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BIOLOGY DIVISION

SEMIANNUAL PROGRESS REPORT

For Period Ending February 15, 1960

Alexander Hollaender, Director
Stanley F. Carson, Assistant Director

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PUBLICATIONS AND LECTURES

Publications. – Manuscripts published and prepared during this half year have varied widely in nature, ranging from the short note to be published quickly because it contains information of timely importance, through the popular medium-length paper, to the very long paper prepared in two languages, and on to the collection of papers making up a book of considerable proportions. As the number of visiting investigators from other countries increases, the number of bilingual papers also grows. The book activity during the period has included one book published (the 1959 Symposium on Enzyme Reaction Mechanisms); one of which Alexander Hollaender is editor – prepared (except for two chapters) in this Division and submitted to the publisher; and another now in final preparation for publication as a supplement to Cancer Research. The last named is written by A. C. Upton, A. W. Kimball, J. Furth, K. W. Christenberry, and W. H. Benedict and is entitled "Some Delayed Effects of Atom-Bomb Radiations in Mice." This is a final report of the delayed somatic effects of radiation in mice exposed at Operation Greenhouse (see page 93 for summary of the report).

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Bernlohr, R. W., and G. D. Novelli	Antibiotic Production as a Function of Spore Formation in *Bacillus licheniformis*	Nature 184, 1256–1257 (1959)
Bollum, F. J.	Thermal Conversion of Nonpriming Deoxyribonucleic Acid to Primer	J. Biol. Chem. 234, 2733–2734 (1959)
von Borstel, R. C.	"A History of Cytology" by Arthur Hughes, Abelard-Schuman, London, 1959, 158 pages	Am. Scientist 47, 392A–394A (1959) (Book Review)
von Borstel, R. C., and M. L. Rekemeyer	Radiation-Induced and Genetically Contrived Dominant Lethality in Habrobracon and Drosophila	Genetics 44, 1053–1074 (1959)
Clayton, R. K.	Hydrogen Donors and Peroxide Concentration in a Respiring Cell Containing Catalase	Biochem. Biophys. Research Communs. 1, 191–193 (1959)
Clayton, R. K.	Purified Catalase from *Rhodopseudomonas spheroides*	Biochim. et Biophys. Acta 36, 40–47 (1959)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Congdon, C. C.	Immunologic Problems in Tissue Transplantation for Recovery from Radiation Injury	In XXI International Congress of Physiological Sciences, Symposia and Special Lectures, Sebastian de Amorrortu and Sons Co. Press, Buenos Aires, Argentina, 1959, p 119–123
Cosgrove, G. E., A. C. Upton, R. A. Popp, and C. C. Congdon	Inhibition of Foreign Spleen Reaction by Inactivation of Donor Cells with Recipient Antigen	Proc. Soc. Exptl. Biol. Med. 102, 525–527 (1959)
Davidson, D.	A Method for Estimating Mitotic Rates in *Vicia* Roots After X Irradiation	Brit. J. Radiol. 32, 612–614 (1959)
Fisher, W. D., N. G. Anderson, and K. M. Wilbur	Studies on Nuclei. II. Effects of X Rays on Deoxyribonucleoprotein from Rat Thymus	Exptl. Cell Research 18, 481–493 (1959)
Fisher, W. D., N. G. Anderson, and K. M. Wilbur	Studies on Nuclei. I. Physical Properties of Deoxyribonucleoprotein from Rat Thymus in 1 *M* Sodium Chloride	Exptl. Cell Research 18, 100–111 (1959)
Gaulden, M. E.	Discussion Parts: Work Conference on Mitogenesis, Argonne National Laboratory, September 1955	In *Mitogenesis*, ed. by H. S. Ducoff and C. F. Ehret, Univ. Chicago Press, Chicago, 1959, p 68–72
Gengozian, N.	Analysis of Serum from Lethally Irradiated Mice Treated with Rat Bone Marrow	J. Immunol. 83, 173–183 (1959)
Goodman, J. W., and L. H. Smith	Life Span of Rat and Mouse Erythrocytes in the Rat-Mouse Chimera (Letter to the Editor)	Nature 183, 1833 (1959)
Grell, R. F., and E. H. Grell	The Behavior of Nonhomologous Chromosomal Elements Involved in Nonrandom Assortment in *Drosophila melanogaster*	Proc. Natl. Acad. Sci. U.S. 46, 51–57 (1960)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Gude, W. D., A. C. Upton, and T. T. Odell, Jr.	Modification of Radiation-Induced Intestinal Injury in Rats by Chilling After Irradiation	Intern. J. Radiation Biol. 1, 247–255 (1959)
Haber, A. H.	Rendering the Germination of Light-Insensitive Lettuce Seeds Sensitive to Light	Physiol. Plantarum 12, 456–464 (1959)
Haber, A. H., and H. J. Luippold	Dormancy Resulting from Gamma Irradiation of Lettuce Seed	Intern. J. Radiation Biol. 1, 317–327 (1959)
Haber, A. H., and N. E. Tolbert	Effects of Gibberellic Acid, Kinetin, and Light on the Germination of Lettuce Seed	In Photoperiodism and Related Phenomena in Plants and Animals, ed. by R. B. Withrow, Am. Assoc. Advance. Sci., Washington, D.C., 1959, p 197–206
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. 34, 376–380 (1959)
Hollaender, A. (Division Director)	Symposium on Enzyme Reaction Mechanisms	J. Cellular Comp. Physiol. 54, Suppl. 1, December 1959
Hollaender, A., and S. F. Carson	Biology Division Semiannual Progress Report for Period Ending August 15, 1959	ORNL-2813
Hollaender, A., and G. E. Stapleton	Ionizing Radiation and the Living Cell	Sci. American 201, 94–100 (1959)
Kimball, R. F.	The Seventh Annual Meeting of the Radiation Research Society	Intern. J. Radiation Biol. 1, 432–433 (1959)
Krieg, D. R.	Induced Reversion of $T4r_{II}$ Mutants by Ultraviolet Irradiation of Extracellular Phage	Virology 9, 215–227 (1959)
Kroeger, H.	The Genetic Control of Genital Morphology in Drosophila: A Study of the External Genitalia of Sex Mosaics	Wilhelm Roux' Arch. Entwicklungsmech. Organ. 151, 301–322 (1959)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Makinodan, T., B. H. Friedberg, M. G. Tolbert, and N. Gengozian	Relation of Secondary Antigen Injection to Time of Irradiation on Antibody Production in Mice	J. Immunol 83, 184–188 (1959)
Oakberg, E. F.	Initial Depletion and Subsequent Recovery of Spermatogonia of the Mouse After 20 r of Gamma Rays and 100, 300, and 600 r of X Rays	Radiation Research 11, 700–719 (1959)
Owen, R. D.	Erythrocyte Repopulation Following Transplantation of Homologous Erythropoietic Tissues into Irradiated Mice	In Les Congrès et Colloques de l'Université de Liège. Vol 12, Colloque International sur les Problèmes Biologiques des Greffes. Université de Liège, 1959, p 1–12.
Popp, R. A., and G. E. Cosgrove	Solubility of Hemoglobin as a Red Cell Marker in Irradiated Mouse Chimeras	Proc. Soc. Exptl. Biol. Med. 101, 754–758 (1959)
Popp, R. A., and L. H. Smith	Electrophoresis of Proteins of Irradiated Mouse Chimeras	J. Natl. Cancer Inst. 23, 395–409 (1959)
Russell, L. B., K. F. Stelzner, and W. L. Russell	Influence of Dose Rate on the Radiation Effect on Fertility of Female Mice	Proc. Soc. Exptl. Biol. Med. 102, 471–479 (1959)
St. Amand, W., and L. H. Smith	Interstrain Ovarian Transplantation in Lethally Irradiated Mice After Bone Marrow Therapy	Nature 184, 1503–1504, Suppl. 19 (1959)
Schwartz, E. E.	Bone Marrow Transplantation and Chemical Protection in the Radiotherapy of Mouse Leukemia	Acta Radiol. 52, 235–240 (1959)
Upton, A. C.	Studies on the Mechanism of Leukemogenesis by Ionizing Radiation	In Ciba Foundation Symposium on Carcinogenesis: Mechanisms of Action, ed. by G. E. W. Wolstenholme and C. M. O'Connor, J. & A. Churchill, Ltd., London, 1959, p 249–273

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Volkin, E.	Ribonucleic Acid Turnover in Phage Infection	In Proceedings of the Fourth International Congress of Biochemistry, Vol VII – Biochemistry of Viruses, Pergamon Press Ltd., London, 1959, p 212–224
Wolff, S.	Radiation Studies on the Nature of Chromosome Breakage	Am. Naturalist 94, 85–93 (1960)

Scientific Society and Traveling Lectures. – Forty-five of the lectures given by Division personnel were before professional society groups at home and abroad, including several long symposium offerings and speeches at several international meetings.

AUTHOR(S)	TITLE	PLACE PRESENTED
Adler, H. I.	The Sequence of Events in Radiation Injury	Meredith College, Raleigh, North Carolina (Dedication of Science Building)
Adler, H. I. (M. S. Engel, and G. E. Stapleton)	Physiology and Radiation Response of a Catalase-Negative Bacterium	Kentucky-Tennessee Branch, Society of American Bacteriologists, Louisville
Anderson, N. G.	Cell Physiology at the Molecular Level	St. Olaf College, Northfield, Minnesota
Anderson, N. G.	1. Chromosomal Condensation	Duke University, Durham, North Carolina
	2. Recent Advances in Fractionation Methods	
Anderson, N. G.	Density Gradient Ultracentrifugation	Society for Experimental Biology and Medicine, Charleston, South Carolina
Anderson, N. G.	Origin of Life	University Symposium: Lectures in Biological Sciences, University of Tennessee, Knoxville

AUTHOR(S)	TITLE	PLACE PRESENTED
Anderson, N. G.	Substructures of the Cell	AAAS (Symposium on "The Organization of the Cell"), Chicago, Illinois
Anderson, N. G.	Toward a Molecular Pathology	Atlanta Diabetic Association, Atlanta, Georgia
Anderson, N. G. (and W. D. Fisher)	Relation of the Physical Properties of Deoxyribonucleoprotein in 1 M NaCl to Nuclear Structure	Faraday Society Symposium on "Aspects of Behavior and Metabolism of the Cell Nucleus," Cambridge, England
Astrachan, L.	Bacterial Viruses — a Tool for Study of Genetic Action at the Biochemical Level	1. Dept. of Biochemistry, Duke University, Durham, North Carolina 2. M. D. Anderson Hospital and Tumor Institute, University of Texas, Houston 3. Dept. of Microbiology, Tulane University, New Orleans, Louisiana 4. Radiation Biology Laboratory, Texas Engineering Experiment Station, College Station 5. Zoology Dept. University of Texas, Austin 6. Division of Basic Health Sciences, Emory University, Georgia 7. Sigma Xi, University of Georgia, Athens

AUTHOR(S)	TITLE	PLACE PRESENTED
Bender, M. A	Chromosome Evolution in Primates	1. Dept. of Biology, Spring Hill College, Spring Hill, Alabama 2. Dept. of Zoology and Entomology, Louisiana State University, Baton Rouge 3. Division of Anatomy, University of Tennessee, Memphis 4. Dept. of Biology, University of Florida, Gainesville
Benedict, W. H.	The Ophthalmic Effects of Radiations of the Electromagnetic Spectrum and Ionizing Particles	Pan-American Congress of Ophthalmology, Caracas, Venezuela
Bollum, F. J.	Deoxyribonucleic Acid Synthesis by Mammalian Enzymes: Some Observations on Primers	Faraday Society Symposium on "Aspects of Behavior and Metabolism of the Cell Nucleus," Cambridge, England
von Borstel, R. C.	On the Nature of Dominant Lethality Induced by Radiation	Associazione Genetica Italiana, Naples, Italy
Burger, C. L. (and W. D. Fisher)	A Comparison of X-Ray, Gamma-Ray, and Fast-Neutron Effects on Nucleoprotein in 1 M NaCl	AAAS, Chicago, Illinois
Canning, R. E. (and N. G. Anderson	Rat Serum Macroglobulins	AIBS (Am. Soc. Zool.), University Park, Pennsylvania
Carson, S. F.	Biotin and Bacterial Carboxylation Reactions	Biology Dept., University of Pennsylvania, Philadelphia

AUTHOR(S)	TITLE	PLACE PRESENTED
Chu, E. H. Y.	Panel Discussion: Conference on Mongolism	National Association for Retarded Children, Cincinnati, Ohio
Clayton, R. K.	Induced Catalase Synthesis in Photosynthetic Bacteria	1. Dept. of Microbiology, Tulane University, New Orleans, Louisiana 2. Dept. of Zoology, University of Texas, Austin 3. Dept. of Biology, University of Houston, Houston, Texas 4. M. D. Anderson Hospital and Tumor Institute, University of Texas, Houston
Cohn, W. E.	Minor Nucleotides of Ribonucleic Acids, the Pseudouridines	1. Dept. of Chemistry, University of Texas, Austin 2. M. D. Anderson Hospital and Tumor Institute, University of Texas, Houston
Cohn, W. E.	Nucleotic Chemistry (2 lectures)	Dept. of Biochemistry, University of Chicago, Chicago, Illinois
Cohn, W. E.	Pseudouridine, an Unusual Nucleoside from RNA	1. College of Physicians and Surgeons, Columbia University, New York City 2. Dept. of Biology, California Institute of Technology, Pasadena 3. Scripps Institute, La Jolla, California 4. Dept. of Biochemistry, Stanford University, Stanford, California

AUTHOR(S)	TITLE	PLACE PRESENTED
		5. Dept. of Biochemistry, University of California, Berkeley 6. Dept. of Biochemistry, University of California, Davis 7. V.A. Hospital, Oakland, California
Congdon, C. C.	Applications of Bone Marrow Transplantation to Special Research Problems (Abstracts of Conferencias Sobre Hematologia, Inst. Hematol., Argentine Acad. Nacional Med., Buenos Aires, p 29–30)	Symposium on Hematology and Hemophilia, Buenos Aires, Argentina
Congdon, C. C.	Bone Marrow Transplantation	1. The Foundation Curie and Medical School, Mendoza, Argentina 2. Biological Society of Santiago, Hematology Group, University of Chile, Santiago 3. Institute for Cancer Research, sponsored by the Peruvian Cancer Society, Lima
Congdon, C. C.	Bone Marrow Transplantation for Whole-Body Irradiation Injury	Terre Haute Academy of Medicine, Terre Haute, Indiana
Congdon, C. C.	Effect of Type of Antigen Injected on Sequential Histologic Changes in Lymphatic Tissue	Committee on Tissue Transplantation of the National Research Council, Washington, D.C.
Congdon, C. C.	The Germinal Center and Antibody Formation	East Tennessee Association of Pathologists, Knoxville

AUTHOR(S)	TITLE	PLACE PRESENTED
Congdon, C. C.	Major Variables in Bone Marrow Transplantation (Abstracts of Conferencias Sobre Hematologia, Inst. Hematol., Argentine Acad. Nacional Med., Buenos Aires, p 29)	Symposium on Hematology and Hemophilia, Buenos Aires, Argentina
Cosgrove, G. E.	Tissue Changes in Irradiated Mammals	Medical College of Virginia, Richmond (AIBS – MEND Lecture)
Davidson, D.	Cell Lineages in Meristems of Irradiated Roots	International Botanical Congress, Montreal, Canada
Doherty, D. G.	Chemical Protection Against Ionizing Radiation	1. Maryville College, Maryville, Tennessee 2. Boston University, Boston, Massachusetts (AIBS – MEND Lecture)
Engel, M. S.	The Autotrophic Oxidation of Ammonium and Hydroxylamine	American Society of Agronomy, Cincinnati, Ohio
Fisher, W. D. (and C. L. Burger)	The Effect of Heat on the Viscosity of Nucleoprotein in 1 M NaCl	AAAS, Chicago, Illinois
Gaulden, M. E.	The Nucleolus and Mitotic Activity	Faraday Society Symposium on "Aspects of Behavior and Metabolism of the Cell Nucleus," Cambridge, England
Grell, R. F.	Effect of Chromosome Four on Segregation of Sex Chromosomes in *Drosophila melanogaster* [Genetics 44, 514 (1959)]	AIBS (Genetics Society), University Park, Pennsylvania
Grell, R. F.	Nonrandom Assortment of Nonhomologous Chromosomes in *Drosophila melanogaster*	AIBS (Symposium on "Mechanisms of Abnormal Genetic Ratios"), University Park, Pennsylvania

AUTHOR(S)	TITLE	PLACE PRESENTED
Haber, A. H.	Physiology of Seed Germination	1. Division of Natural Sciences, Tuskegee Institute, Tuskegee, Alabama (2 lectures) 2. Dept. of Botany, Duke University, Durham, North Carolina
Haber, A. H. (and H. J. Luippold)	Dormancy Induced by Gamma Irradiation of Dry Lettuce Seeds	International Botanical Congress, Montreal, Canada
Hollaender, A.	Radiation Protection and Recovery	1. Biology Division, University of Puerto Rico, Rio Piedras 2. Cancer Institute, Medical School of the University of Puerto Rico, San Juan
Hollaender, A.	1. Radiation Protection and Recovery 2. Work going on in the Biology Division of the Oak Ridge National Laboratory	Asociacion Venezolana para el Avance de la Ciencia, Instituto Venezolano de Investigaciones Cientificas, Universidad Central de Venezuela, Caracas
Hollaender, A. (and A. M. McCarthy)	The Mutagenicity in Aspergillus terreus of Sublethal X-Ray Doses and Its Modification by Chemical Protection [Science 130, 1420 (1959)]	National Academy of Sciences, Bloomington, Indiana
Jagger, J.	Photoreactivation	Symposium on Bioenergetics, Brookhaven National Laboratory, Upton, New York
Khym, J. X. (and W. E. Cohn)	The Controlled Decomposition of Periodate-Oxidized Ribofuranosyl Compounds (Abstracts of Papers, 136th National Meeting of the American Chemical Society, 1959, p 21D)	American Chemical Society, Atlantic City, New Jersey

AUTHOR(S)	TITLE	PLACE PRESENTED
Kikudome, G. Y.	Some Aspects of the Phenomenon of Preferential Segregation in Maize [Genetics 44, 520 (1959)]	AIBS (Genetics Society), University Park, Pennsylvania
Kimball, R. F.	Growth and Development from One Division to the Next in Ciliate Protozoa	1. Biology and Botany Depts., University of Florida, Gainesville (2 lectures) 2. Biology Dept., Florida Southern College, Lakeland
Kimball, R. F.	Recent Advances in the Study of Mutation	1. Botany Dept., University of Florida, Gainesville 2. Biology Dept., The Florida State University, Tallahassee
Kimball, R. F.	1. Quantitative Studies on Cell Development	Southwestern at Memphis, Memphis, Tennessee
	2. Research Careers	
	3. Genetic Effects of Radiation	
Kimball, R. F.	Genetic Effects of Radiation	1. Biology and Chemistry Depts., Memphis State University, Memphis, Tennessee (2 lectures) 2. Tennessee Short-Term Area Institutes for Science and Mathematics Teachers of High Schools and Secondary Schools (1959–60), Memphis, Tennessee
Kimball, R. F.	Nuclear Syntheses and Mutation Induction [Science 130, 1417 (1959)]	National Academy of Sciences, Bloomington, Indiana

AUTHOR(S)	TITLE	PLACE PRESENTED
Kimball, R. F. (N. Gaither, and S. M. Wilson)	The Rate of Loss of Premutational Damage After X Irradiation of *Paramecium aurelia* [Genetics *44*, 520 (1959)]	AIBS (Genetics Society), University Park, Pennsylvania
Kondo, S. (and M. L. Randolph)	Deviations from the Inverse Square Law of Ionization Chamber Readings Against Point Sources	Japanese Physical Society, Hiroshima
Krieg, D. R.	Mutations of Bacteriophage	Dept. of Microbiology, University of Illinois, Urbana
Lindsley, D. L.	The Influence of Gametic Genotype on the Radiation Sensitivity of Mature Sperm of *Drosophila*	1. Zoology Dept., University of Oklahoma, Norman 2. Biology Dept., Centenary College, Shreveport, Louisiana
Lindsley, D. L.	Sex-Linked Recessive Lethals in *Drosophila* That Are Suppressed by the Y Chromosome	Zoology Dept., University of Texas, Austin
Makinodan, T.	An Immunologic Approach to Biologic Problems	1. Biology Dept. and Sigma Xi, Marquette University, Milwaukee, Wisconsin 2. Biology Dept., Alabama College, Montevallo 3. Biology Dept., Centenary College, Shreveport, Louisiana
Makinodan, T.	Use of Lethally Irradiated Isologous Mice as *in vivo* Tissue Cultures for Antibody-Producing Cells	1. Argonne Cancer Research Hospital, University of Chicago, Chicago, Illinois 2. Zoology Dept., University of Wisconsin, Madison
Makinodan, T. (N. Gengozian, and R. E. Canning)	Demonstration of a Normal Serum Macroglobulin Coprecipitating with the Bovine Serum Albumin (BSA)-Chicken Anti-BSA Aggregate [Science *130*, 1419 (1959)]	National Academy of Sciences, Bloomington, Indiana

AUTHOR(S)	TITLE	PLACE PRESENTED
Nicoletti, B. (and D. L. Lindsley)	Reciprocal Translocations Between the X and the Y Chromosome in *Drosophila melanogaster* [Genetics 44, 529 (1959)]	AIBS (Genetics Society), University Park, Pennsylvania
Novelli, G. D.	Bacitracin Synthesis and Spore Formation in *Bacillus licheniformis*	Dept. of Pharmacology, Harvard Medical School, Boston, Massachusetts
Novelli, G. D.	Transplantation Immunity	Medical students, New York University, New York City
Novelli, G. D. (and R. Rabson)	The Incorporation of C^{14}-Leucine into Protein by a Cell-Free Preparation from Maize Endosperm [Science 130, 1422 (1959)]	National Academy of Sciences, Bloomington, Indiana
Oakberg, E. F.	Genetic Effects of Radiation	Radiological Society of North America, Chicago, Illinois
Peck, H. D.	Comparative Metabolism of Pyruvate	1. Medical College of Virginia, Richmond 2. Upstate Medical Center, Syracuse, New York
Peck, H. D.	Reduction of Sulfate by Microorganisms	Medical College of Virginia, Richmond
Peck, H. D.	Sulfate Reduction by *Desulfovibrio desulfuricans*	Division of Biological Sciences, Purdue University, Lafayette, Indiana
Perkins, E. H.	Demonstration of a Linear Relation Between Circulating Antibody Titers and the Number of Cells Participating in the Immune Response	Southeastern Section, Society for Experimental Biology and Medicine, Charleston, South Carolina

AUTHOR(S)	TITLE	PLACE PRESENTED
Phares, E. F. (and S. F. Carson)	A Direct Biotin Function in Succinate Decarboxylation by Bacterial Extracts (Abstracts of Papers, 136th National Meeting of the American Chemical Society, 1959, p 72C)	American Chemical Society, Atlantic City, New Jersey
Popp, R. A.	Growth and Differentiation of Hematopoietic Cells in *in vivo* Cultures	Zoology Dept., University of Michigan, Ann Arbor
Prescott, D. M.	Nuclear-Cytoplasmic Interrelations	AAAS (Symposium on "The Organization of the Cell"), Chicago, Illinois
Rabson, R.	Formation of Glyceric Acid in Plants	International Botanical Congress, Montreal, Canada
Russell, L. B.	Chromosomal Changes Involving Specific Loci in the Mouse	1. Dept. of Genetics, University of California, Berkeley 2. California Institute of Technology, Pasadena
Russell, L. B.	X-Ray-Induced Developmental Abnormalities in the Mouse	American Academy of Cerebral Palsy, Los Angeles, California
Russell, L. B. (and J. W. Bangham)	Variegated-Type Position Effects in the Mouse [Genetics *44*, 532 (1959)]	AIBS (Genetics Society), University Park, Pennsylvania
Russell, W. L.	Dependence of Mutation Frequency on Radiation Dose Rate	Donner Laboratory, University of California, Berkeley
Russell, W. L.	Genetic Effects of Radiation in Mice	1. Dept. of Zoology and Entomology, University of Tennessee, Knoxville 2. California Institute of Technology, Pasadena

AUTHOR(S)	TITLE	PLACE PRESENTED
Schwartz, D.	DNA and Chromosome Structure	Faraday Society Symposium on "Aspects of Behavior and Metabolism of the Cell Nucleus," Cambridge, England
Smith, L. H.	Preservation of Viable Bone Marrow Cells by Freezing (Exhibit)	National Meeting of the American Association of Blood Banks, Chicago, Illinois
Stapleton, G. E.	Cell Physiology and Radiation Sensitivity	Health Physics Section, Puerto Rico Nuclear Center, Mayaguez
Stapleton, G. E.	1. Protection and Recovery from Radiation Damage at Cell Level	U.S. Agricultural Experiment Station, Mayaguez, Puerto Rico
	2. Sequence of Events in Production of Radiation Damage to Living Cells	
Upton, A. C.	Delayed Somatic Effects of Radiation	UT-AEC Seminar, Oak Ridge, Tennessee
Wells, O. C.	1. The Differential Survival of Three Interbreeding Clones of *Tetrahymena pyriformis* After X Irradiation [J. Protozool. 6 (Suppl.), 18 (1959)]	AIBS (Am. Soc. Protozoologists), University Park, Pennsylvania
	2. Increased Viability of Exconjugant Progeny of *Tetrahymena pyriformis* in Response to a Modified Breeding Method [J. Protozool. 6 (Suppl.), 32 (1959)]	
Wells, O. C.	Some Biological Consequences of Ionizing Radiation	Dept. of Science, Western Carolina College, Cullowhee, North Carolina

AUTHOR(S)	TITLE	PLACE PRESENTED
Wolf, F. T.	Pteridine Pigments in Microorganisms and Higher Plants	Symposium on Comparative Biochemistry of Photoreceptor Pigments, Asilomar, California
Wolff, S.	Biochemical Aspects of Chromosome Breakage and Rejoining (Intergenic Mutations)	International Botanical Congress, Montreal, Canada
Wolff, S.	Chromosome Breakage and Rejoining in Irradiated Sperm of Drosophila [Genetics 44, 545 (1959)]	AIBS (Genetics Society), University Park, Pennsylvania
Wolff, S.	Factors Affecting Rejoining of Radiation-Induced Chromosome Breaks	Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Illinois
Wolff, S.	Problems of Energy Transfer in Radiation-Induced Chromosome Damage	Symposium on Bioenergetics, Brookhaven National Laboratory, Upton, New York

Visiting Lecturers. — During the period of this report, the following scientists from laboratories, universities, and research organizations in the United States and abroad have given lectures on the Biology Seminar program.

LECTURER	AFFILIATION	TOPIC
Arnold M. Clark	Department of Biological Sciences, University of Delaware, Newark	Oxygen Poisoning in Insects
Frederick T. Wolf	Department of Biology, Vanderbilt University, Nashville, Tennessee	Pteridine Pigments in Microorganisms and Higher Plants
Donald F. Parsons	Department of Surgery, School of Medicine, Duke University, Durham, North Carolina	Ultrastructure of Cells in Leukemia
Silvio Ranzi	Istituto di Zoologia dell'Universita, Milan, Italy	The Protein Differentiation During the Embryonic Development

LECTURER	AFFILIATION	TOPIC
J. Rotblat and Patricia Lindop	Department of Physics, The Medical College of St. Bartholomew's Hospital, London, England	Long and Short Term Effects of Whole Body Irradiation of Mice as a Function of Age at Irradiation
Cornelia Harte	Universität zu Köln, Köln-Lindenthal, Germany	Physiological Genetics of the Development of Leaves
H. J. Becker	Department of Zoology, University of California, Berkeley	Variegation in the Zeste Alleles of *Drosophila melanogaster*
O. Mühlbock	Institut du Cancer Neerlandais, Amsterdam, The Netherlands	Factors in the Genesis of Mammary Cancer
Guy T. Barry	The University of Tennessee Memorial Research Center, Knoxville	Nonulosaminic Acids
Ronald Mason	Department of Chemistry, University College, London, England	The Energetics of Carcinogenesis
James McRae	Donner Laboratory, University of California, Berkeley	Studies on Wasting Disease
T. Caspersson	Karolinska Institutet, Stockholm, Sweden	Cytochemical Techniques for Cell Population Studies
Eduardo Scarano	Stazione Zoologica, Naples, Italy	A Pyrimidine Deoxyribonucleotide Deaminase in Growing Tissue
L. P. Zill	RIAS, Inc., Baltimore, Maryland	Gas Chromatography of Chloroplast Lipids
R. C. Greulich	Department of Anatomy, University of California Medical Center, Los Angeles	Evidence of Direct and Indirect Radiation Damage to Mice Following Administration of Tritiated Thymidine
John Ormerod	Busch Laboratory of Molecular Biology, Washington University, St. Louis, Missouri	The Function of Organic Compounds in Bacterial Photosynthesis
S. P. Masouredis	University of California Medical Center, San Francisco	Genetic Control of the Rho (D) Antigen Sites of the Human Red Cell
Yvonne Lanni	Department of Bacteriology and Immunology, Emory University, Atlanta, Georgia	DNA Transfer and Invasion by Phage T5

LECTURER	AFFILIATION	TOPIC
Frank Lanni	Department of Bacteriology and Immunology, Emory University, Atlanta, Georgia	Genetic Significance of Microbial DNA Composition
M. T. M. Rizki	Reed College, Portland, Oregon	Developmental Physiology of the tu^w Factor in *Drosophila*
C. W. Emmens	Department of Veterinary Physiology, The University of Sydney, Sydney, New South Wales, Australia	Some Recent Work on Antagonists to Oestrogenic Hormones
S. W. Tanenbaum	Department of Microbiology, Columbia University, New York City	Directed Protein Synthesis; Antibodies and Induced Enzymes
Boris Ephrussi	Centre National de la Recherche Scientifique, Laboratoire de Génétique Physiologique, Seine-et-Oise, France	Genetic Behavior of Respiration-Deficient Mutants in Yeast
G. L. Stebbins	Department of Genetics, College of Agriculture, University of California, Davis	New Light on the Pathway from the Gene to the Character
C. H. Waddington	Institute of Animal Genetics, Edinburgh, Scotland	Ultrastructure in Developing *Drosophila* Cells
W. P. Rowe	National Cancer Institute, National Institutes of Health, Bethesda, Maryland	Epidemiology of the Mouse Polyoma Virus
Paul Mandel	Department of Biochemistry, University of Strasbourg, Strasbourg, France	Free Nucleotides in Tissue
C. S. Rupert	Department of Biochemistry, The Johns Hopkins University, Baltimore, Maryland	Photoenzymatic Repair of Ultraviolet Damage in DNA
R. C. Rustad	Department of Biological Sciences, The Florida State University, Tallahassee	Centriole Damage: A Hypothesis of the Mechanisms of Radiation-Induced Mitotic Delay

LECTURER	AFFILIATION	TOPIC
N. O. Kaplan	Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts	Molecular Heterogeneity and the Evolution of Enzymes
R. B. Setlow	Biophysics Department, Yale University, New Haven, Connecticut	Some Effects of Ultraviolet Light on Macromolecules and Bacteria
J. D. Watson	Department of Biology, Harvard University, Cambridge, Massachusetts	Ribonucleic Acid Within *Escherichia coli*
S. M. Siegel	Physical Chemistry, Union Carbide Research Institute, White Plains, New York	Antioxidants as Biological Agents: Development of a Concept
Margaret Lieb	Department of Biology, Brandeis University, Waltham, Massachusetts	Mutation and DNA Synthesis in *E. coli*
David Lipkin	Department of Chemistry, Washington University, St. Louis, Missouri	The Effect of Various Metal Ions on the Degradation of ATP

Speakers at Professional Meetings, Spring 1960. — The present count of speeches on Biology Division research scheduled to be given before professional societies before the next Semiannual Report time is 56. This is more than double the number scheduled for the period just past (56:24) and considerably above the number scheduled for the same period last year (56:39). Based on past ratios of scheduled papers to those actually given, the number may be expected to rise; for example, 24 scheduled for the last period:45 actually given, and 39 scheduled for the same period last year:44 actually given. Speakers and the occasions of their talks are listed below.

