalock

Previous experiments by Russell and Freeman[9] demonstrated that chronic gamma irradiation had less effect on fertility of female mice than a comparable dose of acute X rays. Since an acute exposure of 50

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[7]Also, W. L. Russell, L. B. Russell, and J. S. Gower, Proc. Natl. Acad. Sci. U.S. 45 (1959) (in press).

[8]C. B. Bridges, Am. Naturalist 56, 51-63 (1922).

[9]L. B. Russell and M. K. Freeman, Radiation Research 9, 174 (1958).

r of X rays destroys all the earlier oocyte stages,[10] it was of interest to determine what effect chronic irradiation would have on these cells.

Adult hybrid (101 × C3H)$F_1$ female mice were exposed to continuous radiation from a $Cs^{137}$ source at dose rates of 0.521, 0.262, and 0.056 rad/hr. Total doses are given in Table 13. Mice of the same age were assigned to the different treatments at random and placed in the radiation field at intervals calculated for completion of total doses at the same time. Controls of the same age were killed at the same time as irradiated animals. Histological procedures were the same as described earlier.[10]

The earliest oocyte stages show the highest sensitivity to chronic gamma exposure, with resistance of the oocyte increasing as the follicle develops (Table 13). Also, loss of oocytes continues throughout the irradiation period, since with the first four oocyte stages, number of survivors decreases with increasing dose. Chronic gamma irradiation is less effective in reducing oocyte numbers than acute X-ray exposure, since survival after 331 rads (Table 13) is much higher than after 50 r of acute X rays.[10] Thus some repair of radiation damage must occur with chronic radiation. Comparison of the reduction in number of oocytes observed in this experiment with the data of Russell and Freeman[9] indicates that the (101 × C3H)$F_1$ female mouse has a supply of ova that is greatly in excess of that required for normal fertility.

The killing of mouse oocytes by radiation still remains an enigma, since these cells are not dividing and, furthermore, are in the dictyate stage of meiotic prophase. In other organisms, oocytes usually survive much higher radiation doses, and in the male mouse, several hundred roentgens are required to kill appreciable numbers of spermatocytes. There also is no indication of whether the increase in resistance with development of the follicle results from changes within the oocyte or from an indirect effect mediated through the follicle. That the mouse oocyte is extremely sensitive to low levels of radiation is demonstrated

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[10]E. F. Oakberg, Biol. Semiann. Prog. Rep. Feb. 18, 1958, ORNL-2481, p 24.

Table 13. Number of "Normal" Oocytes in Female Mice Given Chronic Gamma Irradiation

| Follicle Stage | Experimental/Control Ratios | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.521 rad/hr | | | | | 0.262 rad/hr | | | | | 0.056 rad/hr | |
| | Total Dose (rads) | | | | | Total Dose (rads) | | | | | Total Dose (rads) | |
| | 41 | 82 | 164 | 246 | 331 | 41 | 82 | 164 | 246 | 331 | 41 | 82 |
| 1 | 0.375 | 0.115 | 0.077 | 0.067 | 0.000 | 0.327 | 0.135 | 0.067 | 0.010 | 0.010 | 0.202 | 0.356 |
| 2 | 0.452 | 0.215 | 0.073 | 0.027 | 0.023 | 0.616 | 0.324 | 0.164 | 0.078 | 0.046 | 0.429 | 0.557 |
| 3 | 0.582 | 0.479 | 0.188 | 0.079 | 0.012 | 0.679 | 0.412 | 0.230 | 0.073 | 0.091 | 0.667 | 0.697 |
| 4 | 0.826 | 0.772 | 0.413 | 0.315 | 0.120 | 1.000 | 0.533 | 0.391 | 0.348 | 0.207 | 0.587 | 0.793 |
| 5 | 0.765 | 0.667 | 0.588 | 1.176 | 0.392 | 0.804 | 1.294 | 1.020 | 0.529 | 0.510 | 0.608 | 0.725 |
| 6 | 0.641 | 0.795 | 0.744 | 0.923 | 0.564 | 1.077 | 0.744 | 0.590 | 0.718 | 0.385 | 0.744 | 0.590 |

by the fact that 41 rads given at a rate of 0.056 rad/hr reduces the number of early oocytes to less than half that observed in controls.

## The Acute Lethal Response of the Chick Embryo to X Radiation

R. A. Goff

Introduction. — Although the role of the chick embryo as a tool in radiobiological research is an important one, the available information on its lethal response to X irradiation is insufficient and contradictory. The only study[11] that can be considered a comprehensive survey of the response of the chick embryo throughout its incubation period reports the embryo to be much more resistant that is indicated by the results of a number of investigations[12-17] concerned with short spans of the developmental period. Several factors that none of these studies report may contribute to the discrepant results: (1) stage of development of the embryo at the time it is irradiated (in contrast to its chronological age); (2) natural infertility, inviability, incidence of anomalous embryos, and hatchability of the eggs; (3) season in which the eggs were produced. In the investigation here reported, the importance of the last of these factors has been tested, with proper account being taken of the first two. Some other conditions known to affect the expression of lethality in the chick embryo are: (1) quality of the radiation;[13,15,17] (2) intensity of the radiation;[11,13,15] (3) time after irradiation at which lethal effects are scored;[11,14,15] (4) method of determination and criterion of death; (5) temperature of the eggs while being irradiated.[15] In the present study an attempt was made to use factors in a range where considerable change in the factor did not affect the biological response. Hard X rays (250 kvp, hvl 0.4 mm of Cu)[13,15,17] were used at an intensity of 86–87

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[11]D. A. Karnofsky, P. A. Patterson, and L. P. Ridgway, Am. J. Roentgenol. Radium Therapy 64, 280–88 (1950).

[12]J. M. Essenberg, Radiology 25, 739–44 (1935).

[13]D. E. Lea, Am. J. Roentgenol. Radium Therapy 45, 605–13 (1941).

[14]M. B. Schneller, J. Morphol. 89, 367–95 (1951).

[15]J. Boland, Brit. J. Radiol. 27, 680–87 (1954).

[16]S. R. Glasser and F. T. Brayer, Federation Proc. 14, 58 (1955).

[17]H. I. Kohn and R. F. Kallman, Radiation Research 5, 710–14 (1956).

r/min.[11,13,15] A peak in the incidence of death occurs shortly after irradiation at the intensity of X rays employed. Lethality was, therefore, determined 20 hr after irradiation, this being identified as the period of acute death.[11,15] A criterion of death that is frequently used is absence of heart beat. However, heart beat can persist in early embryos after circulation has failed. Therefore, the criterion of death used was circulatory stasis, it being incompatible with life. Death was determined by direct observation of the embryo, in contrast to candling. The latter method is unreliable, particularly at early and late stages of development and may lead to significant errors. Incubation temperature was not maintained while the eggs were being irradiated; a maximum temperature decline of 4°F occurred. In view of known effects,[15] it is unlikely that this would significantly affect the results. White Leghorn eggs were incubated at 99 3/4°F, 58% relative humidity and staged by the criteria of Hamburger and Hamilton.[18] The total number of eggs irradiated was 1372 in the August series and 896 in the November series. A group of 10–12 eggs was irradiated at each dose level; 5–17 different exposures were made for the determination of each $LD_{50}$. Intervals between doses were from 25 to 50 r in most experiments.

Results. – The $LD_{50}$, 20 hr after irradiation, of two series of embryos, one irradiated in August, the other in November, is shown in Fig. 8. The vertical bars indicate the 95% confidence limits. The $LD_{50}$ of 2-day embryos in the November series was not plotted since insufficient information was gained in this experiment. However, by extrapolation from doses in the high-survival end of the lethal range an $LD_{50}$ of 1250–1300 r was estimated.

The developmental stages that occurred at each chronological age were essentially the same in the two series except in the groups irradiated at 2, 5, and 7 days of incubation, where the series differed by one stage.

Abnormal development caused by the radiation was not evident upon macroscopic examination, the 20-hr period being insufficient to permit the expression thereof. Specific effects on the vascular system were observed and peaks of incidence and severity correlated with developmental

[18] V. Hamburger and H. L. Hamilton, J. Morphol. 88, 49–92 (1951).

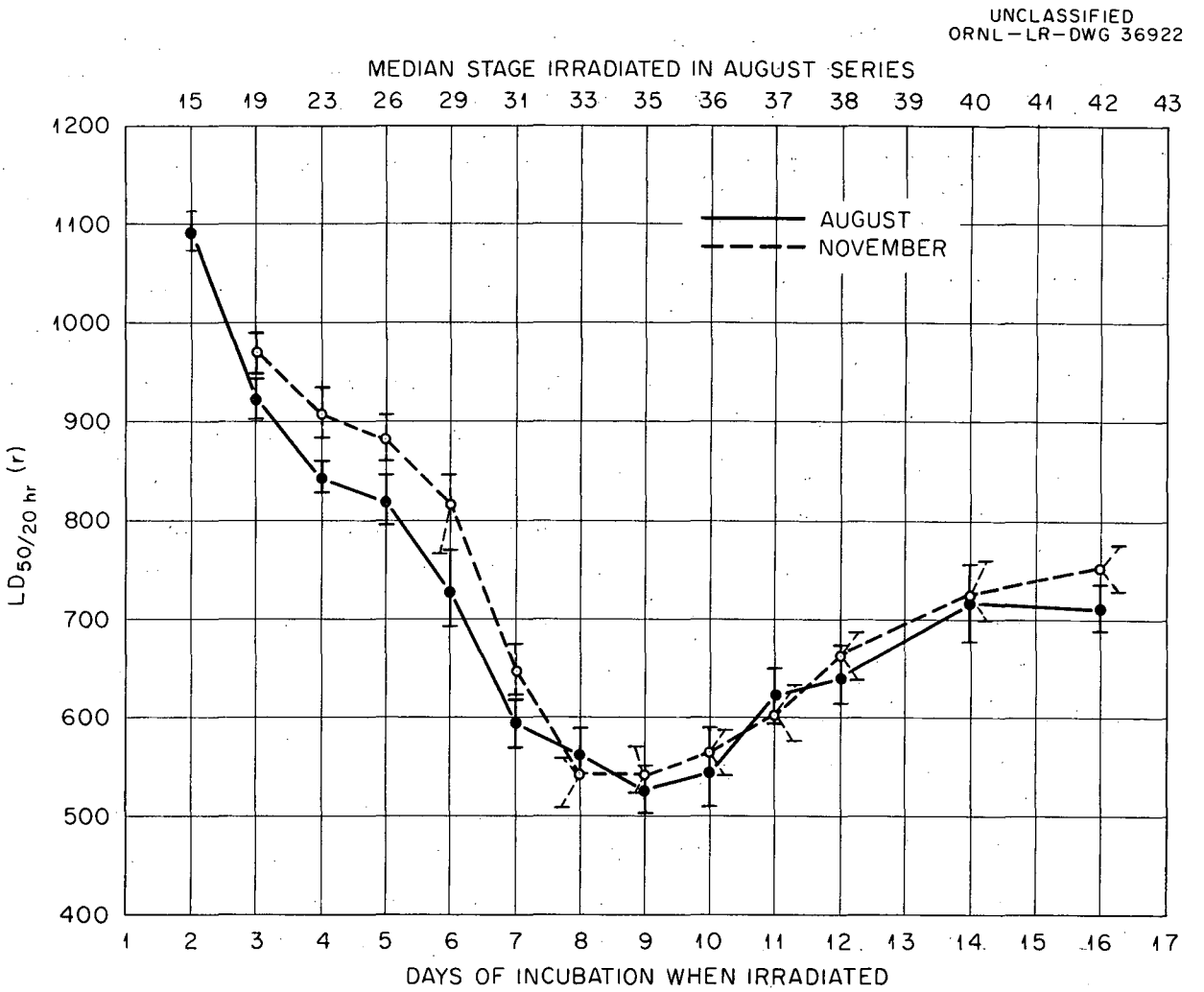


**Fig. 8. The $LD_{50}$ (20 hr After Irradiation) of Two Series of Embryos, One Irradiated in August, the Other in November.**

periods irradiated as follows: hemorrhage in the brain (4–5 days of incubation), mandible (5 days), eyes and legs (6 days), and in down feathers (12 days). General vasodilation and slight edema were responses evident in embryos irradiated after 6 days of incubation. For the most part, these responses occurred only at dose levels that produced some deaths in the acute period.

Discussion. – There was a significant difference between the two seasons tested in the sensitivity of embryos irradiated at 3 through 7 days of incubation. The increased resistance occurred in the November series of embryos, which demonstrated the lowest vitality (Table 14).

Table 14. Percentage Incidence of Factors Related to Vitality of Eggs

| Season | Infertile | Inviable at 12 Days | Abnormal Embryos at 2 Days | Hatchability* | Laying Intensity |
|---|---|---|---|---|---|
| August | 6.0 | 18.5 | 11.1 | 76.1 | 74 |
| November | 9.3 | 24.3 | 11.1 | 58.8 | 62 |

*From the commercial hatchery record.

Correlated with this lower vitality was the fact that the laying intensity of the flock from which eggs were obtained was lower and shell quality was inferior in November.[19] How these factors operate to improve resistance to radiation is not clear. The fact that developmental progression in the two series was in good agreement is evidence against the suggestion that the more resistant embryos were protected by a lower rate of metabolism. The idea that improved hatchability is associated with increased resistance is not supported by this evidence.

It is of interest to compare $LD_{50}$ values of this experiment with the results of other investigators. The present results are in disagreement with the findings of Karnofsky et al.[11] where the $LD_{50}$ at 2–4 days of incubation was over twice the highest shown here, while at 5–7 days the $LD_{50}$ doses were greater by a factor in excess of 1.6. The data on embryos of 8 days of age and older are more comparable. On the other hand, the results reported here are in good agreement with those of other workers using 4-day chick embryos[15,17] under similar experimental conditions. A comparison with studies in which the postirradiation period was very short[12] or considerably more than 24 hr[13,14,16] may not be valid; nevertheless, the results appear to be consistent.

The increase in sensitivity between the 6th day (stage 29) and 7th day (stage 31) of incubation was greater than at any other time except between 2 and 3 days (Fig. 8). When the data from the November series for the 7-day embryos was plotted according to stage of development (stage 30) instead of chronological age, most of this increase appeared to occur between 6 and 6 1/2 days. This demonstrates a possible source

[19]L. W. Taylor (ed.), Fertility and Hatchability of Chicken and Turkey Eggs, John Wiley & Sons, Inc., New York, 1949.

of error in the use of chronological age. There was also one stage difference in age between the 5-day embryos in the two series, but since the rate of change in sensitivity was slower in this period, it made little difference which criterion was used. The estimate of $LD_{50}$ for the 2-day embryos in the November series is made less reliable in that the median stage of these embryos was 14, whereas in the August series it was 15. Little is known of the sensitivity of stage-14 embryos but it is quite likely that this is a period in which it increases rapidly. It is conceivable that differences in stages observed may be the major factor responsible for the discrepant results noted above, particularly regarding the 2- and 3-day embryos. This, combined with the possible differences attributable to a different criterion of death and the uncertainty involved in candling at these early stages may, in part at least, account for the disagreement between the results of different workers.

Days 7–10 of incubation in the August series and days 8–10 in the November series were significantly more sensitive to X rays than were other ages. During this interval, a number of organ systems are undergoing changes in rate of their morphological and functional development, but a precise explanation for this sensitive period is not apparent and it is perhaps premature to attempt one. It should be noted that this period does not correspond with either of the general periods of sensitivity[20] (3–4 days and 19–20 days) in normal development. Similarly, the sensitive periods for the hemorrhaging observed are in need of an explanation. The discreteness of the response suggests that this material may offer the opportunity to elucidate the etiology of the hemorrhagic response so commonly elicited by radiation.

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[20]H. L. Hamilton, Ann. N.Y. Acad. Sci. 55 (Art. 2), 177–87 (1952).

# MICROBIAL PROTECTION AND RECOVERY

## RADIATION PROTECTION AND RECOVERY IN BACTERIA

A. Hollaender
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H. I. Adler
M. S. Engel
A. M. McCarthy
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A. S. Angel

### Growth and Nutrition of a Hemin-Dependent Mutant of *Escherichia coli*

G. E. Stapleton H. I. Adler M. S. Engel

Radiation studies in which a unique *E. coli* mutant strain ($H_7$) was used have been described in previous reports.[2,3] These studies involve the use of the catalase-negative state of this organism when grown in the absence of hemin. As an aid in understanding radiation effects on this organism, its biochemical activity was studied under several physiological conditions but chiefly aerobiosis and anaerobiosis. The inability of *E. coli* ($H_7$) to synthesize its own porphyrin as prosthetic group for respiratory enzymes suggests that cells grown with and without hemin should show a striking difference in their energy requirements for growth (Pasteur effect). Table 15 shows the growth response of these cells on glucose as the source of energy.

As predicted, the cells grown aerobically or anaerobically in the absence of hemin show maximum turbidity at glucose levels above 0.5%, as do anaerobic cultures of cells supplied with hemin. On the other hand, turbidity was maximum in aerobic cultures of cells supplied with hemin at 0.2% or less glucose; also such cells reach a higher final titer than cells under any of the other conditions tested. Aerobiosis seems to be detrimental to the organism cultured without hemin; the effect resembles that observed with some strict anaerobes. There is no evidence that the organism produces $H_2O_2$ under this condition.

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[1]Visiting investigator from abroad.

[2]H. I. Adler, Biol. Semiann. Prog. Rep. Aug. 15, 1957, ORNL-2390, p 46.

[3]H. I. Adler, Radiation Research 9, 451–58 (1958).

Table 15. Dependence of Growth on Concentration of Glucose[a]

| Growth Condition | Optical Density (at 540 mμ) of Overnight Cultures | | | |
|---|---|---|---|---|
| | 0.1% | 0.2% | 0.5% | 1.0% |
| Aerobic[b] plus hemin | 0.80 | 0.96 | 0.96 | 0.96 |
| Anaerobic[c] plus hemin | 0.32 | 0.49 | 0.66 | 0.74 |
| Aerobic minus hemin | 0.07 | 0.21 | 0.41 | 0.42 |
| Anaerobic minus hemin | 0.19 | 0.40 | 0.55 | 0.58 |

[a]All cells grown in 1% peptone + M/15 $PO_4$ buffer with glucose added to the final concentration indicated.

[b]Aerobic – bubbled with air continuously.

[c]Anaerobic – bubbled with 95% He:5% $CO_2$ continuously.

The cells harvested from cultures grown in the presence of hemin can oxidize a number of carbon sources, including pyruvate, succinate, fumarate, malate, α-ketoglutarate, glutamate, and acetate. Cells harvested from media not containing hemin do not oxidize any of these substrates at a significant rate.

In anticipation of more elaborate biochemical studies, we thought it important to have a chemically defined medium for this organism. Half-maximal growth has been obtained on a Lactobacillus medium similar to that suggested by Ravel.[4] Components of the medium are listed in Table 16.

The purines, pyrimidines, and vitamins could be deleted from this medium with no loss of activity. Cystine was the only amino acid that stimulated cell growth with or without hemin. Acetate stimulated growth only for cells cultured in the absence of hemin. The other amino acids, although they increased the rate of growth and shortened the lag phase, had no measurable effect on the final turbidity obtained in such cultures. Figure 9 shows the results of growth experiments with this organism in media lacking hemin. It is clear that cystine is required in all cases. Deletion of acetate reduces the growth in air by 50% but has no effect

[4]J. M. Ravel et al., J. Biol. Chem. 206, 391 (1954).

Table 16. Basal Salts Medium

| Component | Final Concentration (mg/ml) |
|---|---|
| $K_2HPO_4$ | 12.5 |
| $KH_2PO_4$ | 12.5 |
| $MnSO_4 \cdot 4H_2O$ | 0.0004 |
| $MgSO_4 \cdot 7H_2O$ | 0.08 |
| NaCl | 0.0004 |
| $FeSO_4 \cdot 7H_2O$ | 0.0004 |
| $NH_4Cl$ | 0.60 |
| Additional components for chemically defined medium | |
| Sodium acetate | 1.2 |
| Amino acids (18), each | 0.04 |
| Purines and pyrimidines, each | 0.01 |
| Vitamin mixture | |

under anaerobic conditions. Deletion of amino acids reduces the final turbidity to the same extent. Acetate may serve as a precursor of one of the amino acids.

## X-Ray Sensitivity of *Aspergillus terreus* After Treatment with Cysteamine

A. Hollaender A. M. McCarthy

Introduction. — Cysteamine is effective in protecting *Escherichia coli* from the lethal effects of radiation, the DRF being as high as 12 under certain conditions.[5] Treatment of certain strains of *E. coli* with cysteamine before irradiation increases the survival level and decreases the number of mutants that occur per unit dose.[6] In distilled water suspensions of a mutant of *Neurospora* K3/17, Kølmark found that cysteamine was itself toxic and slightly mutagenic and also increased the toxic and

[5]A. Hollaender and C. O. Doudney, in Radiobiology Symposium, 1954 (ed. by Z. M. Bacq and P. Alexander), p 112–15, Butterworths Scientific Publications, London, 1956.

[6]R. L. Girolami and A. Hollaender (unpublished data).

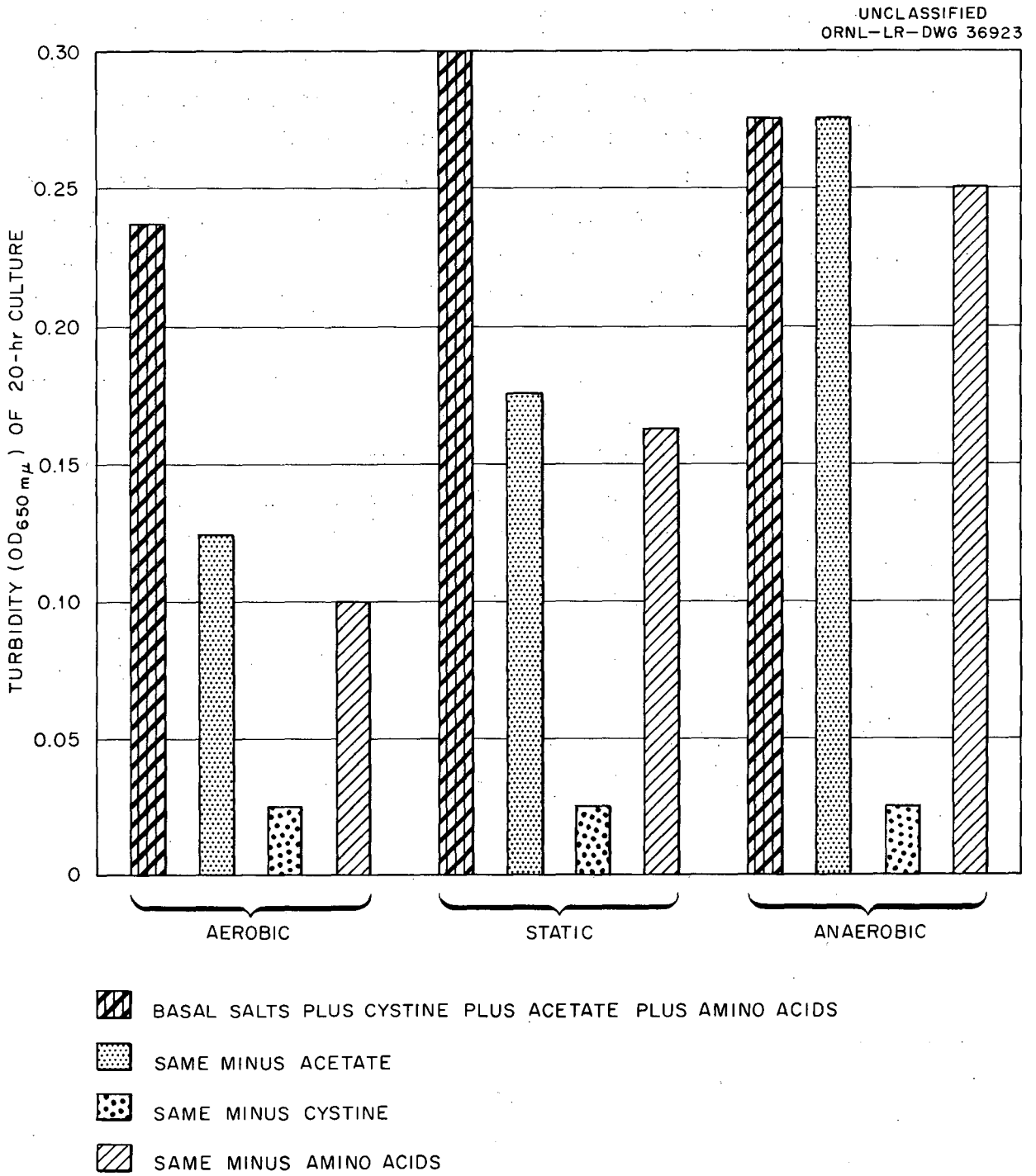


**Fig. 9. Growth of $H_7$ (Minus Hemin) in Chemically Defined Medium.**

mutagenic effects of X rays.[7] This toxicity and mutagenicity was considered somewhat unusual in view of the results with E. coli. The different results obtained with E. coli and Neurospora indicated the advisability of studying the effects of cysteamine on another microorganism. Aspergillus terreus was selected because it mutates at a rather high frequency as a result of irradiation and gives rise to many morphological mutations that are easily identified and that have already been characterized.[8] Yet the spontaneous mutation rate in A. terreus is extremely low.