The Biophysical Society — John Jagger

New York Academy of Science, Conference on Biosynthesis of Proteins — G. D. Novelli

New York Academy of Science, Second Conference on the Mechanisms of Cell Division — N. G. Anderson (coauthors, W. D. Fisher and H. E. Bond)

Symposium on Free Radicals in Biological Systems — M. L. Randolph

Thirteenth Annual Biology Research Conference — L. B. Russell

American Chemical Society, 137th National Meeting — J. X. Khym (coauthor, W. E. Cohn)

American Association for Cancer Research — A. C. Upton (coauthors, E. P. Sniffen and F. F. Wolff)

Federation of American Societies for Experimental Biology — L. Astrachan, F. J. Bollum, R. K. Clayton, C. C. Congdon, G. E. Cosgrove (coauthor, A. C. Upton), J. L. Countryman, D. G. Doherty, W. Friedberg (coauthor, V. Jenkins), K. B. Jacobson, F. T. Kenney, M. Lion (coauthor, L. H. Smith), R. A. McGrath, J. R. Maisin (coauthor, D. G. Doherty), R. J. Mans (coauthor, R. Rabson), T. T. Odell, Jr. (coauthor, T. P. McDonald), H. D. Peck, A. C. Upton (coauthor, E. P. Sniffen), and E. Volkin. Also taking part in a special symposium on "Radiation Protection and Recovery": Alexander Hollaender, T. Makinodan, and J. Maisin (coauthor, D. G. Doherty)

Seventh International Congress of Anatomy — E. C. Horn

Association of Southeastern Biologists – C. C. Hyde (coauthor, M. E. Gaulden) and O. C. Wells

Society of American Bacteriologists – R. W. Bernlohr (coauthor, G. D. Novelli), T. Kameyama (coauthor, G. D. Novelli), H. D. Peck, S. F. Carson (coauthor, E. F. Phares), G. E. Stapleton (coauthors, M. S. Engel and H. I. Adler), M. B. Taylor (coauthors, G. D. Novelli and R. J. Mans), W. H. Taylor (coauthors, K. Tonomura and G. D. Novelli), and C. J. Wust

Radiation Research Society – F. Celada (coauthor, T. Makinodan), G. Cudkowicz (coauthor, G. E. Cosgrove), N. Gengozian (coauthors, R. Carter and W. J. Peterson), J. W. Goodman (coauthor, C. C. Congdon), R. F. Kimball, J. S. Kirby-Smith (coauthors, B. Nicoletti and M. L. Gwyn), D. R. Krieg, T. Makinodan (coauthor, W. J. Peterson), E. H. Perkins (coauthor, M. A. Robinson), R. A. Popp (coauthor, S. A. Sapp), F. J. de Serres, A. C. Upton (coauthor, A. W. Kimball), and S. Wolff

Third International Congress of Photobiology – R. C. von Borstel, J. G. Carlson (coauthors, M. E. Gaulden and J. Jagger), S. Wolff, and Alexander Hollaender

Foreign Travel. – Soon after the beginning of the period of this report, C. C. Congdon and Alexander Hollaender completed a trip to South America. In addition to the papers given at the Congress of Physiological Sciences and the Argentine National Academy of Medicine, Dr. Congdon gave several other lectures. Dr. Hollaender also gave six lectures in Bogota and Lima, not listed in the last report. On a later visit, Dr. Hollaender discussed organization of basic biology and radiation programs with research officials in Caracas, Venezuela. While there, he also gave two lectures at the Universidad Central di Venezuela.

Three members of the Division have visited Puerto Rico. J. S. Kirby-Smith and Alexander Hollaender participated in the Symposium on "Comparative Effects of Ionizing, Ultraviolet, Visible, and Near-Visible Radiations" in San Juan by acting as chairmen of two sessions. Dr. Kirby-Smith was Symposium Secretary. This meeting was sponsored by the Committee on Photobiology, Division of Biology and Agriculture of the National Academy

of Sciences—National Research Council and was supported by the National Science Foundation and the U.S. Atomic Energy Commission. The University of Puerto Rico cooperated in this symposium. On a former visit to Puerto Rico, Dr. Hollaender visited the University of Puerto Rico in San Juan and Rio Piedras and lectured to groups at both locations. During November, G. E. Stapleton visited several laboratories on the Island and lectured at the University in Mayaguez and the U.S. Agricultural Experiment Station in Mayaguez.

Division members giving papers at the Faraday Society conference on "The Cell Nucleus" in Cambridge, England, also visited a number of laboratories in Great Britain and on the Continent. Papers at this meeting were given by N. G. Anderson, F. J. Bollum, M. E. Gaulden, and D. Schwartz.

Another South American trip was taken by W. H. Benedict, who attended the Pan-American Congress of Ophthalmology in Caracas and presented a paper at one of the sessions.

Other foreign travel took four Division members to Montreal to give papers at the International Botanical Congress; these were Douglas Davidson, Sheldon Wolff, Robert Rabson, and A. H. Haber. Sohei Kondo attended a meeting of the Japanese Physical Society in Hiroshima and gave a paper (M. L. Randolph was coauthor).

Thirteenth Annual Biology Research Conference. — The Spring Conference for 1960 convenes at the Mountain View Hotel in Gatlinburg, Tennessee, on April 4 for a four-day session. Members of the organizing committee have arranged an interesting program under the title "Mammalian Genetics and Reproduction." The committee is composed of W. L. Russell, Liane B. Russell, E. F. Oakberg, and W. J. Welshons, all of the Mammalian Genetics and Development section of the Biology Division. The program follows.

MONDAY, APRIL 4

MORNING

Chairman — L. C. Dunn, Columbia University, Nevis Biological Station, Irvington-on-Hudson, New York

Anomalies of Fertilization Leading to Triploidy — C. R. Austin, National Institute for Medical Research, London, England

Genetic Influences on the Morphology and Function of the Gametes — A. W. H. Braden, Commonwealth Scientific and Industrial Research Organization, Prospect, New South Wales, Australia

AFTERNOON

Chairman — Margaret Green, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine

Embryological Phases of Gametogenesis in the Mouse — Beatrice Mintz, The University of Chicago, Chicago, Illinois

Developmental Genetics in the Mouse, 1960 — Hans Grüneberg, University College, London, England

EVENING

Smoker — Host: Union Carbide Corporation

TUESDAY, APRIL 5

MORNING

Chairman — Bernard Amos, Roswell Park Memorial Institute, Buffalo, New York

Genetics in Relation to Reproductive Physiology in Mammals — W. F. Hollander, Iowa State University, Ames

Current Status of Mammalian Immunogenetics — R. D. Owen, California Institute of Technology, Pasadena

AFTERNOON

Excursion — Great Smoky Mountains: The group will leave from the Mountain View Hotel at 2:00 P.M. In case of inclement weather, the program will continue and Thursday morning will remain open for a tour of the Biology Division of Oak Ridge National Laboratory.

EVENING

Reception and dinner

WEDNESDAY, APRIL 6

MORNING

Chairman — C. L. Markert, The Johns Hopkins University, Baltimore, Maryland

The Inheritance of Hemoglobin Types and of Other Biochemical Traits in Mammals — Salome G. Waelsch, Albert Einstein College of Medicine, New York City

Phenogenetic Aspects of Some Hair and Pigment Mutants — H. B. Chase and S. J. Mann, Brown University, Providence, Rhode Island

AFTERNOON

Chairman — Curt Stern, Department of Zoology, University of California, Berkeley

Genetic Analysis of Mutations at Specific Loci — Liane B. Russell and W. L. Russell, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Mammalian Pachytene Chromosome Mapping and Somatic Chromosome Identification — A. B. Griffen, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine

EVENING

Chairman — W. L. Russell, Biology Division, ORNL

Round-Table Discussion — Radiation Effects

THURSDAY, APRIL 7

MORNING

Chairman — Jay L. Lush, Department of Animal Husbandry, Iowa State University, Ames

Genetics of Vital Characteristics of the Guinea Pig — Sewall Wright, University of Wisconsin, Madison

Genetics of Litter Size in Mice — D. S. Falconer, Institute of Animal Genetics, Edinburgh, Scotland

Annual Information Meeting. — The annual visit of the ORNL Advisory Committee for Biology took place November 9, 10, and 11, 1959. On the first day of the meeting, Committeemen, some other visitors, and Division

members met in the large conference room of the Biology Division to hear group leaders review the work in progress. Union Carbide Corporation was host to Division members and the Advisory Committee at dinner on this day. The last two days of the meeting were devoted to consultations and group discussions. Members of the Committee are G. W. Beadle, Department of Biology, California Institute of Technology; Erwin Chargaff, College of Physicians and Surgeons, Columbia University; Curt Stern, Department of Zoology, University of California, Berkeley; E. L. Tatum, The Rockefeller Institute for Medical Research; and P. C. Zamecnik, Massachusetts General Hospital of Boston.

Meeting of Biomedical Directors of the U.S. Atomic Energy Commission. — The Biomedical Directors met in regular session in Oak Ridge on October 5, 6, and 7. Sessions on the first day were held in the Biology Division conference room. S. R. Sapirie welcomed the guests and J. A. Swartout outlined the program of the Oak Ridge National Laboratory. Alexander Hollaender served as chairman of the morning and afternoon sessions, during which several Section leaders summarized the Division activities. The visitors were given a conducted tour of the Division laboratories at the close of the afternoon session.

Bone Marrow—AET Conferences. — The fall 1959 Conference was held September 18 and 19 at the National Cancer Institute in Bethesda, Maryland. The meeting, organized by Dr. Gordon Zubrod of the National Institutes, was well attended. Proceedings of the meeting are being published in Blood, The Journal of Hematology.

As usual, the January Conference was held in the Biology Division of Oak Ridge National Laboratory. The two-day meeting convened on January 8, 1960. Attendance at these conferences is gaining rapidly; about 200 professional people engaged in basic research and clinical application in this field were present.

The next Conference will take place on the first evening of the Federation meetings in Chicago on April 11—15. A symposium on "Radiation Protection and Recovery" to be held on the first day of the Federation will also represent the work and interest of this group. The following speakers will give papers in this symposium: J. Maisin (coauthor, D. G.

Doherty, Biology Division, ORNL), Armed Forces Institute of Pathology; Elizabeth S. Russell, Roscoe B. Jackson Memorial Laboratory; Royal F. Ruth, Carnegie Institution of Washington, Baltimore; T. Makinodan, Biology Division, ORNL; and Alexander Hollaender, who will also serve as chairman.

Education. — Several members of the Division have taught courses offered to professional groups, medical students, or technical personnel.

E. F. Oakberg, G. E. Stapleton, and A. C. Upton served as members of the ORSORT staff during the period. C. C. Congdon, W. D. Gude, and T. T. Odell, Jr., lectured on various phases of radiation biology and techniques during the ORINS Medical Division course on "Radioisotopes in Hematology"; A. C. Upton was chairman of one of the sessions of this course. W. E. Cohn participated as a lecturer in the ORINS Biochemistry Training Course.

R. K. Clayton, J. S. Kirby-Smith, and M. L. Randolph were lecturers in a course in Radiological Physics offered by the Biology Division during the late fall. A course in Genetics, also offered by this Division, is now being given by R. F. Kimball.

G. E. Cosgrove, R. A. Popp, L. B. Russell, and A. C. Upton taught in the spring lecture series to the veterinarians and staff of the UT-AEC Agricultural Research Program. W. L. Russell lectured at the University of North Carolina School of Medicine as a participant in the Medical Sciences Lecture Series for 1959—60. E. F. Oakberg gave a refresher course on "Genetic Effects of Radiation" at the annual meeting of the Radiological Society of North America in Chicago.

The Small College Program. — A conference of undergraduate colleges in the Southeast sponsored by the Oak Ridge Institute of Nuclear Studies in cooperation with Oak Ridge National Laboratory met in Oak Ridge on October 16 and 17, 1959. The following Division members had an active part in the conference: Howard Adler, Alexander Hollaender, and Mary Esther Gaulden. Representatives of the colleges were given a conducted tour of the Biology laboratories.

One appointment on a new phase of the program has been made for the spring term of 1960. In this activity, the student will spend one term of his senior year working in the Biology Division. In the plan that

has been in operation for the past two summers, a selected group of students who have completed their junior year are given experience in the Division during the summer months. A committee of Division members has chosen the trainees for the coming summer.

Program activities for the spring months will include a series of lectures given by Division members to students in the undergraduate colleges and a visit by junior and senior students in Biology from Randolph-Macon Woman's College. The visit to the Biology Division is scheduled for April 8 and 9. The following lecturers will talk to students of the named colleges some time during the spring: L. H. Smith – to Western Maryland, Gettysburg, and Hood Colleges; N. G. Anderson – to Wofford College and others to be arranged; and H. I. Adler – to Women's College of the University of North Carolina and others to be arranged.

CYTOLOGY AND GENETICS

CYTOGENETIC EFFECTS OF RADIATION

R. F. Kimball
D. Davidson[1]
D. M. Green[2]
H. G. Kølmark[1]
D. R. Krieg
D. Schwartz
F. J. de Serres
B. A. Swomley
B. B. Webber[2]
O. C. Wells[2]
S. Wolff
I. R. Cox
R. M. Fulkerson
N. T. Gaither
C. B. Kincaid
H. E. Luippold
K. H. McGrath
S. W. Perdue
A. P. Teasley

Studies on the Refractory Period in Mutation Induction in *Paramecium*

R. F. Kimball N. T. Gaither S. W. Perdue

Introduction. — X irradiation of *Paramecium aurelia* during the second half of the interdivision interval produces only about one-tenth as many recessive lethal mutations as during the first half.[3] The chromosomes must be highly protected against the initial production of radiation damage during this period or else the damage is very efficiently removed or repaired. Studies have been initiated to discover the basis for this refractoriness, and the evidence presently favors the repair or removal hypothesis.

Results. — The following findings have been made: (1) There is a refractory period for recessive lethals induced by α-particles with a difference of approximately sevenfold. (2) The number of apparent recessive lethals induced by high doses of X rays during the refractory period is unaffected by oxygen tension during irradiation or by exposure to streptomycin afterward. Both affect the production of recessive lethals by lower doses of X rays during the first half of the interdivision interval. (3) Studies of the uptake of tritiated thymidine into the macronucleus, confirming earlier microspectrophotometric studies,[4] and of micronuclear size

[1]Visiting Investigator from Abroad.

[2]Research Associate.

[3]R. F. Kimball, N. Gaither, and S. M. Wilson, Biol. Semiann. Prog. Rep. Aug. 15, 1956, ORNL-2155, p 13–14.

[4]R. F. Kimball and T. Barka, Exptl. Cell Research 17, 173–82 (1959).

show that the refractory period normally coincides with the period during which new macronuclear DNA is synthesized and with the postsynthesis, predivision period for the micronucleus, the nucleus in which recessive lethals are detected.

Conclusions. — The evidence for a refractory period with α-particles strongly favors a repair or removal rather than a protection hypothesis since protective agents usually have little effect with radiations of such high LET. Plausibility is added to this conclusion by the earlier finding[5] that streptomycin reduces mutation equally when used as a postirradiation treatment after α-particles or after X rays, showing that α-particle damage is also subject to postirradiation repair or removal. High doses of X rays probably induce changes, for example, abnormal nuclear behavior, that mimic recessive lethals. It is possible that such changes account for the unmodifiable "lethals" at high doses and consequently no, or almost no, true recessive lethals are produced in the refractory period. The effectiveness of repair or removal of initial radiation damage during this period could be attributed either to the doubleness of the micronuclear chromosomes or to some effect of the replicating macronucleus. The latter alternative needs to be seriously considered since the double state of the chromosomes does not cause such complete refractoriness in other organisms which have only one nucleus per cell.

Another Radioresistant Clonal Strain of *Tetrahymena pyriformis*

O. C. Wells

Recently we reported that some of the EU strains of *Tetrahymena pyriformis* are more radioresistant than others.[6,7] Similar studies on another clonal strain of this species, HS(M), show that this strain is even more resistant to the killing action of ionizing radiation. A

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[5] R. F. Kimball, N. Gaither, and S. M. Wilson, Biol. Semiann. Prog. Rep. Aug. 15, 1957, ORNL-2390, p 19–20.

[6] O. C. Wells, Biol. Semiann. Prog. Rep. Feb. 15, 1959, ORNL-2702, p 31–33.

[7] O. C. Wells, Biol. Semiann. Prog. Rep. Aug. 15, 1959, ORNL-2813, p 31–34.

description of certain characteristics of strain HS(M), in comparison to the EU strains, follows as a further contribution to an understanding of radiation killing.

Results. — The effects of X irradiation on strain HS(M) were studied by methods identical to those already described.[6,7] The survival of single-cell isolates after doses of 300 and 400 kr was determined; these doses allow little, if any, survival of EU strains.[6] After 300 kr, 71% of strain HS(M) survived; after 400 kr, 47% survived. Many single-cell isolates of irradiated populations were able to divide many times before death of the cell line; often as many as 200 organisms were produced before death. It is interesting to note that cells irradiated during cytokinesis regressed during the first 24 hr after irradiation, producing vigorous single cells uniform in outline. After a postirradiation lag of two days, these regressed organisms divided several times, but all eventually died.

Measurements of the DNA and RNA content of unirradiated mass populations of strain HS(M) were made by the methods used for similar determinations on the EU strains.[7] The amount of RNA per cell was the same as that of the latter strains. However, strain HS(M) had only half as much DNA per cell as members of the EU strains, which is substantiated by microscopic observations of smaller macronuclei in HS(M) cells. Whole cells of this strain also appear smaller than those of the EU strains.

Discussion. — Several hypotheses may be presented to explain strain-specific radiosensitivity differences within a species. The results of radiobiological studies on *T. pyriformis* strains have made some of these hypotheses improbable but do not yet permit a choice between two major theories: (1) strain-specific differences in protection against radiation damage, or (2) in repair of such damage.

It has already been pointed out that radiosensitivity differences are not explicable on a basis of micronuclear multiplicity, if this multiplicity is considered synonymous with micronuclear ploidy.[6] The present report gives evidence that differences in macronuclear multiplicity cannot account for the observed radiosensitivity differences. Since strain HS(M) has less DNA per cell than the EU strains, it is

reasonable to assume that the macronuclei of members of this strain have fewer multiple targets, thus these cells should be more radiosensitive. Their greater resistance casts strong doubt on the interpretation that macronuclear multiplicity and radiosensitivity are positively correlated.

The Competence of Leaky *ad-3* Mutants of *Neurospora* in Allelic Complementation in Heterokaryons

F. J. de Serres

Introduction. – Previous studies[8] have shown allelic complementation among an unselected sample of 160 *ad-3B* mutants, subdividing them into the following three distinct groups: 148 nonleaky mutants that do not show allelic complementation, a second group of four leaky mutants, and a third group of eight nonleaky mutants that show allelic complementation in any pairwise combination with the second group. Thus, whereas all the leaky mutants (4/4) show allelic complementation, the vast majority of nonleaky mutants (148/156) do not.

Since leaky *ad-3* mutants can grow *without* an exogenous supply of adenine, such mutants must be able to make functional enzyme. There are a number of alternative explanations for the existence of such a class of mutants, but of particular interest is the possibility that leaky mutants result from changes in structure that make the mutant enzyme less efficient than the wild-type enzyme. Since it has been postulated[9] that allelic complementation results from dissociation and reassociation of polypeptide chains of the enzyme molecules with nonidentical defects, if leaky mutants result from various types of restricted alterations in structure, then the incidence of mutants showing allelic complementation should be higher in a *selected* sample of leaky mutants than in the *unselected* samples of mutants tested previously.

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[8]F. J. de Serres, *Biol. Semiann. Prog. Rep. Feb. 15, 1959*, ORNL-2702, p 33–36.

[9]D. O. Woodward, C. W. H. Partridge, and N. H. Giles, Proc. Natl. Acad. Sci. U.S. *44*, 1237–44 (1958).

Results. — Heterokaryon experiments have been made with the test tube method (see the following subject, this report) on selected samples of both leaky and nonleaky mutants induced with a variety of mutagens in forward-mutation experiments on wild-type 74A. The results of such tests thus far have shown that only 5% (12/256) of the nonleaky ad-3B mutants show allelic complementation in contrast to 100% (42/42) of the leaky mutants. No such correlation exists among ad-3A mutants, however; no instances of allelic complementation were found among a sample of 29 leaky and 36 nonleaky ad-3A mutants tested in all possible pairwise combinations in heterokaryons. The patterns of interaction of various combinations of mutants showing allelic complementation have been used to construct a complementation map of this region. The data show a definite correlation between the leaky phenotype and the extent of the functional alteration on a complementation map among ad-3B mutants; most of the nonleaky ad-3B mutants map as multiple cistron mutants, whereas the majority of leaky mutants have only one or a few cistrons inactivated.

Discussion. — Heterokaryon tests on series of allelic ad-3 mutants have shown that loss or impairment of enzymatic activity can be accomplished in a number of different ways. Furthermore, it is apparent that the gene products of such series of mutants do not have identical functional alterations. The patterns of interaction between mutants showing allelic complementation can be used to construct a complementation map of the ad-3 region, and there is tentatively a positive correlation between the positions of individual mutants on both the complementation and genetic maps. Present data are consistent with the hypothesis that the complementation map reflects the structure of a gene product rather than a gene. If the complementation map reflects most directly the structure of the enzyme molecule, then the present data may be interpreted as indicating that leaky ad-3B mutants result from restricted qualitative changes and that these changes can occur in various positions throughout the structure of the molecule. The lack of complementation among leaky ad-3A mutants might be more readily understood if the ad-3A locus carried only a restricted amount of genetic information (i.e.,

for the production of a single polypeptide chain) so that allelic complementation cannot occur. Alternatively, mutation in the ad-3A region might bring about loss or impairment of enzymatic activity by some entirely different mechanism (i.e., changes in rate with no qualitative changes in structure).

A Method for Screening Large Numbers of Allelic Mutants for Pairwise Combinations Showing Complementation in Heterokaryons in *Neurospora*

F. J. de Serres

Introduction. — To test for complementation between various pairwise combinations of allelic mutants, conidial suspensions of two strains are placed alone and mixed (in duplicate) in positions diametrically opposed on the surface of minimal agar in petri plates. A positive reaction is indicated by the growth of a heterokaryon only from the positions where suspensions of conidia have been mixed.[10] For routine screening of the ad-3 mutants obtained from various forward-mutation experiments by the direct method,[11] this method is cumbersome and has proved generally unsatisfactory since results are often equivocal. A concerted effort has been made therefore to perfect a method that could be used for more efficient and accurate routine testing of the hundreds of untested mutants in our present collection.

Results and Discussion. — Pilot experiments with mutants exhibiting allelic complementation showed that such tests could be made with small volumes of suspensions of conidia made up in minimal medium in 10 × 75-mm test tubes rather than on solid minimal agar in petri plates. As a result of many experiments, a simple method has been developed for these tests in which sterile packs of 100 test tubes (10 × 75 mm) are used; they are racked in wire baskets, in a 10 × 10 grid, wrapped in a taut double layer of Saran Wrap. A Cornwall syringe fitted with an 18-gage hypodermic needle is used to inject 1-ml aliquots of each of two conidial suspensions into each tube. The Saran Wrap provides a sterile, transparent covering that permits routine observation of individual tubes over

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

[11] F. J. de Serres and H. G. Kølmark, Nature 182, 1249–50 (1958).

an observation period of from seven to ten days. The 200 holes resulting from inoculation provide humidity control so that growth and conidiation of heterokaryons are confined to a 1.5-cm region above the meniscus of the liquid in each tube. Positive tests with mutants giving a variety of types of allelic complementation have been obtained over a wide range of conidial concentrations ranging from 10^7 to at least 10^4 conidia per tube, and over at least a tenfold difference in inoculum size. With this method routine screening of mutants can be accomplished very rapidly since 5000 and, with practice, as many as 10,000 pairwise combinations can be made in a single experiment. The method permits precise control of conidial concentration per unit volume, a characteristic that has proved useful in providing a means for testing a large proportion of the leaky purple adenine mutants (mutants that grow on minimal medium without an exogenous supply of adenine). Such mutants form a sparse, deeply pigmented mycelium in tubes when conidia are unmixed or in mixtures with mutants giving negative reactions, and dense (nonpurple), typically wild-type appearing mycelium in mixtures with mutants showing positive reactions.

The Effect of Variation in the Levels of Adenine and Arginine on the Recovery of Purple Adenine Mutants in Forward-Mutation Experiments in *Neurospora*

F. J. de Serres H. G. Kølmark

Introduction. – Earlier[12] a direct method for estimating the rates of induced mutation in the *ad-3* region of *Neurospora* was described. The success of the method depends in part on the fact that, under certain conditions, *ad-3* mutant colonies accumulate a purple pigment in the mycelium that readily distinguishes them from nonmutant colonies. Estimates of induced forward-mutation rates might be in error by several orders of magnitude if only a portion of the *ad-3* mutants induced with a given mutagenic treatment were actually recovered. Since it is known that the levels of adenine[13] and arginine (de Serres, unpublished) supplementation influence the degree of pigmentation of individual purple adenine

[12]F. J. de Serres and H. G. Kølmark, Nature 182, 1249–50 (1958).

[13]H. K. Mitchell and M. B. Houlahan, Federation Proc. 5, 370–75 (1946).

mutants, experiments have been performed to determine the effect of variation in the concentration of these supplements on the recovery of induced mutants after some given mutagenic treatment.

Results. – Aliquots of a conidial suspension of wild-type strain 74A irradiated with a dose of 36 kr were incubated in minimal medium in which variation was made both in the concentration of adenine (5 to 30 mg/liter) and arginine (0 to 150 mg/liter). The numbers of purple adenine mutants obtained with each medium variation after seven days' incubation are given in Fig. 1. The data show a marked effect on the number of mutants recovered from aliquots of irradiated conidia with variation in the concentration of both of these supplements. Since the difference in the number of mutants recovered with variation in arginine suggested a concentration effect, in additional experiments variation was made over a wider range. The effect of variation in the level of arginine in these experiments appears to be rather complex, for although with an increase in the level of arginine from 25 to 200 mg/liter (plus 10 mg/liter of adenine) there is a gradual increase in the number of purple adenine mutants recovered, maximum pigmentation is obtained with 100 mg/liter and at higher levels there is a gradual decrease both in pigmentation and in the size of both wild-type and mutant colonies.

Discussion. – These experiments have shown a marked dependence of the number of mutants recovered with a given mutagenic treatment on the incubation medium. Under optimal conditions, as many as 186 mutants could have been recovered in contrast to about 26 if the medium had been supplemented with only 1 mg/liter of adenine (the level used in earlier experiments[12]) resulting in at least a 7.2-fold difference in the estimates of the mutation rate (0.409×10^{-4} in contrast to 2.92×10^{-4}) obtained with a dose of 36 kr.

The biochemical reactions leading to the production of purple pigment by ad-3 mutants are not well understood, and the biochemical basis of the arginine effect on either pigment production or the further restriction of colony size on sorbose containing minimal medium cannot be explained at the present time. Although it is clear that variation in the incubation medium has resulted in an increase in the number of mutants recovered with this direct forward-mutation method, we do not know

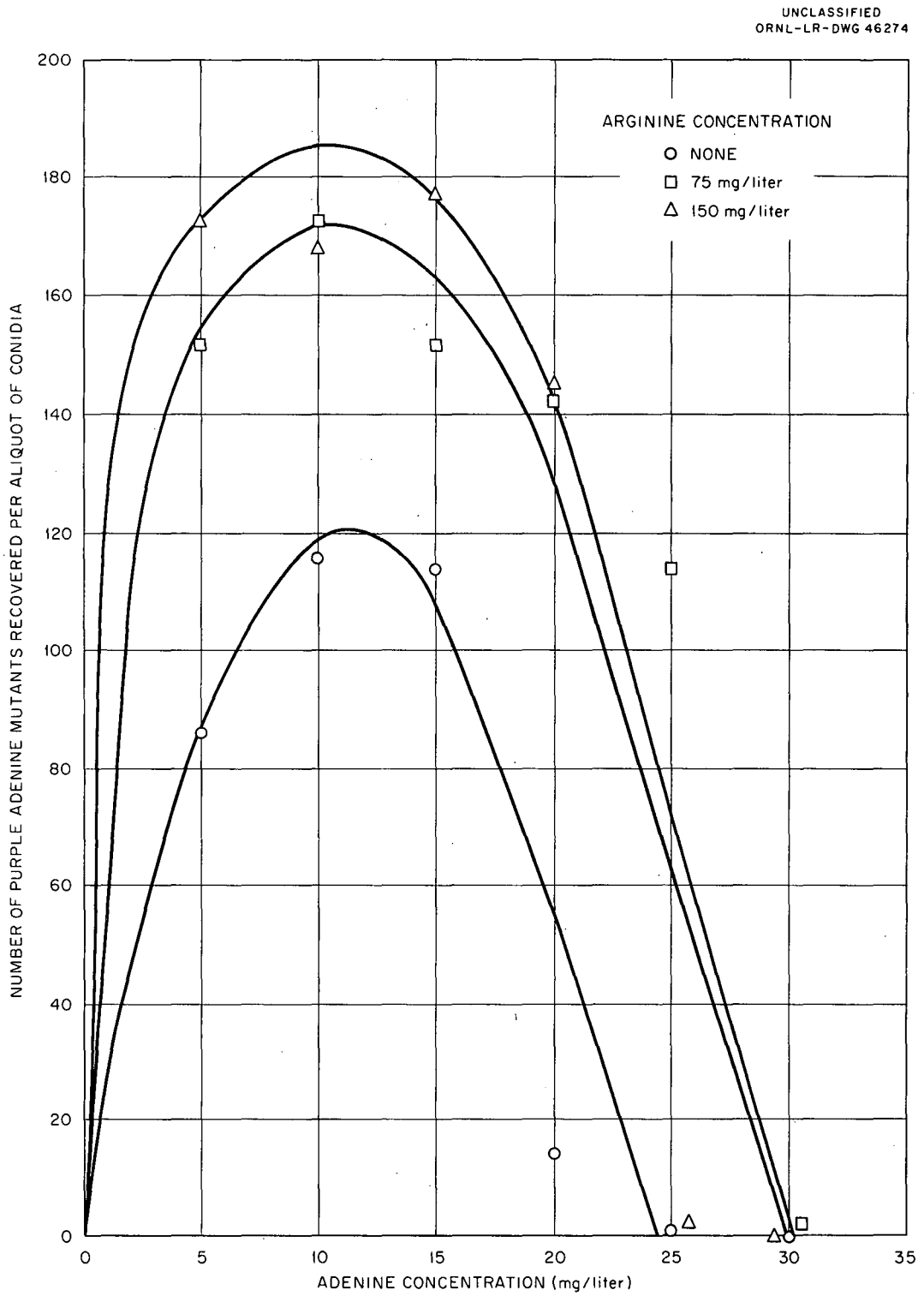

Fig. 1. The Effect of Variation in the Levels of Adenine and Arginine Supplementation on the Recovery of Purple Adenine Mutants.

what portion these are of the total number of mutants actually induced. It should be possible to obtain some information on this point, however, in reconstruction experiments designed to determine the recovery of certain types of known mutants under various experimental conditions.

Protection with Endogenous Anoxia Against the Mutagenic and Lethal Effects of X Rays in *Neurospora crassa*

H. G. Kølmark

Introduction. — A state of anoxia develops under certain conditions when a saline suspension of *Neurospora* conidia is enclosed in an airtight glass tube, owing to a depletion of the limited amount of oxygen by endogenous cell metabolism. These studies were undertaken to determine precisely under which conditions and to what extent endogenous anoxia can modify the effect of X rays in experiments with conidia of *Neurospora crassa*.