Results. — Spores of A. terreus (in saline suspension) treated with cysteamine before irradiation derived some protection against X-ray effects. Evidence of this protection was an increase in the spores' ability to survive and germinate and a reduction in the number of morphological mutants at any given X-ray dose (Table 17). Cells exposed to 100 kr of

[7]H. G. Kølmark, Biol. Semiann. Prog. Rep. Feb. 15, 1958, ORNL-2481, p 10.

[8]K. B. Raper, R. D. Coghill, and A. Hollaender, Am. J. Botany 32, 165–76 (1945).

Table 17. Effect of Cysteamine on Survival and Mutation Production in Aspergillus terreus

| Molar Concentration of Cysteamine | Per Cent Survival | Per Cent Mutations |
|---|---|---|
| 0 | 0.041 | 78 |
| 0.002 | 0.11 | 69 |
| 0.006 | 0.23 | 51 |
| 0.01 | 0.30 | 45 |
| 0.02 | 0.62 | 43 |
| 0.04 | 0.96 | 38 |
| 0.06 | 1.5 | 34 |
| 0.08 | 1.7 | 28 |
| 0.1 | 2.3 | 23 |
| 0.14 | 2.2 | 23 |
| 0.2 | 2.1 | 26 |
| 0.28 | 1.7 | 27 |
| 0.36 | 2.1 | 23 |

250-kv X rays received some protection from concentrations as low as 0.002 M. Protection was maximal with 0.08 to 0.1 M concentrations, at which place it leveled off to a plateau. This was true for both mutation production and survival; it is significant that the plateaus for both were reached at the same concentration level. Cysteamine in concentrations up to 0.36 M, however, produced no evidence of any mutagenic or toxic effect. Treatment with 0.12 M cysteamine before X-ray doses of 40 to 100 kr gave a DRF of 1.8.

Discussion. — Mutation production can be considered a function of survival (Fig. 10); that is, it seems to be directly related to inactivation since the mutation frequency increases as the number of viable spores decreases. At a given survival level, the mutation frequency is the same for both the untreated and cysteamine-treated spores. Hollaender and Stapleton's statement about *E. coli* that "the mutation rate does not necessarily depend on the amount of energy the organisms have absorbed but rather on the number of cells that are able to form visible colonies"[9] seems to apply also to *A. terreus*.

In later experiments with saline suspensions of the same *Neurospora* mutant, Kølmark did find that cysteamine gave some protection, as evidenced by increased survival and fewer mutants found (see p 36, this report).

## Effects of Radiation on Growth and Transforming Principle in *Hemophilus influenzae*

J. H. Stuy

It has been reported[10] that UV irradiation inhibits cell division in the nonsurviving population but has little effect on growth as measured turbidimetrically. Lysis occurs in the nonsurviving population after 80 min postirradiation incubation. There is a dose-dependent inhibition of DNA synthesis for the first 40 min of postirradiation incubation, after which a net increase in DNA is measurable for the next 40 min (i.e., up until lysis occurs). Biological activity, as measured by transforming

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[9]A. Hollaender and G. E. Stapleton, Proc. Intern. Conf. Peaceful Uses Atomic Energy, Geneva, 1955, 11, 311–14 (1956).

[10]J. H. Stuy, presented for publication in J. Bacteriol.

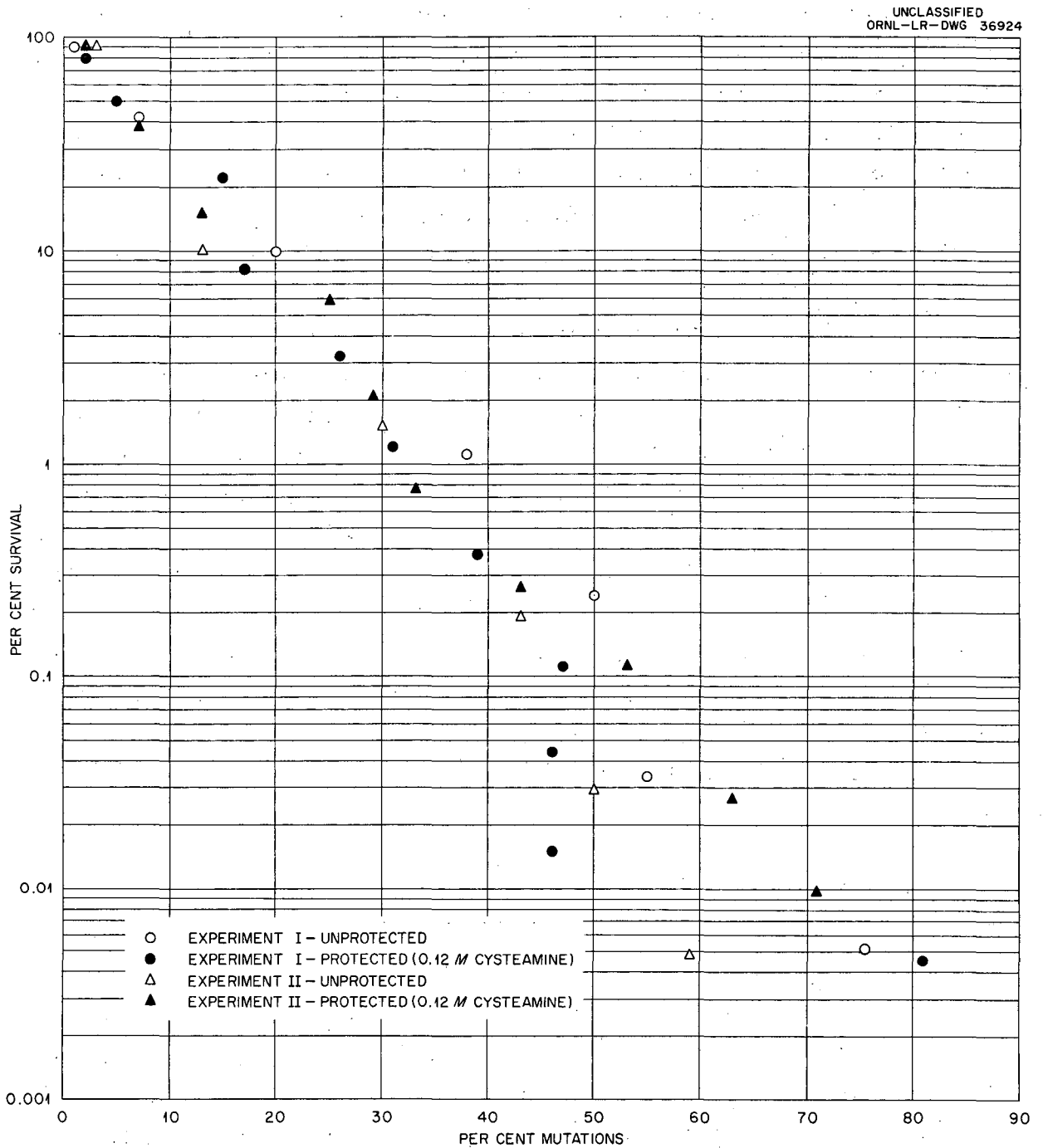


**Fig. 10. Mutation Production in *Aspergillus terreus* in the Presence and Absence of Cysteamine.**

ability, is lost concomitantly with inhibition of DNA synthesis but is rapidly regained. Upon cell lysis, the activity is lost concurrently with DNA depolymerization.

It was of interest to compare these effects of ultraviolet radiation with those brought about by ionizing radiation.

Results and Discussion. — As this work has progressed it has become necessary to improve the procedure for obtaining (1) quantitative liberation of the DNA of donor strains and (2) completely competent cultures of the recipient strain. Techniques have now been devised for measuring almost 100% of the transforming ability of the cellular DNA. Preliminary data from X-ray experiments indicate that chemically assayable DNA and its biological activity increase at about the same rate as does growth of the donor culture (Rd-3M). X-ray doses that reduce the viable population to $10^{-4}$ to $10^{-5}$ immediately inactivate the DNA to 70%. Subsequent incubation of the cells degrades the acid-insoluble DNA to below chemically detectable levels. Biological activity of the DNA is reduced simultaneously to about 10% of normal within 10 min after irradiation. After this the rate of inactivation proceeds more slowly for the next hour or two.

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## X-Ray-Induced Chromosome Aberrations in Diploid Embryonic Fibroblasts of the Chinese Hamster

M. A. Bender

Introduction. — Previous work has shown that the *in vitro* X-ray-induced chromosome breakage rate in diploid "epithelioid" cells derived from both human and spider monkey kidneys is about 0.004 break per cell per roentgen and that the control breakage rate is less than 0.01 break per cell.[3,4] Studies[4] on the femoral bone marrow of the spider monkey suggest that the *in vivo* rates are somewhat lower. In contrast, Puck[5] found that the rates for "fibroblasts" of human origin were enormously higher; the control was about 0.22 break per cell and the average induced rate was 0.027 break per cell per roentgen.

Results. — Fibroblasts from a clonal line derived from an embryo of the Chinese hamster by Puck's technique were examined for spontaneous and induced chromosome breaks by the method used in previous studies on epithelioid cells (Table 18). The average induced breakage rate is 0.008 break per cell per roentgen, or about double the rate previously found for epithelioid cells. The control rate is almost exactly the same as that found by Puck. It is particularly striking that the control aberrations included a number of two-hit types never seen in control epithelioid cells. To determine whether a high aberration rate is typical of this species, we examined bone marrow from an unirradiated Chinese hamster. No aberrations were found in any of the 29 cells examined.

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[1]Consultant.
[2]Visiting investigator from abroad.
[3]M. A. Bender, Science 126, 974–75 (1957).
[4]M. A. Bender, Proc. X Intern. Congr. Genet., p 21–22, 1958.
[5]T. T. Puck, Proc. Natl. Acad. Sci. U.S. 44, 772–80 (1958).

Table 18. Aberrations in Cultured Diploid Embryonic Fibroblasts of the Chinese Hamster 24 hr After Irradiation

| Dose (r) | Cells Scored | Chromatid Deletions | Isochromatid Deletions | Chromatid Exchanges | Other 2-Hit Aberrations | Breaks | Breaks per 100 Cells |
|---|---|---|---|---|---|---|---|
| None | 100 | 8 | 3 | 5 | 0 | 21 | 21.0 |
| 25 | 132 | 22 | 25 | 5 | 2 | 61 | 46.2 |
| 50 | 149 | 29 | 31 | 8 | 6 | 88 | 59.1 |
| 100 | 115 | 21 | 59 | 6 | 9 | 110 | 95.6 |
| 200 | 111 | 21 | 90 | 16 | 9 | 161 | 145.0 |

Discussion. – These results suggest that there may be a large difference in mutability between fibroblasts and epithelioid cells *in vitro*. The existence of such a difference would not be too surprising in view of the other differences between the two cell types, such as the ability of the fibroblast and the inability of the epithelioid cell to produce clones in single cell platings.

## Effect of Lymphatic Tissue Removal on Survival of Lethally Irradiated Mice Treated with Homologous Bone Marrow

R. R. Bigelow

Introduction. – Earlier studies[6] showed that removal of all superficial lymph nodes and the spleen had little or no effect on skin homograft survival. Neither was there any significant effect of major lymphatic tissue removal on survival of irradiated mice given heterologous (rat) bone marrow. The work has now been extended to include homologous bone marrow treatment.

Results. – Twenty-six (101 × C3H)$F_1$ mice given 950 r and 34 to 60 million (L × A)$F_1$ bone marrow cells intravenously showed a mortality of 61% at 30 days and 84% at 60 days. On the day after the lymph node and spleen operation, 36 (101 × C3H)$F_1$ mice were exposed to 950 r and given 34–60 million (L × A)$F_1$ bone marrow cells. Mortality was 66% at 30 days and 77% at 60 days.

[6]R. R. Bigelow, Biol. Semiann. Prog. Rep. Aug. 15, 1958, ORNL-2593, p 49–50.

Discussion. — The reduced mortality during the 30- to 60-day interval after homologous bone marrow treatment of operated irradiated mice is suggestive of a beneficial effect of lymphatic tissue removal, but additional work is needed to substantiate this finding.

## Homologous Fetal Liver for Irradiation Injury in Mice

C. C. Congdon D. B. Duda

Introduction. — Several studies have shown the superiority of homologous fetal blood forming cells over adult bone marrow for long-term survival of lethally irradiated mice. In preliminary experiments, one genetic combination, $C57BL/10D_2 \rightarrow (C3H \times 101)F_1$, did not show fetal tissues to be superior. Additional experiments were made to check this finding.

Results. — Forty-five male and female $(C3H \times 101)F_1$ mice exposed to 950 r and given $C57BL/10D_2$ bone marrow (20–40 million cells) intravenously were all dead by day 60. Twenty-nine of 44 male and female $(C3H \times 101)F_1$ mice exposed to 950 r and given $C57BL/10D_2$ fetal liver (35–39 million cells) were dead at day 90.

Discussion. — These results do not confirm the preliminary experiments, but reaffirm a superiority of homologous fetal tissues over adult tissues for transplantation purposes.

## Erythrocyte Life Span in the Irradiation-Bone Marrow Chimera

J. W. Goodman

Introduction. — Repopulation studies in homologous chimeras showed that the hosts' circulating red cells were completely replaced by donor cells at 30 days.[7,8] Since the normal mouse erythrocyte life span was slightly more than 50 days,[9] host cells must disappear abnormally fast. It seemed advisable to study the disappearance rate of host erythrocytes

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[7]R. D. Owen, Biol. Semiann. Prog. Rep. Aug. 15, 1958, ORNL-2593, p 60–61.

[8]J. W. Goodman, ibid., p 51–52.

[9]L. H. Smith and J. C. Toha, Biol. Semiann. Prog. Rep. Feb. 15, 1958, ORNL-2481, p 31–32.

by a second method. Chromium-51 was used to label host-type erythrocytes, which were injected 24 hr before X-ray and bone marrow treatment. A 100% $Cr^{51}$ reading was obtained just before irradiation, and the disappearance rate of the isotope was observed by sampling tail blood at intervals for seven weeks.

Results and Discussion. — The findings are summarized in Table 19. Rapid disappearance of host-type RBC in the $(L \times A)F_1 \rightarrow (C3H \times 101)F_1$ combination was confirmed, as was the less-rapid disappearance rate in

Table 19. Changes in $Cr^{51}$-Labeled Erythrocyte (Host Type) Disappearance

| Treatment | Host | Time (days) at Which $Cr^{51}$ Activity Has Reached — | | |
|---|---|---|---|---|
| | | 75% | 50% | 25% |
| None | $(C3H \times 101)F_1$ | 10 | 18 | 32 |
| 950 r + normal $(C3H \times 101)F_1$ bone marrow | $(C3H \times 101)F_1$ | 10 | 18 | 32 |
| 950 r + plethoric $(C3H \times 101)F_1$ bone marrow | $(C3H \times 101)F_1$ | 10 | 18 | 32 |
| 950 r + normal $(L \times A)F_1$ bone marrow | $(C3H \times 101)F_1$ | 10 | 11 1/2 | 16 |
| 950 r + plethoric $(L \times A)F_1$ bone marrow | $(C3H \times 101)F_1$ | 10 | 14 | 22 |
| None | $(C57BL \times DBA)F_1$ | 12 | 20 | 30 |
| 800 r + normal $(C57BL \times DBA)F_1$ bone marrow | $(C57BL \times DBA)F_1$ | 10 | 19 | 32 |
| 800 r + normal $(101 \times C3H)F_1$ bone marrow | $(C57BL \times DBA)F_1$ | 14 | 18 | 21 |
| 800 r + normal parent (DBA/2) bone marrow | $(C57BL \times DBA)F_1$ | 12 | 20 | 26 |
| None | DBA/2 | 10 | 21 | 39 |
| 850 r + normal DBA bone marrow | DBA/2 | 10 | 18 | 35 |
| 850 r + normal BALB/c bone marrow | DBA/2 | 10 | 18 | 32 |
| 850 r + normal $(C57BL \times DBA)F_1$ bone marrow | DBA/2 | 10 | 14 | 31 |

donor marrow from $(L \times A)F_1$ mice subjected to artificial polycythemia. Similar results were obtained in the homologous combination $(101 \times C3H)F_1 \rightarrow (C57BL \times DBA)F_1$ but not in the combination BALB/c $\rightarrow$ DBA/2, although the rate at which host RBC disappeared was slightly accelerated after the third or fourth week.

When parent marrow was given to an $F_1$, in this case a DBA/2 $\rightarrow$ $(C57BL \times DBA)F_1$, there was no early rapid disappearance of host type, although after the third week the disappearance rate was somewhat increased. In the reverse situation, $(C57BL \times DBA)F_1 \rightarrow$ DBA/2, accelerated host RBC disappearance was seen by the second week.

Were the mechanism of increased RBC destruction simply an early graft-vs-host phenomenon, one would expect a rapid disappearance of $F_1$ type when X rays and parent marrow were administered but not in the reciprocal combination. The data presented here do not confirm these expectations.

## AET Protection Against Radiation Damage to the Small Intestine of $A_1$ Mice

J. Maisin[10]

Introduction. — The effect of chemical (AET 8.8 mg intraperitoneally) protection on the small intestine of mice exposed to total-body irradiation was determined by measuring intestine length, wet and dry intestine weight, general histology of the mucosa, and number of cells with chromosome breaks in anaphase.

Results. — A transient shortening of the small intestine was observed during the first few hours after 1500 r of X rays. A second progressive shortening of the intestine occurred from the second day until death. In AET-protected mice the progressive shortening of the bowel was not observed. AET protection before 900 r prevented the weight loss of the small bowel. At 1500 and 2000 r there was also marked reduction in intestine weight loss in chemically protected mice compared to nonprotected animals.

The histology of the intestinal mucosa showed a nearly normal appearance in chemically protected mice at four days after 900 r, at five

[10]In collaboration with D. G. Doherty, J. Moutschen, and others.

days after 1500 r, and six or seven days after 2000 r. In control mice given 1500 and 2000 r the mucosa was completely destroyed.

In AET-protected mice at 900 r, 100 cells in anaphase showed 17 chromosome breaks, whereas 57 chromosome breaks were seen in 100 anaphase cells at day 4 after 900 r without chemical protection. These observations were carried out on tissue sections.

Discussion. — These results demonstrate the remarkable protective effect of AET, given intraperitoneally, on the small intestine of irradiated mice.

## Abnormal Differentiation of Crypt Cells in the Mouse Small Intestine After 2500 r of X Rays

R. A. McGrath

Introduction. — As a result of irradiation that produces intestinal death, normal mitosis in the small intestine generative compartment (crypts) stops. Some crypt cells are lost by cytolysis and some by abortive division. The remaining cells were the object of this study.[11]

Results. — Approximately equal groups of $(L \times A)F_1$ mice were killed at 6-hr intervals from the time of exposure to 2500 r of X rays until 96 hr after irradiation. Feulgen- and hematoxylin—eosin-stained sections of the small intestine were studied. At each interval the average cell length on the villus was measured, the number of nuclei per unit volume of tissue was counted, and the nuclear area of cells in different regions of the epithelium was computed.

The change in average cell length on the villus 6–84 hr after radiation is relatively small. A greatly reduced number of nuclei per unit volume with increasing time after exposure and the absence of flattening constitute evidence for cell hypertrophy. Evidence for actual enlargement of nuclei (Table 20) comes from measurement of the average nuclear area of cells in all three zones of the epithelial membrane. Villus cells did not show significant abnormal differentiation. Crypt cells, however, showed extreme hypertrophy. As the crypts disappeared, these large cells migrated onto the villus until they formed the entire cover.

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[11] R. A. McGrath and C. C. Congdon, Intern. J. Radiation Biol. 1, 80–85 (1959).

Table 20. Average Nuclear Area in Three Regions of the Mucosa at Different Intervals After Irradiation

| Time After Irradiation (2500 r) (hr) | Area* ($\mu^2$) ± 10% | | | |
|---|---|---|---|---|
| | Crypt | Neck and Villus Base | | Villus |
| Control | 44 | 31 | | 24 |
| 6 | 58 | 37 | | 35 |
| 12 | 73 | 45 | | 37 |
| 18 | 91 | | Migration → 52 | 38 |
| 24 | 104 | | 58 | 38 |
| 30 | 119 | | 66 | Extrusion from villus |
| 36 | | Migration → 129 | 68 | |
| 42 | Crypt disappears | 137 | 68 | |
| 48 | | 139 | Extrusion from villus | |
| 54 | | 131 | | |
| 60 | | | Migration → 123 | |
| 66 | | | 114 | |
| 72 | | | 104 | |
| 78 | | | 97 | |
| 84 | | | 92 | |
| 90 | | | 89 | |
| 96 | | | 86 | |
| | | | Epithelial cover disappears | |

*Nuclei considered as elliptical.

Discussion. — This study suggests that some crypt cells are killed and lost through cytolysis, others are capable of migration and abnormal differentiation. It is not clear whether the abnormal cell population represents only those cells that were prevented from dividing or also contains cells that may have gone through one or more divisions, thus incorporating abnormal daughter cells into the population.

## Protection of Bone Marrow Cells *in vitro*

L. H. Smith

Introduction. — The study of protective measures on mouse bone marrow cells X irradiated *in vitro* has continued.[12] Attempts are being made to determine the media requirements for the protective action of AET·Cl.

Results and Conclusions. — Several media have been tested including saline, Tyrode's solution, rat plasma, 10% mouse plasma or serum, and 10% bovine serum albumin in Tyrode's solution. The results indicate that the presence of protein in the medium enhances the protective action of AET·Cl. Even distantly related heterologous bovine serum albumin is better than saline or Tyrode's solution and as effective as 10% mouse plasma or serum in saline. Whole mouse plasma or serum, however, seems to provide the optimum environment for the protective action of AET·Cl on irradiated mouse bone marrow cells.

Other studies with mouse bone marrow suspensions have shown that either reducing the temperature of the cells to 0°C during irradiation or adding AET·Cl to the low temperature suspension immediately after irradiation does not protect the cells.

## Retransplantation of Chimera Bone Marrow

O. Vos

Introduction. — In some homologous donor-host combinations embryonic hematopoietic tissues provide a better long-term survival after lethal whole-body irradiation than adult bone marrow. From studies in this laboratory it is known that many changes found in the lymphoid tissue of mice treated with adult bone marrow do not occur in mice treated with fetal hematopoietic tissues. It may be assumed that a difference exists in the immunological reaction between donor and host tissues in the two types of chimeras. Studies of the peripheral blood erythrocytes did not reveal a difference in the repopulation with donor cells between both kinds of chimeras. It seemed worth while to examine the therapeutic effect of bone

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[12] L. H. Smith and P. W. Rueff, Jr., Biol. Semiann. Prog. Rep. Feb. 15, 1957, ORNL-2267, p 42.

marrow from both kinds of chimeras in mice of the original host and donor strains.

Results. — The preliminary experiments showed one obvious difference in the therapeutic effect of both types of chimeric bone marrow cells. A small number of bone marrow cells from chimeras originally treated with fetal hematopoietic cells protected mice of both host and donor strain, whereas a small number of cells derived from chimeras originally treated with adult bone marrow protected only donor strain mice (Table 21).

Discussion. — The difference between the minimal number of isologous and homologous bone marrow cells needed for an adequate 30-day survival of lethally irradiated mice is explained by an early reaction of the host against the graft.[13] The results described here could be explained by a loss of the isoantigenic properties of fetal hematopoietic cells or a loss of their sensitivity to specific antibodies. However, before this conclusion can be proved, new experiments must be done with presensitized and lethally irradiated mice as hosts for the chimera bone marrow and the origin of the produced erythrocytes must be determined.

[13]D. W. van Bekkum and O. Vos, J. Cellular Comp. Physiol. 50, Suppl. 1:139 (1957).

Table 21. Therapeutic Effect of Chimera Bone Marrow Cells in Host and Donor Strain Mice

| Host | No. of Cells Injected $\times 10^{-6}$ | Chimeric Cells Injected — $(101 \times C3H)F_1 \rightarrow (L \times A)F_1$ | | | |
|---|---|---|---|---|---|
| | | Fetal | | Adult | |
| | | No. of Mice | 30-Day Survivors | No. of Mice | 30-Day Survivors |
| $(L \times A)F_1$ | 3.5–4.2 | 20 | 17 | 25 | 16 |
| $(L \times A)F_1$ | 0.2 | 15 | 12 | 23 | 0 |
| $(101 \times C3H)F_1$ | 3.5–4.2 | 21 | 8 | 25 | 12 |
| $(101 \times C3H)F_1$ | 0.2 | 24 | 8 | 25 | 10 |

# RADIATION IMMUNOLOGY

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## Immunochemical Studies of Chicken Precipitin System

T. Makinodan
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Introduction. — It has been well established by Wolfe and his associates[3-5] that chicken precipitin serum, in contrast to that of mammals, has several peculiarities. Of these, high salt dependency seems to be the most perplexing. They found that a salt concentration ten times that of the mammalian antiserum (i.e., 1.5 M NaCl) was most conducive to maximum precipitation of a given soluble antigen and its specific chicken antiserum (at near-equivalence and at antigen-excess regions). Their evidence suggests that the increase in precipitation can be accounted for by a salting-out of the soluble antigen-antibody complexes. This preliminary report presents data showing that, in a BSA-chicken anti-BSA system in 1.5 M NaCl, a serum protein coprecipitates with the antigen-antibody complexes; also, the system exhibits the salting-out phenomenon.