Results. — Conidia of the strain K 3/17 *ad-3A* (38701) were treated, and adenine reverse mutations and survivals were determined by plating and counting of colonies on the appropriate media. The conidia were kept in saline at ice-water temperature during all procedures except as otherwise indicated. During pretreatment and X irradiation, 2.2-ml suspensions of conidia were contained in 3-ml irradiation tubes, sealed with glass stoppers and a thin layer of vacuum grease. Variations in preincubation conditions were brought about by transferring the tubes from an ice-water bath to thermobaths at higher temperatures for various periods of time. All tubes were cooled in ice water at the end of pretreatment and irradiated cold. Untreated series were kept in ice water throughout. With a fixed dose of 48 kr of X rays, variations in each of the factors (temperature, time, and conidial concentration) influenced the level of the lethal response measured in terms of survival, whereas the mutation response was constant. However, in experiments with varied X-ray dose, under optimal conditions of these factors, endogenous anoxia can protect against mutation, with protection factors for both the lethal and the mutagenic effects in the range 1.6 to 1.9. However, a decrease in mutation rate is observed only at doses below 40 kr. Since anoxic and nonanoxic curves intersect at 43 kr, at higher doses the mutation rate is

greatest in the anoxic irradiated conidia. It seems likely that this situation could be caused by a differential killing of induced reverse mutations at high doses of X rays in the presence of oxygen.

Discussion. — These experiments show that the mutagenic and lethal effects of a given dose of X rays can be markedly altered by factors influencing the level of endogenous anoxia. These findings influence the evaluation of experiments with chemical radiation protectors in *Neurospora* since, under certain conditions, endogenous anoxia is present as a background effect.

Complementation Characteristics of Some Ultraviolet-Induced *hist-3* Mutants of *Neurospora crassa*

B. B. Webber

Introduction. — The *hist-3* mutants of *Neurospora crassa* share the following characteristics: They require histidine for growth; they fail to form histidine-independent heterokaryons with a common *hist-3* strain selected as tester; they can be mapped genetically in a definite region (about 0.04 map unit in length) in linkage group I. The mutants may be divided into the following three subgroups: (1) mutants deficient for histidinol dehydrogenase activity; (2) mutants deficient for an unidentified enzymatic step early in the biosynthetic scheme for histidine; and (3) mutants deficient in both activities.

Certain pairs of *hist-3* mutants form a histidine-independent heterokaryon and so seem to be able to carry out both enzyme activities when their nuclei are placed together in the common cytoplasm of a heterokaryon. The use of heterokaryon tests in developing a complementation map have been described.[14] The present experiments utilize this method in the classification of new *hist-3* mutants.

Results. — Complementation tests in which the test-tube method of de Serres (unpublished) was used extended the complementation map of the *hist-3* region.[15] From this map, nine tester strains were selected (labeled A through I in Table 1) and were used in complementation tests

[14]N. H. Giles, p 261–79 in *Proceedings of the X International Congress of Genetics*, vol 1, University of Toronto Press, 1959.

[15]B. B. Webber, unpublished Ph.D. thesis, Yale University, 1959.

Table 1. Classification of Randomly Selected Ultraviolet-Induced Mutants with Respect to Complementation Characteristics

Number of Ultraviolet Mutants	Complementation Relationships of Testers										
	A	B	C	D	E		F *	G *	H *		I *
52											
25							*				
10											
6											*
3											
3											
2							*				
1											
1											
1											
1											
105 total											

with a number of randomly chosen hist-3 mutants derived from ultraviolet-irradiated conidia. The results, presented in Table 1, indicate the frequency with which mutants of each complementation class arose.

Subgroup 1 mutants (deficient only for histidinol dehydrogenase activity) accumulate histidinol in their medium upon growth and therefore can easily be distinguished from subgroups 2 and 3 mutants by Pauly (diazotized sulfanilic acid) tests of the filtrates. The mutants used in the above tests were classified by this means. Subgroup 1 mutants (marked with asterisks in the table) are located at the right hand side of the map (extending no farther left than tester F) and nonaccumulators (subgroups 2 and 3) are mapped partially or completely to the left of tester F. The use of histidinol dehydrogenase assays to distinguish between subgroups 2 and 3 mutants is anticipated.

Discussion. — The present experiments indicate the feasibility of comparing hist-3 mutants of diverse origins by complementation testing.

Also a correlation between enzyme activity deficiency and placement of mutants on the complementation map has been described. These reflect also the order of mutants on the genetic map of the region.[15] The availability of these types of information indicates the unique suitability of the hist-3 mutants for studies of transfer of genetic information involving the correlation of genetic, physiological, and biochemical information and perhaps ultimately information concerning the structure of the enzyme molecule(s) affected by genetic changes at the hist-3 locus.

Genetic Control of Maize Endosperm Proteins

D. Schwartz B. A. Swomley K. H. McGrath

Introduction. – The analysis of the proteins in developing maize endosperm tissue reported in ORNL-2813 has been extended to cover the single mutants su_1 and its allele su_1^{am}, su_2, bt_1 and its allele sh_3, bt_2, sh_1, sh_2, sh_4, ae, h, o_1, o_2, wx and its allele wx^a, du_2, fl_1, and fl_2; a number of the double mutant combinations; the normal nonmutant inbred lines W23, M14, and OH51; and four independently arisen cases of suppression of Sh_1 by Ds.[16] The endosperm proteins were analyzed both electrophoretically on starch gel and immunochemically.

Results. – The results corroborated the conclusion reported previously, that the Sh_1 gene controls the synthesis of the protein component that is present in the highest concentration in the endosperm extract. All the normal and mutant endosperms contain the Sh_1 protein except for the three different $sh_1/sh_1/sh_1$ and four $DsSh_1$,ac lines tested. The protein is present but in reduced amount in $DsSh_1$, endosperms that also carry Ac. Similarly, the Sh_1 protein is present but in reduced amount in sh_4 mutant endosperms that are homozygous for the dominant Sh_1 gene but carry only the recessive sh_4 allele. The results of the electrophoretic and immunochemical analyses are in complete agreement.

The mutants show differences with respect to the protein band (designated G) that occupies a position just forward of the Sh_1 band. This protein appears as a light but distinct band in normal material. The

[16] B. McClintock, Cold Spring Harbor Symposia Quant. Biol. 16, 13–47 (1951).

intensity of this band is increased in the sh_1 mutant. However, in $DsSh_1$,ac endosperm that also lacks the Sh_1 protein, the intensity of band G is similar to that of normal material. This band is not seen in su_1(and su_1^{am}), bt_1(and sh_3) and sh_2 mutant endosperm. It is present in sh_4 endosperm extracts but shows a faster rate of migration. The band is seen in F_1 hybrids between su_1 and sh_2 homozygotes.

Similar investigations have been carried out on six mutants in similar background obtained from Dr. Kramer of Purdue. The mutants su_1, su_2, ae, sh_2, wx, and du_1 were tested in single and double mutant combinations. Band G is not seen in the electrophoretic separation of endosperm extracts of su_1, sh_2, and du_1 but is present in the other three. Analysis of the double mutant combinations suggests gene interaction in the synthesis of band G protein. For example, ae and wx, each of which shows band G in single mutant form, lack this band in the double mutant combination, whereas the combination of ae with su_1 (band G absent) does contain this protein.

Discussion. — The absence of the Sh_1 protein in $DsSh_1$,ac endosperm extracts indicates that Ds suppression operates at the gene level. The reappearance of this band when Ac is introduced into this material adds further support to the conclusion that the Sh_1 protein is under the control of the Sh_1 gene. In the presence of Ac, Ds is frequently removed from its position next to Sh_1 so that the normal phenotype is restored.

The gene interaction in the control of the synthesis of band G is of much interest and could have an important bearing on the problem of genic control of protein specificity. Kramer et al.[17] have shown similar gene interactions in the control of starch synthesis and composition. The presence or absence of this protein component of the endosperm is determined visually by inspection of the starch gel. We are presently working on an immunochemical assay that, if developed, should greatly facilitate the investigation.

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[17]H. H. Kramer, P. L. Pfahler, and R. I. Whistler, Agron. J. 50, 207–10 (1958).

Induced Mutations from UV Irradiation of Bacteriophage: Evidence that the Primary UV Effect Is on Genes

D. R. Krieg

Introduction. – Does the mechanism underlying UV-induced gene mutation involve a primary effect of the irradiation on (1) the genes themselves, or (2) precursor material utilized in their reproduction? Induced phage mutations produced in unirradiated bacteria infected by extracellularly irradiated phage[18] provide a favorable system for the investigation of this question. Since no nonpolymerized precursors of genetic material are irradiated in these experiments, it would seem that alternative (2) is excluded and that the induced mutations must be produced according to alternative (1). However, since the mutations are produced in bacteria that had been infected with several irradiated phage, and the genetic material of some of these may get depolymerized and utilized as precursors in the reproduction of undamaged genes, it might be contended that some form of alternative (2) actually is responsible for the induced mutation in this system. Such an interpretation would predict that induced mutations would be produced even if the "irradiated precursors" were utilized in the reproduction of unirradiated genes.

Results. – In an experiment done to test this prediction, bacteria were infected with about five unirradiated $T4r_{59}$ per bacterium and an equal number that had been irradiated with 25 times the mean lethal dose of UV. It was determined that, under these conditions of mixed infection, at least 95% of the progeny phage receive their r allele from reproduction of the unirradiated phage. Parallel infections were also performed with only the unirradiated phage and with only the irradiated phage. Table 2 presents the relative frequency of revertants at the r locus measured in the progeny of each infection.

Discussion. – In contrast to the prediction based on alternative (2) above, the number of mutants found in the mixed infection was not significantly different from the spontaneous frequency, although reproduction of the irradiated phage by themselves yielded induced mutations. These

[18] D. R. Krieg, Virology 9, 215–27 (1959).

Table 2. Relative Frequency of Revertants

Infecting Phage	Frequency of Revertants Among Progeny Phage
Both irradiated and unirradiated	$1.5/10^7$
Unirradiated only	$1.7/10^7$
Irradiated only	$5.4/10^7$

results support alternative (1): that induced mutations result from the reproduction of irradiated genes.

The Extracellular Nature of the Mutagenic Action of Ethyl Methane Sulfonate on Phage T4

D. M. Green

Introduction. – Induced mutants are observed among the progeny of T4 bacteriophage exposed extracellularly to ethyl methane sulfonate.[19,20] When the progeny yields of single treated individuals are examined for mutants it is found that those progeny containing mutants also contain nonmutant phage. One explanation of the occurrence of mutants in "mixed clones" with nonmutants might be that the treated phage effectively carry the mutagen to the bacterium-phage complex, where it acts to produce mutants during phage growth. Only if such a nonspecific carry-over phenomenon were operative would mutants be expected among the descendants of untreated phage when they multiply in a bacterium together with treated phage. Results of such multiple mixed infections demonstrate that induced mutants are observed only in the descendants of treated phage.

Results. – Mixed infections were performed with revertable r_{II} and nonrevertable r_{II} mutants. This combination of phages allowed efficient detection of revertants of the revertable phage without confusion from either reversion of the carrier phage or recombination between the two phages. Table 3 describes the results of single-step growth experiments of various combinations of mixed multiple infections.

[19] D. M. Green, Biol. Semiann. Prog. Rep. Feb. 15, 1959, ORNL-2702, p 50–52.

[20] D. M. Green, Biol. Semiann. Prog. Rep. Aug. 15, 1959, ORNL-2813, p 48–50.

Table 3. Induced Revertants Among Progeny Phage from Infections of Treated and Untreated Phage

Infecting Phage	Mean Number of Viable Infecting Phage per Bacterium	Revertants ± Std. Error / Revertable Progeny Phage
1. Untreated Revertable	10.3	$(3.86 \pm 1.93)/10^7$
Treated Nonrevertable	3.4	
2. Untreated Revertable	15.7	$(3.64 \pm 1.14)/10^7$
3. Treated Revertable	8.6	$(245 \pm 30)/10^7$
4. Treated Revertable	4.4	
Untreated Nonrevertable	12.7	$(217 \pm 37)/10^7$

Discussion. – It is evident that induced mutants are only observed in the descendants of those revertable phage that have been directly exposed to the mutagen. These experiments rule out a nonspecific transfer of ethyl methane sulfonate as the cause of the occurrence of mutants in mixed clones. Previous observations[19,20] suggest that the mutants arise by "delayed mutation" or by delayed segregation of extracellularly induced mutants.

Far-Red-Induced Mitotic Delay and Chromatid Aberration Yield

S. Wolff H. E. Luippold

Introduction. – Experiments designed to study the increase in X-ray-induced chromatid aberrations caused by post-X-ray treatment with far-red radiation have led to the hypothesis that the far red only delays cell division.[21] Thus when chromosomes are examined at any given time after X irradiation, the cells observed after far red were at a stage of mitosis that was more sensitive to X rays than those not treated with far red. The evidence for this was that only chromatid aberrations that are sensitive to changes in the stage irradiated were increased by post-X-ray far-red treatment, whereas chromosome aberrations were not; and that roots

[21] S. Wolff and H. E. Luippold, Biol. Semiann. Prog. Rep. Aug. 15, 1959, ORNL-2813, p 52–55.

fixed at the transition time when both chromatid and chromosome aberrations were observed showed fewer chromosome aberrations after far-red treatment. The present experiments were performed to further characterize the sensitivity of mitotic stage samples.

Results. – Lateral roots of *Vicia faba* seedlings were treated with 150 r of 250-kvp X rays or the 150 r was followed by 3 hr of far-red radiation. The seedlings were then grown in aerated distilled water. At successive 3-hr intervals after X irradiation, samples of roots were excised, treated with colchicine for 2 hr, and fixed. The results of a typical experiment are presented in Fig. 2.

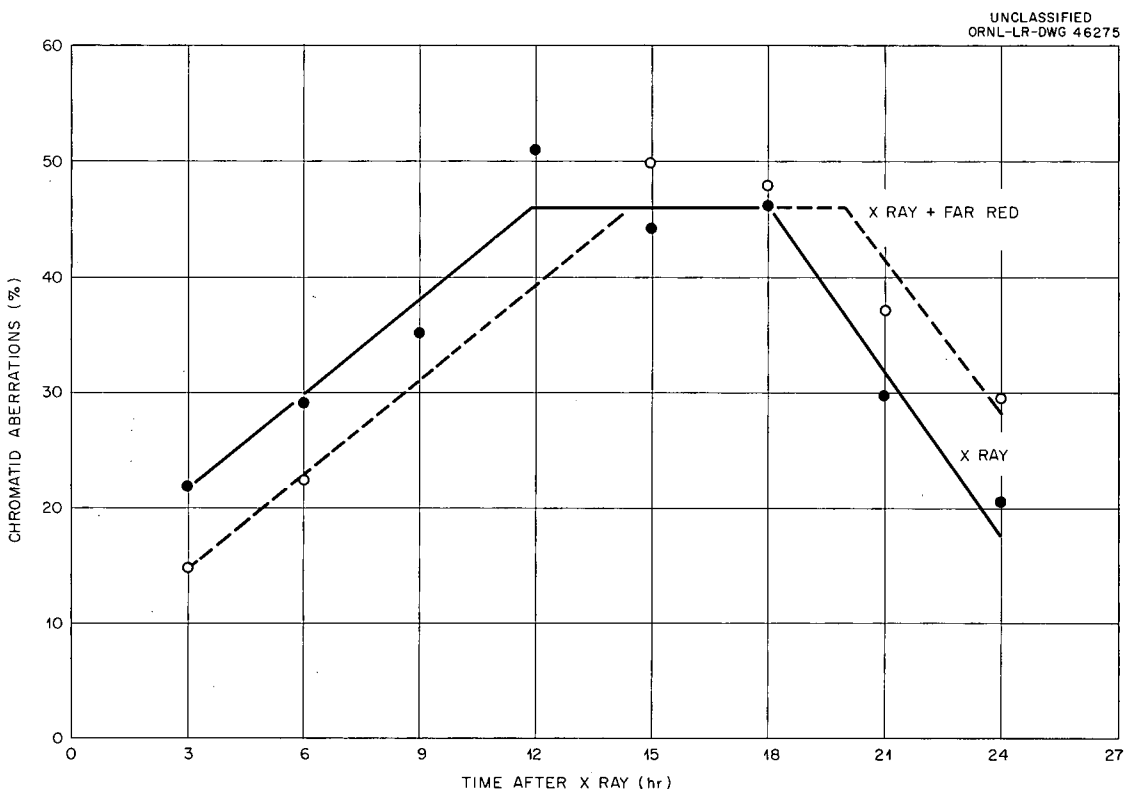

Fig. 2. Aberration Yield at Varying Times After X Rays.

Discussion. – The numbers of chromatid aberrations are relatively low 3 hr after X irradiation, and then increase to a maximum at about 12 hr. They maintain this maximum for several hours and then decrease by 21–24 hr after irradiation. The length of time they maintain the maximum seems to be dependent on the temperature at which the roots are grown. At 22°C it is about 3–6 hr whereas at 19°C it is much longer.

If the roots are treated with far-red radiation for 3 hr after X irradiation, the whole curve seems to be shifted by a time factor of 2–3 hr. Thus, at the early part of the curve, the far-red-treated roots show fewer aberrations than do the X-ray-alone roots. But at later times, when the curves are decreasing from the maximum, the far red results in an apparent increase in the aberration yield.

The results of these experiments are consistent with the hypothesis that the far red seemingly enhances the X-ray-induced chromatid yield only by delaying cell division so that, after far-red treatments, cells are sampled that were at a more sensitive stage at the time of X irradiation.

Chromosome Breakage: Azaserine

D. Davidson

Introduction. – Azaserine is a derivative of the amino acid serine. It induces chromosome breaks in cells of lateral roots of *Vicia faba*.[22,23] The previous studies of its effect have now been extended in order to (1) confirm that it (a) inhibits mitosis and (b) induces chromosome breaks that are localized and not distributed at random; and (2) examine the hypothesis that this compound induces breaks only in nuclei that have passed through the period of chromosome splitting. The azaserine used was a mixture of D and L forms. Fresh solutions, 0.1 mM, 0.01 mM, or 0.001 mM, were made for each experiment. They were used for 1, 3, or 24 hr.

Results. – (1) Mitotic Inhibition. (a) 0.1 mM, 3 hr – Mitosis is completely inhibited for up to 48 hr. (b) 0.01 mM, 24 hr – Mitosis is inhibited. 24 hr + 24 hr in distilled water – Mitosis is inhibited. 3 hr – Mitotic divisions seen 1, 2, 3, and 4 days after treatment. (c) 0.001 mM – Slight inhibition of mitosis in roots treated for 24 hr as compared with those that had been allowed to recover for 24 hr after the same treatment.

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[22] D. Davidson, Biol. Semiann. Prog. Rep. Feb. 15, 1959, ORNL-2702, p 71.

[23] D. Davidson, Biol. Semiann. Prog. Rep. Aug. 15, 1959, ORNL-2813, p 56.

(2) Chromosome Breakage. The frequency of breaks was too low in roots treated with 0.001 m*M* solutions to warrant scoring them. Chromosome breakage was therefore scored exclusively in roots treated with 0.01 m*M* solutions of azaserine. Breaks appear 1, 2, 3, and 4 days after a 3-hr treatment with 0.01 m*M* solutions. They are most frequent in roots fixed on the second day after treatment. On the fourth day they are so infrequent the data have not been included here (Table 4). The relative frequencies of the two different aberrations[22] found in damaged cells are not the same in all fixations; comparison of the results from the three successive fixations reveals two interesting trends: (a) Relative to B' + R', B" + SR decreases in frequency from the first to the final fixation. (b) Consequent upon the change in the relative frequencies of B' + R' and B" + SR, there is a change in the ratio of aberrations affecting S as opposed to M chromosomes. B" + SR occurs mainly in S chromosomes,[22] leading, in fixations with high B" + SR values, to a bias to the S chromosomes. Had aberrations occurred at random the S:M aberration ratio should be approximately equal to the length ratio of 2.5:1.

(3) Time of Action. It has been shown[23] that dicentric chromosomes, the product of B" + R", could occur in roots fixed 48 hr after treatment with azaserine if, and only if, they had also been irradiated; B" + R" did not occur after treatment only with azaserine. Results (Table 4) from fixations later than 48 hr show that the absence of dicentric chromosomes was not due to fixation too soon after treatment.

Table 4. Chromosome Breakage and Reunion Induced in *Vicia faba* Roots by 0.01 m*M* Azaserine

Azaserine 3 hr	Chromosome	Aberrations							Per Cell		B"/SR	B'/R'	Cells	
		B"	B'	C_1SR	C_0SR	mB"	mB'	R'	B"	B'			Damaged	Total
Plus 21 hr	S	71	14	34	44	3	9	19	0.321	0.084	0.916	1.05	82	237
	M	5	6	1	4									
Plus 45 hr	S	104	58	85	82	7	3	58	0.788	0.487	0.653	1.26	116	156
	M	9	15	2	4									
Plus 72 hr	S	19	26	16	11	2	1	33	0.207	0.292	0.71	0.94	30	106
	M	3	5	3	1									

It appears that azaserine is incapable of inducing breaks in the unsplit chromosome of the interphase nucleus. Its action must occur at, or after, the time of splitting. The long delay between treatment and the appearance of the aberrations at metaphase may be attributed to the additional effect of the compound in inhibiting mitosis.

The recent experiments have confirmed that azaserine inhibits mitosis and induces localized breakage; they also provide strong evidence that azaserine is not effective before the period of chromosome splitting.

CELL GROWTH AND REPRODUCTION

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A. R. Whiting[25]
C. C. Hyde

Protein and RNA Syntheses in Enucleated Cells

D. M. Prescott

The only well-established functions of the cell nucleus are concerned with its self-replication and the dissemination of genetic information to the cytoplasm. The problem of the specific biochemical mechanisms that mediate the nuclear contribution of information to the cytoplasm can be approached through comparisons of synthesis capacities of cells with and without nuclei. Any activity present in a nucleated cell but absent in an enucleated cell implies an immediate role of the nucleus in such activity.

By means of microdissection the nucleus can be removed from the amoeba, Acanthamoeba, and the ciliate, Tetrahymena; enucleated cells in a complete nutrient medium survive from one day (Tetrahymena) to two to three weeks (Acanthamoeba). In these and other protozoa survival time of enucleates is roughly proportional to the generation time of the nucleated cell.

In the absence of a nucleus, Acanthamoeba does not incorporate C^{14}-adenine, C^{14}-uracil, C^{14}-orotic acid, H^3-adenosine, or H^3-cytidine into RNA. All these labeled precursors are incorporated by nucleated cells. The incorporation of amino acids by enucleated cells is very weak when

[24]Leave of absence, NSF Senior Postdoctoral Fellowship, Pavia, Italy.
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compared to the similar activity in nucleated cells. The apparent loss of ability of enucleated Acanthamoeba cytoplasm to carry out protein synthesis is contrary to the active amino acid incorporation observed in other enucleated cell types.[26] The situation suggested that enucleated Acanthamoeba may be unable to take up materials from the medium. A study of pinocytosis showed that the process is pronounced in nucleated cells but absent in enucleates. This is consistent with the severe loss of amoeboid motility that occurs immediately upon enucleation. The loss of motility reflects marked changes in the cell cortex (one change, complete cortex solation), which probably also renders the cell surface incapable of pinocytosis. Nutrient uptake by this cell type may normally depend on pinocytosis exclusively. The organizing influence of the nucleus in the maintenance of the sol-gel balance of the cortex, motility, and pinocytosis ability are probably achieved through polyionic properties of the nucleus rather than by the provision of a unique metabolic product.

In Tetrahymena, enucleation does not result in the loss of ciliary motility. Enucleated anterior portions of this cell contain the food vacuole-forming gullet, and posterior enucleated fragments contain the contractile vacuole. Anterior fragments do not live as long as posterior fragments; some observations suggest that the shorter survival time is related to an inability to maintain water balance in the absence of a contractile vacuole. Both types of enucleated fragments have been checked for synthesis activity with essentially the same outcome. Enucleated cells are completely incapable of H^3-cytidine incorporation into RNA, but such incorporation is extremely vigorous in nucleated fragments. The incorporation of C^{14}-amino acids in Tetrahymena is only mildly reduced by enucleation. These experiments on Acanthamoeba and Tetrahymena confirm that the cytoplasm is incapable of independent synthesis of RNA although it continues to synthesize protein in the absence of a nucleus. The data support the thesis[27] that RNA synthesis is an exclusively nuclear function.

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[26] D. Mazia and D. M. Prescott, Biochim. et Biophys. Acta 17, 23–34 (1955).

[27] D. M. Prescott, J. Biophys. Biochem. Cytol. 6, 203–6 (1959).

Ribonuclease Inhibition of Amino Acid Incorporation and Cell Division in *Tetrahymena*

D. M. Prescott

RNase (0.01%) inhibits all cell division and amino acid incorporation in *Tetrahymena* grown in synthetic medium. Suspended in an inorganic salt medium, the cells are ten times more sensitive to RNase. The effects can be reversed by transfer of the cells to RNase-free medium or by the addition of 0.1% (w/v) yeast RNA to the medium. Prolonged treatment with the enzyme causes cytolysis. The effects are presumably achieved by action on cellular RNA. No loss of RNA, however, has been detected in living cells treated with the enzyme, a finding that is in agreement with the inability of RNase to attack the RNA of physiologically intact ribosomal particles *in vitro*. It is conceivable that S-RNA is destroyed by *in vivo* RNase treatment; since S-RNA represents only a small fraction of the total cell RNA, its loss might easily escape detection in measurements of total cell RNA.

DROSOPHILA CYTOLOGY AND GENETICS

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R. D. Wilkerson

Synthesis of Marked Y Chromosomes in *Drosophila melanogaster*

B. Nicoletti D. L. Lindsley

Introduction. – Last year we obtained 87 X-Y translocations that were briefly described in the previous report,[30] where we emphasized their potential usefulness as cytogenetic tools. The fact that the breaks along the X chromosome are distributed in a practically continuous sequence makes it possible to obtain many different deficiencies and duplications. In addition, T(X;Y)'s can be used to build Y chromosomes marked on either arm with genes from the X. To confirm this point we used T(X;Y) No. 148

[28]Visiting Investigator from Abroad.

[29]Consultant.

[30]B. Nicoletti and D. L. Lindsley, *Biol. Semiann. Prog. Rep. Aug. 15, 1959*, ORNL-2813, p 72.

aiming at the production of a Y chromosome marked with the normal alleles of white and Notch (w^+ and N^+).

Results. – T(X;Y) No. 148 has the break points in region 2 D of the salivary X, and in the long arm of the Y; the B^S marker is transferred onto the tip of the proximal part of the X (Fig. 3). The X centromere and proximal heterochromatin of the T(X;Y) was replaced by the base of $XY^L \cdot Y^S$ No. 108-9 or $XY^S \cdot Y^L$ No. 129-16 of Parker (Fig. 3). Males carrying the above recombinants were irradiated with 4000 r of X rays and then mated to w spl rb females in order to delete the majority of the euchromatin of the proximal part of the X indicated by arrows (Fig. 3). This cross yielded about 12,000 males; two carried the desired Y chromosome – one with the markers on the long arm and the other with the markers on the short arm of the Y plus the original y^+ marker on Y^L. Both still carry the B^S marker from the original translocation and an experiment is now in process to eliminate it. Genetic analysis indicates that the constitution of these two fertile Y's is as follows:

$$B^S\ w^+\ N^+\ su\text{-}f^+\ K^S \cdot K^L\ y^+ \qquad\qquad B^S\ w^+\ N^+\ dm^+\ su\text{-}f^+\ K^L \cdot K^S$$

In addition eight other fertile Y chromosomes were recovered carrying the markers B^S and y^+ in different useful combinations.

Discussion. – The two Y chromosomes marked with w^+ and carrying the normal allele for the Notch lethal will be useful for the investigation of complexities of the Notch locus.

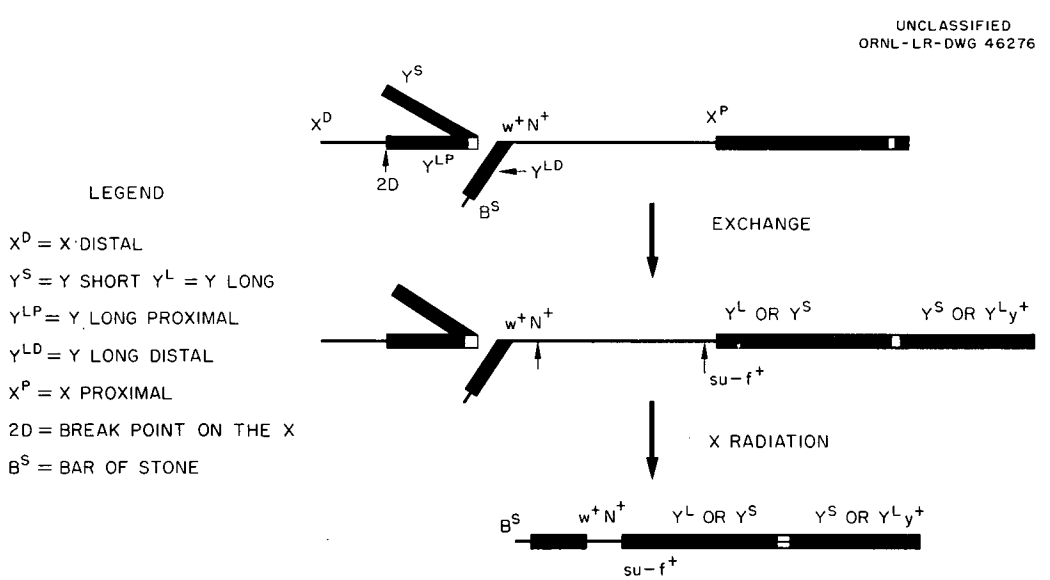

Fig. 3. Sequence of Events for the Production of the Marked Y Chromosome.

MAMMALIAN GENETICS AND DEVELOPMENT

GENETIC AND DEVELOPMENTAL EFFECTS OF RADIATION IN MICE

W. L. Russell, Section Chief

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Mutation Frequency and Radiation Intensity

W. L. Russell E. M. Kelly

Several experiments have been started to investigate some of the implications of our finding that a given dose of chronic irradiation produces fewer mutations than does the same dose of acute irradiation. In the work that resulted in the original finding, the radiation intensities tested adequately were about 90 r/week for the chronic irradiation, and 90 r/min for the acute. Some data were obtained from a 10-r/week irradiation for a total dose of 86 r. In current experiments we are testing dose rates between 90 r/week and 90 r/min, and we are adding to the information obtained both at 90 r/week and 10 r/week.

In one of these experiments, in which the specific locus mutation frequency is being measured in spermatogonia irradiated at an intensity of 10 r/week for a total dose of 300 r (Cs^{137} source), the data now accumulated already permit one important conclusion. The induced mutation rate in this experiment is already significantly higher than the control mutation rate ($\underline{P}$ = 0.0015 for a one-tailed test). The important point is simply that even this low a dose rate of 10 r/week, or

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[1]Consultant.

[2]Visiting Investigator from Abroad.

0.00099 r/min, produces mutations at a frequency which, without much difficulty, could be shown to be higher than the frequency in the controls. This radiation dose rate is, so far as we know, the lowest ever tried in laboratory mutation rate experiments with animals. Furthermore, the cells investigated here (spermatogonia) do show some dependence of mutation frequency on radiation intensity, which is not true for spermatozoa, the cells used in the experiments of most other investigators. Thus we can conclude that, if there is a threshold dose rate below which mutations do not occur, it has not yet been revealed even by the new experiments at a dose rate lower than any ever tested before.

It is important to compare the mutation frequency obtained at 10 r/week with that obtained earlier, and augmented by new data, at 90 r/week. A lower mutation frequency at the lower of these two intensities would suggest that a still lower mutation frequency might be obtained if the dose rate were further reduced. The present point estimate for the mutation frequency at 10 r/week is actually slightly, but not significantly, higher than that for 90 r/week. Thus there is, so far, not only no evidence for a threshold dose rate, but also no indication that further lowering of radiation intensity will result in any more reduction in mutation frequency.

Variegated-Type Position Effects in the Mouse

L. B. Russell J. W. Bangham

Some interesting cases of mottling have occurred among the offspring of X-irradiated wild-type males mated, during their presterile period, to multiple recessive females. Testing of two of these mottleds indicates that they result from sex-linked translocations giving variegated-type position effects. Both mutants were semisterile females that, when mated to the appropriate recessive, produced more mottled progeny, all of which also were semisterile females.

One of the mutants was diffusely mottled with brown. The following explanations for offspring obtained by crossing mottled females to nonrelated brown males are consistent with the results. Mottled females are

heterozygous for brown and a reciprocal translocation between chromosome 8 and the X chromosome, the break in 8 being near the b locus, which carries the wild-type allele. Males heterozygous for this translocation are black, rather than mottled; and completely sterile, rather than semisterile. Sterility is due to degeneration of spermatocytes in pachytene (Oakberg). The segregants bearing the intact chromosomes are fertile brown males and females. Crossing over between the b locus and the breakpoint occurs with a frequency of about 15%. It results in semisterile brown females, sterile brown males, and fertile black animals of both sexes.

The other mottled mutant apparently involves an analogous situation, with the break presumably near the c locus.

These cases indicate that variegated-type position effects occur in mammals and that the X chromosome may be particularly effective in producing them.

Gamma-Ray Sensitivity of Oocytes of Ten-Day-Old Mice

E. F. Oakberg E. B. Blalock

Introduction. — The extreme effects of radiation exposure during the second week of life on subsequent fertility of female mice reported by Russell et al.[3] directed our attention to the apparent high radiation sensitivity of the immature mouse ovary.

Results. — In order to determine the histological basis for this sensitivity, ten-day-old mice were exposed to 1, 3, 5, 7, 10, 15, and 25 r of Co^{60} γ-rays at about 3 r/min. All mice were killed 72 hr after exposure. Ovaries were fixed in Zenker-formol, and 7-μ serial sections stained in hematoxylin azure-II eosin. All oocytes were counted in eight of the largest sections from each ovary. Oocytes were classified into seven stages on the basis of follicle development, stage 1 being the earliest. Mean number of normal oocytes in the three irradiated mice at each dose were expressed as the fraction of the mean count obtained for six controls. An LD_{50} of 8.4 r, with 95% confidence limits of 7.2 and 9.7 r, was estimated for stage 1, and an LD_{50} of 9.4 r, with 95% confidence limits of 6.7 and 14.9 r, was obtained for stage 2 oocytes. The

[3]W. L. Russell et al., Science 129, 1288 (1959).

number observed in stages 3–7 was too low for estimation of the LD_{50}, but sufficient information was obtained to support our previous observation of increased resistance of the oocyte in later stages of follicular development.

Discussion. — We are unaware of any previous estimates of the LD_{50} of early oocytes; however, our results are in general agreement with other studies on radiation sensitivity of the ovary. Dictyate oocytes in early stages of follicular development provide unique material for study of the mechanisms of radiation-induced cell death, since neither DNA synthesis nor cell division is involved.

The Separation of Recessive Lethals at the *Notch* Locus of *Drosophila*

W. J. Welshons E. S. Von Halle

Introduction. — Previous work on the *Notch* locus had demonstrated the separability of two recessive lethals [dominant *Notch* (*N*) mutants] and suggested the possibility that a new lethal was localized between the first two that had been investigated.[4] The tentative linear order of these recessive lethals from left to right on the X chromosome was presumed to be $Notch^{264-40}$ (N^{264-40}), $Notch^{22}$ (N^{22}), $Notch^{Co}$ (N^{Co}), but in order to prove that N^{22} was actually localized between the other two, one would have to separate N^{22} from N^{264-40} and then separate N^{22} from N^{Co}. As indicated in the earlier report, N^{22} was separated from N^{264-40} and was shown to be localized to the right of N^{264-40}; but the experiments with N^{22} and N^{Co} failed to give recombinants, and indicated either that these two mutants were allelic or that N^{22} was a tiny deficiency or aberration which overlapped the locus of N^{Co}.[5] As indicated below, additional experiments have indicated that these two *Notches* are separable and have the linear order depicted above.

Results. — In an attempt to separate N^{22} from N^{Co}, we mated females that were $y\ w^a\ fa\ N^{22}\ +\ +\ /\ +\ w^a\ +\ +\ N^{Co}\ rb$; $SM1,\ al^2Cy\ sp^2\ /Dp\ w^{51b7}$ to

[4]W. J. Welshons, Cold Spring Harbor Symposia Quant. Biol. 23, 171 (1958).

[5]W. J. Welshons and E. J. McCrone, Biol. Semiann. Prog. Rep. Aug. 15, 1958, ORNL-2593, p 44.

w^a fa^{no} spl rb males. The SM1, al^2Cy sp^2 chromosome contains multiple inversions which could be expected to increase crossing over between the X chromosomes, and the Dp w^{51b7} is a small duplication for the w^a to N region that is inserted into the second chromosome, and it has to be used in order to cover the lethality of the N^{22} and N^{Co} combination of lethals. If N^{22} is localized to the left of N^{Co} and separable from it, one would expect to recover + w^a + + + + recombinant chromosomes.

In the earlier experiments no recombinants were detected in approximately 97,394 tested chromosomes, but in the current experiment, in which an autosomal inversion was used in the hope of increasing crossing over, a total of seven recombinants have been found in approximately 112,000 tested chromosomes. In addition to the recombinants, two reversions of N^{Co} and one reversion of N^{22} have been detected.

Conclusion. — It is now possible to conclude that the three Notch mutants are discrete members of a pseudoallelic recessive lethal series, and since reversions of all three mutants have been obtained, they appear to be point mutation mutants rather than induced or spontaneous deficiencies.

Sex Chromatin and Sex Chromosomes in Man

E. H. Y. Chu

Introduction. — The application of cytologic tests for indirectly assessing chromosomal sex and of methods for direct examination of the sex chromosome constitution to the study of abnormalities of sex differentiation has advanced our understanding of the pathogenesis of anomalies of sex in man and contributed to the clinical diagnosis of these afflictions. In this laboratory a systematic analysis of the karyotype of these individuals has been in progress. During the course of these experiments a subject with the syndrome of gonadal dysgenesis and chromatin-positive nuclei was discovered to have a diploid number of 45 chromosomes and an apparent XO sex chromosome constitution.[6]

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[6]Work in collaboration with M. M. Grumbach and A. Morishima, Department of Pediatrics, College of Physicians and Surgeons, Columbia University.

Results and Discussion. — The patient was a 12-year-old phenotypic woman of average intelligence. Her facies and habitus suggested the diagnosis of gonadal dysgenesis, although the neck was not webbed. In addition to some minor anomalies of the ears and hard palate, she had sparse axillary and pubic hair but was otherwise sexually infantile. The titer of urinary gonadotropin was elevated. Her color vision was normal. About 60% of nuclei in many oral mucosal smears showed a conspicuous mass of sex chromatin; the latter did not differ in size, shape, or staining properties from the sex chromatin mass in female nuclei. Thirteen of 250 neutrophiles had a typical "drumstick." More than 65% of intermitotic nuclei in cells grown in tissue culture contained a sex chromatin mass.

A sample of skin and one of bone marrow were obtained and grown in culture. Twenty-one intact cells in metaphase were each found to have 45 chromosomes. The sex chromosome constitution of this patient was XO, lacking a second sex chromosome. No abnormality of the autosomes was detected.

It has been generally assumed, following Barr's initial suggestion, that the sex chromatin in female somatic nuclei is derived from heterochromatic regions of two X chromosomes. However, recent evidence[7] suggests that this hypothesis should be reexamined. Ohno *et al.*[8] showed in squash preparations of liver cells in prophase from female rats that apparently only one X chromosome was distinctly heterochromatic while the other X appeared isochromatic to autosomes; they suggested that only the paternally derived X chromosome was heterochromatic. In our patient, the sex chromatin could not have been derived from two X chromosomes; the presence of female-type nuclei is not inconsistent with the view that the sex chromatin represents the heterochromatic region of a single X chromosome. Studies on prophase nuclei of cells in culture derived from this patient showed clearly a single chromosome having positive heteropyknosis, a situation similar to normal female nuclei. On the other hand, in another patient diagnosed as gonadal dysgenesis, whose cells

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[7] M. L. Barr, Science 130, 679–85 (1959).

[8] S. Ohno, W. D. Kaplan, and R. Kinosita, Exptl. Cell Research 18, 415–18 (1959).

were also found to have 45 chromosomes (XO) but showed negative sex chromatin reaction, negative heteropyknosis in the chromosomes in prophase was found. It is highly suggestive that the derivation of the X chromosome determines whether the heteropyknotic condition is manifested during interphase (as sex chromatin) and prophase (as a single heteropyknotic chromosome).

Analysis of X-Ray-Induced Chromosome Aberrations in Mouse Somatic Cells *in vitro*

E. H. Y. Chu V. C. Monesi

Introduction. — The present study was undertaken in order to have a comparison with the previous observations of X-ray effects on human chromosomes[9-11] and with the proposed study of similar effects on mouse cells *in vivo*.

Methods and Results. — Embryonic skin fibroblast-like cells derived by trypsin dissociation from 15- to 18-day-old (101 ♀ × C3H ♂)F_1 fetuses were grown in culture. Rapid cell proliferation occurs within a day after initial explantation and in the subsequent two or three subcultures. Cells were grown directly on coverslips in depression test tubes (Leighton tubes) for 24 hr and X-irradiated with doses from 25 to 100 r. Cytological examinations of chromosomes were made at 20 and 50 hr after irradiation, utilizing well-spread, colchicine-blocked metaphases. A euploid chromosome number (2n = 40) was found in a great majority of cells and an idiogram has been constructed. Qualitatively, both chromatid and chromosome types of aberrations have been detected. The present experimental procedure has demonstrated the feasibility of obtaining in this material quantitative dose-effect relationship in terms of chromosome aberration frequencies. Such quantitative determinations are in progress.

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[9] M. A Bender, Science 126, 974 (1957).

[10] T. T. Puck, Proc. Natl. Acad. Sci. U.S. 44, 772 (1958).

[11] E. H. Y. Chu and N. H. Giles, Genetics 44, 503 (1959).

RADIATION PROTECTION — LIVING CELLS

Alexander Hollaender

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A. S. Angel
S. D. Haskins

Attempts to Alter the Radiosensitivity of *Escherichia coli* by Metabolic Means

M. S. Engel G. E. Stapleton

Introduction. — We have recently shown that the X-ray sensitivity of *Escherichia coli* can be modified by cultural conditions.[2] Cells grown aerobically in peptone with various glucose-phosphate buffer combinations showed a difference in survival that was directly related to the final pH of the culture in the stationary phase of growth. The extremes in pH obtained varied from 8.3 to 4.8 and the LD_{90} for *E. coli* B/r (Cold Spring Harbor strain) under these conditions ranged from 10 to 50 kr. The resistance was reported to be unrelated to multicellularity, clumping, or multinuclear conditions. Further attempts to characterize the nature of the resistance are here reported. The methods used are similar to those already reported.[2,3]

Results. — The possibility that differences in sensitivity to X rays are caused by a protective compound produced by the cells at low hydrogen ion concentrations was tested in several ways. Cells that had been grown in glucose to a final pH of 4.8 (henceforth called resistant cells) were suspended in 1% KCl, peptone, phosphate buffer at pH 5 and 7, and in the growth medium with no effect by any treatment on the cells' resistance. Cells cultured in peptone to a final pH of 8.3 (henceforth called sensitive cells), when suspended or incubated for varying periods in 1% KCl, peptone, phosphate at pH 5 and 7, peptone plus lactic acid, or the sterile growth medium from resistant cells, exhibited no change in their

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[1]Visiting Investigator from Abroad.

[2]G. E. Stapleton and M. S. Engel, to be submitted to J. Bacteriol.

[3]G. E. Stapleton and M. S. Engel, Biol. Semiann. Prog. Rep. Aug. 15, 1959, ORNL-2813, p 92.

radiosensitivity. The existence of a bound compound has also been investigated, and the protective action of cell-free extracts of both sensitive and resistant cells has not yet yielded unequivocal results.

Resistant and sensitive cells have been introduced to peptone and peptone-glucose plating media to rule out the possibility that postirradiation treatment inhibits cell division in one or the other type of cell. Survival is unaffected by the use of either plating medium, and the cells from the peptone-glucose plates are resistant regardless of the nature of the inoculum cells. When the cells are plated on minimal medium, however, the resistance imparted on low pH cells disappears.

Substrates other than glucose were supplied to cells, and the pH effect was noted when xylose, fructose, galactose, and lactose were used. With glycerol as carbon source, the cells reduced the pH of the medium as in the other cases, but they were not resistant. An interesting observation arising from these experiments was that glycerol *per se* protected resistant and sensitive cells markedly, the degree of protection being directly related to glycerol concentration. When sensitive cells are suspended in 6.9 *M* glycerol a DRF (dose-reduction factor) of 5 was obtained while with resistant cells the DRF varied from 5 to 10. A recent report by Wood[4] reports a DRF of 4 for haploid yeast when protected by similar glycerol concentrations.

Cells cultured at a constant pH of 5.6 and 6.2 were not resistant while in stationary phase of growth; but, when they were allowed to reduce the pH of the medium to 5.0, the shoulder-type survival curve was exhibited. Attempts to induce this resistance in short-term experiments were unsuccessful. Here sensitive cells were given glucose in experiments where the pH was altered from 6.0 to 4.8 with no change in the cells' sensitivity.

The relation of pH to radioresistance was investigated in other organisms. The effect can be measured to varying degrees in strains of *E. coli* variously labeled B and B/r and in the porphyrin-requiring mutant of *E. coli* in the absence or presence of hemin. No such relation

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[4]T. H. Wood, Radiation Research, Suppl. 1, 1959 (Proc. Intern. Congr. Radiation Research, ed. by D. E. Smith), p 332–46.

was noted in Pseudomonas aeruginosa where the LD_{90} was 8 kr over the pH range of 4.4 to 8.5.

Discussion. — These data show that the resistance studied is not caused by a compound that is easily washed out of cells nor can the resistance be readily imparted to sensitive cells. It appears highly unlikely that the selection of a resistant or sensitive mutant could be a factor for reasons that cannot be explained here, owing to space limitation. The substrate specificity in the induction of radioresistance lends credence to the suggestion that an internal protective compound is being produced. It can certainly be stated that the attainment of a low pH will not protect cells as such.

Characteristics of Cultures of Escherichia coli B and B/r

H. I. Adler S. D. Haskins

Introduction. — Much of the current literature in radiation bacteriology reports experiments done on cultures labeled Escherichia coli "B" and "B/r." A survey of this literature and our own experimental experience suggested that the cultures available in various laboratories, although identically designated, differ among themselves and that most of them do not correspond to those originally described and carefully characterized by Witkin.[5,6] This seemed to be particularly true for B/r cultures. The original B/r culture isolated as an ultraviolet-selected mutant of B had, among others, the following characteristics: It was strikingly more resistant than the parent B culture to ultraviolet light, X rays, penicillin, and sodium sulfathiazole. Furthermore, it did not develop the snakelike forms always found in ultraviolet-treated B cultures.

Results. — Figure 4 shows the differences in X-ray sensitivity observed for four B/r cultures obtained from different laboratories but grown and irradiated under identical conditions. It is obvious that the B/r's are a heterogeneous group and that three of them have an X-ray sensitivity very close to that of the group of B cultures and therefore fail to meet one of the important criteria for classification as B/r. The X-ray data suggest that these

[5]E. Witkin, Proc. Natl. Acad. Sci. U.S. 32, 59–68 (1946).

[6]E. Witkin, Genetics 32, 221–48 (1947).

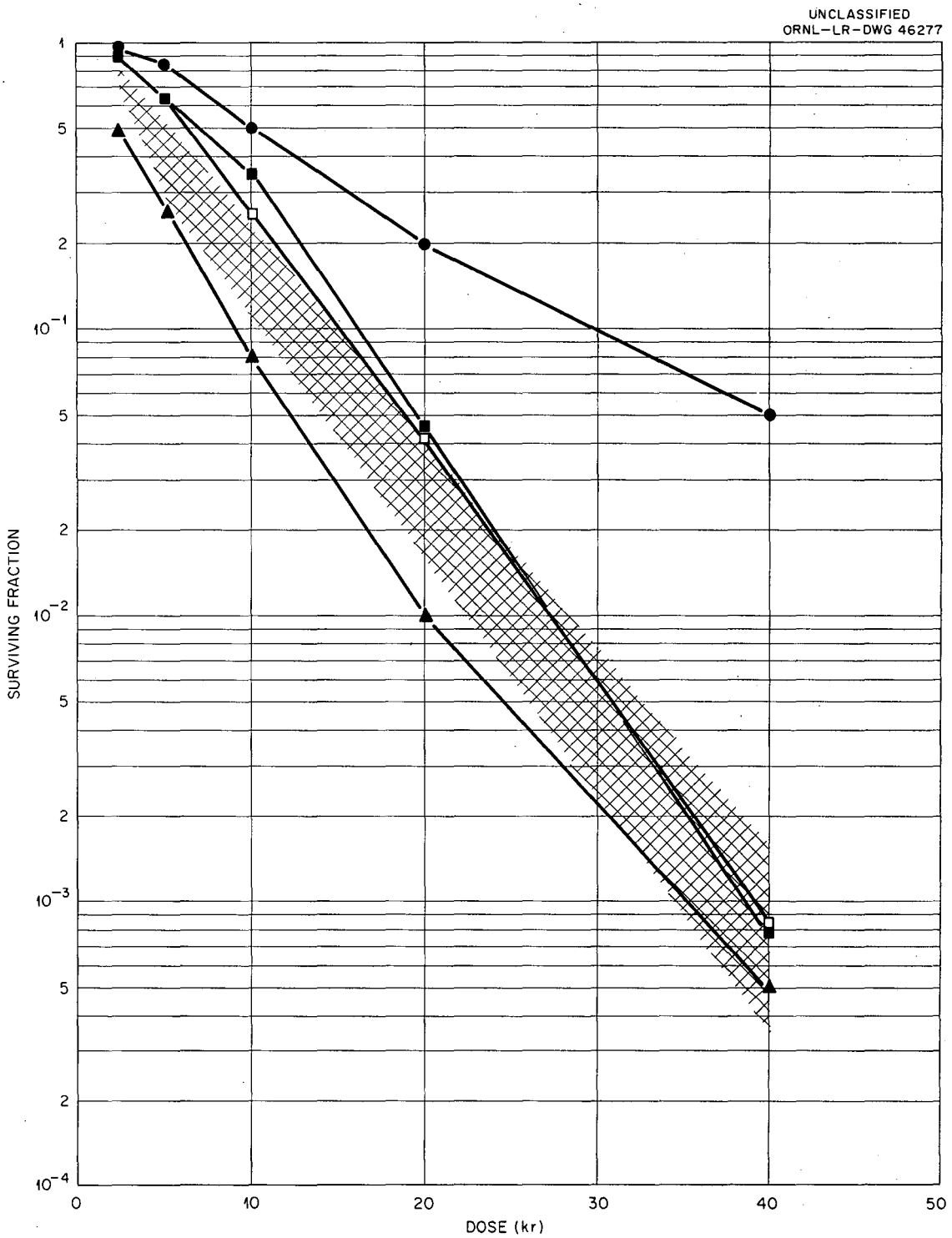

Fig. 4. X-Ray Survival Curves for Four Cultures Labeled *Escherichia coli* B/r Obtained from Different Laboratories. The cross-hatched area represents the range of survival obtained with three cultures labeled *E. coli* B.

three B/r cultures may have "reverted" to B. Experiments have established that this is not the case. They do not form "snakes" after ultraviolet treatment. They characteristically grow to final titers lower than the true B cultures. Furthermore, two of the B/r cultures have been tested for ultraviolet sensitivity and are somewhat more resistant than the true B cultures. The survival curves are sigmoid whereas those for all the B cultures are more nearly exponential.

Conclusions. — The data presented here and other information support the contention that the various B/r cultures currently being used in different laboratories are not genetically or physiologically identical. Furthermore, comparison with the absolute values obtained by Witkin for ultraviolet and X-ray sensitivity of B/r suggest that none of the B/r's tested correspond to the original culture described. Attempts to correlate results obtained in various laboratories undoubtedly suffer from this heterogeneity of experimental material. We suggest that this heterogeneity be acknowledged in the literature and that the designation B/r be reserved only for those cultures that have all the characteristics originally described by Witkin.

AET Modification of X-Ray Sensitivity and Mutagenicity in *Aspergillus terreus*

Alexander Hollaender A. M. McCarthy

Introduction. — It has been previously reported by Kølmark[7] that AET (rearranged at pH 7.0 to form MEG) showed a strong toxic effect on a mutant of *Neurospora* K3/17, as evidenced by the number of survivors obtained when MEG was combined with X rays. A 0.09 *M* concentration of MEG was used combined with an X-ray dose of 60 kr. Under these conditions, the number of mutations also increased. However, Kølmark occasionally observed a small decrease in both the toxic and mutagenic effect of X rays when a 0.01 *M* concentration of AET was used, but this was not regarded as very significant.

Results. — In the present experiments with *Aspergillus terreus* both AET·Br·HBr and AET·Cl·HCl (rearranged at pH 7.0 to form MEG) were used in

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

concentrations varying from 0.002 to 0.08 M. These concentrations of MEG were combined with an X-ray dose of 100 kr. Spores of A. terreus (in saline suspension) were treated with MEG for 30 min before irradiation. With AET·Br·HBr the number of survivors increased and the number of mutations produced decreased up to a 0.01 M concentration of AET. When this concentration of AET was exceeded, the number of survivors showed a decrease, which became more pronounced with increasing concentrations of AET, and at the same time the number of mutations gradually increased.

In the case of AET·Cl·HCl, as in the above, the number of survivors increased and the number of mutations decreased up to a 0.01 M concentration of AET. At this concentration, however, both the number of survivors and of mutations remained constant up to an 0.08 M concentration, which was the highest level tested.

Discussion. – It has been suggested that the differences in the results obtained with the two compounds tested might be accounted for by the nature of the inorganic ion present. At the present time experiments are in progress to determine whether or not this is the case.

MAMMALIAN RADIATION RECOVERY

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X-Ray Survival Curves of a Hypotetraploid Human Cell Line

M. A Bender P. C. Gooch

Introduction. — Puck and his coworkers, using a single-cell plating technique, have investigated the shapes of the X-ray survival curves of a number of human cell lines in tissue culture.[3,4] According to Puck, the experimental curves all fit the expression $S = [1 - (1 - c^{-kD})^n]^m$ (where S = survival, D = dose, n = the multiplicity of sensitive sites, and m = the number of kinds of sites), with n = 2 and k = 0.96. Numerical data are not usually reported by Puck, but there are two cases that can be analyzed. They actually best fit n values in excess of 5. Puck argues that, from his value of n = 2, mammalian cell X-ray killing is caused by chromosome aberration production. Since this mechanism is not expected to yield an n value of 2, we have reinvestigated the shape of X-ray survival curves for a human cell line.

Materials and Methods. — A clonal line derived from normal human heart muscle (GH7A6) was used. The cells were plated in 50-mm Pyrex petri dishes and incubated in a 5–8% CO_2 atmosphere. In order to test the effect of cell number and time of irradiation on the shape of the survival curves, four classes of experiments were performed: A — 100 cells plated per dish at all doses and irradiated after attachment to the glass; B — cell number increased at higher doses in order to obtain

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[1]Consultant.
[2]Visiting Investigator from Abroad.
[3]T. T. Puck and P. I. Marcus, J. Exptl. Med. 103, 653–66 (1956).
[4]T. T. Puck *et al.*, J. Exptl. Med. 106, 485–500 (1957).

an approximately equal number of survivors per plate (Puck's procedure), and irradiated after attachment; C — 100 cells plated at each dose onto an irradiated killed "feeder layer" and irradiated after attachment; D — 100 cells plated at each dose after irradiation in suspension. There were 40 control dishes and 20 experimental dishes at each dose point in each experiment. Doses of 80, 160, 240, 320, and 400 r of X rays of hvl 2 mm of Cu were used. Colonies of 50 or more cells were scored as survivors after ten days incubation.

Results and Discussion. — The plating efficiencies in various experiments ranged from 30 to 60%. The values of k and n for fits of our data to the single-process survival curve (assuming that m = 1) are given in Table 5. There are significant differences in k between various experiments. We do not seem to be controlling all the variables governing

Table 5. Values of k and of n Calculated from Fits of X-Ray Survival Curves for Line GH7A6 with the Single-Process Expression $S = [1 - (1 - c^{-kD})^n]^m$

Type of Experiment		k	n
A. (Equal numbers)	1	0.79 ± 0.066	1.49 ± 0.29
	2	0.51 ± 0.042	1.70 ± 0.29
	3	0.54 ± 0.098	1.61 ± 0.55
B. (Unequal numbers)	1	0.84 ± 0.065	2.36 ± 0.47
	2	0.79 ± 0.091	2.73 ± 0.90
	3	0.54 ± 0.098	1.61 ± 0.55
C. (Equal numbers, fed)	1	0.92 ± 0.044	2.41 ± 0.28
D. (Equal numbers, suspended)	1	0.94 ± 0.12	1.88 ± 0.57
	2	0.96 ± 0.11	2.44 ± 0.80

the sensitivity of the cells. None of the n values are significantly different from 2, in spite of the suggestion that type A experiments have a hit number lower than 2 and the other types a hit number higher than 2. However, g plots of the data[5] show definite inflections, suggesting that

[5]K. C. Atwood and A. Norman, Proc. Natl. Acad. Sci. U.S. 35, 696–709 (1949).

the data would be better fitted with a multiple-process survival curve of the form $S = [c^{-k_1 D}] \cdot [1 - (c^{-k_2 D})^{n_2}]$. Preliminary fitting of our data for cells irradiated in suspension (D) with this expression yields $n_1 = 1$ and $k_1 = 0.47$ for the first component, and $n_2 = 17$ and $k_2 = 0.92$ for the second.

The fact that the g plots of our data show inflections rules out the simple one-process hypothesis suggested by Puck. It will be necessary, however, to perform experiments in which the survival curves are extended several decades lower before the shape of the multiprocess survival curves can be calculated accurately. Even without these calculations it seems clear that X-ray killing of our line of cells *in vitro* cannot be a simple matter of chromosome aberration production.

Lymphatic Tissue Reaction in Normal Mice Given Foreign Bone Marrow

C. C. Congdon P. G. Delker

Introduction. — The detailed anatomical basis for the immune mechanism is poorly understood. Recent studies have shown that the germinal center cells of lymphatic tissue undergo marked changes soon after transplantation antigen injection.[6]

In the present work the effect of foreign bone marrow transplantation antigens was compared with other kinds of antigenic material on germinal center cell response.

Results. — Loss or dissociation of germinal centers in the spleen white pulp is the first change seen in normal mice given a large intravenous injection of foreign bone marrow. The swollen white pulp is then replaced by antibody-forming cells. Nearly all lymphocytes disappear. The antibody-forming cells then disappear and regeneration of the germinal center occurs. Lymphocytes again fill the white pulp. Collections of plasma cells in or near the white pulp can be seen. Rate of delivery of antigenic material possibly controls the amount of plasma cell formation as it does the hyperplasia of germinal centers during their regeneration. After rat bone marrow injection, most of the antibody-forming

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[6] C. C. Congdon *et al.*, Rev. Arg. Cancerol. (1960, in press).

cells have disappeared in four days; with the same amount of homologous bone marrow, these cells take nine or ten days to disappear. Normal mice given rat serum, bovine serum albumin, sheep red blood cell ghosts, Salmonella endotoxin, bacterial polysaccharide, and pertussis vaccine show essentially the same alterations in the splenic white pulp.

Discussion. — Our theory that the germinal centers in lymphatic tissues are the major anatomical structure for the immune mechanism is supported by other evidence in addition to that given above. Ortega and Mellors[7] have demonstrated that germinal center cells in human lymph nodes contain γ-globulin. We have shown that the major uptake of tritiated thymidine in lymphatic tissue is in the germinal center cells.[8] The germinal center structure has a characteristic pattern and orientation in each lymphatic tissue that probably facilitates recognition of antigenic material.

It is of further interest that tissue transplantation antigens in situations where the donor and host are very closely related also promote germinal center cell changes.

The Killing Effect of Blood-Bone Marrow Mixtures Given to Irradiated Mice

J. W. Goodman

Introduction. — Procurement of bone marrow for human use often involves contamination of the marrow with circulating blood cells. Marrow homotransplantation in man after heavy irradiation has been followed in certain instances by transfusion of whole blood from the bone marrow donor. The advisability of such blood transfusions is questioned in the light of the following experimental findings on mice.

Results and Discussion. — Lethally irradiated mice given a mixture of homologous bone marrow and isologous blood after exposure all died within two weeks. Histologic study of mice killed and necropsied daily during the course of this experiment showed evidence of temporary transplantation of bone marrow and then rejection. Marrow failure resulting in hemorrhage and

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[7]L. G. Ortega and R. C. Mellors, J. Exptl. Med. 106, 627–40 (1957).

[8]C. C. Congdon, R. A. McGrath, and W. D. Gude (unpublished data).

infection caused death. Irradiation of the isologous blood, whether in the donor mouse or *in vitro*, before mixing with homologous marrow prevented the killing effect of the blood. Homologous bone marrow alone given to lethally irradiated mice kept 50% alive for 30 days. Gross appearance and histologic study in this group showed the usual lymphatic tissue changes of "secondary disease" or the "foreign bone marrow reaction." Homologous blood mixed with homologous bone marrow as a treatment for lethally irradiated mice caused death of all recipients in 30 days. Gross appearance and histologic study of mice necropsied daily in this group showed marked acceleration of the "secondary disease" without significant loss of the transplanted marrow. These findings demonstrate the deleterious effect of mixing blood with homologous bone marrow for injection into irradiated animals and give further evidence for the existence of an immunologically competent cell in the peripheral blood.

Low-Temperature Protection of X-Irradiated Mouse Bone Marrow Cells

M. B. Lion T. W. McKinley, Jr.

Introduction. – Mouse bone marrow cells exposed to 800 r *in vitro* are no longer able to promote recovery of lethally X-irradiated mice; 2-mercaptoethylguanidine, cysteine, cysteamine, or anoxia are protective to X-irradiated marrow cells.[9] Low-temperature protection of X-irradiated bacteria[10] and yeast[11] has been demonstrated.

Results. – In the present study, mouse bone marrow cells were X-irradiated *in vitro* at -196°C, and their viability determined by their ability to promote survival in lethally X-irradiated mice. Isologous marrow cells, suspended in 15% glycerol in Tyrode's solution, were slow-frozen (1°C/min to -40°C), placed in liquid nitrogen, exposed to 800 r and rapidly thawed, or rapidly thawed and then exposed to 800 r, and injected into mice exposed to a supralethal dose of X rays.

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[9] L. H. Smith, Exptl. Cell Research 13, 627 (1957).

[10] G. E. Stapleton and C. Edington, Radiation Research 5, 39 (1956).

[11] T. H. Wood, Radiation Research, Suppl. 1 (1959) (Proc. Intern. Congr. Radiation Research, ed. by D. E. Smith), p 332.

Discussion. — There is a highly significant difference in the 30-day survival of mice injected with a suspension of 5×10^6 cells irradiated in the frozen state (80 out of 171) compared to the same suspension irradiated after thawing (22 out of 171). Nevertheless, even 800-r irradiation after thawing still leaves a small percentage of cells alive, whereas a liquid suspension not containing glycerol does not lead to any survival of supralethally irradiated mice. A certain protective property of glycerol against radiation seems, therefore, to be indicated.

The above results are qualitative, and a more-accurate description of these phenomena would involve a quantitative assessment of the cell viabilities. An attempt in this direction was made. Serially diluted suspensions of fresh, frozen, and frozen irradiated cells were injected into lethally irradiated mice. When the results are plotted on log probit paper, the best fitting straight lines are parallel. A comparison of the number of cells needed for 50% 30-day survival of the injected mice gives a measure of the relative cell viabilities in the above cases. As the approximate number of living cells in the fresh suspension is known, an absolute assay of treated bone marrow cells reduces to an ordinary biological assay of an unknown against a standard, the only difference being the negative slope of the probit-log dose plot. Factors, such as early death of irradiated mice, if found independent of dose, might therefore be treated as natural mortality and be estimated as such from the data.