Results. — Pooled chicken anti-BSA sera and their antibody-active fractions were analyzed by double serum-agar diffusion, paper electrophoresis, ultracentrifugation, and the standard immunochemical methods before and after precipitation with BSA in phosphate-buffered 0.15 and 1.5 M NaCl solutions (pH 7.0). The high salt-dependent phenomenon is present even though the purity of the antibody-active fraction is increased by a combination of curtain-electrophoretic and salting-out

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[1]Consultant.

[2]Cell Physiology Group.

[3]M. Goodman, H. R. Wolfe, and S. Norton, J. Immunol. 66, 225–36 (1951).

[4]M. Goodman and H. R. Wolfe, J. Immunol. 69, 423–34 (1952).

[5]M. Goodman, H. R. Wolfe, and R. Goldberg, J. Immunol. 72, 440–45 (1954).

methods. This can be seen for example in a 40% saturated ammonium sulfate fraction in the presence or absence of fibrinogen (Fig. 11).

Furthermore, with unfractionated antisera in 1.5 M NaCl, the precipitate formed in antibody- and antigen-excess regions contains the antibody γ-globulin, the BSA antigen, and another serum protein. In contrast, only antibody γ-globulin and the BSA antigen were found when the reaction was run in 0.15 M NaCl. This coprecipitating serum protein, which is present in normal nonimmunized adult chickens, behaves electrophoretically like a β- or γ-globulin, diffuses very slowly in a serum-agar medium, is precipitable in a 30–40% saturated ammonium sulfate solution, and has an $S_{20}$ value of ~20.

Unfractionated antisera at -20°C aged one to four weeks showed a decrease in the apparent antibody titer (micrograms of protein nitrogen per milliliter) in 1.5 M NaCl, but not in 0.15 M NaCl. In an antibody-active fraction lacking this coprecipitating protein, however, the titer did not change at either concentration.

Discussion. – These results support the conclusion of Wolfe and his associates that a salting-out phenomenon of the soluble antigen-antibody complexes takes place. It is evident, however, that a coprecipitating

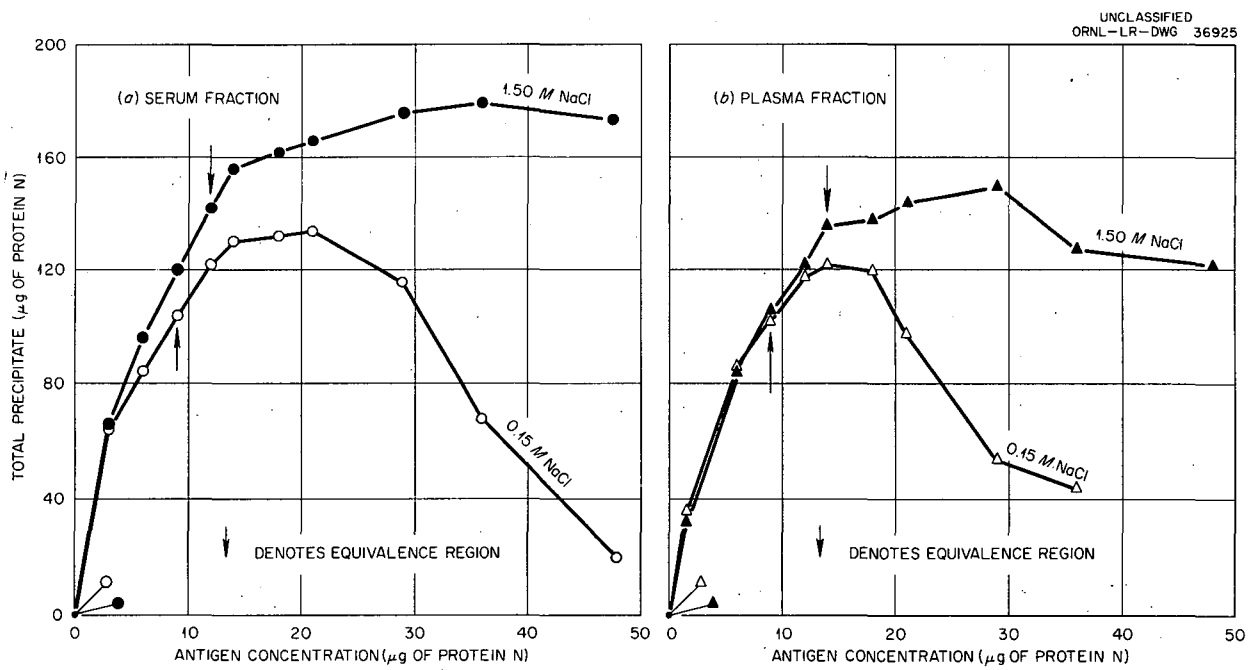


**Fig. 11. The $(NH_4)_2SO_4$ Fraction of Serum vs Plasma.**

serum protein also contributes to the total precipitable material in 1.5 M NaCl.

## Antibody Production in Mice as a Function of Age and Antigen Type

N. Gengozian T. Makinodan

Introduction. — Previous studies on antibody production in normal adult (C3H × 101)$F_1$ mice have shown an equal agglutinin response to sheep and rat RBC antigens. However, in lethally irradiated mice protected with isologous bone marrow and in sublethally irradiated mice with or without isologous bone marrow treatment, the response to the more distantly related antigen, sheep RBC, was greater than to the more closely related antigen, rat RBC.[6-8] From these studies we postulated that X rays injure the mechanism for recognizing and responding to the more closely related antigen more severely than the recognition mechanism for the distantly related antigen; during recovery this difference is expressed by a greater response to the more foreign antigen. This led to the following hypothesis: If, as is generally assumed, X rays cause the immune mechanism to revert to a more immature status, the existence and development of such a recognition mechanism in young mice may become apparent in studies of agglutinin production to foreign antigen.

Results. — In this study, the agglutinin response to sheep, rabbit, and rat RBC antigens was tested in groups of mice 1 day to 30 weeks old. Preliminary studies showed a low level of natural circulating agglutinins to these antigens and a noncross-reactivity of the three antigen-antibody systems. For each age, three groups of mice were injected with sheep, rabbit, or rat RBC antigens, and were bled at intervals of 6, 12, 18, 24, 30, 40, and 50 days. The immune response of each age group for each antigen studied was then expressed in terms of the mean total agglutinin titer ($\log_2$) obtained from 70 individual serum titrations (Fig. 12). One-day-old mice failed to respond to rat RBC but showed a weak

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[6]T. Makinodan, N. Gengozian, and C. C. Congdon, J. Immunol. 77, 250–56 (1956).

[7]N. Gengozian and T. Makinodan, J. Immunol. 77, 430–36 (1956).

[8]T. Makinodan and N. Gengozian, J. Immunol. 81, 513–18 (1958).

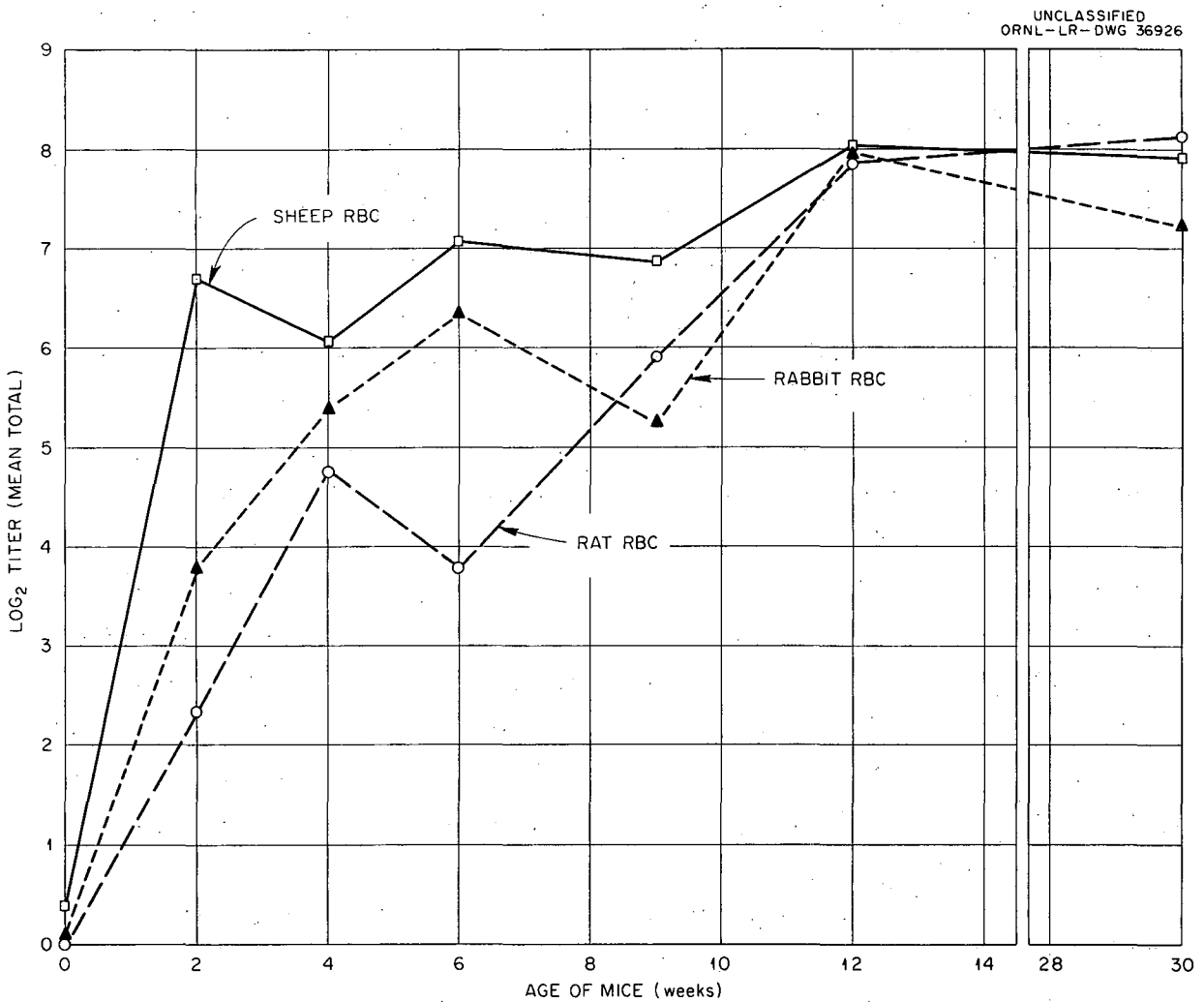


**Fig. 12. Agglutinin Response to Sheep, Rabbit, and Rat RBC Antigens in Mice of Various Age Groups.**

yet definite response to sheep and rabbit RBC antigens. The 2-week-old mice showed agglutinin response to all three antigens, and here the degree of response was dependent on the relation of the antigen to the host, that is, sheep > rabbit > rat RBC. The differential response in favor of the more distantly related antigen was evident also in age groups of 4, 6, and 9 weeks, although in the 9-week-old mice the response to rat RBC seemed to be greater than to the rabbit RBC. Mice 12 weeks of age responded equally to the three antigens. Mice 30 weeks old showed agglutinin production to sheep and rat RBC comparable with that of 12-week-old mice, but their response to rabbit RBC was slightly lower.

Using also the mean peak titers and induction period as criteria of immune status, we found that this differential response in favor of the more distantly related antigen was also evident in various age groups.

The data show the existence of a recognition mechanism and its development with age in the animal. They also indicate that a linear or curvilinear relation with age of the animal and its ability to produce antibodies does not necessarily exist. This is particularly apparent from the greater agglutinin response among 4-week-old mice than among 6-week-old mice to rat RBC antigen (Fig. 12).

Studies on the dependency of the agglutinin response on the antigen dose and type indicate that, in young mice, increasing the amount of antigen injected does not necessarily result in a greater agglutinin production. In contrast, antibody production may be markedly reduced.

Discussion. — The data support the original hypothesis that, with the development of the animal, a recognition mechanism exists whereby the animal's ability to produce antibodies is in part dependent upon the genetic relation of the antigen to the host, that is, a more distantly related antigen may evoke a greater immune response than a more closely related antigen.

## Electrophoretic Studies of Mouse Sera After X Irradiation and Bone Marrow or AET Treatment

I. C. Shekarchi T. Makinodan

Introduction. — Death caused by lethal total-body X irradiation (950 r) can be prevented by preinjection of AET or postinjection of isologous (IBM) or closely related heterologous bone marrow. It has been established that protection afforded by bone marrow injection in these mice is associated with at least a temporary transplantation of the donor cells.[9] In 950 r-RBM mice, a delayed foreign bone marrow reaction characterized grossly by a marked weight loss and death usually occurs after recovery from the immediate effects of radiation (i.e., from the third to the seventh week after treatment). The evidence presented suggests that this reaction is caused by an *in vivo* antigen-antibody reaction.[9] To obtain more information about this reaction, paper

[9]T. Makinodan, J. Cellular Comp. Physiol. 50, Suppl. 1, 327–51 (1957).

electrophoretic studies of serum samples of normal, 950-r, 950 r-IBM, AET-950 r, and 950 r-RBM (101 × C3H)$F_1$ mice were made one to eight weeks after treatment.

Results. – The mean serum protein concentration of normal mice was 8.4 mg of protein nitrogen per milliliter throughout the observation period. Comparable values were obtained after 950 r-IBM and AET-950 r treatments. After 950 r only, the increase was gradual and continued until the time of death. In 950 r-RBM mice, the serum protein concentration decreases during the first three weeks and then increases up to the sixth week. An abrupt decrease in protein concentration was observed during the seventh week, followed by an increase toward the normal value.

Electrophoretic analysis revealed that only in the 950-r and 950 r-RBM mice was the serum protein composition significantly altered. In normal mice, the mean serum albumin concentration was 4.2 mg of protein nitrogen per milliliter. Comparable values were obtained after 950 r-IBM treatment. The concentration was slightly below normal among the AET-950 r mice during the first week but essentially normal thereafter. There was a marked decrease among the 950-r mice until death. This pattern of decrease in albumin was also seen among the 950 r-RBM mice during the first week. It returned to normal during the second week and then gradually decreased up to the seventh week. By the eighth week, the albumin concentration was again returning to normal. The mean serum globulin content for normal mice was 4.2 mg of protein nitrogen per milliliter. Comparable values were obtained after 950 r-IBM and AET-950 r treatment during this period. There was a rapid increase among the 950-r mice until death, which was primarily the result of the increase in the lipoprotein globulins ($\beta$ and $\gamma$). Among the 950 r-RBM mice there was a slight increase during the first week, a decrease during the second week, and a gradual increase to values above normal during the third to the seventh week. Return to normal values was apparent during the eighth week. Again this change in globulin patterns was mainly attributable to the lipoproteins ($\beta$ and $\gamma$).

Discussion. — These results substantiate and extend our previous work.[10] In 950-r mice the increase in serum globulin concentration may be caused by the release of tissue globulin-like proteins by damaged tissues. Mice treated with AET prior to or IBM after 950 r recovered rapidly from the immediate radiation effects, and no major change in the serum protein patterns was seen. The 950-r mice treated with RBM showed striking variation in the serum protein patterns. Previous studies[9] have shown that within the first two weeks after treatment the RBM transplant proliferates rapidly. During this period the serum albumin and globulin concentrations return to essentially normal range. Associated with the delayed foreign bone marrow reaction is an increase in the lipoproteins ($\beta$ and $\gamma$).

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[10] I. C. Shekarchi and T. Makinodan, Radiation Research 7, 449 (1957).

## PATHOLOGY AND PHYSIOLOGY

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### Modification of the Foreign Spleen Reaction in Mice by Paralysis of the Donor Spleen Cells

G. E. Cosgrove, Jr. R. A. Popp E. S. Ledford

Introduction. – The occurrence of a fatal reaction in irradiated $F_1$ hybrid mice injected with parental strain spleen cells has been reported previously. Because this reaction failed to occur in recipients exposed to subthreshold doses of X rays or injected with subthreshold numbers of spleen cells, it is postulated that the pathogenesis of the reaction depends on the initiation at some critical rate of antirecipient antibody production by the graft. Failure of the reaction to occur is tentatively ascribed to immunological paralysis of the graft by excess recipient antigen. To test this hypothesis, spleen cells have been exposed to recipient-type antigen *in vitro* prior to their implantation.

Results. – Young adult (C57BL × 101)$F_1$ mice given a single sublethal dose of 250-kvp whole-body X rays (500–600 r) were divided into three groups and injected intraperitoneally with C57BL spleen homogenate (approximately $150 \times 10^6$ nucleated cells, or 1 spleen-equivalent). In one group, the donor-spleen homogenate was stored overnight in the refrigerator (10°C) with a homogenate of *recipient*-type liver prior to implantation; in another, the donor-spleen homogenate was stored similarly with a homogenate of *donor*-type liver; and in the third, the donor-spleen homogenate was implanted without storage. Storage of the donor spleen with recipient liver inhibited its lethal activity, which was

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unaffected by the other treatments (Fig. 13). Analysis of the hemoglobin of the surviving recipients disclosed that in the majority of animals erythrocytes of donor type were present in large numbers.

Discussion. — These observations support the hypothesis that the foreign spleen reaction may be inhibited if the graft is paralyzed with excess recipient-type antigen. Preliminary results with other strain combinations suggest that paralysis of the graft prior to implantation may have general usefulness in inhibiting foreign bone marrow as well as spleen reactions.

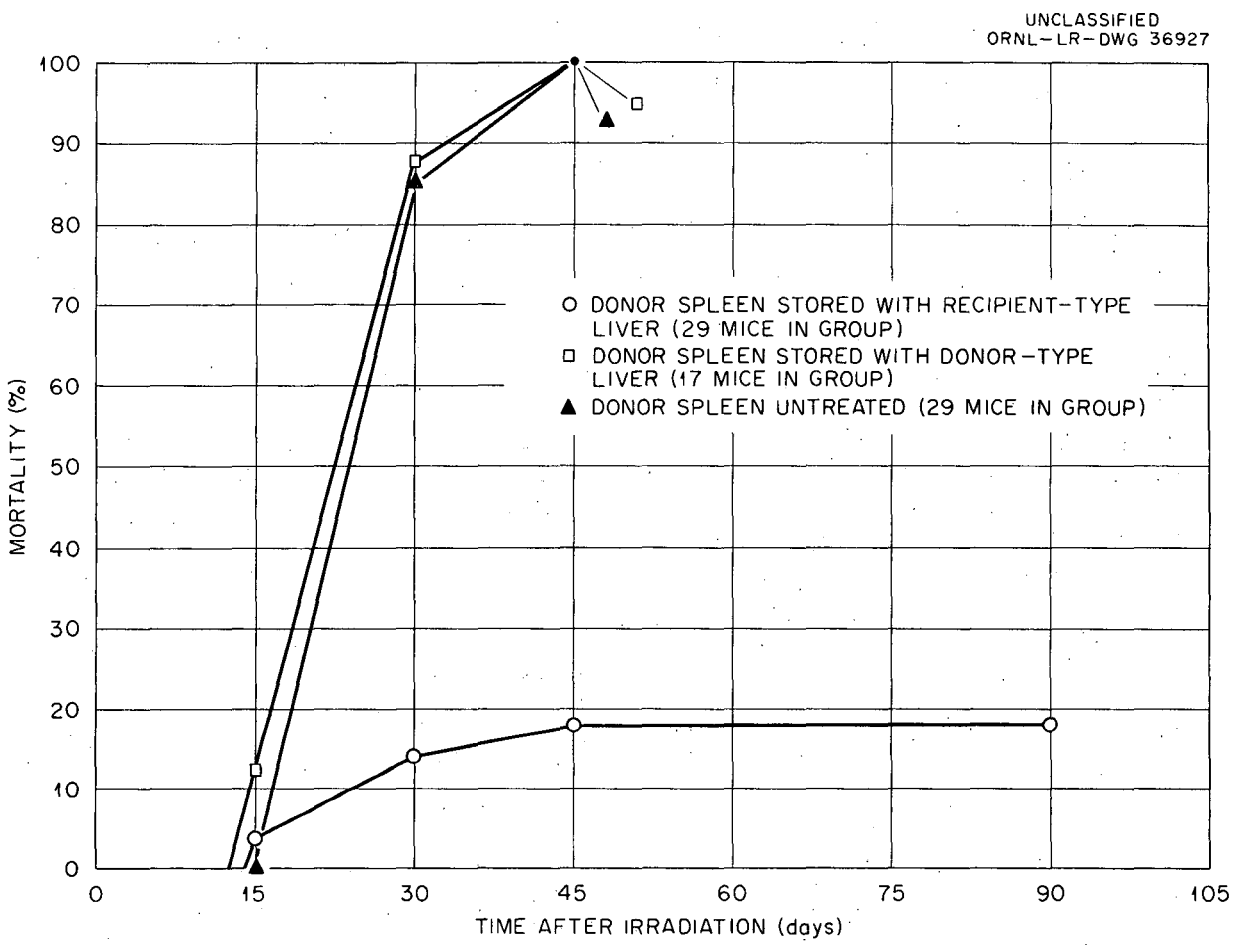


**Fig. 13. Mortality in Irradiated (C57BL × 101)$F_1$ Mice Injected with Paralyzed and Nonparalyzed C57BL Spleen Cells. X-ray dose, 500–600 r.**

Solubility of Hemoglobin as a Red Cell Marker in Irradiated Mouse Chimeras

R. A. Popp G. E. Cosgrove, Jr. D. Reynolds

Introduction. – Agar[3] and starch gel[4] electrophoresis of hemoglobin, as well as serological markers,[5] have been used to demonstrate the transplantation and growth of erythrocyte progenitors in lethally irradiated mice. Homozygous and heterozygous-diffuse types of hemoglobin cannot, however, be distinguished from one another electrophoretically. This report describes solubility differences between homozygous-diffuse, heterozygous-diffuse, and single type mouse hemoglobins which can be used to identify erythrocytes containing any of these various types.

Results. – Hemoglobins studied were: (1) homozygous-diffuse hemoglobin from C3H, 101, RF, DBA/2, and (101 × C3H)$F_1$ mice, (2) single hemoglobin from C57BL, C57L, 11G/R1, and $pc^{ch}$ mice, and (3) heterozygous-diffuse hemoglobin from (C57BL × 101)$F_1$, (C57BL × DBA/2)$F_1$, and (C57L × A)$F_1$ mice. Oxyhemoglobin from washed red cells was converted to carbon monoxyhemoglobin (HbCO) and the solubility of HbCO was determined in $K_2HPO_4$–$KH_2PO_4$ buffer at pH 6.5.[6] Preparations stood for 18–24 hr at room temperature before they were filtered through Whatman No. 1 filter paper. The optical density of the HbCO filtrates read at 575 mμ differed for red cells of C57BL, 101, and (C57BL × 101)$F_1$ mice (Fig. 14). The corresponding hemoglobin genotypes of the various other strains exhibited similar respective solubilities.

Discussion. – Since comparable results were obtainable when a few drops of whole blood from the tail vein were placed in distilled water and the solution was bubbled with carbon monoxide before phosphate buffer was added, red cells of irradiated mouse chimeras were readily identified by the solubility of their hemoglobin in 3.08 M phosphate buffer (Table 22). The advantage of the use of solubility over electrophoretic mobility is also shown in Table 22; although transplants of strain

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[3]W. Welling and D. W. van Bekkum, Nature 182, 946–47 (1958).

[4]R. A. Popp, G. E. Cosgrove, Jr., and R. D. Owen, Proc. Soc. Exptl. Biol. Med. 99, 692–94 (1958).

[5]R. D. Owen, Radiation Research 9, 164 (1958).

[6]Y. Derrien, Biochim. Biophys. Acta 8, 631–40 (1952).