The data accumulated so far are not numerous enough to allow a decisive statistical analysis to be made, but provisionally about 1.2–1.5×10^5 fresh cells, 4×10^5 glycerol-frozen cells, and about 2.5×10^6 glycerol-frozen and irradiated cells are needed for 50% 30-day survival in lethally irradiated mice. If the viability of cells in the fresh suspension is taken as 100%, the cell viability in the frozen suspension would be 25–35%, and in the frozen irradiated suspensions it would be about 5%.

Cytological Study of the Intestines of Mice Exposed to X Rays

R. A. McGrath

Introduction. — X-ray-induced abnormal enlargement of mouse intestinal crypt cells is an effect of radiation[12] reaching its maximum 48 hr after exposure. Greater enlargement occurs with increasing radiation dose.[13]

Results. — Repeated injection of isotopically labeled thymidine, cytidine, or leucine into irradiated mice and using autoradiographs to assay the amount of precursor incorporated into cells showed that abnormal enlargement of intestinal cells was associated with increase in total cellular protein and probably RNA, together with a dilution of the existing DNA. It was also noted that nucleolar size and leucine and cytidine incorporation decreased with increasing differentiation of the cells on the villus.

Discussion. — Although many details vary, the enlargement process in mouse intestinal cells is probably comparable to a similar effect found in bacteria,[14] where UV exposure causes enlargement (filament formation) with increased dry weight and without increase in DNA.

There is no satisfactory explanation for the observation that increasing radiation exposure causes increased abnormal enlargement.

Protection of Mouse Bone Marrow by Inorganic Compounds During Freezing and Thawing

Phan The Tran

Introduction. — Our previous experiments showed that mouse bone marrow cells remained viable after slow-freezing in the presence of organic molecules other than glycerol. Many polyalcohols, mono- and disaccharides are effective.[15,16] It was usually assumed, however,

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

[13]R. A. McGrath, Intern. J. Radiation Biol. (1960, in press).

[14]R. A. Deering, J. Bacteriol. 76, 123–30 (1958).

[15]M. A Bender, Phan The Tran, and L. H. Smith, J. Appl. Physiol. (in press).

[16]Phan The Tran and M. A Bender, J. Appl. Physiol. (in press).

that only organic molecules would protect cells against freezing and thawing damage since Lovelock[17] had tested a number of neutral organic solutes on red blood cell preservation. It was not known whether neutral inorganic solutes would also protect. Experiments were performed to test this point. The methods used are described elsewhere.[15,16]

Results. — The results are summarized in Table 6. Only the data for optimum concentration of each compound are given. Twenty mice were used

Table 6. Thirty-Day Survival of Lethally Irradiated Test Mice After Infusion with 10^6 Nucleated Bone Marrow Cells Frozen and Thawed in Solutions of Inorganic Salts

Bone Marrow	Eosin-Negative Cells (%)	30-Day Survival (%)
None		0
Fresh	84	75
Frozen in saline	3	0
Frozen in Tyrode's solution	5	0
Frozen in NaI, 0.025 M	35	50
Frozen in NaBr, 0.006 M	16	55
Frozen in $NaNO_3$, 0.013 M	12	95
Frozen in $NaNO_2$, 0.05 M	12	5
Frozen in Na_2SO_4, 0.003 M	6	60
Frozen in NaSCN, 0.006 M	15	40
Frozen in $Na_2S_2O_3$, 0.1 M	6	35

at each of eight concentrations (0.006 to 0.4 M) of each compound. There were always concentrations other than the optimum that gave some survival. There were no survivors among the 210 mice used as X-ray controls, but 75% of the 288 control mice that received fresh bone marrow survived more than 30 days. Marrow frozen in either physiological saline or in Tyrode's solution gave no survivors out of 81 and 58 mice, respectively. Of the seven salts selected because they give neutral solutions, six (NaI, NaBr, $NaNO_3$, Na_2SO_4, $Na_2S_2O_3$, and NaSCN) gave fairly high 30-day

[17] J. E. Lovelock, Biochem. J. 56, 265 (1954).

survivals at some concentration, whereas the seventh ($NaNO_2$) gave low 30-day survival. The eosin uptake measurements appear to be of little value in assessing the recovery-promoting power of cells frozen in these solutions.

A small amount of the protective compound was of course injected into each test mouse along with the test cells. The dose of compound varied from a minimum of 0.056 mmole/kg up to 12.8 mmoles/kg. It is extremely unlikely that this quantity could have any marked effect on the test mice.

Discussion. — The ability of inorganic salts to act as protective agents for the preservation of bone marrow may not be of practical importance, since many thoroughly tested organic compounds are known. The present data may eventually be of value in formulating a general theory of the mechanism by which compounds protect mammalian cells during freezing and thawing.

Homologous Leukemoid Leukocytes in the Treatment of Radiation Lethality

L. H. Smith

Introduction. — Injection of homologous leukemoid leukocytes (HLL) into lethally X-irradiated mice improves 30-day survival in some strain or hybrid combinations[18] but not in others.[19] This report describes results of experiments with HLL.

Results. — Female (101 × C3H)F_1 mice were exposed to 950 r of X rays. After irradiation mice were given (1) no treatment, (2) homologous bone marrow (HBM) from BALB/c donors, (3) HLL from BALB/c donors, or (4) both HBM and HLL. Table 7 shows 30-day survival values for each group. The data show that HLL alone do not improve 30-day survival and that HLL + HBM negate the beneficial effects of HBM alone.

Discussion. — Goodman (this report) reported that HBM + homologous whole blood kills most irradiated recipients by day 25, suggesting a transplantation of immunologically competent cells (contained in whole

[18] R. M. Merwin, Proc. Soc. Exptl. Biol. Med. 101, 9–12 (1959).

[19] L. H. Smith and C. C. Congdon, Arch. Pathol. 63, 502–7 (1957).

Table 7. The Effect of Homologous Bone Marrow and Leukemoid Leukocytes (BALB/c) on Mean Death Time and 30-Day Survival of Lethally Irradiated (950 r) (101 × C3H)F_1 Mice

Treatment (BALB/c Donor)	Number of Mice	Mean Death Time (days)	30-Day Survival (%)
None	21	10.5	0
Bone marrow cells (10×10^6)	20	18.0	55
Leukemoid leukocytes (200×10^6)	25	11.0	0
Bone marrow cells (10×10^6) + leukemoid leukocytes (200×10^6)	16	11.6	0

blood) that form antibodies against host tissues. The same mechanism may be responsible for the killing effect of HLL + HBM observed in the present study.

Effect of Lymphatic Tissue Removal on Skin Homograft Survival

D. C. Swartzendruber R. R. Bigelow

Introduction. — Billingham et al.[20] reported that removal of regional lymph nodes draining a homograft site had no effect on survival of skin homografts in mice. It appears that lymphatic channels to the remaining lymph nodes are quickly reestablished and the residual lymphatic tissue causes the rejection. We have removed a much larger portion of the organized lymphoid tissue than reported above in attempts to modify the homograft reaction in mice.

Results. — A total of 104 adult (C3H × 101)F_1 mice received (C57L × A)F_1 skin homografts. Forty-eight of the above served as sham-operated or normal controls. The average homograft survival time for the control group was approximately ten days. The superficial lymph nodes and spleen were removed three to seven days after skin homografting in a second group of 41 mice. The range of skin homograft survival for the second group was

[20]R. E. Billingham, L. Brent, and P. B. Medawar, Proc. Roy. Soc. (London) B143, 58–80 (1954).

10 to 32 days; the average rejection time was 17 days. Fifteen animals were subjected to a more extensive lymphadenectomy in a third group. All the lymph nodes except thoracic and Peyer's patches were removed, plus the spleen. The range of homograft survival was 10 to 19 days, average rejection time 14 days, for the third group.

Extensive lymphadenectomy had little or no effect on the accelerated second set homograft rejection. Sixteen mice were tested.

Discussion. — Removal of a large portion of the lymphatic tissue has a mild effect on skin homograft survival in mice. The increase in survival is extremely variable. The results are interpreted to mean that very few immunologically competent cells are necessary for rejection of a skin homograft. They also indicate that transplantation antigens can easily get to the residual lymphatic tissue through quick regeneration of lymphatic vessels or through absorption into the blood stream.

RADIATION IMMUNOLOGY

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Quantitative Assay of Radiosensitivity of Antibody-Forming Cells

T. Makinodan W. J. Peterson

Introduction. – Studies on the effect of varying doses of X rays on antibody response have been limited in the past to use of intact animals. Extrapolation of the data obtained from such studies to characterize the relative radiosensitive status of antibody-forming cells, although commonly done, is valid provided that it can be shown that the number of these cells is not a variable. Thus a study was initiated to determine the radiosensitivity of antibody-forming cells relative to that observed among intact mice.

Results. – The model used was one we had devised previously,[4] in which heavily X-irradiated isologous mice are used as *in vivo* tissue cultures for a known number of spleen cells from treated donors. A linear $\log_2$ relation between antibody titer and nucleated spleen cell number was obtained; the cell dose ranged from 0.19×10^6 cells to 48×10^6 cells. Thus it is possible, for example, to detect the activity of only 2% of the 48×10^6 spleen cells. Subsequent studies on spleen cells from sublethally X-irradiated mice showed that 50, 90, and 98% of the cells are injured by 100, 300, and 500 r, respectively.

Discussion. – It was found previously that X-ray doses up to 700 r had no detectable effect (mean peak titer, mean total titer) on secondary

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[1]Visiting Investigator from Abroad.

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[4]T. Makinodan *et al.*, in *Proceedings of the Symposium on Mechanisms of Antibody Formation (Prague-Liblice, May 1959)*, in press.

antibody-responding mice.[5] Furthermore, 12×10^6 spleen cells were shown to be the minimum number of cells from preimmunized donors in lethally X-irradiated isologous recipients to give a response comparable to that observed among nonirradiated secondary responders.[6] Since 500 r can abolish 98% of the antibody-forming activity of spleen cells and since this dose of X rays has no detectable effect on intact secondary antibody responders, it would appear that there exist a minimum of $\sim 600 \times 10^6$ cells comparable to spleen cells in an intact preimmunized mouse.

Effect of Time and Route of Antigen Injection on Primary and Secondary Antibody Response After Transfer of Isologous Spleen Cells to Lethally Irradiated Recipients

E. H. Perkins M. A. Robinson

Introduction. – Previous studies[4,6,7] in which lethally irradiated mice were used as *in vivo* tissue cultures for quantitative study of antibody production demonstrated that antibody titer is directly related to the number of antibody cells participating in the "immune" response. The purpose of this study was to determine the optimum time and route of antigen injection in this model.

Results. – After transfer of either nonsensitized or sensitized isologous spleen cells and injection of antigen (1 ml of 1% sheep RBC) into lethally irradiated recipient mice, antibody titers were independent of the route (intravenous or intraperitoneal) of immunization; that is, the difference in titers did not appear to be significant, although the intraperitoneal route of antigen injection consistently gave higher titers than the intravenous route. The time of antigen injection after spleen cell transfer was critically important when nonsensitized spleen cells were transferred to the irradiated recipient. Maximum antibody response was obtained when antigen was injected immediately after transfer of spleen cells, that is, within 2 hr. Delay in antigen injection for 1, 2, or 5

[5]T. Makinodan and M. G. Tolbert, Biol. Semiann. Prog. Rep. Aug. 15, 1959, ORNL-2813, p 118.

[6]N. Gengozian, T. Makinodan, and I. C. Shekarchi, Federation Proc. 18, 568 (1959).

[7]E. H. Perkins and T. Makinodan, Radiation Research 11, 459 (1959).

days resulted in a delay in appearance of antibodies in the circulation and a marked reduction of mean total titer. In contrast, when sensitized spleen cells were transferred into irradiated recipients a comparable antibody response was obtained when antigen was injected 0, 1, 2, or 5 days after spleen cell transfer.

Discussion. – An important ancillary finding in this study was the difference in antibody response by nonsensitized and sensitized spleen cells to antigen at various time intervals after spleen cell transfer. This finding has given insight into the role and functional capacity(ies) of antibody-forming cells and potential antibody-forming cells when acted upon by physiological factors unrelated to antigenic stimulus. Clarification of optimum time and route of antigen injection now permits a study to determine the amount of antigen necessary to initiate antibody response as a function of the number of spleen cells transferred. With the establishment of this important factor, a study on the ability of an antibody-forming cell to respond to one or more antigens can be undertaken.

Transplantation of Heterologous Chimera Spleen Cells in Irradiated Recipients

N. Gengozian R. R. Carter W. J. Peterson

Introduction. – Transplantation of donor hematopoietic tissue (homologous or heterologous) in lethally irradiated recipient mice has been established by various investigators, who have positively identified donor-type erythropoietic, lymphopoietic, and myelopoietic elements in the circulation and tissues of the recipients injected with bone marrow. The contribution of donor vs host cells, however, to the immune mechanism of the irradiated recipients particularly with regard to the secondary disease phenomenon occurring in these animals is still under debate; that is, whether the host is reacting immunologically against the graft or the graft against its foreign host. The studies reported here are an attempt to resolve this problem in irradiated mice treated with bone marrow.

Results. – $(C3H \times 101)F_1$ mice were exposed to either 710 or 950 r total body irradiation and injected with 140×10^6 rat bone marrow cells. At various intervals after this treatment, the mice were splenectomized;

spleens were teased, made into a cell suspension, and injected into (a) lethally irradiated mice that had also received isologous bone marrow, or (b) midlethally irradiated rats. The latter animals were killed at intervals after treatment, and the serum was collected and analyzed for circulating antibodies to rat RBC or mouse RBC. In all cases, antibodies to rat RBC could be demonstrated in serum from mice that had received the transplanted chimera spleen cells; antibody activity to mouse RBC in the serum of irradiated rats receiving the chimera spleen cells was negative.

Discussion. — Demonstration of antibody activity to rat RBC and not mouse RBC by the transplanted chimera spleen cells further substantiates the thesis that a host-vs-graft reaction is occurring in heterologous chimeras.

Relation of Route of Sensitization to Challenging Dose on Active Anaphylactic Shock in Mice

P. Urso T. Makinodan

Introduction. — A general procedure for actively sensitizing mice to bovine serum albumin (BSA) in the induction of active anaphylactic shock is to inject a water-in-oil emulsion of the antigen (BSA-adjuvant) that includes killed tubercle bacilli intracutaneously, subcutaneously, intramuscularly, or intraperitoneally. The mice are subsequently challenged intraveneously with the antigen about 30 to 50 days later, when optimum fatal shock occurs. As yet the effect of route of sensitization on fatal anaphylactic shock in mice has not been reported. This report describes results of fatal anaphylactic shock obtained in mice challenged after sensitization to BSA-adjuvant by the subcutaneous (SC) and intraperitoneal plus subcutaneous (IP-SC) routes.

Results. — Twelve-week-old (C57BL × 101)F_1 mice were sensitized with 0.5 ml of BSA-adjuvant containing 20 mg of the antigen; 35–47 days later, they were challenged with 0.5 ml of varying doses of BSA by a single intravenous injection. Table 8 shows the results of three separate experiments. With IP-SC sensitization, mortality from anaphylactic shock is directly proportional to the challenging dose. Challenge with 20–100 mg resulted in 70% mortality, whereas challenge with 0.2 and 0.02 mg resulted

Table 8. Mortality of (C57BL × 101)F_1 Mice Challenged with Varying Doses of BSA After Sensitization to the Antigen by Two Different Routes

Challenging Dose of Antigen (mg)	Route of Sensitization	
	(IP-SC) (% Fatal Shock)	SC (% Fatal Shock)
100	71.4	20
50	70	0
20	70	0
10		0
4.7		0
2.45		14.4
2.0	40	30
1.4		10
0.2	25	70
0.02	16.6	50

in 25 and 16.6% mortality, respectively. On the other hand, in mice actively sensitized by a subcutaneous injection, mortality from anaphylactic shock is inversely proportional to the challenging dose. Only 2 of 24 mice died when challenged with 20–100 mg of BSA, but with 0.2 mg 70% died and with 0.02 mg 50% died.

Discussion. – There is a definite correlation between the challenging dose of antigen and the route of sensitization. With an increase in challenging dose an increase in the frequency of fatal anaphylaxis occurs when mice are sensitized via the IP-SC routes and a decrease in the frequency occurs after sensitization by the SC route. Work is now in progress to determine the relation of amount of circulating antibodies at the time of challenge, the route and dose of sensitization, and the challenging dose to fatal anaphylactic shock.

The Immune Status of Lethally Irradiated Mice Treated with Isologous and Homologous Adult Bone Marrow and Homologous Fetal Hematopoietic Tissue

N. Gengozian T. Makinodan I. S. Urso[8]

Introduction. – A previous study on the immune capacity of lethally irradiated mice treated with isologous, homologous, and heterologous adult bone marrow revealed a subnormal immune response among animals treated with the foreign marrow.[9] The present report is a study of the relative immune status of lethally irradiated mice that had received homologous fetal liver tissue as contrasted to those receiving homologous and isologous adult bone marrow.

Results. – Lethally (900 r) irradiated (C57L × A)F_1 mice were used as recipients of (a) homologous (101 × C3H)F_1 fetal liver, (b) homologous adult bone marrow, and (c) isologous adult bone marrow. One hundred days after treatment, RBC were 100% donor type. The long-term survivors (greater than 100 days) among the three different groups were then injected intraperitoneally with 1 ml of 1% rat RBC, sacrificed 18 days later, and the serum collected for antibody titration. The $\log_2$ titer for circulating agglutinins in the three groups and a normal control group were as follows: (a) fetal homologous, 4.0, (b) adult homologous, 1.6, (c) adult isologous, 4.8, and (d) normal (C57L × A)F_1, 7.4.

Discussion. – The data show that the immune response among mice treated with homologous fetal hematopoietic tissue is comparable to those treated with adult isologous bone marrow and significantly greater than that obtained in mice treated with adult homologous bone marrow. It may be noted, however, that the responses of the former two groups appear to be significantly less than the normal control group of mice, suggesting that transplantation of a compatible graft in the irradiated recipient does not necessarily connote a normal physiologic status.

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[8]Formerly, Mammalian Radiation Recovery.
[9]N. Gengozian *et al.*, Proc. Natl. Acad. Sci. U.S. *44*, 560–65 (1958).

A New Method for the Study of Hematopoietic Transplantation Antigens

F. Celada T. Makinodan

Introduction. — In simplicity, sensitivity, reproducibility, and quantitative evaluation of the antigenic stimuli of homologous hematopoietic transplant in mouse, none of the methods used previously was considered satisfactory. For this reason a new model has been developed that seems to be highly sensitive and reproducible.

Results. — A known number of spleen cells from homologous donors are injected intravenously into X-rayed recipients (800 r). The test antigen (sheep RBC) is injected intraperitoneally immediately, and the animals are bled six days later for antibody titration. The donor mice are preimmunized with the antigen two weeks before the removal of their spleens. The data show a linear relation between antibody titer and the amount of injected spleen cells. No response was detected in the controls that received only X rays and the antigen. The range of linearity extends from 1.5×10^6 to 96×10^6 cells. Thus, for example, it should be possible to detect the activity of as high as 99% of the injected cells. If the recipient animal is sensitized with donor-type bone marrow cells in 12, 6, or 3×10^6 nucleated cells ten days before irradiation and spleen cell transfer, no antibody-forming activity is detected, indicating a complete failure of take, or at least inhibition of protein-forming activity of the transferred cells (Fig. 5).

Discussion. — The data concerning the dose of immunization of the recipient with intact donor bone marrow cells indicate that the minimal amount of sensitizing cell is less than 3×10^6 cells. When the critical dose is established, it will be possible to screen for transplantation antigens from donor cell extracts. The antibody-forming activity should vary inversely with the relative antigenic potency of the donor material.

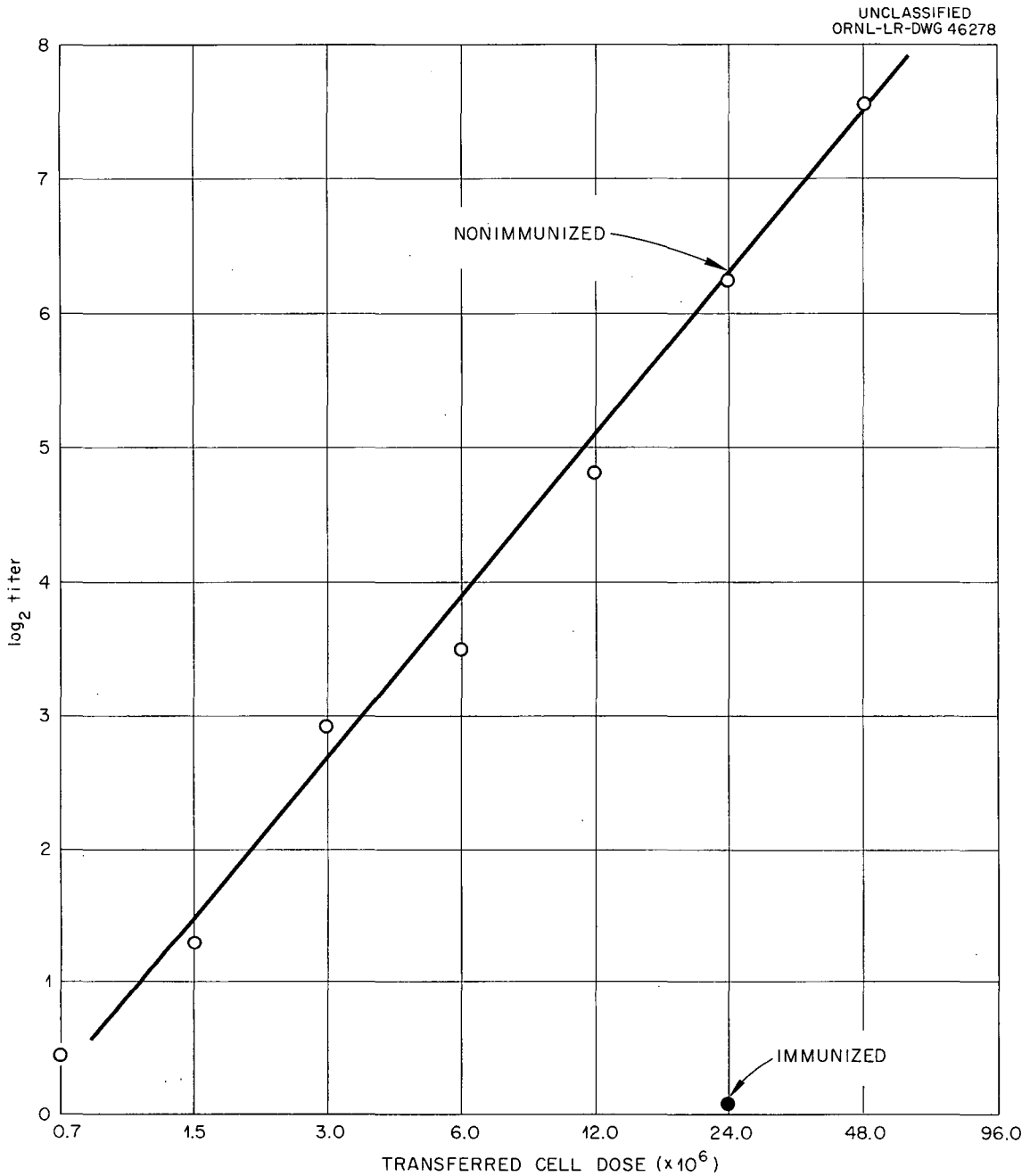

Fig. 5. Antibody-Forming Capacity of Homologous Spleen Cells as Affected by Pretreatment of Recipients.

PATHOLOGY AND PHYSIOLOGY

A. C. Upton, Section Chief

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Lifespan of Blood Platelets in Mice

T. T. Odell, Jr. T. P. McDonald

Introduction. — The aim of these experiments was to determine the lifespan of blood platelets in the peripheral blood of the mouse. Little information is available about mouse blood platelets, although this species is widely used in experimental work.

Methods and Results. — Male BDF_1 mice were injected daily by the intraperitoneal route with 1 μc of S^{35}-labeled sulfate per gram, the object being to maintain a relatively constant body pool of the isotopic compound. In the last two of five experiments, 0.6 μg of cold carrier sulfate per microcurie of $S^{35}O_4$ was added to the injection solution. Platelets were separated from the blood of serially killed animals, numerical and radioactivity counts were made on platelet suspensions, and the average activity per platelet was calculated from these values. The radioactivity of platelets separated from peripheral blood rose rapidly during the first four days and was erratic thereafter without a definite upward or downward trend, but with a wide variation (Fig. 6). The mean value of platelet radioactivity of all mice sampled on days 5 through 10 was 441×10^{-10} counts/min/platelet. The daily rate of increase to this

[1]Consultant.
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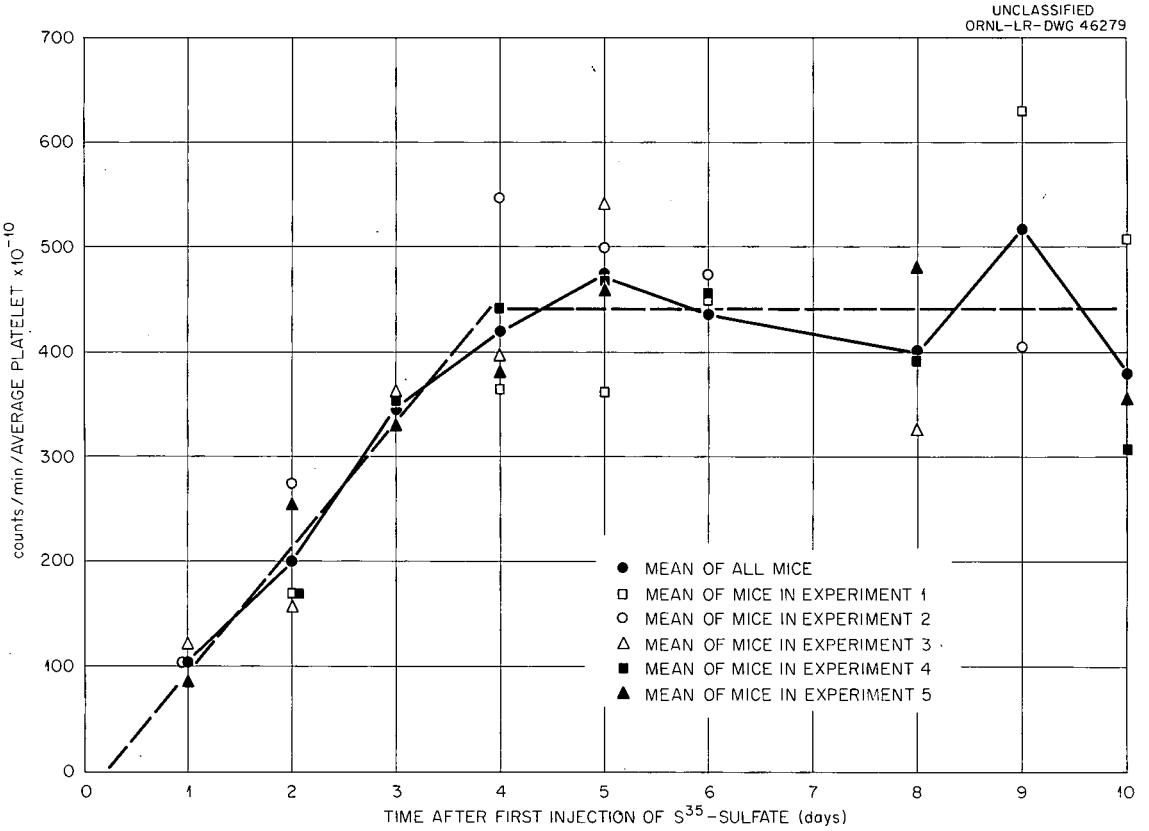

Fig. 6. Radioactivity of Platelets of Mice Injected Daily with S^{35}-Sulfate.

level during the first four days was about 120×10^{-10} counts/min/platelet, or 27.2%. The plasma activity remained relatively constant throughout the experimental period, especially in later experiments when cold carrier sulfate was added.

Discussion. — In similar experiments in rats, the radioactivity of platelets increased for several days, as in the present experiments, and remained constant thereafter. The initial period of increase suggested to us a gradual replacement of unlabeled platelets in the peripheral blood by newly labeled platelets from hemopoietic tissue, and as a corollary that platelet labeling occurred in hemopoietic tissue before release of platelets into the peripheral circulation. The later plateau suggested an equilibrium; that is, replacement of previously labeled platelets by newly labeled ones, and thus no further increase in total platelet activity. Although there was great variation among animals in the mouse experiments, the existence of a plateau of platelet radioactivity like that

in the rat seems likely. According to the results, the daily rate of increase of radioactivity in the peripheral platelet mass was 27.2% per day, from which may be derived an estimate of platelet lifespan of 3.7 days.

Coleukemogenic Effects of Myleran on Lymphoma Development in Irradiated RF Mice

E. P. Sniffen W. D. Gude

Introduction. — Male RF mice were injected with Myleran before or after whole-body irradiation in a study of the influence of bone marrow cellularity on susceptibility to leukemia induction.

Results. — Myleran increased the yield of thymic lymphomas in irradiated mice, depending on the time of injection, but decreased the yield of myeloid leukemias correspondingly (Table 9). No significant

Table 9. Leukemia Incidence in Myleran-Injected Mice

X-Ray Dose[a] (r)	Other Treatment[b]	Number of Mice[c]	Leukemia Incidence (%)	
			Myeloid	Thymic Lymphoma
150	Myleran 15 days before irradiation	73	6	29
150	Myleran 5 days before irradiation	74	34	26
150	Myleran 5 days after irradiation	73	12	22
150	Vehicle 5 days after irradiation	79	30	5
300	Myleran 15 days before irradiation	71	4	38
300	Myleran 5 days before irradiation	70	22	34
300	Myleran 5 days after irradiation	78	26	15
300	Vehicle 5 days after irradiation	73	31	16
0	Myleran alone	66	3	9
0	Vehicle alone	75	5	3

[a]250 kvp, 0.44 mm of Cu hvl, 80–90 r/min.

[b]Myleran, 0.4 mg/mouse, intravenously in dimethyl acetamide vehicle.

[c]Irradiated at ten weeks of age.

effect on the leukemia incidence in nonirradiated mice was evident.

Discussion. — Because of its myelotoxic properties, Myleran was expected to enhance the induction of myeloid leukemias instead of lymphomas. The basis for the observed results is therefore puzzling, particularly since Myleran has now been found to exert minimal effects on thymic weight. Further study of the action of Myleran on the turnover of cells in the thymus and lymph nodes is in progress.

Protein Metabolism in the Rat-Mouse Chimera

W. Friedberg V. K. Jenkins O. V. Müderrisoğlu

Introduction. — A greater fractional rate of loss of injected labeled protein (HSA-I^{131}) was noted in 950 r-RBM mice (mice inoculated with rat bone marrow after 950 r) than in similarly irradiated mice protected with leg shielding.[3] This difference has been investigated further to determine the time at which it can first be detected and its pathogenesis.

Results and Discussion. — As early as 12 days after irradiation the fractional rate of loss of HSA-I^{131} is greater in 950 r-RBM mice than in those treated with isologous bone marrow (950 r-IBM mice) but no such difference has been observed between four and seven days after treatment. Increased catabolism may account for the elevated fractional rate of protein loss in 950 r-RBM mice, but another possibility — leakage of protein through the wall of the gastrointestinal tract and subsequent breakdown or excretion — is also under investigation. After intraperitoneal injection of HSA-I^{131}, small amounts of nondialyzable radioactivity are detected in the pooled 24-hr feces of both normal controls and 950 r-RBM mice, but neither the amounts nor the differences between the two groups appear significant. In studies with I^{131}-labeled polyvinylpyrrolidone (PVP-I^{131}), the feces of 950 r-RBM mice were more radioactive than those of normal control mice, suggesting increased permeability of the intestinal or gastric wall or loss via the bile duct. However, the significance

[3]W. Friedberg and D. E. Reynolds, Biol. Semiann. Prog. Rep. Aug. 15, 1959, ORNL-2813, p 132–34.

of the PVP-I^{131} results with respect to protein loss depends on the molecular size of the PVP in the feces and on the role of the liver in the excretion of PVP and protein.