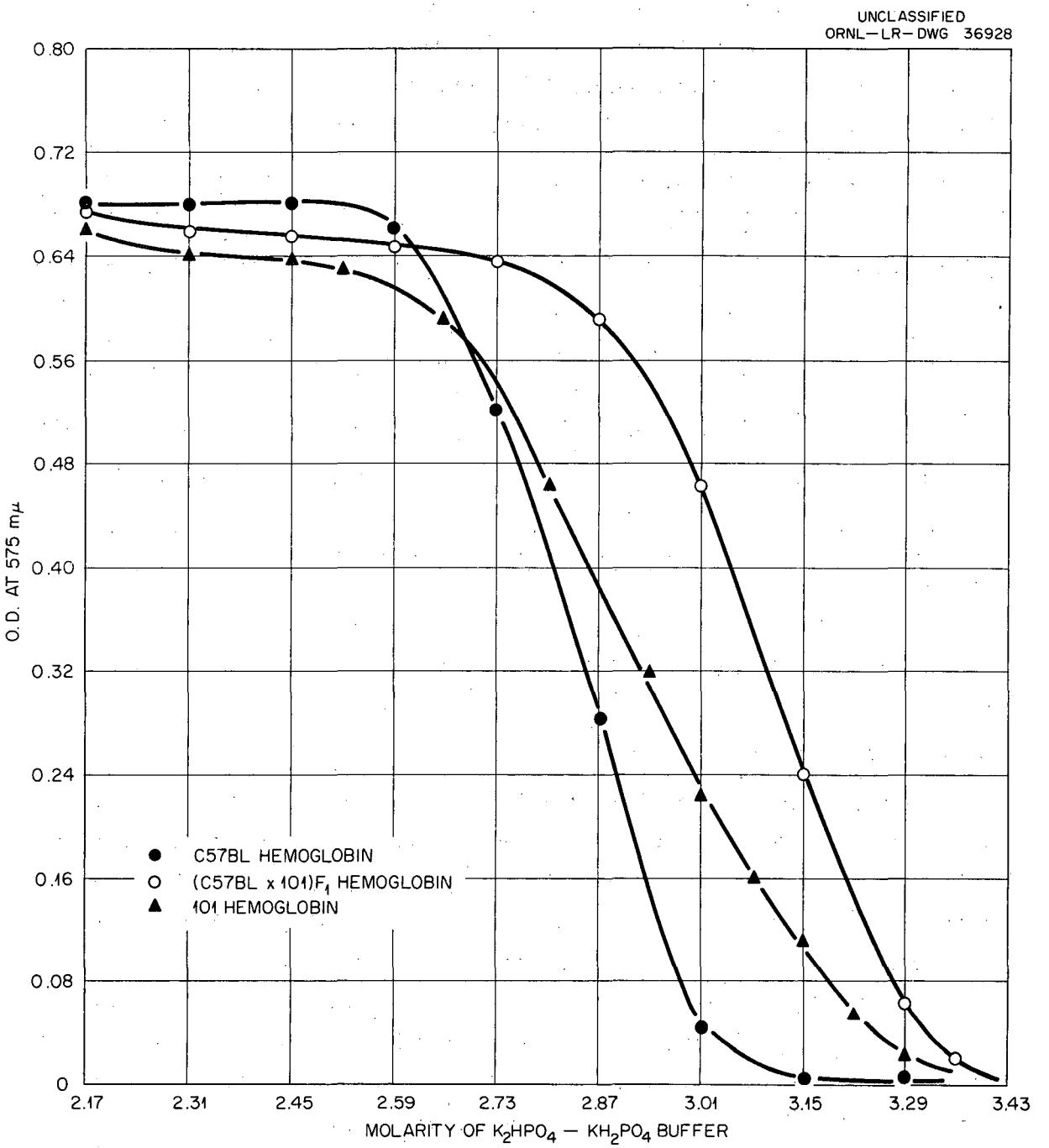


**Fig. 14. Salting-Out Curves for Genetically Different Mouse Hemoglobins.**

101 cells in group B and absence of such cells in group C cannot be determined by electrophoresis, they can be readily distinguished by hemoglobin solubility. This method will therefore be of great value in transplantation studies with homografts of bone marrow and spleen.

Table 22. Identification of Hemoglobin in Irradiated Mouse Chimeras

| Group | No. of Mice | Recipient Strain | Donor Strain | Chimera Hemoglobin | |
|---|---|---|---|---|---|
| | | | | Electrophoretic Mobility* | Optical Density** |
| A | 7 | (C57BL × 101)$F_1$ | C57BL | Single | 0.039 ± 0.011 |
| B | 17 | (C57BL × 101)$F_1$ | 101 | Diffuse | 0.18 ± 0.008 |
| C | 26 | (C57BL × 101)$F_1$ | C57BL or 101 | Diffuse | 0.40 ± 0.012 |

*As determined by starch gel electrophoresis.

**Of HbCO filtrate.

## Influence of Age at Irradiation on Susceptibility of RF Mice to Radiation-Induced Leukemia

E. P. Sniffen W. D. Gude

Introduction. — Earlier investigations indicated that newborn RF mice were relatively refractory to the induction of myeloid leukemia by X rays despite marked susceptibility to lymphoma induction. The present experiment was carried out to study the susceptibility of these mice at various ages during the first two months of life.

Results. — Susceptibility to induction of myeloid leukemia increased gradually during the first several weeks after birth, reaching a maximum at about 70 days of age (Table 23). Conversely, susceptibility to induction of thymic lymphoma declined after the first month of life, being minimal by 70 days of age.

Discussion. — The explanation for these age variations in susceptibility is being sought in experiments now in progress. It appears noteworthy that the rate of cell turnover in the bone marrow and thymus varies with age in the same manner as the susceptibility of these tissues to leukemogenesis. The significance of this correlation remains to be determined.

Table 23. Influence of Age at Irradiation on Susceptibility to Radiation-Induced Leukemia in RF Mice

| Age at Exposure to 300 r (days) | No. of Mice Exposed | Leukemia Incidence (%) | |
|---|---|---|---|
| | | Myeloid | Thymic Lymphoma |
| 1–3 | 123 | 5 | 21 |
| 7–9 | 44 | 7 | 14 |
| 14 | 105 | 25 | 12 |
| 28 | 84 | 40 | 13 |
| 40 | 104 | 43 | 19 |
| 70 | 66 | 52 | 8 |
| 180 | 111 | 32 | 9 |
| Nonirradiated control | 312 | 3 | 5 |

## Effects of Radiomimetic Drugs on Hematopoietic and Intestinal Tissues of Rats

T. T. Odell, Jr. B. C. Caldwell

Introduction. — The effects of certain drugs on the hematopoietic system and lymphatic tissues are being compared with those of irradiation in the study of homotransplantation and in the search for agents that will promote susceptibility to bone marrow homografts.

Results. — Sprague-Dawley, CC, or DD rats were injected intravenously with a single maximum sublethal or lethal dose of one of the drugs shown in Table 24 and were serially killed for histologic examination. Preliminary data on the damaging effects of these drugs on hematopoietic and intestinal tissues are summarized in Table 24. The greatest depression of lymphatic tissue was induced with chlorambucil. All four agents had a marked effect on bone marrow cellularity, 4M20 apparently producing the most severe and lasting damage. The two nitrogen mustard derivatives and dimethylmyleran brought about degeneration of crypt cells and reduction in mitotic activity in the intestinal epithelium, but this was not seen in rats injected with 4M20.

Table 24. Toxic Effect of Drugs on Hematopoietic and Intestinal Tissues

| Drug* and Dose Given | Time After Treatment (days) | No. of Rats | Degree of Injury** | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Lymph Nodes | Thymus | Spleen | | Bone Marrow | Intestine |
| | | | | | White Pulp | Red Pulp | | |
| 4M20 | 2 | 2 | + | - | + | +++ | +++ | |
| 100 mg/kg | 3 | 2 | + | ++ | + | +++ | +++ | - |
| | 4 | 2 | - | + | | +++ | +++ | - |
| | 5 | 2 | - | +++ | | +++ | +++ | - |
| | 7 | 1 | + | +++ | +++ | +++ | +++ | - |
| Chlorambucil | 1 | 2 | + | ++ | + | + | + | + |
| 8.5 mg/kg | 2 | 2 | +++ | +++ | +++ | +++ | +++ | ++ |
| | 4 | 2 | +++ | +++ | +++ | +++ | ++ | ± |
| | 8 | 2 | +++ | +++ | | + | + | - |
| Melphalan | 1 | 2 | + | + | + | | + | ++ |
| 11 mg/kg | 2 | 2 | + | ++ | + | + | +++ | +++ |
| | 4 | 2 | + | +++ | + | ++ | +++ | + |
| | 8 | 2 | ++ | +++ | ++ | +++ | + | - |
| | 11 | 2 | - | ± | + | + | - | - |
| Dimethylmyleran | 2 | 2 | + | - | - | ++ | ++ | + |
| 21 mg/kg | 3 | 2 | + | + | - | ++ | +++ | +++ |
| | 4 | 2 | + | ++ | ++ | +++ | +++ | +++ |

*4M20 is 4-(p-dimethylaminostyryl)quinoline, prepared by Dr. C. T. Bahner. Dimethylmyleran, chlorambucil, and melphalan were sent to us by Professor Alexander Haddow. Dr. Donald S. Searle of Burroughs Wellcome and Co. (U.S.A.) Inc. supplied melphalan.

**Symbols indicate degree of injury: -, normal; +, slight; ++, moderate; +++, marked; blank, no record.

Discussion. — These observations indicate that, of the four drugs used, chlorambucil has the greatest effect on the lymphatic tissue and therefore might be the most effective in depressing the immune system. So far, however, bone marrow homotransplantation in rats treated with chlorambucil has not been successful. The marked differences in the toxicity of the various agents for different organs is quite striking and suggests the possibility that combinations of these agents, with or without radiation and AET or without either, may have potential value in conditioning recipients for homografts of bone marrow.

## Protein Metabolism in the Rat-Mouse Chimera

W. Friedberg D. Reynolds

Introduction. — The wasting disease characteristic of rat-mouse chimeras is associated with abnormalities of the serum protein electrophoretic pattern. A study of the kinetics of protein metabolism in these animals has therefore been undertaken.

Results. — After $I^{131}$-labeled human serum albumin (HSA-$I^{131}$) was injected into mice, repeated whole-body radioactivity measurements were made on individual animals (pretreatment with stable iodide reduced radioiodine retention sufficiently for protein elimination rates to be determined). Decrease in radioactivity (physical decay corrected) seemed to follow first-order kinetics, the biological half life (T/2) being approximately 26 hr (24–30 hr) in 12 normal (101 × C3H)$F_1$ females. In seven rat-mouse chimeras, the T/2 of HSA-$I^{131}$ was 14–19 hr 31 days after 950 r of X radiation and rat bone marrow implantation. Serum albumin-globulin ratios in the chimeras were lower than normal because of decreased albumin concentration, often associated with increase in the concentration of one or more globulin fractions, as has previously been reported.[7] The degree of chimerism per mouse was 50–80%, as determined by a quantitative test for circulating rat erythrocytes.[8] Short-term effects of 950 r of X radiation were studied in eight mice protected with AET and in nine mice shielded over one femur. The T/2 of HSA-$I^{131}$ was about the same in both groups (AET, 20–25 hr; leg shielding, 21–23 hr) four days after irradiation but slightly less than in unirradiated controls (24–30 hr); however, the statistical analysis of the data collected in these experiments has not been completed.

Discussion. — The finding of a shorter T/2 of protein in chimeras, as compared with normal mice, suggests increased catabolism, although tests for intact protein in the excreta have yet to be made. Further studies will be carried out to test the hypothesis that there is a general increase in protein catabolism in the chimera associated with a greater increase in the anabolism of certain serum globulin fractions.

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[7] T. Makinodan, J. Cellular Comp. Physiol. 50, Suppl. 1, 327–51 (1957).

[8] W. St. Amand and L. H. Smith, J. Appl. Physiol. (in press).

## Influence of Head Irradiation on Survival of Mice

J. W. Conklin

Introduction. — Although shortening of the life span by irradiation is ascribed to no single radiation injury, the relative importance of effects on various organs is not known. A series of mice exposed to localized X irradiation of the head for study of cataract formation has provided the following data on longevity.

Results. — RF male mice exposed to 250-kvp X rays at a rate of 100 r/min were shielded in lead cylinders in such a way that only the head was irradiated directly, the dose to the body being less than 2% of the incident radiation. The mice were exposed in groups of 20 per treatment sample at an age of ten weeks. At each dose level, one group was injected with AET, 9.9 mg in buffered solution, 10 min before irradiation, and another group received an equal volume of physiological saline concurrently. The median survival time was affected very little, if at all, at dose levels below 1000 r (Table 25), but at higher levels the life span was markedly reduced, the predominant cause of death being starvation from loss of teeth.[9] The mice given AET exhibited less shortening of life than those given saline.

Discussion. — It is evident that the effects of head irradiation account in only very small measure for the life-shortening action of

[9]A. C. Upton et al., Radiation Research 8, 475–79 (1958).

Table 25. Effects of Head Irradiation on Longevity of RF Male Mice

| X-Ray Dose (r) | Median Survival Time (months) | |
|---|---|---|
| | AET-Treated | Saline-Treated |
| 1800 | 13.0 | 0.5 |
| 1500 | 17.5 | 10.5 |
| 1000 | 19.5 | 18.0 |
| 750 | 19.0 | 19.0 |
| 500 | 19.5 | 19.0 |
| Control | 19.5 | 20.5 |

sublethal doses of whole-body radiation. These results agree with those of Kallman and Kohn,[10] who also noted that partial-body irradiation was relatively ineffective in shortening the life span. Whether the life-shortening effects of total-body irradiation represent merely the sum of effects on individual organs or whether a more complex interaction of effects is involved remains to be determined.

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[10]R. F. Kallman and H. I. Kohn, Science 128, 301 (1958).

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## Electrophoretic Analysis of Soluble Tissue Proteins

N. G. Anderson H. D. Swanson

Introduction. — The soluble or supernatant proteins of rat liver, kidney, brain, and testis have been analyzed electrophoretically to see what differences and similarities exist, and to provide a normal base line suitable for comparison with preparations from experimentally treated animals.

Methods and Results. — Soluble protein preparations were prepared from perfused rat organs by centrifuging 0.25 M sucrose homogenates 1 hr at 104,000 × g. The clear supernatant solutions were dialyzed against an NaCl-sodium phosphate buffer, pH 7.5, 0.1 ionic strength, and analyzed in the Spinco model H electrophoresis apparatus. Four to seven independent runs were made for each tissue. As shown in Fig. 15 a characteristic complex pattern was obtained for each organ studied. The average mobility of each low point between peaks was found, and the areas between these mobility values were determined. Comparison between organs showed that the soluble fraction of brain contains material, about 5% of the total, with a mobility greater than that of any discernible component of the other organs. Many of the peaks of liver and kidney, but not other organs, have similar mobilities. To express the average mobility of the protein preparations, the curves were divided into halves and the mobility of the dividing line determined. The values, expressed in T units, were: liver 2.0, kidney 3.0, testis 4.1, and brain 5.2.

Conclusion. — Characteristic electrophoretic patterns were obtained for the soluble phase of four organs of the rat. These were analyzed in

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[1]Consultant.
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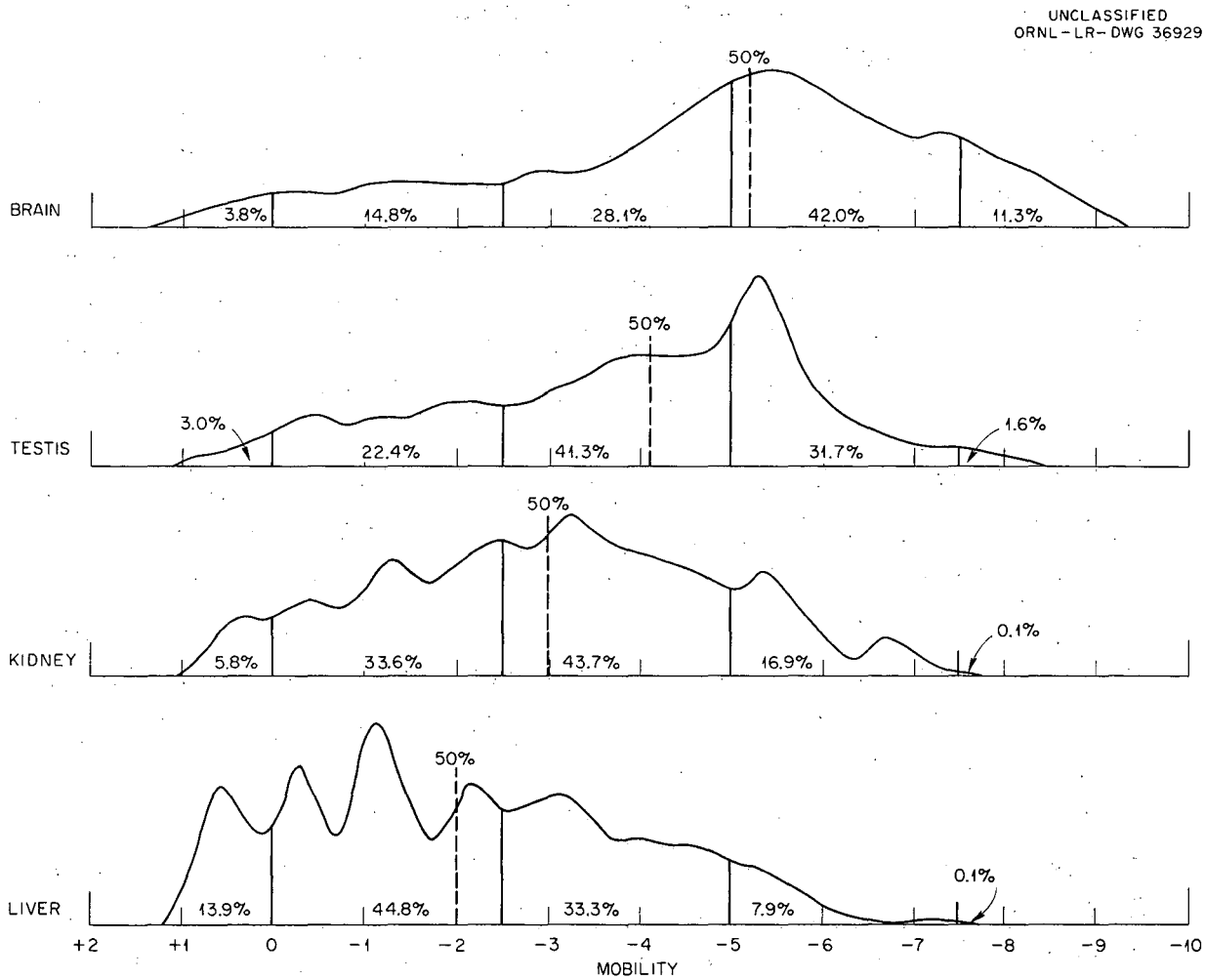


**Fig. 15. Electrophoresis Patterns of Mobility of Brain, Testis, Kidney, and Liver Homogenates.**

such a way that small changes could be noted, and repeated a sufficient number of times to give an indication of the normal variation. Comparison between organs showed both qualitative and quantitative differences.

## Flagella of *Polytoma uvella*

C. J. Brokaw

Introduction. — Several workers have found that flagellated cells, after extraction in solutions containing glycerol, saponin, or digitonin, each plus EDTA and salts, can be reactivated by placing them in solutions containing ATP. The present study is an attempt to define the structural and chemical requirements for the activity of flagella.

Results. — Flagella can be removed cleanly from the orange biflagellate alga *Polytoma uvella* by suspending the cells in a solution containing 0.2% saponin and 0.05 M $MgCl_2$, at room temperature (25–27°C). These flagella do not have a mitochondrial sheath or other complications and are therefore good material for a study of the actual contractile organelle. The flagella usually continue to beat for 5 to 10 sec after removal. No effects of ATP on the beating of flagella removed by this and a number of other chemical techniques have yet been obtained. Measurements of the ATPase activity of the flagella have therefore been undertaken.

Homogeneous preparations of intact flagella have been prepared from *Polytoma* by treating the cells with saponin and $MgCl_2$, followed by differential centrifugation. Counting the flagella in a bacterial cell-counting chamber, under dark-field illumination, reveals yields of approximately 50%. Liberation of inorganic phosphate after incubation with ATP at room temperature was measured by the standard Fiske-SubbaRow method. Results have been extremely variable.

Preparations of flagella that show reasonably high ATPase activity lose their activity after washing; this activity is not recovered in the wash solution. When the washed flagella are recombined with the original supernatant saponin-$MgCl_2$ solution, they regain much of their original ATPase activity. Further study is being directed at understanding this effect and determining whether these, or earlier, measurements of the ATPase activity of *Polytoma* flagella give any information about the rate of utilization of ATP during the normal activity of the flagella, and the access of ATP to the contractile elements after saponin treatment.

# NUCLEIC ACID CHEMISTRY

W. E. Cohn F. J. Bollum
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## Pseudouridine

W. E. Cohn A. J. Bandy J. X. Khym

Introduction. – The so-called "fifth" nucleotide of RNA, identified as the phosphate ester of 5-ribosyluracil[2] (now termed "pseudouridine," or ψ-uridine[3]) has been further investigated with regard to its unusual chemical properties.

Results and Discussion: Isomeric Forms Produced by Acid. – Since the previous report,[2] a fourth nucleoside isomer has been discovered in acid-treated material (N HCl, 100°C). Previously, a total of three forms were known. These were separable by anion exchange in borate-containing solutions and are termed A, B, and C in the order of appearance. The last of these is the "normal" (presumably ribofuranoside) form, whereas the A form accumulates on prolonged acid treatment. On prolonged acid treatment (~12 hr), an equilibrium of about 75% A, 8% B, and 17% C is reached among the three forms.

Paper chromatography has now shown that the A peak is composed of two substances – $A_S$ (slow) and $A_F$ (fast). These have characteristic spectral differences, resembling C and B, respectively, and – like B – are interconvertible in acid. Although $A_S$ is apparently the form first produced by acid treatment of C, $A_F$ slowly accumulates until it equals the amount of $A_S$ present. The amounts present as a function of time of acid treatment are shown in Fig. 16. We may postulate the equilibria and sequences as one of the following:

$$C \rightleftharpoons B \rightleftharpoons A_S \rightleftharpoons A_F \qquad (1)$$

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[1]Visiting investigator from abroad.

[2]W. E. Cohn, A. J. Bandy, and D. G. Doherty, Biol. Semiann. Prog. Rep. Aug. 15, 1958, ORNL-2593, p 77–78.

[3]W. E. Cohn, Biochim. Biophys. Acta (in press).

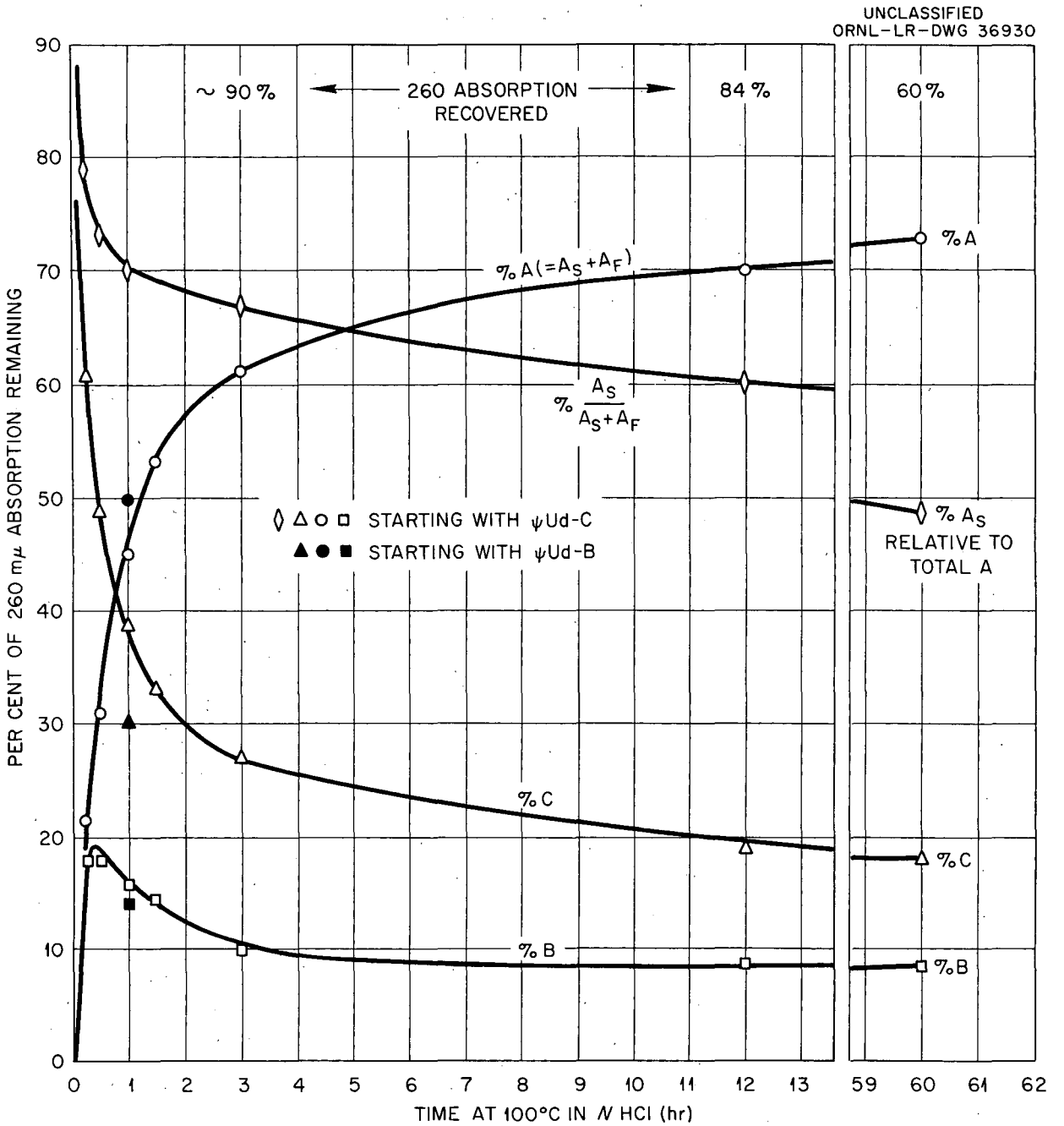


**Fig. 16. Relative Amounts of Pseudouridine Isomers After Acid Treatment of Pseudouridine (ψUd).**

$$C \rightleftharpoons B \begin{matrix} \nearrow A_F \\ \searrow A_S \end{matrix} \qquad (2)$$

$$C \rightleftharpoons \begin{matrix} \nearrow A_S \\ \searrow \end{matrix} B \rightleftharpoons A_F \qquad (3)$$

Sequence (1) seems unlikely since more C than B is formed from either $A_S$ or $A_F$, or than the other A isomer. Sequence (3) seems favored by the strong spectral resemblances between $A_S$ and C, and between $A_F$ and B. (The spectra of the last two strongly resemble the hydrogenated product, in which one mole of $H_2$ breaks the same cyclic sugar bond that is probably broken by prolonged hydrolysis.)

It seems reasonable to approach the problem of the structures of the isomers by quantitative periodate oxidations and by nuclear magnetic resonance spectra on purified material; the production of the required amounts is under way.

The —NH— groups of the pyrimidine ring may be methylated by diazomethane. A study of the sequence and amount of methylation[4] of pseudouridine (and analogues) indicates (a) the existence of at least one intermediate form and perhaps two, (b) a doubled enhancement of mobility on paper in organic solvents of the final methylated product as compared to those pyrimidine compounds that can add only one methyl group. This is consistent with the 5-ribosyluracil structure proposed for pseudouridine.

Three problems remain, however. (a) It is difficult to obtain evidence for more than one singly methylated pseudouridine. The one usually encountered appears to be the 3-methyl derivative; only once, in several tries, has one that appears to be the 1-methyl derivative been found. (b) Two forms of completely methylated pseudouridine are found, and no immediate explanation is at hand. An answer may lie in the examination of the separated methylated $A_F$, $A_S$, B, and C forms (see above) and in the periodate oxidation of the various methylated derivatives. (c) One new methylated derivative of unusual spectral properties has been observed.

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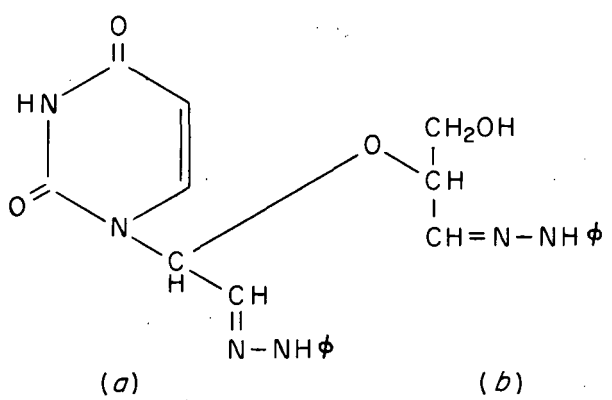
[4]Experiments performed by D. G. Doherty, Chemical Protection and Proteolytic Enzyme Studies Group.

The route of degradation, by a sequence of alternating periodate oxidations and borohydride reductions, described previously gave a small yield of 5-hydroxymethyluracil from pseudouridine. Improvement of the yield and identification of the various fragments successively removed requires an understanding of the various steps. An investigation along these lines has been undertaken. In brief, we propose to identify the various carbohydrate residues split off as their hydrazones or osazones.

The oxidation of uridine with periodate yields a dialdehyde that, on treatment with phenylhydrazine, yields the osazone of uracil hydroxymethyldiglycollic aldehyde (I); this has been prepared in good yield.




I

Hydrolysis in acetic acid and excess phenylhydrazine should yield uracil and the phenylosazones of glyoxal (a) and of glyceraldehyde (b). The first two products were indeed isolated, but the last appeared to be degraded to a tar. However, distillation from mineral acid of the original dialdehyde, before reaction with phenylhydrazine, did yield uracil and methylglyoxal. The latter, which results when glyceraldehyde is distilled, was identified as its osazone.

When these two degradative procedures were applied to pseudouridine, results consistent with its formulation as 5-ribosyluracil were obtained. The mineral acid distillation of the periodate oxidation product (presumably the dialdehyde) yielded methylglyoxal. We hope to find a uracil-glycollic aldehyde compound in the residue. The acetic acid-phenylhydrazine degradation yielded, in constrast to uridine, no glyoxal osazone, but a similar tar, and a yellow water-soluble substance. The failure to

find the 2-carbon fragment is consistent with its attachment to the 5-carbon rather than, as in uridine, to the 1-nitrogen. We hope to find it, and the uracil, in the yellow aqueous fraction as uracil 5-glycollic aldehyde osazone or hydrazone.

## Enzymatic Synthesis of Deoxyribonucleic Acid

F. J. Bollum

The primary objective of this investigation is to determine whether DNA synthesis catalyzed by purified enzymes is a process of molecular replication. A necessary intermediate step is to find biological sources and develop procedures for obtaining DNA-synthesizing enzymes. It has not been possible to find such sources by direct demonstration of DNA accumulation in crude cell-free extracts because (1) DNA synthesis is a minor process in the total economy of the cell and is probably carried out by a rather low concentration of enzyme molecules (1–100 per cell) and (2) there seems to be a surfeit of DNA-depolymerases and deoxynucleotide-degrading enzymes present in the cell. It has therefore been necessary to utilize less-direct methods for demonstrating DNA synthesis and, in every successful case, high specific activity radioactive precursors have been used. Further purification and concentration of any promising enzyme source is then required before the direct demonstration of DNA synthesis can be attempted.

The investigation in progress concerns a deoxynucleotide-polymerizing enzyme from calf thymus. This enzyme has been purified 30- to 50-fold from the soluble protein fraction of thymus gland. The fractionation procedures used include isoelectric precipitation, ammonium sulfate fractionation, a new chromatographic procedure for removal of contaminating ribonucleic acid, and refractionation with ammonium sulfate. Gel and solvent fractionations have also been successful but are not yet included in the routine preparative procedure.

The enzyme was purified by following the incorporation of $H^3$-thymidine triphosphate into acid-insoluble deoxypolynucleotide in the presence of "primer" DNA, $Mg^{++}$, and nonradioactive deoxyadenosine, deoxyguanosine, and deoxycytidine triphosphates. That the latter nucleotides

are required by virtue of their being incorporated into deoxypolynucleotide has now been established by preparation of all the deoxynucleotide triphosphates with $P^{32}$ in the ester phosphate and demonstrating their conversion to $P^{32}$-deoxypolynucleotides.

At the present stage of purity the enzyme polymerizes $P^{32}$-deoxynucleotides at the rate of 120 mμmoles per hour per milligram of enzyme protein under optimum conditions. The purification procedure has also released an alkaline DNase (heretofore unknown in thymus) that does not permit one to realize maximum reaction rates in prolonged incubations because of primer degradation. It has, however, been possible to demonstrate chemically detectable increases in DNA phosphorus, indicating that the polymerizing enzyme from calf thymus is catalyzing net DNA synthesis.

## Nucleic Acid Metabolism of *Chlorella*

T. Iwamura

By a technique of synchronous culture, the life cycle of the alga, *Chlorella*, is separable into mass increase and cell multiplication. DNA is vigorously synthesized just before cell division, whereas RNA is synthesized parallel with increase of cell mass.[5] Significant changes in base compositions of RNA and DNA fractions of the cells were observed during the life cycle.

The current work is directed toward examining detailed patterns of the acid-soluble nucleotides and RNA's and DNA's in physically separated cellular parts from such synchronized cells at various stages of the life cycle. The results obtained so far are as follows:

1. The acid-soluble nucleotide fraction of unsynchronized algal cells consists mainly of adenylic and uridylic acids. In synchronized cells, during active synthesis of DNA, some portion of the triphosphate region disappears, suggesting that that portion may be precursor for DNA.

2. The algal cells were successfully disrupted with French's cell disintegrator. The disrupted cell materials can be separated mainly into two parts: a yellowish supernatant and green particles, presumably

[5] J. Myers and T. Iwamura, Arch. Biochem. Biophys. (in press).

disrupted chloroplasts. Each fraction contains both RNA and DNA demonstrable by chemical tests.

The DNA of the supernatant fraction seems also to be demonstrated by ultracentrifugal analysis as a sharp spike located in a region similar to that of the DNA of bacterial cells.

Preliminary assays show that both nucleic acids in the two fractions differ in their base compositions.

# NUCLEIC ACID ENZYMOLOGY

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## Nucleic Acid Metabolism in Phage-Infected *Escherichia coli* B

E. Volkin L. Astrachan M. H. Jones K. B. Hudgins

Introduction. – Uninfected *Escherichia coli* B grown in the presence of $P^{32}O_4$ synthesizes RNA resulting in uniform specific activities for the four constituent mononucleotides, even immediately after isotope is added. A striking departure from this uniformity is observed after T2 infection. The relative specific activities of the RNA mononucleotides are unequal at any time after infection when radioactive orthophosphate is assimilated. They show a striking similarity to the molar base composition of the related deoxynucleotides in T2 DNA. The parallelism of host RNA mononucleotide labeling with the composition of the infecting phage DNA is evident also upon T7 infection.

Since alkaline hydrolysis of RNA produces the 2',3'-mononucleotide isomers, it can be argued that any interpretation of specific activities based on these products is spurious. Thus, if the route of RNA biosynthesis is via 5'-nucleotide intermediates, the phosphorus associated with these precursors would be joined in internucleotidic linkage at the 3'-ribose carbon of the adjacent nucleotide. About three out of four of the adjacent structures would contain a different base. As a result of alkaline hydrolysis, radioactive phosphorus originally associated with one nucleoside may be found with a different nucleoside. Additional complications in interpretation would arise in analyzing whole RNA that may consist of mixed species of specific radioactivity. We therefore used a nucleic acid precursor, formate-$C^{14}$, which is involved in purine base synthesis and yields results independent of the mechanism of phosphoryl cleavage of the RNA.

Results. – Uninfected *E. coli* in exponential growth, T2-infected *E. coli*, and T7-infected *E. coli* were compared with respect to the existence

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of turnover of the carbon isotope in RNA and, in particular, with respect to the distribution of isotope in the individual RNA mononucleotides. Sodium formate-$C^{14}$ was added 2 min after bacteriophage infection. Ten minutes later the specific activity of the extracellular formate was reduced 100-fold by the addition of unlabeled sodium formate. Samples for chemical fractionation were removed at intervals during assimilation of high-specific-activity formate and after dilution of radioactivity. The experiment with uninfected bacteria was comparable with respect to initial bacterial titer, duration of the assimilation, and the sampling times for fractionation.

Because of the following points of agreement, these RNA data strengthened our interpretations of the $P^{32}$ experiments. First, turnover of RNA in both T2 and T7 phage-infected systems is demonstrable with formate-$C^{14}$, whereas no RNA turnover can be demonstrated in the uninfected culture. Second, comparison of the two purine nucleotides clearly shows that (1) in the T2-infected cell, the initial specific activities of adenylic acid are about twice those of guanylic acid; (2) in the T7-infected cell, adenylic acid is about 10 to 15% higher in specific activity than guanylic acid; and (3) in uninfected bacteria, the specific activities of adenylic and guanylic acids are virtually the same. Moreover, the specific activities of adenylic and guanylic acids in the T2 system approach each other during the period of isotope loss. The results, then, are in complete accord with data obtained when $P^{32}$ was used as a tracer in such systems.

Discussion. — The results with base (or nucleoside) labeling seem to be exactly the same as those obtained by isolating phosphorus-labeled 3'-mononucleotides. This might indicate a scheme of RNA biosynthesis via 3'-nucleotide intermediates in phage-infected systems. It is, however, quite possible that a hydrolytic method yielding 5'-mononucleotides from RNA of the phage-infected cell could give the same isotope specific activity pattern as that obtained by alkaline hydrolysis of the RNA. Such experiments are now in progress.

Nucleic Acid Metabolism in *Escherichia coli* B

J. L. Countryman E. Volkin M. H. Jones

Introduction. — It is well known that, when bacterial cells are taken from an old culture and inoculated into fresh medium, there is a time lapse, the lag phase, before a constant rate of cell division is established. During this time, however, there is an increase in cell mass as a result of extensive synthetic activity, the major part of which, initially, is the synthesis of RNA. In previous studies with *E. coli* B we observed that RNA synthesis begins almost immediately after inoculation, and its rate increases rapidly throughout the lag phase. By the end of this period the total amount of RNA increases 300 to 500%. DNA synthesis begins some minutes later than does that of RNA and proceeds at a slower rate.

Results and Discussion. — Mass cultures for chemical determinations and isotope experiments may be obtained in which no cells divide for 80 to 100 min after inoculation. At that time there is a 70 to 80% increase in cell number as measured by direct count within 20 to 25 min. The cells increase in size during this period, often elongating markedly, and sometimes very long filamentous forms may be seen.

Cells from a 24-hr culture, grown in a synthetic salt medium containing 0.4% glucose and buffered with Tris, are chilled at 6°C for 45 min, spun out, and diluted tenfold in fresh synthetic medium. After 10 to 15 min aeration at 37°C for thorough suspension of the cells, glucose is added and samples of the culture are removed at intervals for optical density readings, plate counts, direct counts (with a Petroff-Hausser bacterial counter), and chemical determinations. For the chemical determinations, samples are removed, and the cells are spun out, washed, and chemically fractionated. When $P^{32}$ labeling is to be studied, $H_3P^{32}O_4$ is neutralized and added to the medium before inoculation. The cells take up little or no isotope until the glucose is added.

The incorporation of $P^{32}$ by such a culture was studied in one experiment. There was no increase in cell number for about 85 min, and $P^{32}$ was present in the medium throughout this period. Within 5 min the acid-soluble pool attained a high specific activity. This value increased about

30% during the next 35 min and remained constant during the remainder of the experiment. Phosphorus-32 was incorporated into both RNA and DNA from the beginning, but into RNA at a much faster rate. This was most pronounced during the first 45 min when the ratio of RNA-$P^{32}$/DNA-$P^{32}$ (calculated from the specific activity of the medium) rose very rapidly. At about 45 min, this ratio leveled off abruptly, suggesting a marked increase in DNA synthetic activity at this time. The total RNA phosphorus increased 380% by the time the cells began to divide; the DNA, 280%.

These observations suggest that a culture in this physiological state offers a system in which a maximum synthesis of RNA (relative to other syntheses) and its possible relation to other syntheses can be investigated.

## CHEMICAL PROTECTION AND PROTEOLYTIC ENZYME STUDIES

D. G. Doherty J. D. Bales, Jr.

### Structure and Activity in Radiation-Protective Sulfhydryl Compounds

D. G. Doherty J. D. Bales, Jr.

Introduction. — The evaluation of protective compounds by gross mortality at various radiation doses gives little clue as to what biological systems are protected by the chemical agents and no quantitative information directly correlating the compound with a particular system. To obtain such information, Urso *et al.*[1] devised a simple system for examining bone marrow in irradiated animals. AET was studied at one compound dose level and found to reduce the effect on the marrow by about a factor of 2 at 900 and 450 r. This method was used in the present study to determine the effect of varying levels of AET and routes of administration on the marrow as well as the effects of APMT on the marrow system.

Results and Discussion. — As can be seen from Fig. 17a, protection of the marrow is related to the amount of compound administered; the marrow of the animal receiving the higher AET dose recovers earlier. When AET is given orally at the maximum dose — 640 mg/kg (compared to the maximum intraperitoneal dose of 360 mg/kg), the marrow depression is much less although the recovery to normal is a little slower. At 450 r the same oral dose of AET gave a fivefold increase in marrow count compared to the untreated controls (Fig. 17b). At 1400 r this dose of AET did not influence the drop in marrow count but did allow a slow recovery to a low normal by the fifteenth day. There was no effect of AET on the marrow of unirradiated animals at the maximum levels of 360 mg/kg given intraperitoneally or 640 mg/kg orally.

In contrast to AET, APMT at any oral dose level did not seem to have any effect on the initial bone marrow depression (Fig. 17c). Recovery at all dose levels did not take place until the fourth or fifth day and was essentially complete by the eleventh or twelfth day. At 1400 r no recovery of the marrow was observed, and all the animals died — as was to be

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[1]P. Urso *et al.*, Blood 13, 665–76 (1958).

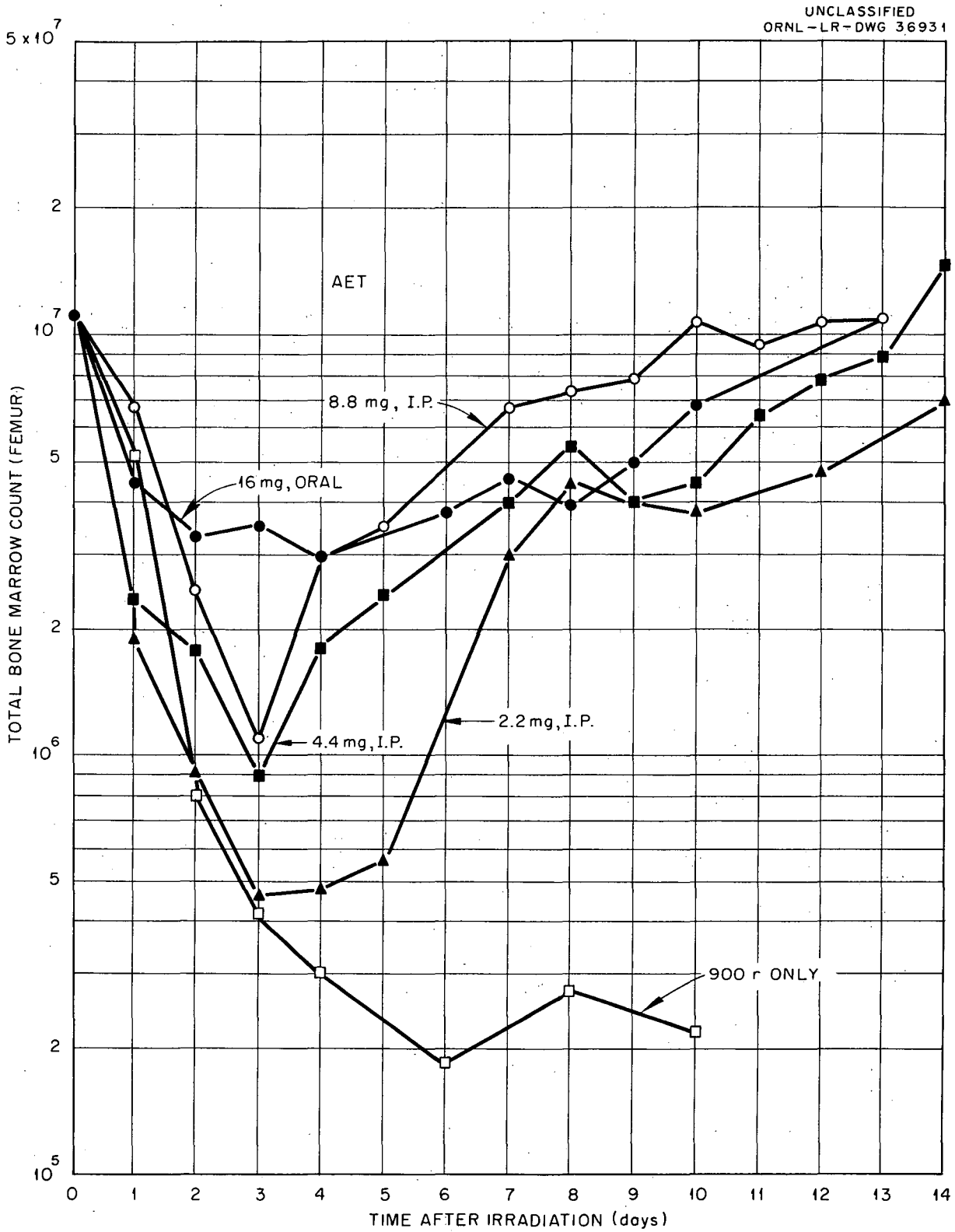


**Fig. 17a. Recovery of Bone Marrow of Chemically Pretreated (AET), Irradiated Mice.**

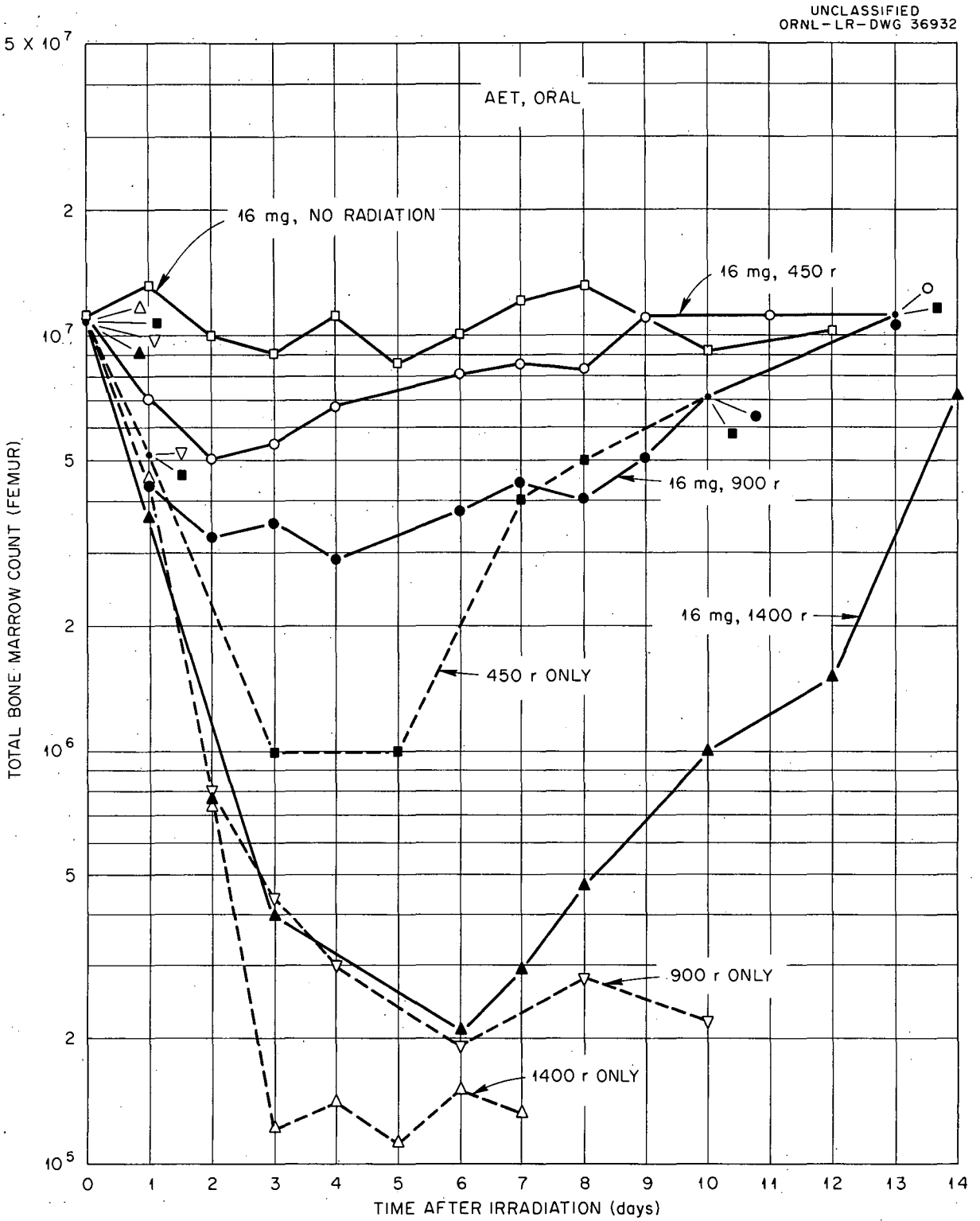


**Fig. 17b. Recovery of Bone Marrow of Chemically Pretreated (AET Oral), Irradiated Mice.**

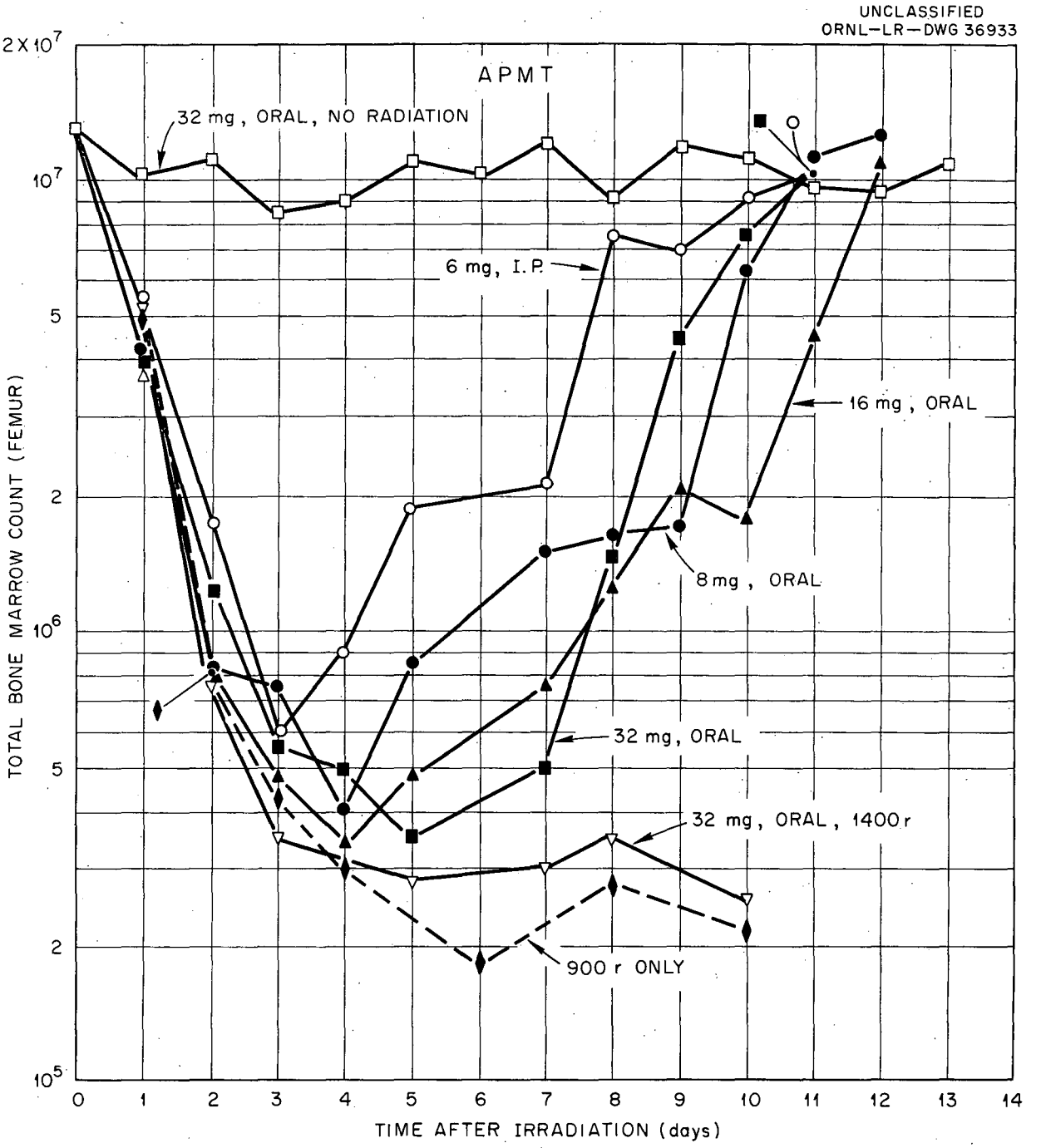


**Fig. 17c. Recovery of Bone Marrow of Chemically Pretreated (APMT), Irradiated Mice.**

expected from previous radiation $LD_{50}$ experiments. Intravenous administration of the compound, however, brought about a rapid recovery similar to that seen with AET. It is interesting to note that, although the orally treated animals showed a slow recovery of their marrow, they were otherwise grossly in good condition and were over their irradiated weight by the seventh or eighth day while the marrow was still depressed. It would seem then that the protective effect of APMT must primarily be on some other system than the marrow.