Long-Term Survival of Mice Protected with Isologous Bone Marrow and AET

G. E. Cosgrove
C. C. Congdon[4]
D. G. Doherty[5]
A. W. Kimball[6]
M. L. Davis

Introduction. – Protection of female (101 × C3H)F_1 mice against 1100–1900 r of X radiation by AET, isologous bone marrow (IBM), or AET and IBM together was reported previously.[7] Since that time groups exposed to 700 r of 250-kvp X rays (~ $LD_{25/30}$) have been similarly treated and observed throughout life.

Results. – In agreement with the earlier results, AET and bone marrow apparently gave some degree of protection against life shortening and leukemia induction (Table 10), although the effects are probably not significant statistically.

Discussion. – Although the survival time was increased by AET and bone marrow, the degree of dose reduction was not so great as that observed earlier at high radiation dose levels. Whether this denotes that the protective efficacy of AET is influenced by the dose of radiation or other factors remains to be elucidated. As in the earlier experiment, little protection against nephrosclerosis was afforded by AET, suggesting that the kidney is not protected despite a high relative concentration of AET in this organ. This paradox calls for further study of the microscopic distribution of AET within kidney by means of microautoradiography.

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[4]Mammalian Radiation Recovery.

[5]Chemical Protection and Proteolytic Enzyme Studies.

[6]Biometrics.

[7]G. E. Cosgrove et al., Biol. Semiann. Prog. Rep. Feb. 18, 1958, ORNL-2481, p 44.

Table 10. Incidence of Delayed Radiation Effects in Mice Protected with AET and IBM

X-Ray Dose (r)	Protective Treatment	Number of Mice	Median Survival (months)	Thymic Lymphoma (%)	Total Leukemia (%)	Nephrosclerosis (%)
0	None	149	28.5	0.7	5	1
700	None	69	18.5	3	10	13
700	IBM*	89	19.5	0	0	13
700	AET**	84	20	1	5	8
700	AET,** IBM*	89	20	0	4	11

*Isologous bone marrow cells, 1 femur-equivalent, injected intravenously soon after irradiation.

**S,2-Aminoethylisothiuronium bromide hydrobromide, 9.0 mg, injected intraperitoneally a few minutes before irradiation.

Studies on the Mouse Hemoglobin Locus

R. A. Popp W. St. Amand[8]

Introduction. — Studies of differences in mouse hemoglobins[9,10] have been extended to explore the position of the hemoglobin locus in the mouse genome.

Results. — Hemoglobins were classified as either diffuse or single, on the basis of their patterns after electrophoresis in starch gels. Strain NB (p c^{ch} with single hemoglobin) and SeAB (P C with diffuse hemoglobin) mice were mated to obtain animals crossed to p c^{ch} mice with single hemoglobin for coupling analyses. Similarly, F_1 progeny of BALB/c, CFW, or FU (with diffuse hemoglobin) and C57BL/6 (with single hemoglobin) mice were crossed to p c^{ch} mice with single hemoglobin for repulsion analyses. A total of 1065 progeny were classified for p, c^{ch}, and hemoglobin traits.

[8]University of Mississippi, University, Miss.

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

[10]R. A. Popp and G. E. Cosgrove, Proc. Soc. Exptl. Biol. Med. 101, 754–58 (1959).

The recombination frequency between albinism and hemoglobin was 2.49 ± 0.52 in males and 5.13 ± 0.58 in females. A three-point analysis indicated that the linear order of the loci was p-c-hemoglobin. It was further noted that single and diffuse hemoglobins exist in a population of wild mice sampled.

Discussion. — Presumably each hemoglobin fraction found in mice with genetically determined diffuse hemoglobin is controlled by different genetic units, since the molecules differ in at least some physicochemical properties, for example, electrophoretic mobility and solubility. Genes for the diffuse hemoglobin pattern are probably very tightly linked, however, since recombinant hemoglobin patterns have not as yet been found. Establishment of the position of the hemoglobin locus in linkage group I makes it an especially useful genetic marker since, with the use of solubility analyses,[10] even mice heterozygous for different hemoglobins can be classified for hemoglobin type. The study also suggests that the hemoglobin differences found in laboratory mice were directly derived from similar differences in populations of wild mice rather than by mutations during captivity.

Mortality and Changes in Irradiated F_1 Hybrid Mice Injected with Parental Adult Liver Cells

G. Cudkowicz G. E. Cosgrove

Introduction. — Since the foreign spleen reaction was inhibited when donor-type spleen cells were exposed *in vitro* to recipient-type liver cells,[11] the effect of implanting liver cells alone under the same conditions has been investigated.

Methods and Results. — Young adult (C57BL × 101)F_1 mice were injected intraperitoneally or intravenously with C57BL or 101 fresh liver-cell suspensions (approximately 1/4–1/10 liver equivalent) within 2–3 hr after whole-body exposure to 600 or 900 r of 250-kvp X rays; the groups receiving 900 r were also inoculated intravenously with isologous or parental bone marrow (10×10^6 nucleated cells). Liver-cell suspensions were derived from perfused and unperfused tissue. Within two to three weeks after implantation of parental liver cells a fatal disease developed (Fig. 7), and

[11] G. E. Cosgrove *et al.*, Proc. Soc. Exptl. Biol. Med. 102, 525–27 (1959).

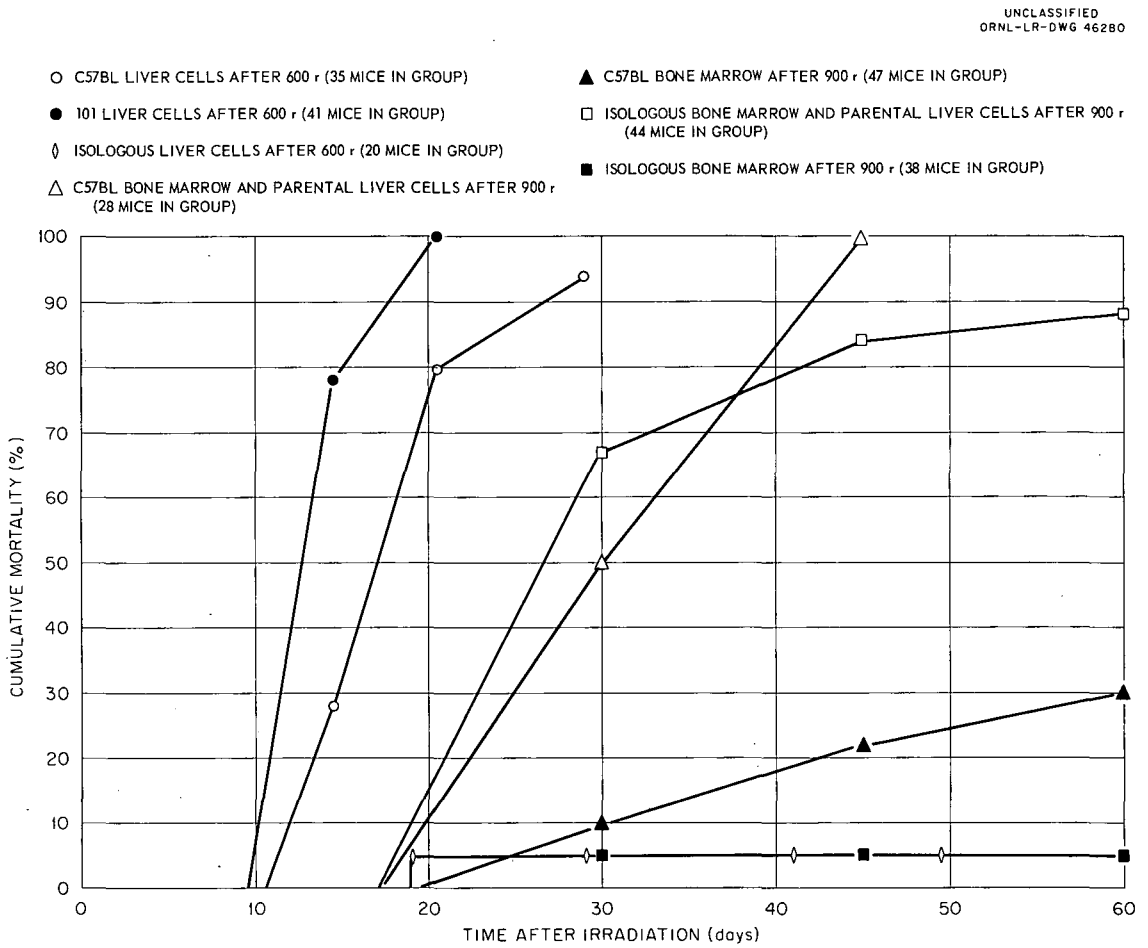

Fig. 7. Mortality in Irradiated (C57BL × 101)F_1 Mice Injected with Parental Liver Cells.

was only delayed by simultaneous injection of isologous or parental bone marrow. The disease was characterized by histologic changes similar to those observed in the foreign spleen reaction, with replacement of the lymphoid follicles by proliferating reticuloendothelial cells. No mortality or related histological changes have been observed in irradiated F_1 hybrids given isologous (F_1) liver cells or liver cells from irradiated (950 r) parental donors, in nonirradiated F_1 hybrids given parental liver cells, or in irradiated C57BL mice (500 r) or 101 mice (550 r) given F_1 hybrid liver cells.

Discussion. – The fatal reaction caused by implantation of adult parental liver cells in irradiated (C57BL × 101)F_1 mice is ascribed to an

immune reaction of immunologically competent grafted cells against the recipient. The identity of these cells remains to be established.

Late Somatic Effects of Atom-Bomb Radiation in Mice Exposed at Operation Greenhouse

A. C. Upton
A. W. Kimball[12]
K. W. Christenberry
W. H. Benedict, Jr.

Introduction. — Results of this experiment, some of which were mentioned in a preliminary report,[13] have now been prepared for final publication in the open literature.

Results. — The major observations are as follows: Male and female (C57L × A/He)F_1 mice surviving instantaneous exposure to ionizing radiation from an experimental nuclear detonation were observed until natural death for delayed effects of irradiation. Among the effects observed was shortening of the lifespan, which varied with the radiation dose. The shortening of life was not attributable to increased mortality from any specific cause but was correlated with premature onset of all diseases associated with natural senescence. Although the effects of radiation on the incidence and severity of diseases of old age varied markedly from one disease to another, all diseases were advanced in onset to essentially the same extent by any one dose of radiation, with the exception of thymic lymphoma. The onset of thymic lymphoma was hastened considerably more than that of any other disease, particularly in heavily irradiated mice, which also had an elevated incidence of this neoplasm. There was no consistent over-all relation between the frequency of neoplasia and the radiation dose. The incidence of certain neoplasms (thymic lymphoma, adenocarcinoma of mammary gland, pituitary adenoma, adrenal adenoma, Harderian gland adenoma, hepatoma, ovarian tumor, and granulocytic leukemia) was increased by irradiation, whereas the incidence of others (pulmonary adenoma, mammary gland sarcoma, nonthymic lymphoma) was decreased. In no instance

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[12]Biometrics.

[13]K. W. Christenberry et al., Biol. Semiann. Prog. Rep. Feb. 15, 1954, ORNL-1693, p 49–53.

was there a linear relation between tumor incidence and dose. Most neoplasms were less common after large doses of radiation than after intermediate doses, suggesting that neoplasia was inhibited by excessive radiation injury. Depigmentation of the hair, which was detected as early as three months after irradiation, progressed at a rate and to an extent that varied with the dose, but it was not observed in lightly irradiated mice or in the controls. Cataract of the optic lens, which was also noted within 90 days after irradiation, progressed at a rate and to an extent that varied with dose. The radiation cataracts differed in location from the opacities occurring spontaneously in senile controls. Atrophy of the iris, which developed spontaneously in senescent controls, occurred prematurely in irradiated mice and progressed in severity with the dose. Radiation nephritis, or nephrosclerosis, was common in mice receiving more than 400 rads but was rare below this dose level. In a few mice with advanced nephrosclerosis, polyarteritis was noted in the kidney and elsewhere. Miscellaneous infectious and inflammatory lesions, which were relatively rare in controls, were not increased in frequency by irradiation. Subcapsular ovarian cysts and hydrometra, which were relatively common in aging controls, were reduced in incidence in irradiated females, possibly through sterilization of the ovary. Loss of incisor teeth occurred in a relatively high proportion of aging males and was nearly ten times as common in males as in females. Its frequency was not significantly affected by irradiation. For most of the effects observed, neutrons were more effective than gamma rays. Because, however, of uncertainties in dosimetry and the relatively small numbers of neutron-exposed animals, precise estimation of the relative effectiveness cannot be made from the results of this experiment.

Discussion. — The wealth of information about delayed somatic effects of radiation supplied by this investigation is of great theoretic as well as practical value, and because of the significance of this information, the study would appear to have amply justified its enormous cost. Not only were many of the effects observed hitherto unrecognized, but the results obtained have far-reaching implications in the fields of radiobiology, cancer, and gerontology, and will be helpful in guiding future research.

CELL PHYSIOLOGY

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H. E. Bond[1]
B. T. Cole[2]
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R. E. Canning
J. A. Levinson
C. B. Norris
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H. D. Swanson[4]

Effects of Products Formed After Ultraviolet Irradiation of Methyl Linolenate on Mouse Cells and Cells of *Allium cepa*

B. T. Cole
N. G. Anderson
E. H. Y. Chu*
D. Davidson**

Introduction. – This study was initiated in an effort to separate various substances formed as a result of UV irradiation of pure methyl linolenate by gas liquid chromatography, to correlate these materials with the thiobarbituric acid test, and to assay them for toxicity. Fatty acid was irradiated with an AH4 lamp without filter, operating at 110 v and 5 amp. Irradiated material was passed through the Aerograph GLC column packed with diethyleneglycol succinate and separated into three peaks. Several aliquots of 2 μl each were passed through, and material from the peaks was trapped in liquid nitrogen as distinct fractions. The material was diluted in 2 ml of 5% ethyl alcohol and added to tissue culture cells, which were harvested at 1-1/2, 3, 6, and 24 hr.

Results. – The amounts of color formed on incubation with thiobarbituric acid (i.e., TBA value) were 0.026, 0.029, 0.026, and 0.014 for material collected from peaks 1, 2, 3, and 4, respectively. Peak 2 material clearly showed a mitotic inhibition effect and deeply staining cytoplasm plus an atypical "network" kind of cell growth beginning at 3 hr.

Additional preparations were made for studies on chromosome breakage by using both fatty acid ester and a tissue containing large amounts of

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unsaturated fatty acids. Aliquots of linolenate and brain tissue were irradiated for 1 hr 15 min, extracted with water, and allowed to stand overnight in the refrigerator. The aqueous extracts of irradiated methyl linolenate were diluted as shown in Table 11, and thiobarbituric acid values determined. Aqueous extracts of irradiated material were used to treat root tips of Allium cepa. The cytological effects are being studied.

Table 11. TBA Values of Various Aqueous Dilutions of Materials Formed by Irradiation of Pure Linolenate and Brain Homogenate

Material	Dilution	TBA Value
Unirradiated linolenate	1.5–10	0.038
Irradiated linolenate	1.5–10	0.510
Irradiated brain homogenate	1.5–10	0.050
Irradiated linolenate	10–20	0.218
Irradiated linolenate	10–50	0.093
Irradiated linolenate	10–100	0.068
Irradiated linolenate	10–150	0.054
Irradiated linolenate	10–250	Negative

Discussion. – The toxic effects of materials formed by UV irradiation of pure, unsaturated fatty acids, demonstrated by Wilbur et al.[5] in eggs of lytechinus, may be associated with factors separable by gas liquid chromatography. The reduction in germination, mitotic aberrations, chromosome breakage, and appearance of viable mutations found by Swaminathan et al.[6] in seeds soaked in peanut and mustard oils may well be attributable to substances formed by degradation of highly unsaturated fatty acids upon incubation at room temperature.

Separation of Lipid Components of Rat Liver Microsomes

B. T. Cole N. G. Anderson

Introduction. – This study was initiated in order to determine lipid constituents of microsome fractions separated from rat liver cells by standard procedures.

[5]K. M. Wilbur et al., Anat. Record 120, 708 (1954).

[6]M. S. Swaminathan and A. T. Natarajan, J. Heredity 50, 177–87 (1959).

Results. – Microsomes were extracted in cold 95% ethyl alcohol overnight. Unfilterable material was further extracted with hot alcohol in a Soxhlet apparatus for 3 hr, and the resultant ether-soluble materials from both extractions were pooled. Lipids were separated into eight major classes (Fig. 8) by gradient elution from a silicic acid column according to a method described by Hirsch and Ahrens;[7] 2372 mg of microsomes yielded a total lipid of 711.7 mg, thus the fraction proved to be 30% of the total mass. Of the lipid fraction, 4% appeared to be esters of cholesterol, 9% triglycerides, 5% nonesterified fatty acids, 5% unesterified cholesterol,

[7]J. Hirsch and E. H. Ahrens, Jr., J. Biol. Chem. 233, 311–20 (1958).

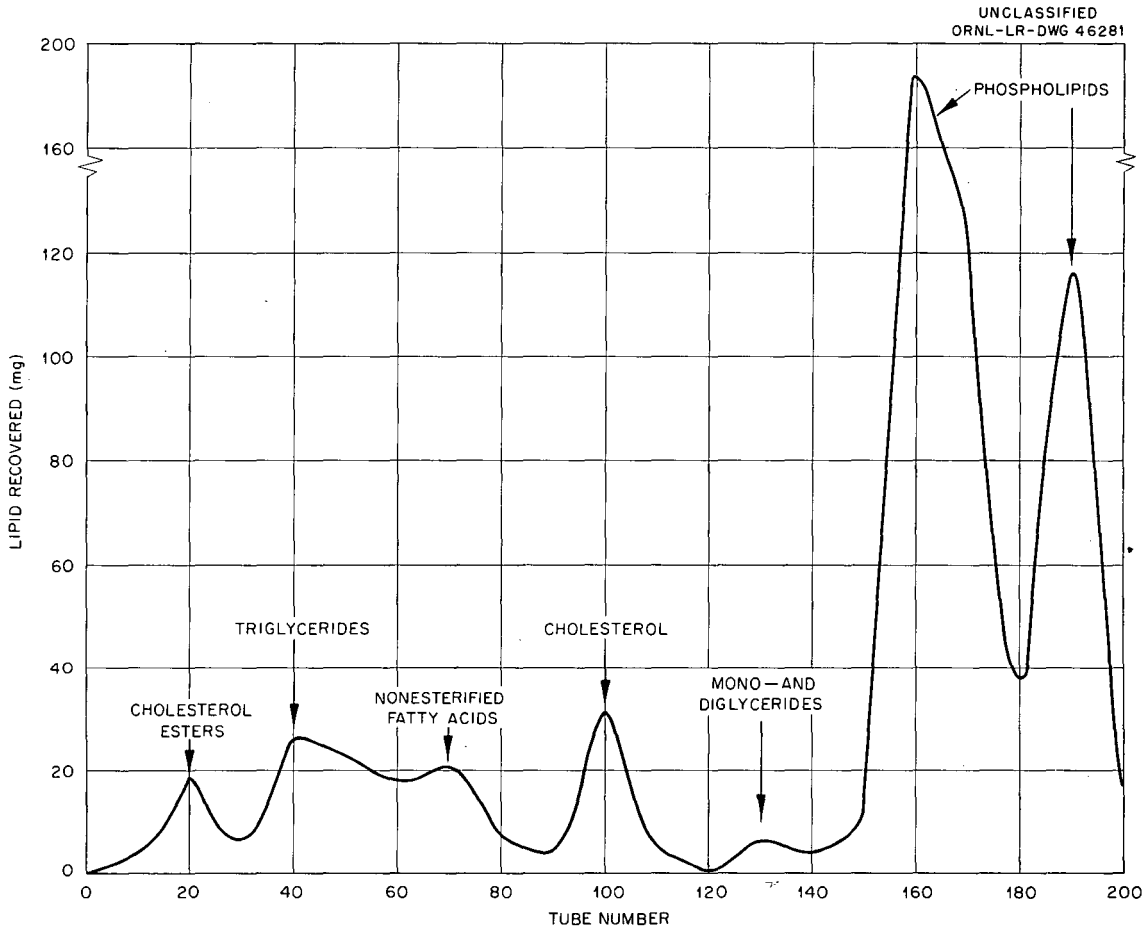

Fig. 8. Lipids Recovered from Microsome Fraction of Liver Cells. Silicic acid column. Microsomes extracted with hot alcohol (Soxhlet) and then eluted from column with ether, solutions of petroleum ether and ethyl ether, and methanol. Total lipids, 711.7 mg.

2% mono- and diglycerides, and 75% phospholipids. The phospholipids came off the column in two distinct peaks. Separations into cephalins, lecithins, and sphingomyelins have not been accomplished.

Discussion. – Similar separations followed by analysis of constituent fatty acid components by gas liquid chromatographic techniques are being carried out. We hope in this manner systematically to analyze various cell fractions for quantitative amounts of lipid constituents from normal, experimental, and pathological tissues.

Separation of Fatty Acids and Products Formed by Ultraviolet Irradiation of Linolenate by Gas Liquid Chromatography

B. T. Cole

Introduction. – This study represents an effort to characterize the peroxide products of fatty acid autoxidation in terms of their peak characteristics after gas liquid chromatography.

Results. – An Aerograph gas chromatographic analysis unit has been installed and utilized for the separation of pure methyl esters of linoleic, linolenic, and oleic acids by a diethyleneglycol succinate packing in a 7-1/2-ft, stainless steel, coiled column of 1/4 in. internal diameter. Retention times for linolenate, linoleate, and oleate were calculated to be 6 min 56 sec, 5 min 18 sec, and 1 min 58 sec, respectively, with column operating at 205°C, flow rate of 85 ml/min, filament current 265 ma, and chart speed of 1 in./min (see Fig. 9).

One gram of linolenate was irradiated for 1 hr, and 2-μl samples were injected into the column. After irradiation, five peaks appeared, and retention times were measured to be as follows: peak 1, 17 sec; peak 2, 30 sec; peak 3, 40 sec; peak 4, 2 min 20 sec; peak 5 (apparently unaltered linolenate), 7 min 52 sec. In the analysis of irradiated material, the column was operating at a temperature of 210°C, flow rate of 67 ml/min, filament current 265 ma, and chart speed of 1 in./min.

Discussion. – A more sensitive instrument having an ionization chamber type detection system has been constructed and will be available for use in the near future. We hope that other peaks will appear, and that they may be separated more discretely and trapped in liquid nitrogen in sufficient quantity to carry out various tests for toxicity.

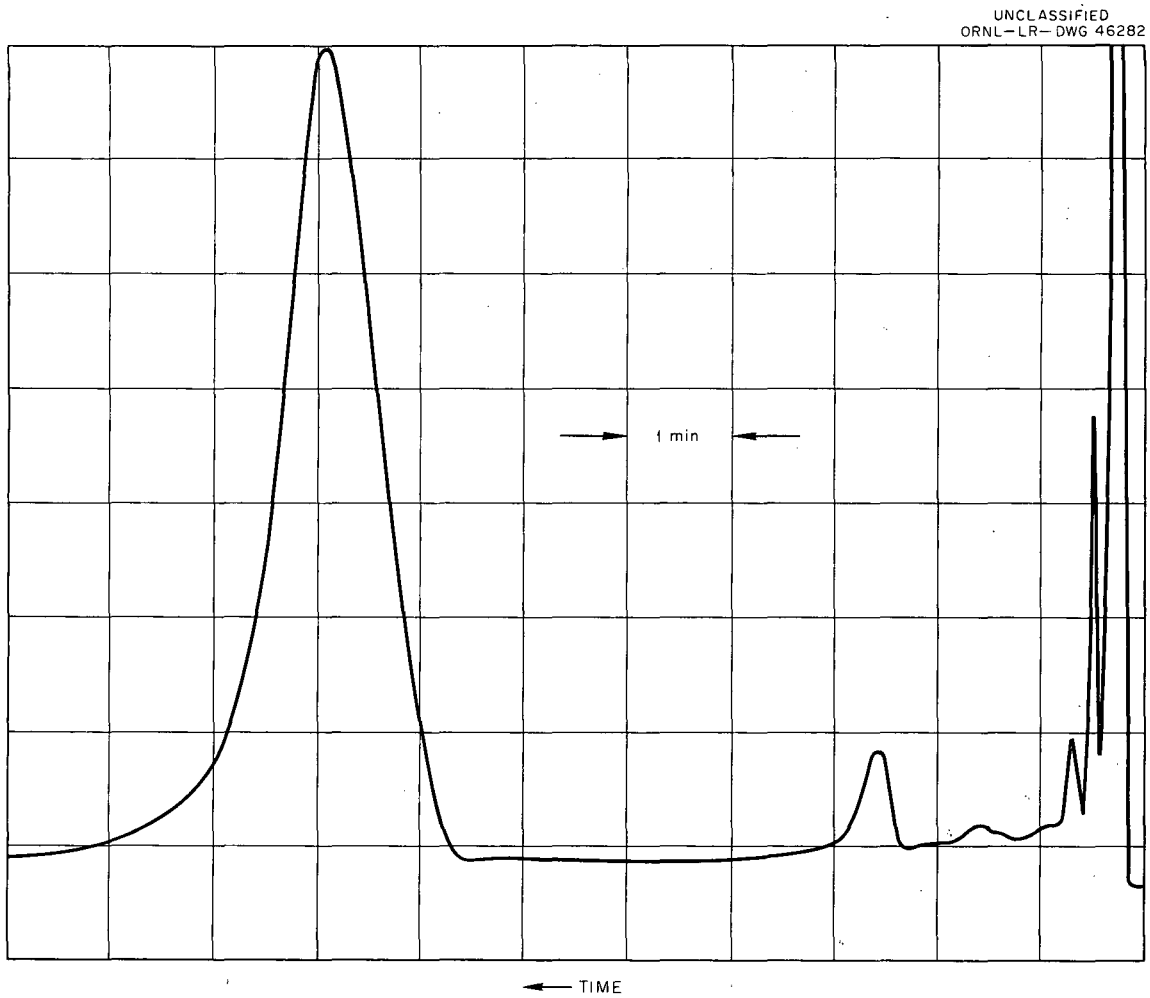

Fig. 9. Gas Chromatogram of Irradiated Linolenate. Column packing, diethyleneglycol succinate; temperature, 210°C; flow rate, 67 ml/min; filament current, 265 ma; chart speed, 1 in./min.

Isolation and Characterization of Soluble Tissue Proteins

H. E. Bond N. G. Anderson

Introduction. – This investigation was initiated for the purpose of isolating and characterizing the soluble proteins from normal tissues. Rat liver proteins are being used at present, but the methodology, when satisfactorily completed, will be applied as far as practicable to all other tissues. Emphasis is being placed on the use of the cellulose cation- and anion-exchange materials to accomplish the initial separation of the

proteins. When it seems feasible, additional methods of protein fractionation will be used. The isolated components are characterized by their physical, chemical, and enzymatic properties; they will be coded accordingly for the purpose of classification. The amino acid composition will be determined, and antibodies will be prepared against the materials for use in further studies.

Results. — To the present time, the study has been confined to perfecting the necessary methods. A gradient-elution system has been established for DEAE-cellulose that will allow separation of rat-liver soluble proteins into 13 identifiable components containing 86.2% of the nitrogen placed onto the column. Ultraviolet spectrophotometry is used to identify the different components on the chromatogram. Since an area of the chromatogram has not yet been resolved, the gradient system is undergoing further refinement. Preliminary investigations have been started to characterize the proteins in a poorly resolved basic fraction from the DEAE-cellulose chromatogram. The electrophoretic mobilities have been obtained for three components in this fraction, and the sedimentation coefficients have been obtained for two components. Experiments are currently being conducted to establish a satisfactory gradient-elution schedule for CM-cellulose, with which we hope to further resolve the basic fraction.

Discussion. — Work from this laboratory has indicated that differences in nuclear size found in the various tissues of an animal may be caused by a variation in cellular basic proteins. The basic proteins from the rat liver have been isolated as a group and are presently being resolved into individual components. When this is completed, the specific protein or proteins responsible for the effect can be fully characterized, and the mechanism more fully understood.

Effects of Subzero Temperatures on Cells

P. Mazur

Introduction. — Low temperatures are receiving increasing attention in diverse areas of biology, ranging from cytology where they are used to fix tissues[8] to radiation biology where they have been used to study the

[8]N. Feder and R. J. Sidman, J. Biophys. Biochem. Cytol. 4, 593–602 (1958).

relation between radiation effects and the state of cellular water.[9] But the chief applications have been in the long-term preservation of cells and tissues.[10] In spite of the rather wide and varied interest in low temperatures, little is known of how they affect cells at a subcellular and a molecular level, and little is known of the physical and biological factors that determine whether or not a cell survives such exposure. The purpose of the present investigation, then, is to determine these effects and to elucidate the underlying biological and physical factors.

Results. – Attention has been concentrated on how the survival of microorganisms is related to physical and temporal factors, such as the rate of cooling, minimum temperature of exposure, rate of warming, and physical state of the surrounding water.[11] All these factors have marked effects on yeast, the organism of current interest. Particularly striking are the influences of temperature and the physical state of water, as indicated in Fig. 10. Survivals remain close to 100% whenever the extracellular water is unfrozen or when it is frozen but the cells are not cooled below -10°C. In contrast, exposure to -30°C or lower for periods of 1 min or less results in 1% survival or less. Survival is also importantly affected by the rate at which the cells are cooled and the rate at which they are warmed. Most harmful are rapid cooling (50°C/min) and slow warming (1°C/min); most beneficial are slow cooling (1°C/min) and rapid warming (500–1000°C/min).

Discussion. – These findings and others seem to be best accounted for by the following hypothesis: that death is associated with intracellular ice formation. According to this hypothesis, intracellular freezing occurs only below -10°C (the cell remaining supercooled above this temperature) and then only when the extracellular water is frozen. Extracellular freezing does not by itself cause death but it does somehow seem to initiate intracellular freezing below a certain critical temperature. The investigator has suggested[11] that the external ice does this by growing through

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[9]T. H. Wood and A. L. Taylor, Radiation Research 6, 611–25 (1957).

[10]A. U. Smith, in Biological Applications of Freezing and Drying, ed. by R. J. C. Harris, Academic Press Inc., New York, 1954.

[11]P. Mazur, Ann. N.Y. Acad. Sci. (1960, in press).