## Prolongation of Homograft Survival in Mice Protected with AET and Treated with Isologous Bone Marrow

D. G. Doherty C. C. Congdon[2] J. D. Bales, Jr.

The histologic recovery of lymphatic tissue after lethal (900 r) whole-body irradiation in mice pretreated with AET or posttreated with isologous bone marrow is nearly complete 30 days after exposure.[1] Recovery of the ability to form hemagglutinins to rat and sheep red blood cells after similar treatment also requires ~30 days to achieve significant titers.[3] However, the response of chemically protected or isologous bone marrow-treated irradiated mice to homologous antigens has not been specifically studied. Injury to the recognition factor for homologous antigens might be greater than for heterologous types.[3] Evidence in favor of this has been obtained by the prolongation of $(C57L \times A)F_1$ skin homografts on $(101 \times C3H)F_1$ mice exposed to 905 r of total-body X radiation and given $10 \times 10^6$ isologous bone marrow cells. The $(C57L \times A)F_1$ skin graft persisted in 13 mice for 36 to 41 days; this was followed by depigmentation of the hair and a slow rejection of the graft. The homografts were rejected in the control mice, 23, in from the ninth to the thirteenth day, none persisting longer than that. At 800 r half the grafts in the treated mice were rejected by the twentieth day, the remainder behaving similar to the 950 r grafts. At 700 r 80% of the grafts were rejected by the fourteenth day and the balance persisted to the thirty-fifth day. In maximally protected AET-treated animals, the

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[2]Mammalian Recovery Section.

[3]T. Makinodan, I. C. Shekarchi, and C. C. Congdon, J. Immunol. 79, 281–87 (1957).

grafts persisted for 20–32 days and then were slowly rejected. In animals treated with less AET, there were indications of a longer graft survival although few animals survived 950 r.

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## Synthesis of Ornithine Transcarbamylase by Concentrated Cell Suspensions

P. Rogers, Jr.

Introduction. – As a preliminary step in a study of enzyme formation in disrupted preparations of *Escherichia coli*, the effect of cell concentration on the synthesis of ornithine transcarbamylase (OTC) was investigated. The rationale for these experiments was that a multicomponent system, such as protein synthesis, must be studied under concentrated conditions, since the final products of any such system are expected to decrease exponentially with dilution.

Other workers have investigated conditions whereby bacteria can be grown to high concentration of cells by using cellophane membranes to separate the growing culture from additional medium,[5,6] by increasing rate of aeration,[7] by using a biphasic agar broth culture,[8] and by adding various absorbents.[5] With the possible exception of Hestrin *et al.*,[9] there have been no reports of attempts to induce specific enzyme synthesis by using crowded cells or protoplasts. Our studies reveal that OTC synthesis occurs at high cell or protoplast concentration only when pH is directly controlled or when sufficient surface is provided to allow exchange between the cell suspension and additional medium.

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[2]Research Associate.

[3]Life Insurance Medical Research Fund Fellow.

[4]Visiting investigator from abroad.

[5]A. N. Gorlick, D. D. Mead, and E. H. Kelly, J. Bacteriol. 61, 507 (1951).

[6]G. Vinet and V. Fredette, Science 114, 549 (1951).

[7]C. G. Smith and M. J. Johnson, J. Bacteriol. 68, 346 (1954).

[8]E. A. Tyrrell, R. E. MacDonald, and P. Gerhardt, J. Bacteriol. 75, 1 (1958).

[9]S. Hestrin, S. Avineri-Shapiro, and M. Aschner, Biochem. J. 37, 450 (1943).

Results and Discussion. – Cells of E. coli W released from arginine suppression[10,11] were incubated at 37°C for 60 min at concentrations from $2 \times 10^9$ cells/ml (0.38 mg of protein per ml) to $2 \times 10^{11}$ cells/ml (23.4 mg of protein per ml). The amount of OTC synthesized decreased exponentially as the concentrations of cells increased beyond $10^{10}$ per ml. Similar results were obtained with the induced synthesis of β-galactosidase. Glycine $C^{14}$ incorporation into RNA, DNA, and protein was greatly impaired in a concentrated cell suspension as was utilization of glycerol as a carbon source. In addition, the pH dropped from 7.6 to 5.5 in most experiments.

By placing the concentrated suspension in contact with a large reservoir of medium, it was possible to obtain OTC specific activities up to 50% of those in dilute suspensions of cells. On solid surfaces such as agar, heat-denatured serum albumin, heat-denatured E. coli protein, and starch, OTC synthesis occurred in cell suspensions as high as $2 \times 10^{11}$ per ml. Suspensions of concentrated cells or protoplasts placed between two dialysis membranes that are kept in contact with an agitated liquid medium reservoir also synthesize OTC rapidly.

Since it was shown previously that OTC synthesis does not occur to any extent below pH 5.0,[12] attempts were made to maintain the pH of concentrated cell suspensions above this value. High buffer concentrations of 0.5 M $K_2HPO_4$ or 0.5 M Tris were shown to inhibit all synthesis of OTC by cells at all concentrations. Lower buffer concentrations would not effectively combat acid production. Direct titration of the concentrated cell suspension during incubation at 37°C with 1 N KOH allowed a high rate of OTC formation. The concentrated cells show the same quick burst of synthesis as do dilute cell suspensions but reach only one-third the final level. Experiments in which solid $CaCO_3$ was added to cell suspensions to combat acid production were partially successful. Synthesis of OTC occurred for the first 30 min of incubation in high cell concentrations at a good rate. The $CaCO_3$ had no effect on the rate of OTC formation in dilute cell suspensions.

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[10] J. DeMoss and G. D. Novelli, Biol. Semiann. Prog. Rep. Aug. 15, 1957, ORNL-2390, p 83.

[11] P. Rogers and G. D. Novelli, Biol. Semiann. Prog. Rep. Feb. 15, 1958, ORNL-2481, p 62.

[12] P. Rogers and G. D. Novelli, Biochim. Biophys. Acta (in press) (1959).

# The Biosynthesis of Bacitracin

R. W. Bernlohr

Introduction. – An investigation of the biosynthesis of the polypeptide antibiotic, bacitracin, was undertaken with the hope that this compound would provide a convenient model for an understanding of the more complex problem of protein biosynthesis. It is further anticipated that these studies may cast some light on the biological role of this odd class of compounds in the physiology of the organisms that produce them.

Results. – *Bacillus lichenformis*, the bacitracin-producing strain, will incorporate D-phenylalanine-$H^3$ and D-ornithine-$H^3$ into bacitracin during growth. The antibiotic is usually produced after about five days of growth. This is long after the cells have reached their maximum density. It was also observed that the pH of the culture medium must drop from an initial 7.2 to about 5.5 and then be returned to 7.2 before the antibiotic is produced. Any manipulations of the culture that altered or prevented these pH changes resulted in failure to produce antibiotic. For these reasons, a careful examination of cultural conditions necessary for bacitracin production has been undertaken. One striking observation is that bacitracin is produced only under cultural conditions that eventually lead to spore formation. The antibiotic seems to be produced just prior to spore formation.

Discussion. – The polypeptide antibiotics, bacitracin, polymyxin, gramicidin, circulin, subtilin, and tyrothricin are all produced by different species of the genus *Bacillus*, and cultural conditions in every case indicate a similar relation of sporulation to antibiotic production. In addition, the mode of action of each polypeptide of this group appears to be the same, for example, an inhibition of cell wall metabolism. It is hoped that a better understanding of the metabolism of one antibiotic-producing microorganism may help in determining the same information for the others.

## Attempts To Stimulate Incorporation of Amino Acids into Rat Liver Microsomes by Microbial Extracts

G. D. Duda

Introduction. – As previously reported,[13] bacterial extracts are capable of incorporating $C^{14}$ amino acids into their protein to only a small extent and even this was not readily reproducible. In rat liver microsomes, however, the incorporation of $C^{14}$ amino acids is both more extensive and reproducible. Consequently an examination was made of the effect of various bacterial extracts on the incorporation of 1-$C^{14}$-leucine into rat liver microsomes.

Results and Discussion. – Extracts of *Photobacterium fischeri*, *Alcaligenes faecalis*, and *Azotobacter* were prepared by the means previously described. Freshly prepared and lyophilized rat liver microsomes were both used in the test system. The effect of the bacterial extracts on the incorporation of $C^{14}$-leucine into the microsomes was compared to that of crude rat liver supernatant and the "pH 5 enzymes." In no case did the bacterial extracts substitute for or act as a stimulant to the rat liver supernatant.

Amino acid incorporation in bacterial extracts is an elusive phenomenon and may require more highly organized structural units than we have heretofore used. It was observed that the insoluble residue of broken preparations of *A. faecalis* incorporated $C^{14}$-valine. However, the possibility that a small number of whole cells were present cannot be discounted.

## Enzyme Systems in Developing Chick Embryo

K. B. Jacobson

Introduction. – During embryonic growth, the synthesis of polymers (nucleic acid and protein) occurs. The systems responsible for the synthesis may be studied to elucidate the mode of polymer formation. Several preliminary investigations have been made in the tissues of the chick embryo.

Results and Discussion. – The observation of an exchange of inorganic pyrophosphate with ATP may be interpreted as the activation of amino acid,

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[13] G. D. Duda and G. D. Novelli, Biol. Semiann. Prog. Rep. Aug. 15, 1958, ORNL-2593, p 84.

if the exchange is dependent on amino acids, and it may be interpreted as synthesis of RNA when it occurs in absence of amino acids.[14] In the liver and brain homogenates, the amino acid-stimulated exchange declined ninefold from the eleventh day of incubation to one day after hatching. The exchange in absence of amino acid declined similarly. In the soluble cytoplasm fraction, the exchange in absence of amino acid did not decline but rose somewhat in both liver and brain, whereas the amino acid-dependent exchange of this fraction declined, paralleling the activity in the homogenate. The interpretation of the amino acid-independent exchange is complicated by the lack of knowledge of the variation in free amino acid in the enzyme preparation. The rate of growth (weight increase) of the brain is much faster than that of the liver during this period, yet the specific activity of the two exchange reactions in the homogenate declines at similar rates in both organs.

The exchange of orthophosphate with ADP may represent activity of polynucleotide phosphorylase. This exchange reaction remained much lower (1/10–1/20) than the ATP-PP exchange during the 11 to 21 days of chick embryo development. The significance of this fact is difficult to assess unless polynucleotide phosphorylase was functioning as a degradative enzyme or the exchange reaction was measuring some other activity.

An enzyme not involved in polymer formation that cleaves nicotinamide from DPN is called DPNase. The activity of this enzyme in the brain was reported by Burton[15] to rise sharply and reach adult level soon after the birth of the rat. The chick embryo differs from the rat in that the 11-day chick embryo has brain DPNase activity that already is 50% of the adult value. The liver, on the other hand, has no detectable DPNase until after the fourteenth day but at hatching has twice the adult level. The interpretation of such data cannot be sure since one may only speculate that the function of DPNase is to regulate DPN- and TPN-requiring reactions, such as oxidative phosphorylation, glycolysis, and fatty acid synthesis.

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[14] C. W. Chung and H. R. Mahler, J. Am. Chem. Soc. 80, 3165 (1958).
[15] R. M. Burton, J. Neurochem. 2, 15 (1957).

## Nuclear Phosphorylation

K. B. Jacobson

Introduction. — Creasy and Stocken[16] reported that the nuclei of radiosensitive tissues of the rat generated high-energy phosphate bonds when incubated at 0°C immediately after isolation. They further noted that low doses of X radiation (100 r) abolished this activity.

These observations are significant from the standpoint of radiation damage to tissue, and, since these same tissues proliferate rapidly, this system may provide an insight into growth and development as well.

Results. — The ability of spleen nuclei to generate high-energy phosphate bonds has been confirmed qualitatively but not quantitatively. The abolishment by X-ray treatment (200 r) has not occurred in the limited number of rats so far examined.

## An ATP-Dependent Oxygen Exchange Between Orthophosphate and $H_2O^{18}$

M. P. Stulberg

Introduction. — During a study of glycine activation by an extract from *Photobacterium fischeri*, a glycine-independent, ATP-dependent oxygen exchange between inorganic phosphate ($P_i$) and $H_2O^{18}$ was observed.[17] The exchange activity was dependent on the presence of ATP and occurred concurrently with ATP hydrolysis. Since the extract also catalyzed a $P_i^{32}$-ATP exchange, it seemed possible that the observed oxygen exchange could be a result of the combined action of "ATPase" and $P_i^{32}$-ATP exchange. In order to check this possibility, further purification of the oxygen exchange activity was attempted.

Results. — A procedure of cell extraction (precipitation of nucleic acid from the extract with streptomycin sulfate, and ammonium sulfate and DEAE cellulose column fractionation) produced a fraction that was approximately 50-fold purified, based on "ATPase" activity. This fraction retained the ability to catalyze the ATP-dependent oxygen exchange between

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[16] W. A. Creasy and L. A. Stocken, Biochem. J. 69, 17 (1958).

[17] M. J. Cormier, M. P. Stulberg, and G. D. Novelli, Biochim. Biophys. Acta (in press).

$P_i$ and $H_2O^{18}$ in spite of the complete removal of the $P_i^{32}$-ATP exchange activity.

The exchange activity can be measured by (1) the disappearance of $O^{18}$ from $O^{18}$-labeled $P_i$ and (2) the appearance of $O^{18}$ into $P_i$ from $H_2O^{18}$. The rates of "ATPase" and oxygen exchange activities are of the same order of magnitude. Further investigation of the hydrolytic activity of this purified fraction revealed a nonspecificity toward substrate. The system demonstrates phosphatase activity toward adenosine, inosine, guanosine, cytidine, and uridine monophosphates, as well as the corresponding triphosphates. However, the purine compounds are hydrolyzed at a much faster rate than the pyrimidine compounds. As yet, the ratio of "ATPase" to "AMPase" activities has remained constant throughout fractionation. β-Glycerophosphate is inactive as a substrate.

Discussion. – The ability of this fraction to catalyze an oxygen exchange between $H_2O$ and $P_i$ only in the presence of a phosphorylated substrate without demonstrating a $P_i^{32}$-ATP exchange clearly differentiates it from oxygen exchanges previously reported.[18–21] These properties also indicate the presence of a phosphorylated intermediate that can rapidly exchange its phosphate oxygens with those of water. A simple reversible binding and release of $P_i$ cannot account for these observations. Further work is in progress toward elucidating the role of ATP and other phosphorylated compounds and the effects of known inhibitors on this exchange activity.

## The ATP-Dependent Reduction of Sulfate with Hydrogen in Extracts of *Desulfovibrio desulfuricans*

H. D. Peck, Jr.

Introduction. – Enzymatic systems that reduce sulfate seem to be widespread in nature since large numbers of organisms are able to satisfy their nutritional requirements for sulfur with sulfate. The ability to reduce

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[18] M. Cohn, Biochim. Biophys. Acta 20, 92 (1956).

[19] H. M. Levy and D. E. Koshland, Jr., J. Am. Chem. Soc. 80, 3164 (1958).

[20] M. Cohn, J. Biol. Chem. 230, 369 (1958).

[21] P. D. Boyer, W. W. Luchsinger, and A. B. Falcone, J. Biol. Chem. 223, 405 (1956).

sulfate is most highly developed in certain microorganisms, such as Desulfovibrio desulfuricans, in which inorganic sulfate functions in anaerobic respiration as the terminal electron acceptor. Although whole cells of D. desulfuricans readily reduce sulfate to hydrogen sulfide in the presence of molecular hydrogen, the inability of cell-free extracts to reduce sulfate has been observed repeatedly.[22,23] The inhibition of sulfate reduction in whole cells by molybdate and selenate suggested that it was necessary to react sulfate with ATP to form a nucleotide bound sulfate, which then was the substrate for sulfate reductase.[24]

Results. – Cell-free extracts were prepared from heterotrophically grown cells of D. desulfuricans by passage of a 30–40% suspension of cells once through a French pressure cell. The presence of the enzyme, sulfurylase, which forms adenosine-5'-phosphosulfate (APS) from ATP and sulfate was observed in these extracts by the rapid liberation of inorganic phosphate from ATP upon the addition of molybdate, chromate, or tungstate. The utilization of hydrogen for sulfate reduction in extracts was demonstrated to require both ATP and sulfate. Inhibition of sulfate reduction in cell-free extracts as well as in whole cells by molybdate, tungstate, and chromate indicates that the formation of APS is the first step in sulfate reduction.

At pH 8.0, there is 1 μmole of hydrogen utilized per micromole of ATP added to the reaction mixture, and the product of the reaction has been identified as sulfite. At pH 6.0, more than 1 μmole of hydrogen is utilized per micromole of ATP added to the reaction mixture, and here the product of the reaction seems to be hydrogen sulfide. For each micromole of hydrogen consumed at pH 8.0, 2 μmoles of inorganic phosphate is liberated from ATP and 1 μmole of sulfite is formed. The stoichiometry of the reaction suggests that APS is not only the first intermediate in sulfate reduction but also the substrate for sulfate reductase. This conclusion is further supported by the observation that APS is the only nucleotide labeled in the presence of $S^{35}$. In addition, hydrogen is consumed in the presence of crude preparations of APS in the absence of ATP.

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[22] J. R. Postgate, J. Gen. Microbiol. 14, 545 (1956).

[23] M. Ishimoto et al., J. Biochem. (Tokyo) 41, 537 (1954).

[24] L. G. Wilson and R. S. Bandurski, J. Biol. Chem. 233, 975 (1958).

Cytochrome $c_3$ is required for the coupling of hydrogenase with sulfate reductase, and seems to be the natural carrier. The one-electron dye, methyl viologen, will also couple these two enzymes, and cannot be replaced by tri- or diphosphopyridine nucleotide.

Discussion. – An ATP-dependent reduction of $S^{35}$-labeled sulfate to sulfite[25,26] and to hydrogen sulfide[26] has been reported to occur in cell-free extracts of baker's yeast. In both cases 3'-phosphoadenosine-5'-phosphosulfate (PAPS) was suggested to be the substrate for sulfate reductase. APS is the first intermediate in sulfate reduction by extracts of *D. desulfuricans*, and the data are consistent with the idea that APS rather than PAPS is the substrate for sulfate reductase. The possibility remains, however, that APS itself is not the substrate for sulfate reductase, but rather transfers its sulfate to an unknown sulfate carrier on which the sulfate is then reduced to sulfite.

## Properties of Substances Producing Early Changes in Spleens of Lethally Irradiated Mice

G. D. Novelli A. N. Best P. F. Lincoln

Introduction. – Congdon[27] has discussed a characteristic histologic reaction that occurs in the white pulp of bone marrow-treated mice 24 hr after irradiation. Our investigation is an attempt to isolate and characterize the chemical substance responsible for the change.

Results and Discussion. – Since Congdon observed that only one lot of horse serum from Cappel Laboratories gave a particularly strong reaction, horse serum was used as a starting material for the isolation. The substance was found to be heat stable (10 min at 100°C), nondialyzable, incompletely precipitable by saturated ammonium sulfate, and completely precipitable by four volumes of acetone. The acetone-precipitable material could be digested with pepsin, trypsin, and papain and still retain its ability to elicit the reaction. At the present time the assay for the substance does not permit precise quantitation, and we therefore cannot

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[25] L. G. Wilson and R. S. Bandurski, J. Am. Chem. Soc. 80, 5576 (1958).

[26] H. Hilz and M. Kittler, Biochim. Biophys. Acta 14, 287 (1954).

[27] C. C. Congdon, Biol. Semiann. Prog. Rep. Aug. 15, 1958, ORNL-2593, p 50.

say how much of the substance is recoverable by the various procedures. The information presently available on the properties of the substance indicates that it may be in the class of compounds called mucopolysaccharides. Anticipating that we might soon exhaust our supply of the one lot of horse serum that contains this material, we investigated other sources. A similar substance (or substances) seems to be present in rat liver microsomes, rat liver mitochondria, and extracts from certain microorganisms. This project will continue until the substance is isolated and characterized.

## Biochemical Properties of Antigens Responsible for Bone Marrow Transplantation Immunity

A. N. Best
P. F. Lincoln
T. Makinodan[28]
G. D. Novelli

Introduction. – This is a continuation of a joint effort of the Enzymology and Radiation Immunology Groups to isolate and characterize the antigens involved in bone marrow transplantation immunity.[29]

Results and Discussion. – Because the yield of mitochondria is much greater than the yield of microsomes, we turned our attention to mitochondria as a source material for the isolation. By sonic disruption of mitochondria, we prepared a soluble active material. The active material could be precipitated by acetone but not with ammonium sulfate. The substance continues to be relatively heat stable. Thus the soluble fraction of sonic treated mitochondria was heated for 10 min at 100°C, and the coagulated protein was removed by centrifugation. The active material was then precipitated with acetone and was dissolved in saline. The yield of solid material was very low; but the observation that the substance can be extracted with water at 100°C suggested that a better yield may be obtained by direct extraction of whole liver without a preliminary centrifugal fractionation. Experiments are in progress to explore various ways of extracting the active component so that the amount of starting material can be increased.

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[28]Radiation Immunology Group.

[29]A. N. Best, T. Makinodan, and G. D. Novelli, Biol. Semiann. Prog. Rep. Aug. 15, 1958, ORNL-2593, p 87.

## Biosynthesis of Protein and RNA in *Escherichia coli* After Ultraviolet Irradiation

T. Kameyama

Introduction. – It is well known that levels of UV radiation that cause nearly complete inhibition of DNA synthesis do not have a drastic effect on the biosynthesis of RNA and protein.

Our preliminary studies of the effect of UV on RNA and protein biosynthesis in *Escherichia coli* indicated that there were great differences in metabolic turnover and chemical composition of RNA as compared with normal cells.[30] The results obtained by adding chloramphenicol suggested that synthesis of RNA after UV irradiation was strikingly dependent on a certain protein biosynthesis within a definite incubation period. Moreover, the most sensitive effect of UV radiation was the suppression of the RNA component of a RNA-protein fraction isolated by zone electrophoresis. This suppressed peak was gradually restored during further continuous incubation of the culture.

These results support the hypothesis that synthesis of a certain RNA or protein is one of the prerequisites for reduction of survival and UV-induced mutation.[31,32]

Results and Discussion. – *E. coli* B was grown in Tris-glucose medium containing minimal salts. The collected cells, in logarithmic growth phase, were irradiated in physiological saline. Irradiation reduced the viable cell count by 99.5–99.9%. The irradiated cells were washed and resuspended in reaction medium for enzyme biosynthesis and labeling of protein or nucleic acids with $S^{35}$ or $P^{32}$. After incubation the cells were broken by sonic oscillation. The sonicate was fractionated by DEAE cellulose column. The most prominent difference from normal can be detected in a fraction of ribonucleoprotein on the elution pattern. This is completely consistent with our previous result.