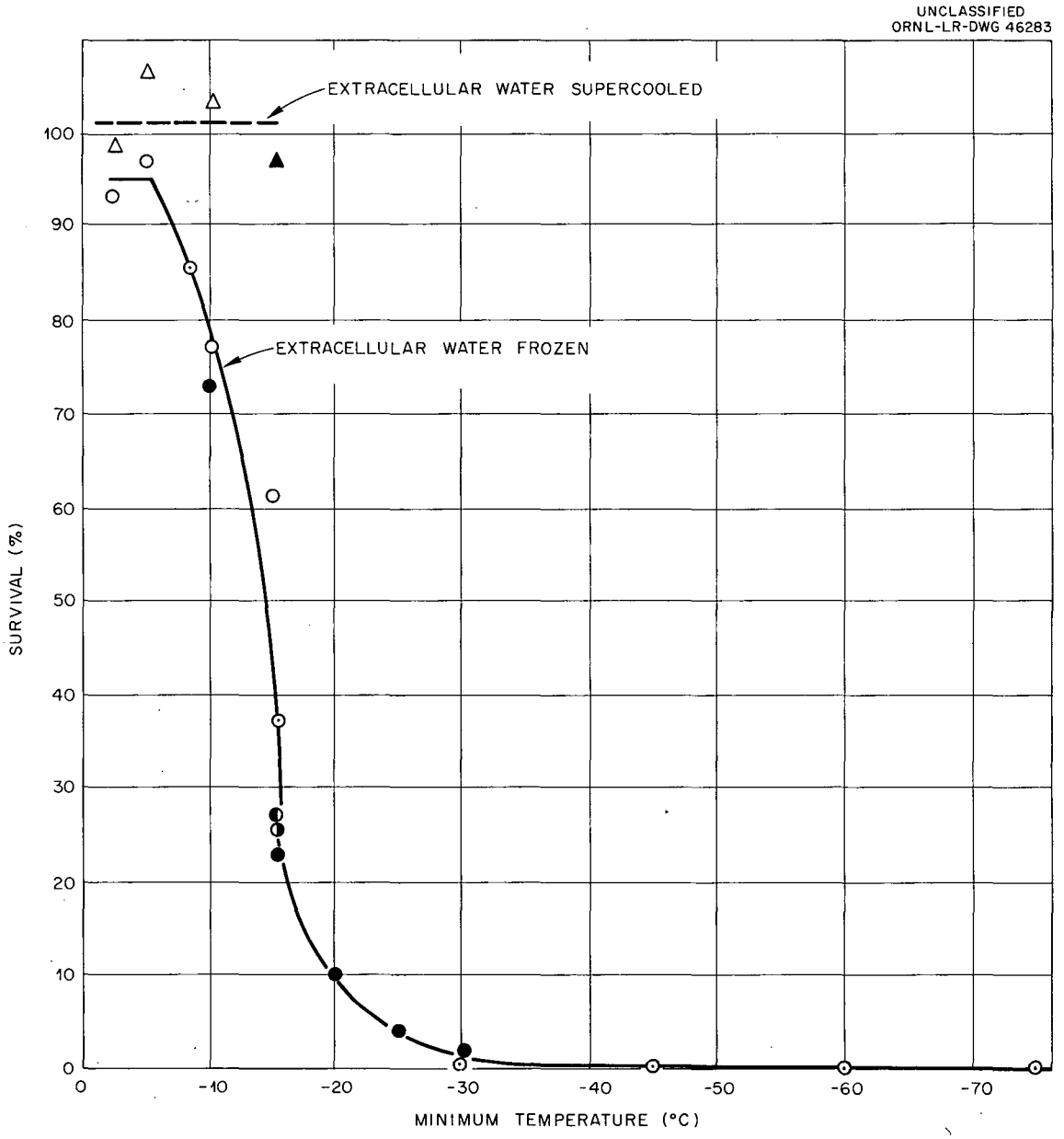

Fig. 10. Recovery of Cells of Saccharomyces cerevisiae After Rapid Cooling to Indicated Temperatures and Subsequent Slow Warming.

pores or aqueous channels in the cell wall or cell membrane and seeding the supercooled water within the cell. Permeability studies on other cells have indicated that such pores are extremely minute. If in yeast they were 25 Å in radius (and this is probably a high figure), only a crystal of this radius or smaller could grow through the pore. It is a physical fact, however, that the melting point of such a crystal would no longer be 0°C but

would be about -15°C. Thus, only at -15°C or below, could an external crystal grow through the wall or membrane and initiate intracellular freezing. If this hypothesis proves to be the correct explanation of the critical dependency of survival on temperature, there is the intriguing possibility that freezing studies can be used to estimate the size of the aqueous channels in cell walls and membranes.

Effect of Cytoplasmic Proteins on Nuclear Size

H. D. Swanson

Introduction. — Electrophoretic analysis on the proteins of the soluble phase of several rat organs[12] demonstrated a difference in the average net charge of the proteins from the several organs, as reflected in the mobility at pH 7.5. The average mobilities found were 2, 3, and 5.2×10^{-5} $cm^2/v/sec$, for liver, kidney, and brain, respectively. Average nuclear size also differs in these organs, and a correlation between nuclear size and average charge of soluble proteins is suggested. In liver and kidney, which have relatively basic protein, the average volume of isolated diploid nuclei is about 600 μ^3, in brain, with relatively acid protein, about 1100 μ^3. In addition, it has been found that in some tumors which have greatly enlarged nuclei, the proteins of the soluble phase are more acid than in the corresponding normal tissues.[13] Experiments were performed to investigate a possible influence on nuclear size of the cytoplasmic proteins.

Results. — Isolated nuclei of rat brain and rat liver were incubated for five days at 0°C in solutions of the soluble phases from liver and brain, which had been dialyzed against a buffered sucrose solution and adjusted to the same protein concentration. After incubation for five days, brain nuclei in brain protein were unchanged in size, but the volumes of those in liver proteins had decreased by 30%. Liver nuclei in liver protein did not change in size, but those in brain protein increased 20% in volume. The extent of the change was less after shorter periods of incubation.

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[12] N. G. Anderson and H. D. Swanson, Biol. Semiann. Prog. Rep. Feb. 15, 1959, ORNL-2702, p 126.

[13] S. Sorof and P. P. Cohen, Cancer Research 11, 376–82 (1951).

Discussion. – These results indicate that the size of the nucleus in the cell is determined, at least in part, by the characteristics of the cytoplasmic proteins, and that one of the important characteristics may be the net charge of proteins. It is suggested that basic proteins may form complexes with DNA, shrinking the nuclear gel, and that there may be more of such basic proteins in cells having small nuclei than in those having large nuclei. That the effects studied here may be important in differential activation of genes during development is suggested by the work of Kroeger,[14] who found that a new pattern of "puffing" or regional swelling is induced in salivary chromosomes of *Drosophila* by transplantation into egg cytoplasm. Experiments are now in progress to study the effect of specific proteins on nuclei.

Rat Serum Proteins

R. E. Canning T. Makinodan[15] N. G. Anderson

Introduction. – Previously a rat serum macroglobulin was isolated and characterized by free, paper, agar, and starch electrophoresis, immunoelectrophoresis, and by the analytical ultracentrifuge. This report concerns the preparation of antigen reagents against other purified serum proteins by the same methods.

Results. – By a combination of ammonium sulfate precipitation, paper curtain electrophoresis, and immunochemical methods, it has been possible to isolate and prepare antiserum against six different protein components. Three of these are macroglobulin, fibrinogen, and albumin. The other three components have yet to be characterized. Preliminary observations suggest that one of these is the fastest diffusing protein in agar-gel and has a mobility like that of albumin in electrophoresis. The second one appears to be a γ-globulin, and the third has a mobility electrophoretically comparable to that of a macroglobulin but a diffusion rate in agar-gel comparable to a γ-globulin. Further studies are being carried out to characterize these proteins.

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[14] H. Kroeger, Biol. Semiann. Prog. Rep. Aug. 15, 1959, ORNL-2813, p 69–70.

[15] Radiation Immunology.

Discussion. — The isolation of individual serum proteins and the subsequent preparation of antisera against them will enable us to determine whether there are identical proteins in rat tissues and to check the appearance of these proteins during embryonic growth and aging. By immunochemical assay with antisera against purified proteins it should be possible not only to detect its presence or absence but also its concentration. Our initial program is therefore concerned with the preparation of pure antiserum reagents against single proteins.

NUCLEIC ACID CHEMISTRY

W. E. Cohn
F. J. Bollum
J. X. Khym
A. J. Bandy
G. E. Houts

Minor Nucleotides in Ribonucleic Acids

W. E. Cohn A. J. Bandy

I. Introduction. – Continuing the investigation of pseudouridine and its isomeric forms, periodate oxidation has been applied to the four isomers, A_F, A_S, B, and C, described in the previous reports.[1,2]

Results. – The results indicate that whereas C (the "natural" ribofuranosyl form) and B – formed from C in alkali as well as acid, consume 1 mole each, both A isomers consume 2 moles each. The latter indicates a pyranose structure. This is consistent with the behavior of these substances in ion-exchange chromatography in the presence of borate. Although a mechanism of furanose-pyranose interconversion is not difficult to imagine, the existence of two forms of each, differing in chromatographic properties and in ultraviolet absorption spectra, is totally unprecedented. The nuclear magnetic resonance spectra of several related substances are being obtained in the hope of unraveling this mystery.

II. Introduction. – To establish whether pseudouridine exists preferentially at the ends of RNA chains or occupies a random distribution throughout the chain, L. Astrachan[3] and I have hydrolyzed a sample of RNA by snake venom diesterase (to yield the 5' nucleotide) as well as by alkali (to obtain the 2' and 3' nucleotides).

Results. – The results indicate that the 5' nucleotide can be obtained, a point that had not previously been established, and that the yield in this form was equal to the yield of the 2' and 3' nucleotides of pseudouridine in the alkaline digest. Thus there appears to be no substantial difference in distribution of this nucleotide in RNA chains. It was also observed that

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[1]W. E. Cohn, Biol. Semiann. Prog. Rep. Aug. 15, 1959, ORNL-2813, p 152–53.

[2]W. E. Cohn, A. J. Bandy, and J. X. Khym, Biol. Semiann. Prog. Rep. Feb. 15, 1959, ORNL-2702, p 129.

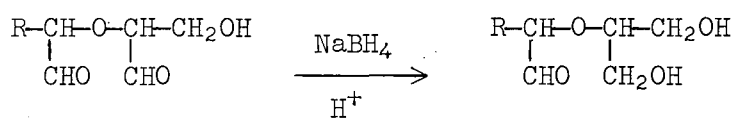
[3]Nucleic Acid Enzymology.

ribothymidylic acid was obtained from both digests and in amounts not differing one from the other. Therefore it too conforms to the conclusion reached regarding distribution.

Reactions of Periodate-Oxidized Nucleosides

J. X. Khym

It has been shown that periodate-oxidized nucleosides, which can be considered as derivatives of diglycolic aldehyde, react quantitatively with alkaline sodium borohydride to produce the corresponding alcohol derivatives.[4] A selective reduction of a single aldehyde group of the dialdehydes occurs, in accordance with the given equation



if the reduction with sodium borohydride is carried out in dilute acid. That only the group distal to the purine or pyrimidine ring is preferentially reduced (90–95% yield) was determined by the nature of the products formed by the degradation with phenylhydrazine.[5] A quantitative yield of purine or pyrimidine base, the phenylosazone of glyoxal, and free glycerol were identified from the phenylhydrazine reaction mixture.

The remaining aldehyde group of these monoaldehyde compounds is easily reduced by adding solid sodium borohydride to a neutral solution of these derivatives. The product of this reaction is the same as that produced by the alkaline reduction of the dialdehydes with sodium borohydride. After this procedure the compounds are stable to phenylhydrazine.

The alkaline and acidic reduced products behave differently on paper in butanol-water and on Dowex-1-chloride columns. Their properties are shown in Table 12.

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[4]J. X. Khym, Biol. Semiann. Prog. Rep. Aug. 15, 1959, ORNL-2813, p 153.

[5]W. E. Cohn, A. J. Bandy, and J. X. Khym, Biol. Semiann. Prog. Rep. Feb. 15, 1959, ORNL-2702, p 129.

Table 12. Chromatographic Properties of Alkaline and Acid Reduced Periodate-Oxidized Nucleosides

Initial Nucleoside	R_f Relative to Uracil in Butanol-Water			Peak Value (ml) from Dowex-1-chloride*	
	Alkaline Reduced	Acid Reduced	Dialdehyde Before $NaBH_4$	Alkaline Reduced	Acid Reduced
Adenosine	0.45	0.52	1.22	25	275
Cytidine	0.35	0.49	1.11	No separation with exchanger	
Guanosine	0.12	0.18	0.52	150	350
Uridine	0.58	0.87	1.57	100	400

*Elution with NH_4OH-NH_4Cl buffers: adenosine compounds, 200 ml of 0.1 M NH_4OH followed by 0.2 M NH_4OH–0.025 M NH_4Cl; guanosine compounds, 0.2 M NH_4OH–0.05 M NH_4Cl; uridine compounds, 0.2 M NH_4OH–0.025 M NH_4Cl.

DNA Synthesis by Mammalian Enzymes

F. J. Bollum G. E. Houts

Introduction. — Studies on the primer requirement of a DNA synthesizing enzyme from calf thymus gland have demonstrated that neither high molecular weight nor native, hydrogen-bonded structures are necessary for priming action insofar as the formation of new diester bonds is concerned.[6] Small synthetic polydeoxynucleotides (kindly furnished by H. G. Khorana, British Columbia Research Council, Vancouver, B.C.) were therefore tested in the hope that a simple primer would provide more insight into the detailed mechanism of primer action.

Results and Discussion. — Polythymidylates containing three to seven nucleotide residues have now been found to be active as primers for the polymerization of single deoxynucleoside triphosphates and two types of reaction are observed.

With a hydrogen-bonding substrate, such as dCTP or dATP, a single discrete compound is formed in the presence of each thymidylate polymer.

[6]F. J. Bollum, Biol. Semiann. Prog. Rep. Aug. 15, 1959, ORNL-2813, p 154–55.

This result suggests that small polynucleotides prime the synthesis of complementary small polynucleotides of magnitude similar to the primer. With nonhydrogen-bonding substrates, dTTP or dGTP, a series of compounds is formed from each primer, suggesting that only end-group addition is occurring.

Structure analysis of the polymeric products formed is now in progress. The type of reaction observed will be helpful in establishing the mechanism of priming in DNA synthesis by polymerase enzymes and useful for synthesizing small polydeoxynucleotides.

NUCLEIC ACID ENZYMOLOGY

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

RNA Function in Phage-Infected Bacteria

E. Volkin M. H. Jones

Introduction. – Experiments in progress are intended to define the biological function of RNA in phage infection in relation to specific DNA and protein syntheses. Isotope data indicate that, as a result of phage infection, a new species of RNA is synthesized having an apparent composition similar to the infecting phage's DNA. This RNA, which seems to be localized in a very large particle or on the bacterial cell membrane, undergoes rapid metabolic turnover, especially in the initial stages after infection. It can be demonstrated that, under conditions such as chloramphenicol inhibition or deprivation of a required amino acid, where protein and DNA syntheses are blocked, some of the phage-specific RNA will nevertheless accumulate. With the use of a double mutant of *E. coli* B, requiring both adenine and arginine, we are now able to control separately RNA and protein syntheses in the phage-infected system. The mutant is B94, kindly furnished by Dr. Joseph Gots of the University of Pennsylvania.

Results. – The mutant B94 will not support the production of $T2r^+$ or T7 in the absence of either adenine or arginine. In addition, no phage synthesis (nor bacterial growth) will take place in the presence of arginine plus deoxyadenosine. This observation demonstrates that the organism is unable to convert the deoxynucleoside to adenine and deoxyribose.

When cells in logarithmic growth are well washed then infected with $T2r^+$ or T7 in a minimal medium containing adenine but lacking arginine, synthesis of RNA specific to the phage-infected system will take place. No phage production accompanies this incubation period. However, if the cells are now washed free of adenine then placed in a medium containing arginine (or especially arginine plus deoxyadenosine), phage synthesis will ensue. It should be recalled that these latter two media are completely incapable of supporting phage production by themselves.

It can be shown, by removing the cells from the culture containing adenine alone at various time intervals, that the extent of subsequent

phage production in the arginine plus deoxyadenosine medium follows a reasonable correspondence to the amount of specific RNA formed in the primary incubation. This quantity reaches a maximum at around 90 min incubation in adenine. After the incubation in adenine only, the infected cells can be further incubated in deoxyadenosine alone without phage production, but will retain their ability to produce phage when finally given arginine and deoxyadenosine (or arginine alone). This type of experiment shows that phage production in the latter media is not a result of some endogenous free adenine, since such adenine would have been utilized (or removed) during the intermediate incubation in deoxyadenosine.

One of the unusual (and somewhat unfortunate) properties of these experiments is the nature and extent of phage production after removal of the adenine. That is, cells that under normal conditions undergo lysis inhibition and yield after some hours burst sizes of around 200, under these special conditions produce only about five to ten T2 per infected cell, and this production takes place totally within 30 to 40 min during the incubation in arginine plus deoxyadenosine. This may be explained by the fact that the RNA synthesized in the first culture phase may become quickly exhausted, and without adenine present in the subsequent incubation there is no opportunity for RNA renewal. In this respect it is noteworthy that, after a primary adenine alone incubation, when adenine is then added to the culture containing arginine plus deoxyadenosine, phage yields at early times are much greater than those produced by the appropriate control cultures which had not received a preincubation in adenine.

Discussion. — These experiments demonstrate that the synthesis of a specific type of RNA must occur as a primary prerequisite to phage production. It seems reasonable to suppose that the RNA is associated with the synthesis of specific proteins — phage protein or specifically induced enzymes or both. Experiments designed to test this hypothesis are under way.

Composition of Metabolically Active RNA from Phage-Infected Bacteria

L. Astrachan K. B. Hudgins

Introduction. — Investigations of the RNA formed in *E. coli* after infection with bacteriophage have consistently indicated that the composition of this RNA is very similar to the DNA of the infecting bacteriophage. This information was derived from experiments in which the RNA was labeled with P^{32}-orthophosphate and then hydrolyzed with alkali to yield 2',3' mononucleotides. The specific radioactivities of these mononucleotides varied exactly in proportion to the base composition of the infecting phage DNA. When C^{14} was used to label the base and sugar portions of RNA, the data again pointed to the similarity between RNA formed after infection and the DNA of the infecting bacteriophage. Although this relation has been established for two bacteriophages — T2 and T7 — further experiments were designed to test the reality of the relation.

Results and Discussion. — The first experiments arose from the fact that bacteriophage "ghosts" (bacteriophage particles without DNA) show many of the properties of complete bacteriophage in that they can infect bacteria, inhibit RNA synthesis, and eventually kill and lyse the bacteria. They cannot, however, induce the bacteriophage to synthesize new bacteriophage particles or even bacteriophage DNA. The data we have obtained demonstrate that whatever RNA is synthesized after infection with "ghost" particles does not resemble the DNA of the parent bacteriophage. After infection with "ghosts," the small amount of RNA synthesized has a composition characteristic of the host bacteria. These data indicate that the process of infection, with its attendant inhibition of RNA synthesis, cannot account for the synthesis of the "phage-like" RNA. For the "phage-like" RNA to be synthesized, phage DNA must be present.

The second group of experiments, centering around the reality of the "phage-like" RNA, have been discussed in the previous semiannual reports. It was reasoned that, if a new RNA is formed from uniformly labeled precursors, evidence for its resemblance to phage DNA should also derive from experiments in which the RNA was hydrolyzed to 5' mononucleotides rather than 2',3' mononucleotides. As reported then and confirmed since, the data indicate that a "phage-like" RNA is formed but it is necessary

to assume that the precursors to this RNA do not immediately have uniform specific activities. It is to this assumption that we have devoted our attention. At present, we have established nearly quantitative methods for the isolation from the acid-soluble fractions of each of the ribonucleotide mono-, di-, and triphosphates. Experiments to compare the specific activities of the possible precursors with that of the "phage-like" RNA are now in progress.

In summary, phage DNA must be present for the synthesis by an infected host of "phage-like" RNA. Methods have been developed for the isolation and assay of less than micromole quantities of nucleotide mono-, di-, and triphosphates from acid-soluble fractions of bacteria.

CHEMICAL PROTECTION AND PROTEOLYTIC ENZYME STUDIES

D. G. Doherty J. D. Bales, Jr.
F. C. Grimm[1] C. K. Myers

Chemical Protection

D. G. Doherty J. D. Bales, Jr.
J. Maisin* C. K. Myers

Introduction. — Although considerable information has been accumulated on the effectiveness of various protective compounds in reducing the short-term mortality of irradiated mice, much less is known about their relative effectiveness or distribution in specific systems in the animal. The response of the bone marrow and intestinal systems of chemically treated irradiated mice was therefore examined by various histological and cytological techniques in an effort to explain the differences in protective activity at supralethal radiation dose levels of 2-aminoethylisothiourea (AET) and related compounds.

Results. — The recovery of the bone marrow was measured by the procedure of Urso *et al.*[2] AET used as a control gave results comparable to those previously reported[3] with the marrow count beginning to recover after the third day and reaching normal levels by the eighth day. Effects of the rearrangement products of AET, 2-aminothiazoline, bis(2-guanidoethyl) disulfide, as well as 3-aminopropylisothiourea (APT), 2-aminopenthiazoline, bis(3-guanidopropyl) disulfide, 2-mercaptoethylamine (MEA), 3-mercaptopropylamine, bis(2-aminoethyl) disulfide, bis(2-aminopropyl) disulfide, serotonin, and AET-serotonin combinations were examined in mice given 900, 1200, and 1400 r. The disulfides were given both intraperitoneally and orally. The thiazolines were relatively ineffective at 900 r in promoting bone marrow recovery, the marrow count remaining low until the ninth to eleventh day and returning to normal by the fourteenth day. The guanido

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*Mammalian Radiation Recovery.
[1]Research Associate.
[2]P. Urso *et al.*, Blood 13, 665 (1958).
[3]D. G. Doherty and J. D. Bales, Jr., Biol. Semiann. Prog. Rep. Feb. 15, 1959, ORNL-2702, p 140.

disulfides were slightly better, the ethyl compound being more effective than the propyl compound, with the counts becoming normal by the twelfth day. The aminoethyl and propyl disulfides were completely ineffective; the marrow count did not return to normal until about the twenty-fifth day. At higher radiation doses, none of the thiazolines and disulfides showed a protective effect. MEA, at a comparable dose to the control AET, gave little marrow recovery until the tenth day and normal values by the sixteenth day. At twice this dose, the process was accelerated by about four days, although it never equaled the rapid recovery of AET-treated marrow. Mercaptopropylamine was less effective than the ethyl compound but better than the disulfides and thiazolines. The mercaptoamines were ineffective at 1400 r; both AET and APT gave a slow recovery to normal by the sixteenth day. Serotonin alone at 900 r gave a slow bone marrow recovery similar to the low dose of MEA. In combination with AET at 1500 r, the recovery was similar to AET alone. Raising the serotonin dose to the maximum possible did not increase the recovery rate.

The histology and mitotic activity of the intestinal crypt cells of mice pretreated with AET, MEA, and AET-serotonin combinations were examined. The best protection at 1500 r was obtained with AET- or MEA-protected mice, although all the MEA-treated mice died by the eighth day. Oral doses of AET or MEA also protected the intestinal tract. As little as 4 mg of oral AET protected the intestines of 1500 r mice, but none lived past day 6. Addition of 2 mg of serotonin gave better intestinal protection and increased the survival time. Optimal doses of AET or AET-serotonin gave the same protection at 1500 r, and at 2500 r the combined treatment gave some intestinal recovery by the fifth day although no mice survived beyond the sixth day. Tritium-labeled AET was prepared, purified by ion-exchange chromatography, and its distribution studied. Autoradiographs 5 min after an intraperitoneal injection showed diffuse labeling higher in the bone marrow, spleen, and intestine and lower in other organs. Labeling of the nuclei, cytoplasm, cell membranes, and mitotic figures was also observed.

Conclusion. — The bone marrow observations are in good agreement with the radiation LD_{50} mortality studies in that those compounds that failed to provide good marrow protection at 900 r also gave LD_{50}'s in the 1200 r

range or below. It seems clear from these results that, contrary to previous reports, the disulfides are less effective protective agents than the free thiols. In the intestinal system the maximum dose reduction with AET was about 2, in accord with mortality findings and the recovery of the bone marrow. The rapid recovery of normal mitotic activity along with the presence of AET within the cells and nuclei indicates that AET provides protection to intracellular systems.

Enzyme-Substrate Complex Formation

F. C. Grimm D. G. Doherty

Introduction. – The equilibrium binding technique,[4] previously developed in this laboratory, has been valuable for obtaining information on the nature and functioning of the catalytic site of a proteolytic enzyme, α-chymotrypsin; in theory, it should be applicable to nonproteolytic enzyme systems. Since little is known about the active site of oxidative enzymes, ox heart malic dehydrogenase was selected for this study. Its low molecular weight (~ 20,000 obtained for a partially purified enzyme[5]) and readily available enzyme rich source of supply make it particularly suitable for an investigation of the number and chemical nature of the substrate binding groups at this protein's active centers. Initial efforts have been directed toward the preparation of pure, homogeneous, and if possible crystalline enzyme and a search for a suitable equilibrium type substrate or competitive inhibitor.

Results. – A 115-fold purification of the enzyme has been achieved yielding 610 mg of a 60% pure malic dehydrogenase from 5 kg of minced ox heart representing an over-all 16% yield. The five-step procedure devised involves an acetone powder extract, ammonium sulfate fractionation, ethanol fractionation, ion-exchange chromatography, and zone electrophoresis on a starch column. A comparison of turnover numbers, under the same conditions as used previously,[5] has shown that this preparation is twice as active as any previous preparation. Inhibitor studies thus far have shown that

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[4]D. G. Doherty and F. Vaslow, J. Am. Chem. Soc. 74, 931 (1952); F. Vaslow and D. G. Doherty, J. Am. Chem. Soc. 75, 928 (1953); and D. G. Doherty, J. Am. Chem. Soc. 77, 4887 (1955).

[5]D. D. Davies and E. Kun, Biochem. J. 66, 307 (1957).

competitive inhibition can be obtained with thiomalate, mesotartrate, and maleate when they are present in tenfold excess over the amount of substrate. At similar levels DL-α-phenylmalate, the two racemic β-phenylmalates, the two racemic β-methylmalates, tartronate, D-tartrate, fumarate, malonate, succinate, glutarate, L-chlorosuccinate, and L-aspartate were ineffective as inhibitors. In accord with previous findings mesotartrate was found to be a poor substrate. The K_m value for malate at pH 10 with this preparation was found to be 7.0×10^{-3} M.

Discussion. — The good yield of highly active enzyme from ox heart is encouraging in that it makes possible for the first time the preparation of enzyme in sufficient quantity for complete purification by other methods. Although the inhibitors found so far are suitable for binding studies, other structural modifications of the substrate are in process with the hope of finding a stronger and more useful competitive inhibitor.

ENZYMOLOGY

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Protein Biosynthesis

Introduction. — This area of research continues to represent a major effort of this group. In addition to studies of reaction mechanisms in the activation of amino acids, in their transfer and incorporation into proteins,[4,5] the scope of the project has been expanded to include enzyme synthesis by cell-free systems and negative-feedback control and hormonal control of enzyme synthesis. These problems are being investigated with bacteria, plants, and animals. Since this project will continue to be actively investigated, this and subsequent reports will be restricted to the highlights of results accumulated in the previous six-month period.

Results and Discussion. — In studies on the induction of tyrosine-α-ketoglutarate transaminase in rat liver by tyrosine or cortisone,[6] an effort has been made to determine whether the induced increase in enzyme activity represents *de novo* synthesis of enzyme protein molecules or simply activation of an enzymically inactive protein precursor. The transaminase was highly purified from rat liver by extensions of methods previously described[7] and used to prepare highly specific antisera.

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[1]Research Associate.

[2]U.S. Public Health Fellow.

[3]Visiting Investigator from Abroad.

[4]G. D. Novelli and A. N. Best, Biol. Semiann. Prog. Rep. Aug. 15, 1956, ORNL-2155, p 54.

[5]G. D. Novelli and A. N. Best, Biol. Semiann. Prog. Rep. Feb. 15, 1957, ORNL-2267, p 69.

[6]F. T. Kenney, Biol. Semiann. Prog. Rep. Aug. 15, 1959, ORNL-2813, p 168.

[7]F. T. Kenney, J. Biol. Chem. 234, 2707 (1959).

Early experiments comparing the antigenic content of livers of adapted and of control animals indicated that, although the enzyme activity of the former group was six times that of controls, the antigen content was unchanged. More-conclusive results have come from experiments with adrenalectomized animals as controls combined with attempts to label the enzyme with C^{14}-leucine. Both groups of animals were injected with cortisone and C^{14}-leucine 5 hr before sacrifice. The soluble liver proteins were obtained by ultracentrifugation, and enzyme activity, antigen content, and radioactivity of the precipitated antigen were measured. The transaminase activity of the adapted group averaged about 30 times that of controls, whereas the antigen content was the same in both groups. Radioactivity of the precipitated antigen was the same in both groups and was slightly lower than that of the total soluble proteins. The results indicate that *de novo* enzyme synthesis is not the mechanism involved in this increase in activity. Investigations concerning the mechanism of the increased enzyme activity are under way.

In a continuation of studies on the effect of ultraviolet light (UV) on the induced synthesis of β-galactosidase in *Escherichia coli*,[8] we have observed that UV light suppresses the induction of β-galactosidase synthesis as well as the biosynthesis of nucleic acids and protein. Suppression of enzyme induction, but not of general protein and nucleic acid synthesis, is preferentially reversed by white light. By electrophoreses on Geon, evidence has been obtained for the existence of both free and particle-bound β-galactosidase. The appearance of the particle-bound enzyme is suppressed by UV and enhanced by white light. Removal of inducer causes a migration of the particle-bound enzyme to the cytoplasm. These results suggest that the particle-bound enzyme is precursor to the soluble enzyme and may reflect a role of the particle in enzyme synthesis. The latter possibility is under investigation.

In studies on the incorporation of amino acids into protein by cell-free preparations of rat liver, attention has been focused on the transfer of amino acid charged S-RNA to microsomal protein and the possible role

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[8]T. Kameyama, Biol. Semiann. Prog. Rep. Aug. 15, 1959, ORNL-2813, p 184.

of GTP in this process. Preliminary results indicate that S-RNA can be effectively charged with C^{14}-amino acids by the pH 5.0 fraction of rat liver supernatant. Such charged S-RNA is stable to freezing and thawing for several months. With unwashed microsomes the transfer of amino acids from S-RNA to microsomes is a very fast and efficient process. We have obtained a 50% transfer in 10–15 min, but at this time the reaction ceases. Why the reaction stops when charged S-RNA is still present is not known. If the microsomes are washed or treated with Lubrol, the transfer of amino acids is greatly reduced. Attempts to restore the transfer activity with fractions of the supernatant have not been entirely successful. The possible participation of GTP in the transfer reaction has been investigated with GTP labeled with P^{32} in the 5' phosphate. When such labeled GTP was incubated with rat liver microsomes in the presence or absence of S-RNA, there was no indication of a stable linkage of GMP or GDP with microsomes or S-RNA. When the products of the reaction were chromatographed, GDP was the only product identified. This suggests that, in the transfer reaction, the involvement of GTP probably requires the transfer of the terminal phosphate in such a way that does not allow for an exchange with orthophosphate.

A system has been developed from germinating maize seedlings that actively incorporates amino acids into protein. This system is somewhat different in requirements and behavior from that previously described from developing maize endosperm.[9] The system consists of the 100,000 × g particle isolated from homogenates of two-day-old seedlings made in 0.05 M Tris pH 7.5, 0.001 M $MgCl_2$, and 0.45 M sucrose. In the presence of ATP, GTP, $MgCl_2$, and an energy generator, the particles incorporate more than 0.5% of the added C^{14}-leucine. Incorporation is stimulated by the addition of a pH 5 fraction prepared from rat liver. When the particles are washed with the grinding medium they exhibit absolute requirements for $MgCl_2$, ATP, rat liver pH 5 fraction, and are stimulated by GTP and an energy generator. Maize S-RNA stimulates incorporation by washed maize particles but not to the extent of the maize pH 5.0 fraction. Thus this

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[9]R. Rabson, Biol. Semiann. Prog. Rep. Aug. 15, 1959, ORNL-2813, p 172.

preparation differs from the one obtained from pea seedlings[10] in requiring soluble factors as well as the polynucleotide.

Metabolic Reaction Mechanisms

Introduction. – Another major effort of the Enzymology group is directed toward an understanding of the basic enzymic mechanisms used by cells to carry out the fundamental reactions of energy production and biosynthesis. An understanding of these processes is requisite to our understanding of the growth process and for collaborative efforts with other groups in the Biology Division. Since a variety of independent enzymic reactions are being investigated, we will present in these reports only those results that seem to have some significance.