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[30] T. Kameyama, J. Ono, and K. Suzuki, 9th Symposium on Biological Sciences, Japan (in press).

[31] E. M. Witkin, Cold Spring Harbor Symposia Quant. Biol. 21, 123 (1956).

[32] F. L. Haas and C. O. Doudney, Proc. Natl. Acad. Sci. U.S. 43, 871 (1957).

The UV effect on enzyme biosynthesis is under investigation. A considerable difference of UV effect will be expected between production of ornithine transcarbamylase (OTC) and β-galactosidase and it may reflect the different kind of participation of RNA in the biosyntheses of these enzymes, respectively.[33]

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[33]P. Rogers and G. D. Novelli, Biol. Semiann. Prog. Rep. Aug. 15, 1958, ORNL-2593, p 83.

# TRACER STUDIES ON INTERMEDIARY METABOLISM

S. F. Carson E. F. Phares
M. I. Dolin R. K. Clayton
M. V. Long

## Induced Synthesis of Catalase in *Rhodopseudomonas spheroides*

R. K. Clayton

Introduction. — Synthesis of catalase is induced in the photoheterotroph *Rhodopseudomonas spheroides* upon contact with air. Under gentle aeration in the light, the air acts as an inducer of catalase synthesis, but the general cellular metabolism is based on photosynthesis. This system holds promise for the study of enzyme synthesis; it is hoped that synthesis of the apoenzyme and attachment of the prosthetic group can be studied separately. Current work is aimed at characterizing the enzyme and describing its induced synthesis phenomenologically.

Results and Conclusions. — The catalase of *Rps. spheroides* has been purified and characterized. It is almost identical with catalase from *Micrococcus lysodeikticus* with regard to molecular weight, activity toward hydrogen peroxide, and optical properties. It is more reactive toward alkyl peroxides and peroxidatic hydrogen donors than the catalase from *M. lysodeikticus*.

The inducer of catalase synthesis is hydrogen peroxide generated intracellularly when air is present. This conclusion is based on two findings: (1) Methyl hydrogen peroxide, which is decomposed by catalase in a peroxidatic reaction yielding no oxygen, is an effective inducer. (2) The synthesis of catalase induced by $O_2$, by $H_2O_2$, or by $CH_3OOH$ is inhibited by methanol, ethanol, and *n*-propanol. The effectiveness of these inhibitors is consistent with their activities in peroxidatic reactions which destroy $H_2O_2$ and $CH_3OOH$.

A $C^{14}$-incorporation experiment is in progress; its purpose is to determine whether the induced synthesis of catalase in *Rps. spheroides* involves a *de novo* synthesis of protein.

## Flavoprotein Respiration

M. I. Dolin

Studies on the highly purified diaphorase of Streptococcus faecalis[1] have been continued. (The physiological significance of diaphorase activity has been treated in detail elsewhere.[2]) Representatives of all known classes of flavoprotein oxidants have been tested as potential electron acceptors for the enzyme; however, the only oxidants found so far are 2,6-dichlorophenol indophenol, ferricyanide, quinones, and naphthoquinones. The high turnover (73,000 moles of DPNH oxidized per minute per mole of bound FMN) and low $K_s$ ($5 \times 10^{-6}$ M quinone) found when p-benzoquinone is used as electron acceptor suggest that a naturally occurring quinone may be the physiological oxidant of the enzyme. Water-insoluble quinones such as vitamin $K_1$ and coenzyme $Q_{10}$ are inactive as electron acceptors; however, menadione (vitamin $K_3$), which may be considered a water-soluble model of vitamin K, as well as water-soluble quinones related in structure to coenzyme Q, show considerable activity.

Balances and product determinations have been carried out for representative one- and two-electron acceptors. In addition, the second-order rate constants for combination of reduced enzyme with oxidants have been determined for all the active electron acceptors. The values for the second-order constant (1/mole/min) range from $1 \times 10^{10}$ for p-benzoquinone to $2 \times 10^{6}$ for aurantiogliocladin.

Work on the enzyme-substrate complex of DPNH peroxidase is being continued in order to obtain information concerning the nature of the active site. Experiments dealing with the binding of DPN adducts to the enzyme indicate that there are at least two binding sites on the enzyme, one of which can be occupied without affecting the visible region of the flavin spectrum.

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[1]N. P. Wood and M. I. Dolin, Bacteriol. Proc. (1959) (in press).

[2]M. I. Dolin, vol II, chap. 10 in The Bacteria, ed. by I. C. Gunsalus and R. Y. Stanier, Academic Press, Inc., New York (in press).

## Bacterial Succinic Decarboxylase System

E. F. Phares S. F. Carson
M. V. Long

Resolution of several soluble enzymes and a small-particle fraction (the "$CO_2$ enzyme") of the succinic decarboxylase system from Micrococcus lactilyticus has been described.[3] Resolution and purification of another soluble enzyme component of the system, CoA transphorase, has been accomplished by ion-exchange chromatography of cell-free extracts of M. lactilyticus on DEAE cellulose. Gradient elution with sodium acetate has produced extremely well resolved, sharp enzyme activity zones. DEAE-cellulose ion-exchange chromatography has also been used to improve the resolution of the extremely labile succinic decarboxylase from Propionibacterium pentosaceum. Resolution has been improved, but without an increase in specific activity, by allowing only a very short contact with the ion exchanger. The sensitivity and accuracy of the succinic decarboxylase assay ($CO_2$ estimation) have been substantially increased by use of tracer carbon and subsequent measurement of $C^{14}O_2$ by gas counting. The combination of the purified CoA transphorase and "$CO_2$ enzyme" fraction with the improved $CO_2$ measuring techniques, provides the means for a more precise study of the cofactor and other nonenzymatic requirements as well as the mechanism of the decarboxylase system.

## Malic-Lactic Transhydrogenase

E. F. Phares

A malic-lactic transhydrogenase, isolated from Veilonella gazogenes, has been reported.[4] The enzyme can dehydrogenate malate or lactate with pyruvate or oxalacetate, respectively, as the hydrogen acceptor. The transhydrogenase did not function as a malic or lactic dehydrogenase, with DPN as the hydrogen acceptor, and thus was not merely a combination of those two dehydrogenase activities. The enzyme has been found in high concentration in a related organism, Micrococcus lactilyticus, and has

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[3]S. F. Carson and E. F. Phares, Bacteriol. Proc. 58, 121–22 (1958).
[4]E. F. Phares, Abstracts of Papers, 130th Meeting of the American Chemical Society, 1956, p 62C.

been purified tenfold in a single step by ion exchange on DEAE-cellulose. Further purification has been obtained by classical procedures, yielding a preparation free of DPN-linked malic and lactic dehydrogenases and fumarase. The purified material was used in mechanism studies with tritium-labeled malate as a substrate, in order to see whether the transhydrogenase effects a direct transfer of the nonexchangeable hydrogen atom (attached to the carbinol carbon of malate) to lactate. The tritium-labeled malate was prepared by catalytic reduction of enol-oxalacetic acid with pure tritium, thus labeling the nonexchangeable hydrogen attached to the carbinol carbon of malate. This labeled malate was incubated with the transhydrogenase in the presence of pyruvate. Analysis of the incubation products failed to reveal tritium-labeled lactate as a product. This result can be interpreted as evidence that a direct transfer of hydrogen does not occur. However, consideration of the enormous isotope effect of tritium suggests that the reaction needs to go virtually to completion (more than 99%[5]) before the isotope effect is overcome. Since the reaction is easily reversible, complete substrate utilization is difficult to obtain. The use of a more favorable tracer, deuterium, may be necessary in order to answer the question of direct transfer.

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[5]C. J. Collins (unpublished).

A. H. Haber H. J. Luippold
W. A. Arnold

## Nonrespiratory Gas Released from Seeds During Moistening

A. H. Haber N. Brassington[1]

Introduction. – Several investigators have observed that many species of seeds seem to have a relatively high respiratory quotient (RQ) soon after they begin to take up water and that the RQ decreases and finally levels off with increasing time of imbibition. Stiles and Leach[2] considered such RQ variations indicative of shifting metabolic pathways within the seed. Crocker and Barton[3] emphasized that gaseous exchanges of intact seeds do not necessarily reflect actual metabolism. James[4] suggested that these variations in observed RQ might be explained by irregularities in oxygen absorption. Our results show that gas, presumably air adsorbed within the seeds, is given off as the tissues become wet. This physical effect may provide another explanation for the shifting values of the apparent RQ during imbibition.

Results. – Seeds or other materials were placed in the main compartment of a Warburg flask with distilled water in the side arm. Flasks were allowed to equilibrate for at least 25 min, and then the water from the side arm was tipped onto the seeds. Curves A, B, and C of Fig. 18 illustrate gas output of Grand Rapids lettuce seed immediately after wetting. That this is not an artifact produced by the tipping process is shown by the steady behavior with 0.1 ml of glass beads similarly treated (curve D). The seeds in contact with a water-saturated atmosphere for 60 (curve B) or 120 min (curve C) seemed to produce some gas before the water was tipped from the side arm. The total net gas produced (curves A, B, C) before and after tipping seemed to be constant, suggesting that the release of

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[1]Biology Student Trainee, Summer 1958.

[2]W. Stiles and W. Leach, Respiration in Plants, Methuen, London, 1952.

[3]W. Crocker and L. V. Barton, Physiology of Seeds, Chronica Botanica, Waltham, Mass., 1953.

[4]W. O. James, Plant Respiration, Clarendon Press, Oxford, 1953.

gas is a physical phenomenon. Within the limits of experimental error, the results were the same with 0.2 ml of 20% KOH as with 0.2 ml of distilled water in the center well, indicating that little or no $CO_2$ was produced. This phenomenon appears to be essentially completed several minutes after the wetting (see Fig. 18). Respiratory gas exchange during these few minutes is probably negligible, since we have found $Q_{O_2} \approx 1$ μl/min per 100 seeds at 37°C for fully imbibed (4 1/2 hr) seeds. The amount of gas (STP) released from Grand Rapids lettuce seeds after wetting approximately doubled with each 10° rise in temperature from 7 to 37°C. Similar experiments were performed with different materials. Nonviable seeds, which neither germinated nor respired, showed the effect, as did dried tea leaves and potato starch. Therefore the phenomenon observed with viable seeds seems to be a general property of dry plant material.

Discussion. — The gas output observed when dried material was moistened cannot be explained by simple displacement of air at atmospheric pressure, since in this case the total pressure of the system would remain unchanged. Thus the gas was either trapped under pressure or it was adsorbed upon surfaces within the material. Because of the variation of

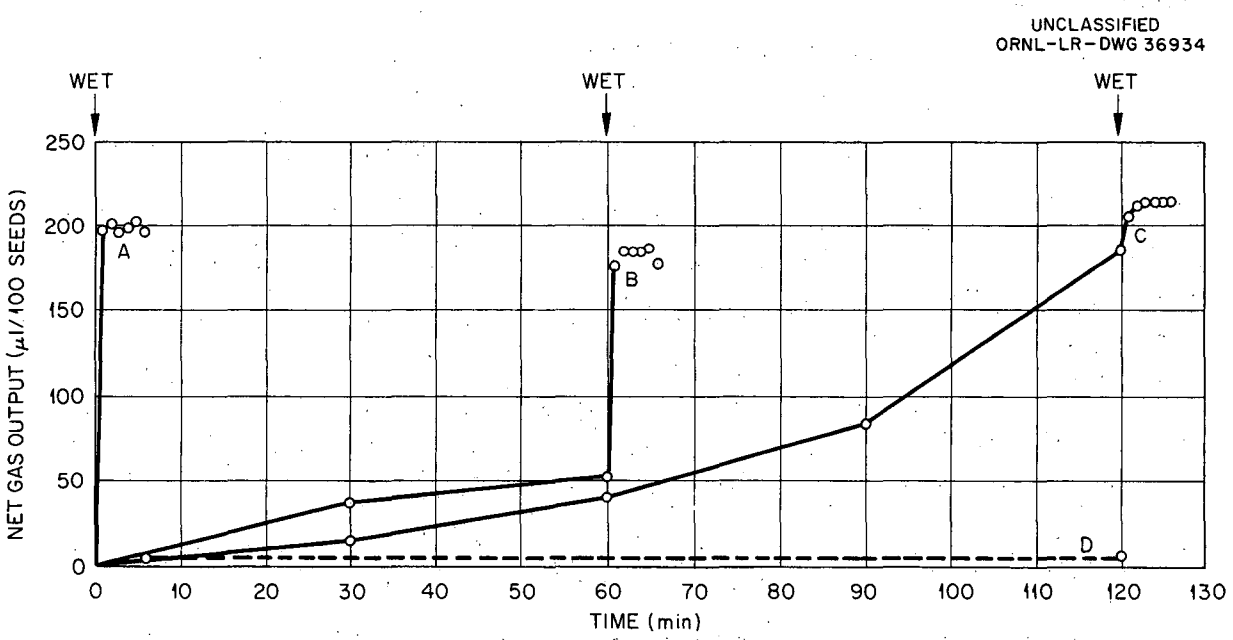


**Fig. 18. Gas Released from Lettuce Seeds at 37°C. Seeds were in water-saturated atmosphere until moistened at indicated times.**

output with temperature and also because the volume of gas released at 37°C was approximately twice the total volume of the dry seeds, we favor the explanation in terms of colloidal adsorption.[5] The pertinence of this physical phenomenon to the experimental determination of RQ values is obvious. Nonrespiratory evolution of gas as described here would lead to calculation of mistakenly high RQ values. Figure 18 suggests that this nonrespiratory phenomenon can occur over a period of several hours when seeds are moistened slowly. To illustrate our argument, we note that peas have been reported to have the exceptionally high RQ of 6.0 after a 4-hr soaking, with a decrease to 1.8 after 8 hr, and a further decrease with increasing time.[3,4] Allerup[6] showed, however, that peas require approximately 18 hr to complete their imbibition of water. Thus it may be suggested that nonrespiratory gas output during wetting accounted for the high apparent RQ values reported after 4 and 8 hr. These experiments suggest certain precautions for studies of respiration of intact seeds and similar biological material. Examination of the literature suggested that this unrecognized nonrespiratory gas output in many instances led to mistakenly high apparent RQ values before water imbibition had been completed.

A more detailed account of this work will be published soon.[7]

## Photoreversible Control of Germination of Damaged New York Lettuce Seeds by Red and Far-Red Light

A. H. Haber H. J. Luippold

Introduction. — Although the germination of New York lettuce seed is normally insensitive to light at 22°C, such seeds are rendered photoblastic by damage from X radiation, freezing, mannitol, or dinitrophenol.[8] We have studied the photosensitivity induced by the injurious treatments using filter systems for the isolation of red (590–680 mμ, 4.9 ergs•mm$^{-2}$•sec$^{-1}$) and far-red (710–1150 mμ, 93 ergs•mm$^{-2}$•sec$^{-1}$) light.

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[5] J. W. McBain, Colloid Science, D. C. Heath and Co., Boston, 1950.

[6] S. Allerup, Physiol. Plantarum 11, 99 (1958).

[7] A. H. Haber and N. Brassington, Nature (in press).

[8] A. H. Haber, Biol. Semiann. Prog. Rep. Aug. 15, 1958, ORNL-2593, p 91–92.

Results. — Table 26 shows that the photoblastism induced in New York seeds by X rays appears to be of the red, far-red photoreversible type found in photoblastic varieties of lettuce seed. Similar results were found with seeds damaged by freezing, mannitol, or dinitrophenol instead of X radiation. From such data, values of $X^2$ for the four degrees of freedom were partitioned into four orthogonal components designed to assay the significance of (1) the germination-inhibiting effect of far-red light after red-light treatment as compared with no light exposure after red-light treatment and (2) the germination-promoting effect of red light after far-red treatment as compared with that of no light exposure after far-red treatment. For any of the four injurious types of treatment, far-red light significantly inhibited germination when administered after red light, and red light significantly promoted germination when administered after far-red light.

Discussion. — These results point to a further parallel between the interactions of X radiation and light in the germination of lettuce seed on the one hand, and in the production of chromatid aberrations in *Vicia* on the other.[8,9] The photoblastism induced in this normally light-insensitive variety of lettuce appears to be of the same type as that found in varieties normally photoblastic. Thus we might speculate that there may be some inherent, perhaps genetic, damage that results in greater photosensitivity for some varieties.

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[9]C. C. Moh and R. B. Withrow, Radiation Research 10, 13–19 (1959).

Table 26. Photoreversal of Light Effects on X-Irradiated New York Lettuce Seeds

1 kr/min, 250 kvp, during first 45 min in darkness

| Light Treatment* | Percentage Germination (24 hr, 22°C) |
|---|---|
| None | 16 |
| Red | 29 |
| Far-red | 13 |
| Red + far-red | 9 |
| Far-red + red | 33 |

*2 hr each, begun immediately after X irradiation.

A more detailed account of the work described in this and in our previous report[8] will soon be published.[10]

## Photosynthesis

W. A. Arnold

Experiments in the last two years have shown that the chlorophyll in chloroplasts acts as a semiconductor in a number of ways. During these studies a new instrument was developed to explore the semiconduction of thin films. It consists of a small electrical condenser, with one or more layers of the material to be studied on one of the plates. The condenser is connected between ground and the input of a vibrating reed electrometer. Upon illumination of the material, the electrometer shows a charge, if electrons are transferred between layers or between one layer and the metal plate.

The last six months have been spent in improving the instrument and in the study of chloroplasts with it.

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[10]A. H. Haber, Physiol. Plantarum (in press).

# BIOPHYSICS

J. S. Kirby-Smith J. Jagger
M. L. Randolph S. Kondo[1]
D. L. Parrish

## Electron Spin Resonance

M. L. Randolph J. S. Kirby-Smith

Introduction. — The study of radiation-induced electron spin resonances has been continued on several closely interrelated topics. These include (1) RBE-LET relations in crystalline amino acids; (2) investigation of changes in shape and magnitude of the resonance patterns with time, including long-term effects over several months as well as the more-immediate and important changes soon after irradiation; and (3) studies designed to determine whether the long-lived resonances observed in crystalline solids, such as the simple amino acids, arise from true molecular free radicals or from structural abnormalities such as lattice defects or other solid-state phenomena. All three types of investigation are of fundamental importance to an understanding of electron spin resonance phenomena in biological and chemical systems. They also have a direct empirical value in studying the effect of radiation.

Results and Discussion: RBE-LET relations. — The previous report[2] on the RBE-LET relation for glycine has been reconfirmed and qualitatively generalized to three more amino acids. The latest values obtained are given in Table 27. In each case the sensitivity to the high LET radiations (neutrons and alpha particles) is significantly less, rather than greater, than to the lower LET spectra of X rays or gamma rays.

Decay and changes in resonance patterns after irradiation. — The decay of six amino acids has been studied quantitatively on samples given about 18 Mrads by a $Co^{60}$ gamma-ray source and subsequently observed after

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[1]Visiting investigator from abroad.

[2]J. S. Kirby-Smith and M. L. Randolph, Bull. Am. Phys. Soc. 2, Ser. II, 278 (1957).

Table 27. Relative Efficiency for Production of Resonances in Crystalline Amino Acids by Different Ionizing Radiations

| Material | Radiation | | | |
|---|---|---|---|---|
| | 250-kvp X Rays, 0.44 mm of Cu hvl | $Co^{60}$ Gamma Rays | 14-Mev Neutrons | $Po^{210}$ Alpha Particles |
| Glycine | | | | |
| (previous values)* | | 1.0 | 0.7 | 0.1 |
| (latest values) | | 1.0 | 0.7 | 0.3 |
| DL-α-Alanine | 1.00 | 1.2 | 0.5 | |
| Glycylglycine | 1.00 | 0.8 | 0.56 | |
| Glutamic acid | 1.00 | 1.0 | 0.22 | |

*J. S. Kirby-Smith and M. L. Randolph, Bull. Am. Phys. Soc. 2, Ser. II, 278 (1957).

storage in air at room temperature for periods as long as 95 days. Methods of measurement have been given previously.[3,4] All measurements have been standardized vs diphenylpicrylhydrazyl samples, and the following results indicate good instrumental stability. The 95% confidence intervals found for 9 calibrations vs one sample and 14 vs the other give the areas of the absorption curves per microgram of sample as 1.054 ± 0.065 and 1.076 ± 0.061, and the peak-to-peak heights of the derivative curves as 2.27 ± 0.04 and 3.32 ± 0.11. All the curve shapes for irradiated amino acids are complex rather than simple, which suggests the presence of multiple kinds of resonant sites. Furthermore, the general trend is toward complexity increasing with time. Whereas, in general, the maximum peak-to-peak heights of different curves for a particular irradiated amino acid are roughly proportional to the area of the absorption curve, or number of resonances since each absorbs very nearly the same microwave energy, progressive changes in patterns that alter this proportionally do occur. For glycine the derivative curve peak-to-peak height initially decays much

[3]M. L. Randolph and D. L. Parrish, Biol. Semiann. Prog. Rep. Aug. 15, 1958, ORNL-2593, p 96.

[4]A. D. Conger and M. L. Randolph, Radiation Research (in press).

more rapidly than the area of the absorption curve, which leads to the loss of resolution between the central peak and the first satellites of the absorption curve, as Zimmer reported.[5] The results of empirical attempts to reduce our decay data to analytic forms are given in Table 28. The stability of $\alpha$-alanine is impressive, and one might think of correlating it with the previous observation[3] that the components of the absorption curve of $\alpha$-alanine are in the expected binomial ratio. However, examination of the derivative curves for these very heavily irradiated samples indicates that the patterns are unexpectedly complex. The appreciable rise in the number of glutamic acid resonances at 20 days is surprising, and is being remeasured. If this result is real, an explanation would be to postulate excited energy states in the crystals that are not detected by electron spin resonance methods until they decay to a lower energy level.

The yield values directly obtained from this high dose experiment, in terms of resonances per electron volt dissipated, are, with the exception of glycylglycine, considerably less than obtained previously.[3] Possible explanations for this finding are that, although molecular saturation is excluded by the 100:1 ratio of molecules to resonances, structural saturation may be evident at this level; or that appreciable decay, which we may not measure properly, occurred during the protracted irradiation of 15 days. For the proline and DL-leucine samples, decay-corrected calculations of the yields have been made as follows: The exponential decay given in Table 28 is assumed to apply throughout the experiment, and the rate of production of resonances during irradiation is taken as a constant, $\alpha$. Then the number of resonances at the end of the irradiation will be

$$N = \frac{\alpha}{\lambda}\left(1 - e^{-\lambda t}\right) , \qquad (1)$$

which is $\alpha t$ if the exponent is small or is $\alpha/\lambda$ if it is large. For the leucine the latter holds, and for the proline the exponential is significantly different from $(1 - \lambda t)$. Correcting observed data to the end of

[5]K. G. Zimmer et al., Strahlentherapie 103, 3–15 (1957).

Table 28. Disappearance Characteristics of Radiation-Induced Resonances in Crystalline Amino Acids

| Material | Empirical Form of Decay (t, time in days) | |
|---|---|---|
| | Area of Absorption Curve | Height of Differential Curve |
| Glycine (2 samples), 18 Mrads | $t^{-0.17}$ for $6 \leq t \leq 95$<br>$e^{-0.0040t}$ for $20 \leq t \leq 95$ | $t^{-0.23}$ for $6 \leq t \leq 95$<br>$e^{-0.0029t}$ for $20 \leq t \leq 95$ |
| 8.7 Mrads | $t^{-0.22}$ for $0.2 \leq t \leq 33$ | |
| Glycylglycine | $t^{-0.046}$ (?) for $6 \leq t \leq 95$ | $t^{-0.046}$ (?) for $6 \leq t \leq 95$ |
| DL-Leucine | $t^{-0.85}$ for $1 \leq t \leq 20$<br>$e^{-2.4t}$ for $0.025 \leq t \leq 1$ | |
| L-Proline | | $e^{-0.042t}$ for $0 \leq t \leq 40$ |
| DL-α-Alanine | Less than 10% decay in 95 days | |
| Glutamic acid | About 25% rise at 20 days, 10% above initial value at 95 days | |

irradiation and using Eq. (1), one determines the rate of production of resonances and then the average energy dissipated per resonance. For proline this calculation decreases the value from 613 to 460 ev/resonance and for leucine gives a value of 7.4 ev/resonance. Although this value is probably inaccurate, it suggests a very high frequency of resonances induced in these crystals by ionizing radiation and emphasizes the need for precise experiments immediately after irradiation.

Present limitations in our experimental arrangements permit observation of resonances no sooner than 5 or 10 min after irradiation, but even after this interval interesting changes in irradiated molecules are observed. Tradescantia pollen given doses of the order of $10^6$ rads shows an increase in differential peak height and a narrowing in pattern from 10 to 30 min after exposure, followed by a relatively rapid decay, which is dependent on the amount of water present in the material. These results indicate that the resonance method can be used in the study of radical kinetics, at least in some biological systems.

Nature of the magnetic centers in irradiated crystalline amino acids. — At present it is not possible to state definitely the relative importance of true free radicals (such as $CH_2$ in the case of irradiated glycine) and lattice imperfections in giving rise to the observed resonances in irradiated crystalline amino acids. Our results on the RBE-LET dependence, which follow closely the case for induction of fluorescence centers in glass, a decrease observed in resonances produced in fine particles compared with single crystals, and a similar decrease in magnetic centers induced in glycine adsorbed presumably in noncrystalline form on silica gel all support the hypothesis that at least some of the magnetic centers are of lattice origin. Further work on the glycine-silica gel system is in progress in an attempt to answer this important question.

## Finite Size Ion Chambers and the Inverse Square Law

S. Kondo M. L. Randolph

The previously reported[6] theoretical and experimental check of the variation of readings of finite size ion chambers placed near a point source of photons has been completed. A complete set of calculations for cylindrical chambers of any ratio of diameter and length placed either endwise or sidewise toward the source has been performed on the Oracle. Quite satisfactory agreement with the theory was found not only for the previously reported cylindrical chamber placed endwise but also for a cylindrical chamber placed sidewise and for a spherical chamber, all under very severe conditions. The theory also agrees with the precise measurements made by Mayneord and Roberts 20 years ago.[7] The optimum ratios of diameter to length for cylindrical chambers to show minimum variations from the inverse square law because of finite chamber size are also predicted by the theory.

## Silver-Activated Phosphate Glass Dosimeters

S. Kondo

Introduction. — Silver-activated phosphate glass after exposure to X rays acquires the ability to fluoresce when excited by UV.[8] The amount of this luminescence is linearly proportional to the dose. Commercially available glass rods 1 mm in diameter and 6 mm long have been used in alpha-particle and neutron dosimetry and in measurement of the internal dose distribution in mice exposed to X rays.

Results and Discussion. — The glass rods exposed to ionizing radiations are excited by UV of 3650 Å, and the amount of the orange luminescence is measured with a fluorometer made of a photomultiplier tube and a typical d-c amplifier. The linear relation between the luminescent output of the exposed rods and the exposure doses has been confirmed for X rays, $Co^{60}$ gamma rays, $P^{32}$ beta rays, $Po^{210}$ alpha particles,

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[6] S. Kondo and M. L. Randolph, Biol. Semiann. Prog. Rep. Aug. 15, 1958, ORNL-2593, p 95.

[7] W. V. Mayneord and J. E. Roberts, Brit. J. Radiol. 10, 365 (1937).

[8] J. H. Schulman et al., J. Appl. Phys. 22, 1479 (1951).

thermal neutrons, and 14-Mev neutrons. The reproducibility of the glass readings holds within ± 3% accuracy for every case. The luminescent output first increases with time after irradiation, reaches maximum in about 1 hr, and then decreases with time so slowly that it decays only about 5% in a month.

The most serious disadvantage of this glass dosimeter is a sensitive dependence on gamma-ray energy. In spite of this, the depth dose curve of X-ray dose distribution in Lucite is equal, within ± 2% experimental error, for the glass readings and Victoreen r-meter readings. Thus the readings of glass rods implanted in an X-rayed animal are taken as true doses once they are normalized to the X-ray energy. With this method, we found no appreciable deviation from homogeneous dose distribution among spleen, liver, intestine, kidney, testis, and backbone of a mouse exposed to 250-kvp X rays with a 3-mm Al filter. For larger animals, some deviation may be expected.

To compare the response of the glass dosimeter to $Co^{60}$ gamma rays with that to any other kind of radiation, we should use the term "response per rad in glass" or its equivalent. Figure 19 summarizes the results for a number of different radiations. The glass response to thermal neutrons is almost all attributable to beta rays of 1.2-Mev average energy emitted from the silver nuclei activated by the thermal neutrons so that some fraction of beta rays originating inside the rods escape. A correction for these escaping beta rays has been taken into account for the value given in Fig. 19. The calculation of rads in glass exposed to 14-Mev neutrons can be done, for the present, only for the lower and probable upper values because of the lack of some neutron cross sections for high Z elements of the glass.

Figure 20 shows the difference in glass response to 14-Mev neutrons between rods with and without a Lucite cover. The difference is caused by the protons recoiled by the neutrons from the Lucite and plotted also in Fig. 19 as the response per rad in glass for recoil protons of 7 Mev average energy. From Fig. 19 we may conclude that the relative response of the silver-activated glass decreases with increasing LET in the opposite direction to the common RBE connected with chromosome breakage but in the same direction as for the paramagnetic response of some organic

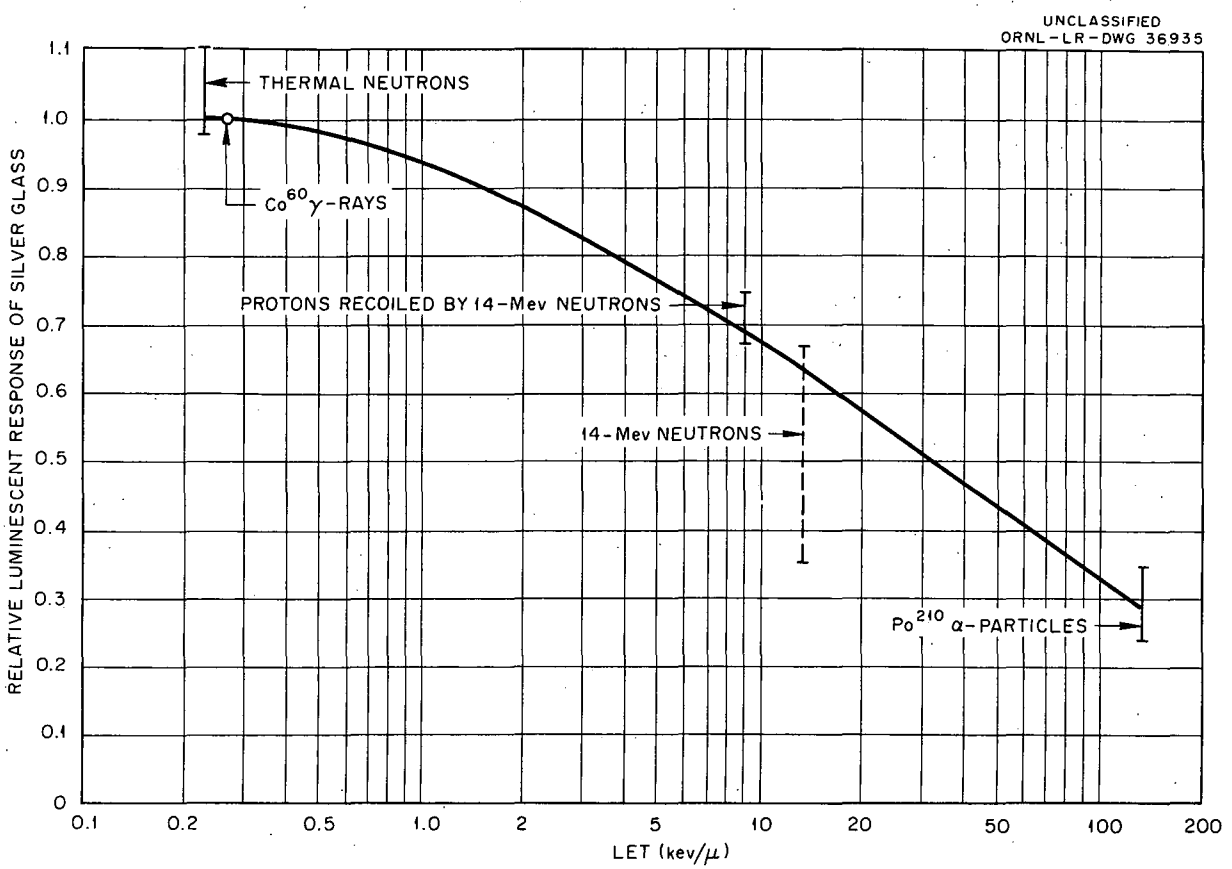


**Fig. 19. Relative Responses of Silver-Activated Phosphate Glass per Rad of Thermal Neutrons, Recoil Protons, 14-Mev Neutrons, and $Po^{210}$ Alpha Particles Compared with the Response per Rad of $Co^{60}$ Gamma Rays. The fluctuation in the values is not caused by the inaccuracy of the glass dosimeters but rather by the uncertainty in estimation of rads.**

materials.[9] Once we know the relative response of glass vs LET of ionizing radiations, we may, in principle, use a single phosphate glass to make the absolute measurement of any kind of ionizing radiation in reference to the known response to $Co^{60}$ gamma rays. The small size of the glass rod, its good accuracy, and the small decay in reading over months offer promising fields of application.

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[9]M. L. Randolph and J. S. Kirby-Smith, "Electron Spin Resonance," this report.

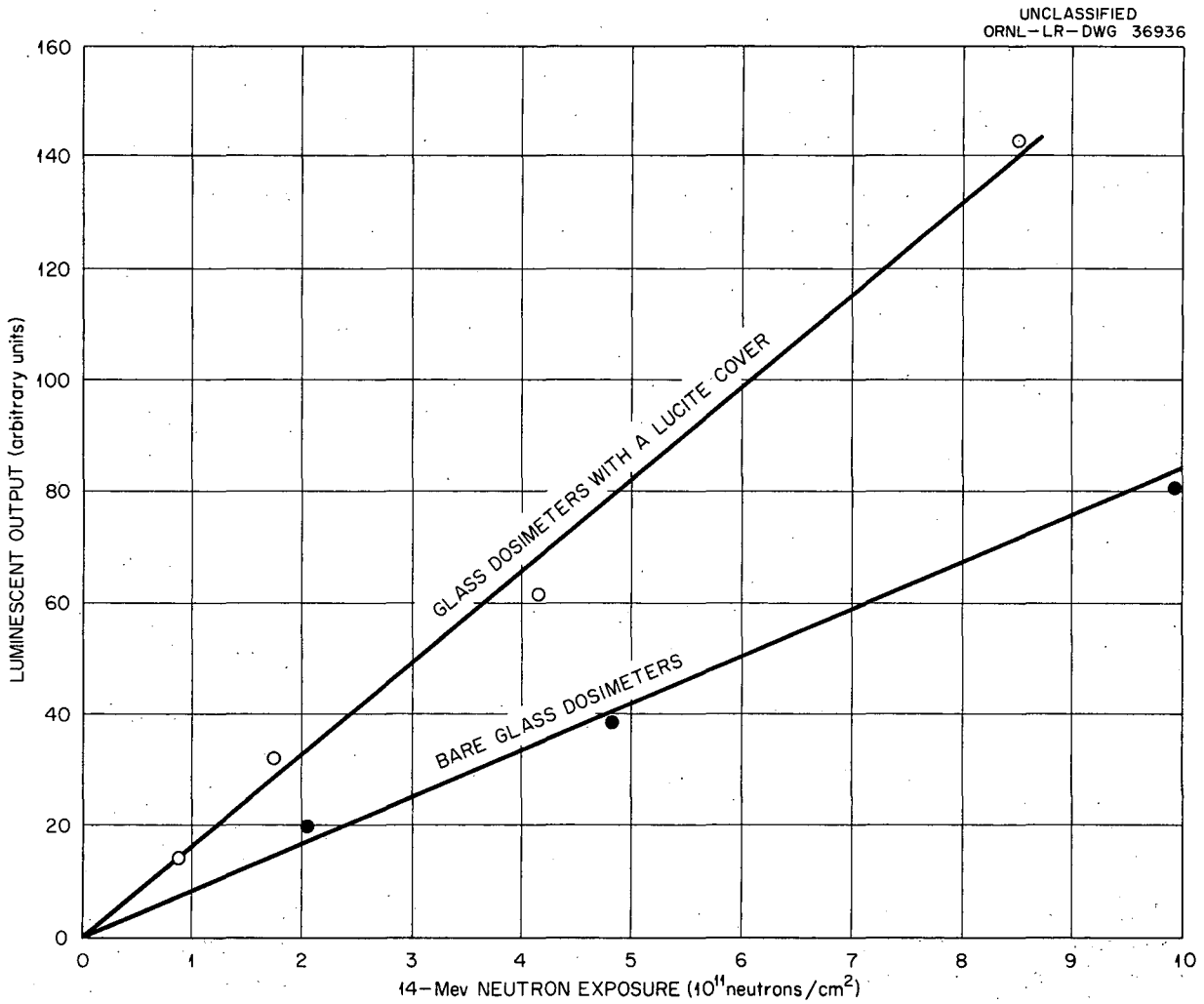


**Fig. 20. The Difference in Glass Response to 14-Mev Neutrons Between Glass Rods With and Without a Lucite Cover.** Fluorometer sensitivity: 132 = luminescent output produced by a glass rod exposed to 1000 r of $Co^{60}$ gamma rays; average exposure dose rate: $\sim 10^{10}$ neutrons$\cdot$cm$^{-2}\cdot$min$^{-1}$.

Photoprotection Against Ultraviolet Damage in *Escherichia coli* B

J. Jagger

*Introduction*. – Photoprotection (PP) is protection of a biological system from UV damage by illumination with light of longer wavelength *before* the UV irradiation. Many negative experiments on PP have been reported,[10] and it is clear that it is not as universal as photoreactivation. However, the phenomenon has been clearly demonstrated in *Escherichia coli* by Weatherwax,[11] who found it in strain B but not in B/r, and by Miki,[12] who found it in strains B and K12 (λ), but not in B/r and K12S. The present report gives preliminary data on the characterization of this phenomenon in *E. coli* B.

*Results*. – *E. coli* B was grown at 37°C for 16 hr with aeration in nutrient broth. The cells were washed, diluted, illuminated, and irradiated at 23° in *M*/15 phosphate buffer. Illumination was done at 3000–6000 Å and UV irradiation at 2537 Å. After treatment, cells were placed on either nutrient agar (plain agar) or nutrient agar containing 0.8% NaCl (salt agar), and incubated at 37° for 20 hr. UV survival was much lower on salt agar, in agreement with the report of Alper and Gillies.[13] Figure 21 shows that PP is a striking phenomenon for cells plated on salt agar but is a considerably smaller effect for cells plated on plain agar (DRF of roughly 0.25 and 0.7, respectively, at 20% survival). PP is seen to occur principally for low doses of UV. At higher doses, the curves become parallel, suggesting that no further PP is taking place. Finally, at very high UV doses, PP disappears. For the lower UV doses, PP seems to raise the survival on salt agar to the level found without PP on plain agar. Because the effect is so much greater on salt agar, this was used in most subsequent experiments.

Figure 22 shows PP at 23, 30, and 37°C. The curves do not differ beyond experimental error and appear to be quite parallel, although there may be small differences in the initial lag. This is in contrast to

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[10] J. Jagger, Bacteriol. Rev. 22, 99–142 (1958).

[11] R. S. Weatherwax, J. Bacteriol. 72, 124–25 (1956).

[12] K. Miki, Japan. J. Bacteriol. 11, 803–12 (1956).

[13] T. Alper and N. E. Gillies, J. Gen. Microbiol. 18, 461–72 (1958).

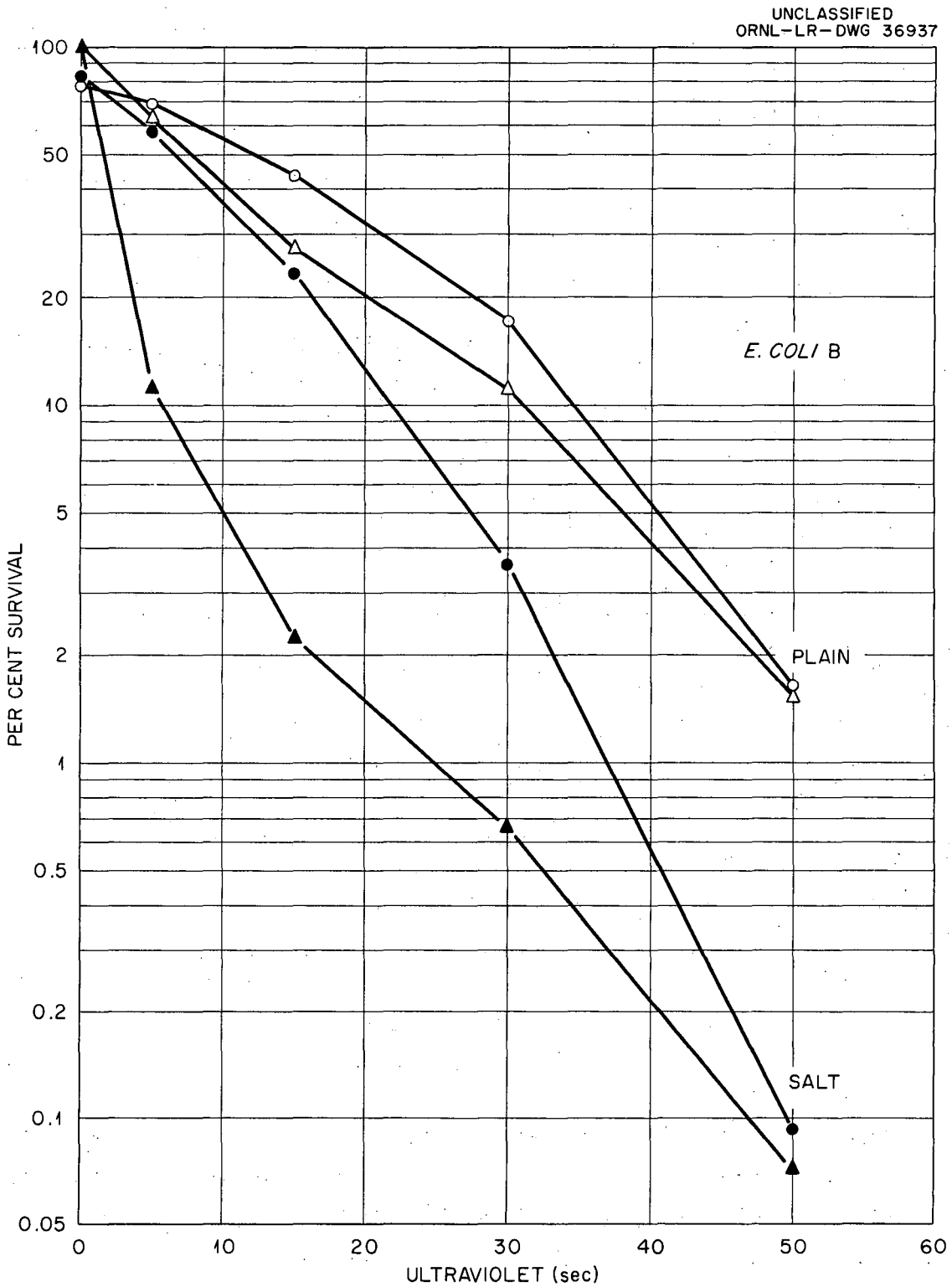


**Fig. 21. Ultraviolet Survival as a Function of UV Dose, with Plating on Plain Agar or Salt Agar.** In each group, the lower curve is the survival with no photoprotection and the upper curve with optimal photoprotection. Sum of two identical experiments.

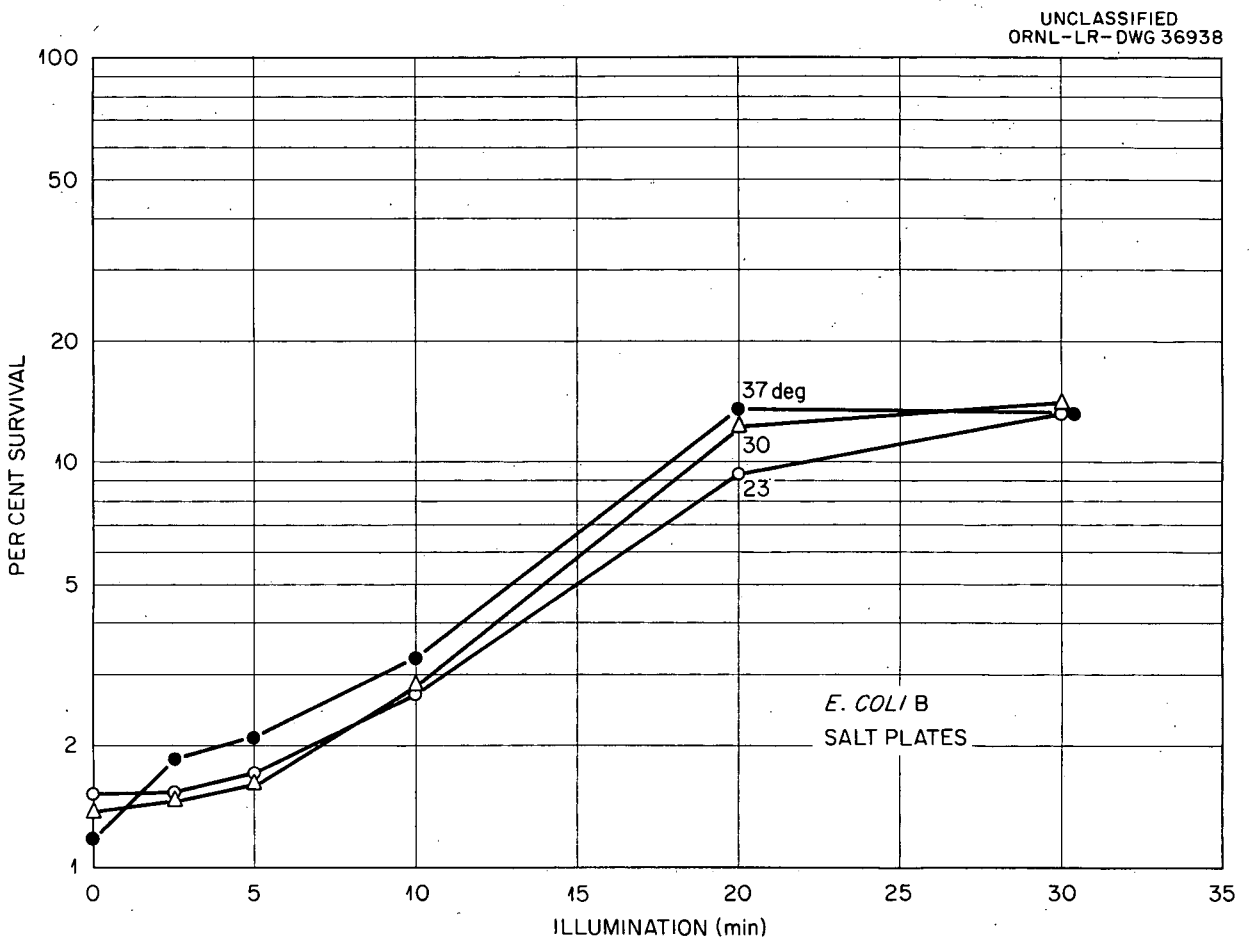


**Fig. 22. Ultraviolet Survival as a Function of Photoprotecting Dose at Three Different Temperatures. The curves do not differ beyond experimental error. Sum of two identical experiments.**

photoreactivation, which shows a $Q_{10}$ in this range of about 2.[10] It can also be seen from the form of these curves that PP is a multihit phenomenon of rather high hit number. This is again in contrast to photoreactivation, which shows hit numbers less than 3 in most bacterial systems.[10]

Experiments in which the cells were held in buffer for various times between illumination and irradiation show that PP lasts for a long time and shows a roughly exponential decay with time. For cells held at $10^6$ cells/ml in buffer at 23°C (salt plating), the decay half time is about 36 hr for cells that have received optimum illumination. This behavior is comparable to that found with photoreactivation, and it is to be expected that cells kept at 0° would retain some protection for weeks.

The UV dose for $10^{-2}$ survival on salt agar without PP is about 260 ergs/mm$^2$. At this level, the dose of illumination for optimum PP is about $10^7$ ergs/mm$^2$. This is about 50 times that required for optimum photoreactivation.

Discussion. – These studies have not yet progressed far enough to permit fruitful discussion of mechanisms. It is clear that PP does not utilize the same mechanism as photoreactivation. However, it is possible that the two processes overlap to some extent.

## GLOSSARY

| | |
|---|---|
| AET | S,2-aminoethylisothiourea |
| AMP | adenosine monophosphate |
| APMT | S,3-aminopropyl-N'-methylisothiourea |
| APS | adenosine-5'-phosphosulfate |
| ATP | adenosine triphosphate |
| BSA | bovine serum albumin |
| DEAE | diethylaminoethyl |
| DEB | diepoxybutane |
| DNA | deoxyribonucleic acid |
| DPN | diphosphopyridine nucleotide |
| DPNH | reduced diphosphopyridine nucleotide |
| DRF | dose reduction factor |
| EDTA | ethylenediaminetetraacetic acid |
| FMN | flavin mononucleotide |
| HSA | human serum albumin |
| IBM | isologous bone marrow |
| LET | linear energy transfer |
| MEA | 2-mercaptoethylamine |
| OTC | ornithine transcarbamylase |
| PAPS | 3'-phosphoadenosine-5'-phosphosulfate |
| $P_i$ | inorganic phosphate |
| PP | inorganic pyrophosphate; photoprotection |
| RBC | red blood cells |
| RBE | relative biological effectiveness |
| RBM | rat bone marrow |
| RNA | ribonucleic acid |
| RQ | respiratory quotient |
| TPN | triphosphopyridine nucleotide |
| UV | ultraviolet |

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