Results. – Studies on the mechanism of reduction of sulfate with molecular hydrogen in extracts of *Desulfovibrio desulfuricans* have been continued.[11] Since this organism can grow autotrophically with H_2, CO_2, and SO_4,[12] it is certain that the reduction of SO_4 to H_2S with molecular hydrogen yields the metabolic energy necessary for growth. If these cells are like all other cells the transfer of electrons in the reduction of sulfate should be coupled with the formation of ATP from inorganic phosphate. Because of the well-known uncoupling of oxidative phosphorylation by 2,4-dinitrophenol (DNP), we tested the effect of this compound on sulfate reduction. The reduction of sulfate with hydrogen by whole cells is completely inhibited by DNP, but not the reduction of thiosulfate. In cell-free extracts, neither sulfate reduction with added ATP nor thiosulfate reduction are inhibited by DNP. Methyl viologen, a dye that stimulates reduction of sulfate and thiosulfate in extracts by shunting electrons past the natural electron carriers, completely inhibits sulfate reduction but not thiosulfate reduction in whole cells. Peck[13] has established that the initial step in sulfate reduction requires the activation of sulfate

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[10] G. C. Webster, Arch. Biochem. Biophys. 85, 159 (1959).

[11] H. D. Peck, Jr., Biol. Semiann. Prog. Rep. Aug. 15,1959, ORNL-2813, p 170.

[12] K. R. Bultin, M. E. Adams, and M. Thomas, J. Gen. Microbiol. 3, 46 (1949).

[13] H. D. Peck, Jr., Proc. Natl. Acad. Sci. U.S. 45, 701–8 (1959).

by ATP as shown below.

$$ATP + SO_4^{--} \longrightarrow APS + PP$$

APS is then reduced to yield AMP and sulfite. In contrast, sulfite and thiosulfate are reduced by H_2S without requiring ATP. Thus the inhibitory effect of DNP and methyl viologen on sulfate reduction in whole cells is a result of an inhibition of ATP formation and a consequent failure to activate sulfate. This system is being further examined to obtain direct evidence for phosphorylation coupled with electron transport.

The inhibition of acetylation of arylamines by amethopterin, reported previously,[14] has now been shown to be competitive with the acetyl donor. Further studies on the characteristics of this enzyme reveal a substrate specificity with regard to the acetyl donor that is related to the electronegativity of the substituent on the benzenoid ring. When the electronegativity is greatest the ability to donate the acetyl group is greatest. Thus in considering para-substituted acetanilides the reaction rate decreases as follows:

$$NO_2 > Cl = Br > H, CH_3$$

Preliminary studies on the inhibition of the acetyl transfer indicate that certain purines are also effective inhibitors. In most cases a similarity of the effective purines and the pteridine structure of amethopterin exists and is likely to lead to the identification of the active locus in the amethopterin molecule.

A study of the enzymic formation of tryptophan from indoleglycerol phosphate by tryptophan synthetase from Neurospora crassa has been initiated. A specific antiserum has been prepared that inhibits enzyme activity and also precipitates the enzyme. It has been found that pyridoxal phosphate (B_6alP), the coenzyme for the reaction, protects the enzyme from antibody neutralization if added before the antiserum. Measuring protection of enzyme by B_6alP with this technique has revealed a variation in the degree of protection when extracts of different mutants are tested. Since the tryptophan synthetase reaction is a highly complex one, the results obtained with antibody suggest a heterogeneity in this reaction. By comparing indole disappearance, tryptophan synthesis, and formation of indoleglycerol phosphate in the partially inhibited system a clearer picture may be obtained.

[14] K. B. Jacobson, Biol. Semiann. Prog. Rep. Aug. 15, 1959, ORNL-2813, p 179.

MICROBIOLOGY

ENZYME AND TRACER STUDIES ON BACTERIAL METABOLISM

S. F. Carson M. V. Long
E. F. Phares C. Smith
R. K. Clayton

Bacterial Methylmalonyl-CoA Isomerase

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

Introduction. — The two principal steps in bacterial succinate decarboxylation are (1) conversion of succinyl-CoA to methylmalonyl-CoA by extracts from *Propionibacterium pentosaceum* (methylmalonyl isomerase), and (2) decarboxylation of methylmalonyl-CoA to propionyl-CoA and CO_2 by a washed particle preparation from *Micrococcus lactilyticus* (a biotin-requiring decarboxylase). Extracts of the *Micrococcus* contain also the isomerase. In reaction (1) succinyl-1,4-C^{14}-CoA is converted to C^{14}-labeled methylmalonyl-CoA, and in reaction (2) methylmalonyl-2-C^{14}-CoA is decarboxylated to C^{14}-labeled propionyl-CoA.

Experimental. — An assay for reaction (1) as an isolated system has been developed, involving the conversion of methylmalonyl-CoA-1-C^{14} to succinyl-CoA-C^{14}. Radioactivity in succinate is assayed after treatment of the incubation mixture with hot, fuming nitric acid, followed by dilute, neutral permanganate. Several properties of the system have been studied. The optimum pH of the reaction is 6.7, in agreement with the previous results from measurement of CO_2 production from succinyl-CoA in a less highly purified system; however, the new test system is not stimulated by high salt concentrations and by anaerobic incubation conditions (nitrogen atmosphere and sulfhydryl compounds). The reaction is unaffected by propionyl-CoA; for example, no dilution of succinate specific radioactivity was obtained when unlabeled propionyl-CoA was included with labeled methylmalonyl-CoA, and conversely no significant incorporation of C^{14} was noted when labeled propionyl-CoA was incubated in the presence of unlabeled methylmalonyl-CoA. These results certainly indicate an

intramolecular isomerization rather than an intermolecular "transcarboxylation" as proposed by Beck and Ochoa[1] for the mammalian system. The participation of the vitamin B_{12} coenzymes[2] in this reaction has also been demonstrated. Untreated, crude cell extracts, or those treated with charcoal and exposed to a strong tungsten light, are stimulated up to twofold by the addition of boiled extracts; cell extracts treated with protamine and charcoal are stimulated over fivefold. Purification of the enzyme by ammonium sulfate precipitation or by zone electrophoresis is increased by addition of boiled extracts. Probably much of the observed instability of this enzyme was caused by loss of the coenzyme. The purification procedure described by Barker *et al.* for the coenzyme in *Clostridium tetanomorphum* appears to apply also to the coenzyme in *P. pentosaceum*.

Induced Synthesis of Catalase in *Rhodopseudomonas spheroides*

R. K. Clayton C. Smith

Introduction. — Oxygen and hydrogen peroxide induce a dramatic synthesis of catalase in *Rhodopseudomonas spheroides*.[3] The enzyme-protein is synthesized *de novo*.[4] This system is being investigated to identify intermediate steps in the over-all process and to elucidate the function of catalase in a photosynthetic bacterium.

Results and Discussion. — The effect of sodium azide in enhancing induced catalase synthesis, studied quantitatively, has led to the conclusion that H_2O_2 is an intermediate when O_2 is the inducer.[3] The intracellular formation of H_2O_2 from O_2 is very efficient in bright light and may therefore play a role in the photooxidative killing of photosynthetic cells. With a view to studying this question, a mutant of *R. spheroides* was isolated that maintains a high constitutive catalase content (2% of the dry cell mass). This mutant is slightly more sensitive to 250-kv

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[1]W. S. Beck and S. Ochoa, J. Biol. Chem. 232, 931 (1958).

[2]H. A. Barker *et al.*, J. Biol. Chem. 235, 181 (1960).

[3]R. K. Clayton, J. Cellular Comp. Physiol., in press.

[4]R. K. Clayton, J. Biol. Chem., in press.

X rays than the wild type: LD_{50} (mutant) = 1.7 kr; LD_{50} (wild type) = 1.9 kr.

When added to R. spheroides, H_2O_2 is destroyed rapidly by catalase and by a noninducible peroxidase.[5] While present, it promotes an energy-dependent formation of an internal inducer which in turn promotes the synthesis of catalase. The internal inducer (I_i) is stable for many hours in darkness at 0°C; its destruction is accelerated by bacteriochlorophyll-absorbed light. The formation of I_i does not require a reaction between H_2O_2 and catalase, and its destruction is not related stoichiometrically to the synthesis of new catalase. The time course of induced catalase synthesis after exposure to H_2O_2 seems to reflect the formation and dissipation of I_i. The greater inducibility of stationary-phase cultures, as compared with growing cultures, can be related to a slower dissipation of I_i. In terms of a repressor hypothesis, in which inducers act to antagonize repressors of enzyme synthesis, the dissipation of I_i could be in fact the synthesis of more repressor.

The over-all process of induction is inhibited by chloramphenicol but not by azaguanine or bromouracil, suggesting that RNA synthesis is not involved, whereas protein synthesis is. The effect of chloramphenicol on separate parts of the sequence

$$H_2O_2 \rightarrow I_i \rightarrow \text{catalase synthesis}$$

has not yet been studied.

Attempts are in progress to extract I_i from one batch of cells and cause it to induce catalase synthesis in another.

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[5]R. K. Clayton, J. Cellular Comp. Physiol., in press.

A. H. Haber H. J. Luippold
W. A. Arnold D. M. Furr[1]

Separation of the Mechanisms Initiating Cell Division and Cell Expansion in Lettuce Seed Germination[2]

A. H. Haber H. J. Luippold

Introduction. — After imbibition of water by seeds under favorable circumstances, the growth by which the embryo becomes a young seedling occurs by both expansion of cells originally present in the dormant embryo and mitotic divisions and results in an increase in cell number. We attempted to separate the inception of cell division from that of expansion during lettuce seed germination.

Results and Discussion. — 1. At 26°C lettuce seed germinates rapidly, and the beginnings of cell division and cell expansion roughly coincide in time.

2. When γ-irradiated seeds are sown on a solution of kinetin at 26°C, germination, although greatly delayed compared to that of unirradiated controls, precedes cell division by several days.

3. Results similar to those with irradiated seeds were found with unirradiated seeds germinating at 10°C. The distribution of mitoses among root tips of newly germinated seeds at 10°C suggested that mitoses occurred randomly among them.

4. When seeds were germinated at 26°C in solutions of mannitol, cell divisions preceded germination by many days. High concentrations of mannitol prevented germination of seeds that nevertheless underwent mitotic activity.

5. At 30°C, only a very small percentage of seeds germinate. Most seeds, however, undergo cell division. The distribution of mitoses among radicle meristems of nongerminating seeds showed that mitoses were not occurring randomly among the unexpanded radicles. Rather, there was a tendency for an all-or-none characteristic in the occurrence of mitoses within

[1]ORINS Fellow.

[2]A. H. Haber and H. J. Luippold, Plant Physiol. 35, in press (1960).

individual radicles. As time progressed, the mitotic activity in these nongerminating seeds diminished.

These results indicate that: (a) the initiation of cell expansion and that of cell division are controlled by separate mechanisms during germination; (b) rootlet protrusion during germination results from cellular expansion, whereas cell division plays little or no role; (c) the mechanisms by which kinetin and other agents stimulate lettuce seed germination are related to the initiation of cellular expansion and not to cell division.

A more detailed account of this work will be published in the March issue of Plant Physiology.[2]

Effects of Gibberellin, Kinetin, Thiourea, and Photomorphogenic Radiation on Cell Division in Nonsprouted Lettuce Seeds

A. H. Haber H. J. Luippold

Introduction. — Lettuce seed germination can be stimulated, probably by distinct mechanisms, by any of the three widely studied morphogenic agents: gibberellin, kinetin, or red light.[3] It has been shown that cellular expansion is both necessary and sufficient and that cell division is neither necessary nor sufficient for the initiation of lettuce seed germination.[2] Thus it is now possible to study the effects of germination-stimulating agents on mitotic activity in embryos uncomplicated by germination and growth from expansion. In this report, mitotic activity as it is affected by gibberellic acid (GA), kinetin, and light, as well as by thiourea, which also stimulates lettuce seed germination, is studied in radicles of nonsprouted lettuce embryos.

Results and Discussion. — At various temperatures superoptimal for lettuce seed germination, we studied the effects of four germination-stimulating agents on cell division in radicles in the absence of germination and growth from cell expansion. Only kinetin stimulated cell division in the absence of germination. Studies of heat inactivation and respiration at high temperatures showed that kinetin does not generally protect seeds from high-temperature injury. Consequently kinetin must be considered a true accelerator of

[3]A. H. Haber and N. E. Tolbert, p 197 in Photoperiodism and Related Phenomena in Plants and Animals, American Association for the Advancement of Science, Washington, D.C., 1959.

mitosis in these seeds. Since no binucleate cells are observed, kinetin must also be an accelerator of cell division in this system. Neither GA nor red (compared with far-red) light affected mitotic activity in nonsprouted seeds under the same conditions for which they stimulated germination. This suggests that these agents do not affect growth by direct actions on cell division, in agreement with previous studies in which a complementary system was used (growth occurs by cell expansion in the absence of any cell division), showing that the GA-induced growth stimulation in young wheat seedlings could be attributed solely to effects on cell expansion and not to any effects on cell division.[4] Thiourea inhibits cell division in nonsprouted seeds in the same experiment in which it later stimulates germination.

These findings further support the conclusion that cell expansion and cell division are initiated by different mechanisms during germination, since some agents that stimulate cell expansion have no effect on cell division in the absence of growth from cell expansion (e.g., GA or red light) and may even inhibit cell division in the absence of growth from cell expansion (e.g., thiourea).

Together with our previous studies on respiration[5] and fixation of carbon from bicarbonate[6] in relation to physiological dormancy, these results lead to the conclusion that the definitive characteristic of physiological dormancy in seeds is a subtle block that specifically prevents the initiation of cell expansion. This is pertinent to radiation studies, since such physiological dormancy can result from γ-irradiation.[5]

A more detailed account of this work has been accepted for publication in Plant Physiology.

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[4]A. H. Haber and H. J. Luippold, Am. J. Botany 47, in press (1960).

[5]A. H. Haber and H. J. Luippold, Intern. J. Radiation Biol. 1, 317 (1959).

[6]A. H. Haber and N. E. Tolbert, Plant Physiol. 34, 376 (1959).

Metabolism of Chlorogenic Acid in Tobacco

D. M. Furr

Introduction. – Chlorogenic acid (CA), an ester of caffeic and quinic acids, is the most abundant phenol of tobacco and has been reported to occur in many other species.[7,8] Oparin[9] has suggested that CA may act as a respiratory chromogen, and Bonner[10] has suggested that caffeic acid and related compounds may be of importance as hydrogen-transporting systems in plant respiration. Recent interest in CA has centered on its being a substrate for polyphenoloxidase.[11,12] The darkening of injured fruit may be caused by oxidation of CA.[13] It may also be an antifungal factor and one cause of natural immunity.[14] Despite the widespread occurrence of CA, its metabolic roles have been little studied. The purpose of the present work is to investigate the metabolism of CA in tobacco.

Results. – Carbon-14-labeled glucose, fructose, tyrosine, phenylalanine, and CA were fed to young seedlings, leaf discs, intact leaves, and stem callus-tissue cultures for various lengths of time in light, in darkness, and in light plus ultraviolet radiation. Combined hot 80% alcohol and hot water extracts of these tissues were chromatographed and the chromatograms were radioautographed. The radioactive spots are being identified by R_f values and spray reagents and the radioactivity is being counted.

Thus far results show that tyrosine and phenylalanine are interconverted, and that either labeled compound can give rise to labeled CA. When C^{14}-phenylalanine is fed to young seedlings, more radioactivity gets into CA than into tyrosine. When leaves were fed C^{14}-tyrosine or C^{14}-phenylalanine and then extracted with hot 80% ethanol and hot water, venation patterns of radioautograms of leaves indicated that the insoluble

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[7] K. Gorter, Arch. Pharm. 247, 184 (1909).
[8] R. H. Goodwin, Plant Physiol. 4, 283 (1953).
[9] A. Oparin, Biochem. Z. 182, 155 (1927).
[10] J. Bonner, Plant Biochemistry, Academic Press Inc., New York, 1950.
[11] E. H. Hess, Arch. Biochem. Biophys. 74, 198 (1958).
[12] E. C. Sisler and H. J. Evans, Plant Physiol. 4, 255 (1958).
[13] C. Weurman and T. Swain, Nature 172, 678 (1953).
[14] E. Vallee, Acta Chem. Scand. 11, 395 (1957).

radioactivity is in cell wall material. Either C^{14}-glucose or C^{14}-fructose fed to leaves for 24 hr yielded 2–4% of the total radioactivity in the extract as labeled CA.

C^{14}-chlorogenic acid was biosynthesized by feeding $C^{14}O_2$ to photosynthesizing tobacco leaves. Hydrolysis of this C^{14}-CA to quinic and caffeic acids yielded all of the detectable radioactivity in quinic acid. When this C^{14}-CA was fed to tobacco leaves for 24 hr, 30% of the radioactivity was found in glutamic acid. It was also found that CA was metabolized more rapidly in darkness than in light, and that the radioactivity was found mostly in phenylalanine, tyrosine, threonine, glutamic and aspartic acids, arginine, and asparagine.

Photosynthesis

W. A. Arnold R. K. Clayton

Washed chromatophores from Rhodopseudomonas spheroides, dried on glass plates, exhibit changes in absorbancy when illuminated with light absorbed by bacteriochlorophyll. The spectrum of these changes can be correlated with absorption bands of bacteriochlorophyll and carotenoids. The "carotenoid part" of the spectrum can be abolished by exposing the film to ethanol vapor, which does not actually remove the carotenoids from the film. The phenomenon appears at 77°K, with little change except that the rise and decay of the response is much faster than at 300°K. The kinetics and the dependence on light intensity are consistent with the idea that electrons, excited to a conduction band in a chlorophyll lattice and then trapped, are responsible for the changes in absorbancy. It may be possible to correlate this optical effect with delayed light emission in the same material. The behavior at 2°K will be investigated.

BIOPHYSICS

J. S. Kirby-Smith
M. L. Randolph
J. Jagger
M. L. Gwyn
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The Induction of Chromosomal Aberrations in *Tradescantia* Pollen by Combined X-Ray and Ultraviolet Treatment

J. S. Kirby-Smith B. Nicoletti[2] M. L. Gwyn

Introduction. — Although a considerable body of information exists on the induction of chromosomal aberrations in *Tradescantia* pollen by ultraviolet and ionizing radiations, except for a report by Swanson[3] that 2537-Å ultraviolet radiation can modify X-ray-induced aberrations in growing pollen tube cultures and some fragmentary work carried out in this laboratory by Lovelace,[4] little is known of the action of these agents in common. Studies also made in this laboratory (Kirby-Smith and Craig)[5] indicate that the primary mechanisms of ultraviolet radiation action are markedly different from those involved from ionizing radiation. Accordingly, a careful study of the combined action of these two agents may be expected to yield interesting results and possibly cast considerable light on the action of both types of radiations.

Results. — Preliminary results have confirmed these expectations, and a very pronounced synergism in the action of the different radiations has been observed. Pollen was irradiated under controlled conditions of humidity, given pre- and posttreatments of 2650- and 2804-Å radiation in combination with 150 rads of 250-kvp X rays. The results of a typical experiment are shown in Table 13. In both the pre- and posttreatments the time interval between the ultraviolet and X-ray exposure was 15 min. For an interval of 1/2 hr the effect is much less than that shown above; in particular, for the X-ray + UV case almost no synergistic effect was

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[1]AEC Radiological Physics Fellow.

[2]Drosophila Cytology and Genetics.

[3]C. P. Swanson, Genetics 27, 491 (1942).

[4]R. Lovelace, Genetics 37, 602 (1952) (abstract).

[5]J. S. Kirby-Smith and D. L. Craig, Genetics 42, 176–87 (1957).

Table 13. Aberration Frequencies for Single and Combined Radiation Treatments

Treatment	Aberration Frequency (isochromatid aberrations per cell)
X rays alone	0.14
UV alone	0.02
X rays + UV	0.48
UV + X rays	0.74

found, that is, X + UV is only slightly above the sum of individual X-ray and UV irradiations given alone.

Discussion. — These experiments show definitely an extremely strong time-dependent interaction between UV- and X-ray-induced chromosomal damage. They also suggest a number of further investigations now in progress involving such parameters as the dependence on UV and X-ray dose levels and wave lengths, as well as the time interval between different treatments. The results already obtained do indicate, however, that at least a significant portion of the primary potential UV damage that can interact irreversibly with subsequent X-ray-induced effects spontaneously recovers. Much experimental work remains to be done before a satisfactory working hypothesis for this phenomenon can be constructed. It is possible that free radicals may play some role, since rather long-lived radicals, that is, 1/2-hr half life, can be observed in irradiated *Tradescantia* pollen.

Application of Analog Computer to ESR Spectroscopy

M. L. Randolph H. G. Jones

Introduction. — For quantitative electron spin resonance (ESR) studies, such as determination of the concentration of diverse resonances produced by radiation, the quantity of interest is the area of the microwave power absorption curve. However, the output of high-sensitivity ESR spectrometers approximates the derivative of the absorption curve. Hence, using

a commercially available analog computer, we integrate the spectrometer output twice.

Results. — Analysis of typical results for rather wide ESR patterns indicates that the precision (taken as the standard error/average value) of direct measurements is about 8%, which is reduced to about 2% by a simple baseline correction. Figure 11 is an example of derivative, area, and integral curves plotted simultaneously.

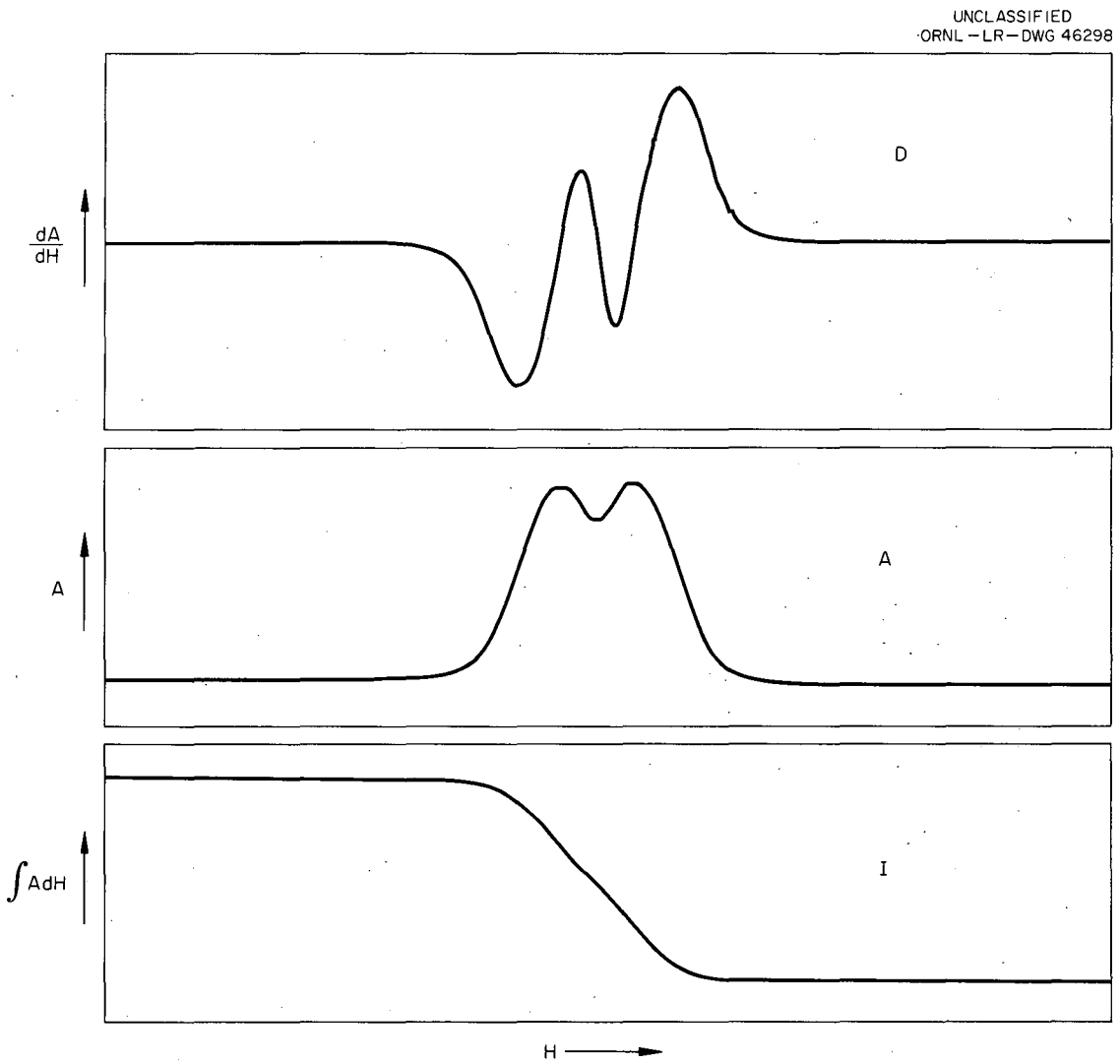

Fig. 11. Simultaneous Plots of Derivative of ESR Absorption Curve, D, Absorption Curve, A, and Integral of Absorption Curve, I, Each vs Magnetic Field, H. Data taken reading from right to left.

Discussion. — The addition of a doubly integrating analog computer to an ESR spectrometer markedly increases the rate of quantitative, as contrasted with structural or analytic, spin resonance studies. Because such a system adds technical complexities, the minimum detectable signal for area measurements is greater than for differential measurements at instrument settings permitting good resolution of peaks. By greatly increasing the oscillatory magnetic sweep voltage, however, this disadvantage can at least in part be offset since the linear proportionality between area and sweep extends much higher than between derivative curve height and sweep. The seemingly improbable possibility that greater ultimate sensitivity may be obtained thus is being investigated.

Radiation-Induced Free Radicals in Macromolecules

W. B. Nix

Introduction. — Three studies on radiation-induced free radicals in macromolecules have been made: (1) a determination of the relative effect of radiations of different linear energy transfer (LET), (2) a study of decay rates of those free radicals, and (3) a study of the effect of heat denaturation on sensitivity for free radical formation.

The substances studied were salmine (protamine) sulfate, chondroitin sulfate (a polysaccharide), and gelatin, all in the dry form. Radicals are induced by irradiation with Co^{60} γ rays, 250-kvp X rays filtered with 3.1 mm of Al, 5-Mev polonium α-particles, and 14-Mev D-T neutrons. The free radicals so induced are detected by electron spin resonance spectrometry.

Results. — 1. LET Study. Area measurement of the absorption envelopes for both gelatin and chondroitin indicated that X rays and these neutrons induced, respectively, 70 and 50% as many free radicals per unit dose as did γ rays. Visual inspection indicates that the absorption curves are not all of the same shape. This is confirmed by a comparison of peak-to-peak heights of the absorption derivative curves; that is, for chondroitin — γ defined as 1.00, X rays 0.7, neutrons 0.4; for gelatin — γ 1.00, X rays 0.9, neutrons 0.4. Studies with α irradiation are in progress.

2. Decay Study. For all these substances studied, the number of X-ray-induced free radicals decayed with time. Observation of derivative peak heights shows only that all have two-component decay schemes. The half lives of the components are approximately as follows: salmine, 9 and 78 hr; gelatin, 26 and 147 hr; chondroitin, 25 and 368 hr.

3. Denaturation. Salmine sulfate was denatured by boiling in water for 1 hr then heat drying. X-ray-induced free radicals measured from derivative peak heights were about three and one-half times as concentrated (per rad) as in undenatured material. Decay rates were also changed. The decay scheme still had two components, but the half lives were changed to about 35 and 390 hr.

Discussion. — The results of the LET experiment confirm, at least qualitatively, those of Randolph and Kirby-Smith:[6] that is, sensitivity for free radical induction decreases with increasing LET of the radiation. A mechanism that might be described as waste of energy by high LET radiations has been postulated.[7]

The decay schemes were determined from derivative curve peak heights only. The areas of all the absorption envelopes have not been determined, and it is possible that they may be changing at rates different from those given above. By visual observation, though, the curve shapes do not seem to be changing.

The observation of increased sensitivity with denaturation confirms that of Blyumenfel'd and Kalmanson.[8] Their explanation is that denaturation breaks the hydrogen bond subchains, thus disrupting the channels whereby an electron may migrate through the molecule to pair with another unpaired electron.

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[6]M. L. Randolph and J. S. Kirby-Smith, Biol. Semiann. Prog. Rep. Feb. 15, 1959, ORNL-2702, p 167.

[7]J. S. Kirby-Smith and M. L. Randolph, Symposium on the Immediate and Low Level Effects of Ionizing Radiations (Venice, June 1959), in press.

[8]L. A. Blyumenfel'd and E. A. Kalmanson, Biofizika 3(1), 87–91 (1958).

Photoprotection from Ultraviolet Killing in *Escherichia coli* B

J. Jagger

Introduction. – Photoprotection (PP) is the protection from ultraviolet radiation (UV) lesions in a biological system with light of wave length longer than that of the damaging radiation. The fact that it is a protection implies that the light is administered before the beginning of the UV irradiation. Studies of this phenomenon in the bacterium *E. coli* B have continued, the techniques described in an earlier report[9] being used.

Results. – It has been found that PP (1) is a much more striking effect when the cells are plated on agar containing NaCl; (2) is important only at low UV doses, at moderate UV doses (yielding 10^{-2} survival); it shows a high hit number and has a definite lag period; (3) shows dose reduction by factors of 2–4, at low UV doses; (4) decays slowly, showing a 50% decay in 28 hr for cells kept in phosphate buffer at 23°C; (5) requires doses of PP light so high as to be slightly lethal, a dose giving optimal PP kills about 20% of the control cells; (6) is effective with wave lengths in the region 3000–5000 Å; (7) does not protect from killing by 250-kvp X rays; (8) as a function of dose, is virtually independent of temperature during illumination in the range 3–37°C (Q_{10} of 1.05); and (9) as a function of dose, is independent of dose rate when this is varied by a factor of 16.

Discussion. – With respect to results 3, 4, 6, and 7, PP is similar to photoreactivation. With respect to all the other results, however, it differs strikingly from photoreactivation. In particular, the independence of temperature and dose rate leads to the conclusion that, in contrast to photoreactivation, limiting thermal reactions play little if any part in the early events in PP.

Half Lives of Polonium-Beryllium Fast Neutron Sources

M. L. Randolph H. G. Jones

Introduction. – There have been various vague hints that Po-Be fast neutron sources may not decay directly as does the α-particle emitter.

[9] J. Jagger, Biol. Semiann. Prog. Rep. Feb. 15, 1959, ORNL-2702, p 176.

Such irregularity is usually imagined to be caused by variations in the mixing of polonium and beryllium. Having made rather extensive measurements on three such sources, all encapsulated at Mound Laboratory, we sought to determine whether our data showed significant deviation from the expected decay.

Results. – Three sources, three locales of differing neutron scattering, and two long counter fast neutron detectors were used. The half lives obtained when the seven sets of data were submitted to least-squares analyses and sets of data statistically averaged were: Po^{208}-Be, 1041 ± 6 days vs the latest Po^{208} half life 1058 ± 1 day;[10] 100-curie Po^{210}-Be, 139.1 ± 0.7 days and 20-curie Po^{210}-Be 140.4 ± 2.2 days vs accepted Po^{210} half life of 138.4 days. For the Po^{208} source, corrections were made for Po^{209} contaminant. These measurements extended for from 200 to 1800 days and in one case encompassed ten half lives.

Discussion. – For various reasons, the real precision of the values is somewhat less than calculated, and hence we cannot assert that there is a real difference between fast neutron and α-particle decay of such sources. No systematic nonlinearity of decay was demonstrated. The Po^{208}-Be source has been used as a standard for calibrations with the Biology Division Cockcroft-Walton accelerator. The standard error of about 3% found for individual measurements furnishes a check on the precision of fast neutron measurements in this Division. The 100-curie Po^{210}-Be source is that used for chronic neutron irradiation by the Pathology and Physiology Section.

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[10] K. C. Jordan of Mound Laboratory, Miamisburg, Ohio, private communication.

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