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SEMIANNUAL PROGRESS REPORT

For Period Ending August 15, 1959

Alexander Hollaender, Director
Stanley F. Carson, Assistant Director

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PUBLICATIONS AND LECTURES

Publications. – During the period covered by this report 68 papers and 27 abstracts have been published. In press are 77 papers, 36 abstracts, and a symposium – "Enzyme Reaction Mechanisms."

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Anderson, N. G.	Studies on Isolated Cell Components. X. Structure Formation by Clear Liver Protein Solutions	Exptl. Cell Research 16, 42–48 (1959)
Anderson, N. G., and R. E. Canning	Studies on Isolated Cell Components. XII. An Ultracentrifugal Analysis of Soluble Proteins from Rat Liver, Brain, Kidney, and Testis	Exptl. Cell Research 17, 465–471 (1959)
Anderson, N. G., R. E. Canning, M. L. Anderson, and R. H. Shellhamer	Studies on Isolated Cell Components. XI. Rat Blood Proteins	Exptl. Cell Research 16, 292–308 (1959)
Arnold, W., and H. K. Maclay	Chloroplasts and Chloroplast Pigments as Semiconductors	In Brookhaven Symposia in Biology, No. 11: The Photochemical Apparatus, Its Structure and Function. Brookhaven Natl. Lab., 1958, p 1–9
Astrachan, L., and E. Volkin	Effects of Chloramphenicol on Ribonucleic Acid Metabolism in T2-Infected *Escherichia coli*	Biochim. et Biophys. Acta 32, 449–456 (1959)
Atwood, K. C., and S. L. Scheinberg	Isotope Dilution Method for Assay of Inagglutinable Erythrocytes	Science 129, 963–964 (1959)
von Borstel, R. C., and D. L. Lindsley	Insect Embryo Chromosome Techniques	Stain Technol. 34, 23–26 (1959)
Brosseau, G. E., Jr.	Crossing Over Between Y Chromosomes in Male Drosophila	Drosophila Info. Serv. 32, 115–116 (1958)
Brosseau, G. E., Jr., and D. L. Lindsley	A Dominantly Marked Y Chromosome: YB^S	Drosophila Info. Serv. 32, 116 (1958)

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Cohn, W. E.	5-Ribosyluracil, A Carbon-Carbon Ribofuranosyl Nucleoside in Ribonucleic Acid (Preliminary Note)	Biochim. et Biophys. Acta 32, 569–571 (1959)
Congdon, C. C.	Recovery from Radiation Injury, with Special Consideration of the Use of Bone Marrow Transplantation	In Progress in Hematology, Vol. 2 (ed. by L. M. Tocantins), Grune & Stratton, New York, 1959, p. 21–46
Conger, A. D., and M. L. Randolph	Magnetic Centers (Free Radicals) Produced in Cereal Embryos by Ionizing Radiation	Radiation Research 11, 54–66 (1959)
Cormier, M. J., M. P. Stulberg, and G. D. Novelli	Mechanism of the Carboxyl Activation of Glycine in Extracts of *Photobacterium fischeri* (Short Communication)	Biochim. et Biophys. Acta 33, 261–263 (1959)
Cosgrove, G. E., Jr., A. C. Upton, E. E. Schwartz, C. C. Congdon, and T. Makinodan	Effects of Immunized Parental Strain Bone Marrow on Lethally Irradiated F_1 Hybrid Mice	Proc. Soc. Exptl. Biol. Med. 100, 417–420 (1959)
Countryman, J. L., and E. Volkin	Nucleic Acid Metabolism and Ribonucleic Acid Heterogeneity in *Escherichia coli*	J. Bacteriol. 78, 41–48 (1959)
Dent, J. N.	A Technique for Removing Thyroid Glands from Anuran Larvae (A Note)	Endocrinology 64, 314–316 (1959)
Dolin, M. I.	The Oxidation of Reduced Diphosphopyridine Nucleotide by *Clostridium perfringens*. I. Relation of Peroxide to the Over-All Reaction	J. Bacteriol. 77, 383–392 (1959)
	The Oxidation of Reduced Diphosphopyridine Nucleotide by *Clostridium perfringens*. II. Purification of the Oxidase; Relation to Cytochrome c Reductase	J. Bacteriol. 77, 393–402 (1959)
Fabergé, A. C.	Production by Alpha Particles of Functionally Stable Broken Chromosome Ends in Maize	Genetics 44, 279–285 (1959)

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Furth, J., A. C. Upton, and A. W. Kimball	Late Pathologic Effects of Atomic Detonation and Their Pathogenesis	Radiation Research, Suppl. 1, 243–264 (1959); Proc. Intern. Congr. Radiation Research (ed. by D. E. Smith)
Gengozian, N., W. J. Peterson, and T. Makinodan	Killing Effect of Rat Bone Marrow in Sublethally Irradiated LAF_1 Mice	Cancer Research 19, 534–537 (1959)
Grell, E. H.	The Autonomy of Reciprocal Eye Transplants Between pn and K-pn	Drosophila Info. Serv. 32, 123–124 (1958)
Grell, R. M.	The Effect of X Chromosome Loss on Variegation	Drosophila Info. Serv. 32, 124 (1958)
	New Mutants	Drosophila Info. Serv. 32, 80 (1958)
Haber, A. H., and N. Brassington	Nonrespiratory Gas Released from Seeds During Moistening (Letter to the Editor)	Nature 183, 619–620 (1959)
Hollaender, A.	Opening Remarks – First International Congress of Radiation Research	Radiation Research, Suppl. 1, xi–xiii (1959); Proc. Intern. Congr. Radiation Research (ed. by D. E. Smith)
Hollaender, A., and S. F. Carson	Biology Division Semiannual Progress Report for Period Ending February 15, 1959	ORNL-2702

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Hollaender, A., C. C. Congdon, D. G. Doherty, T. Makinodan, and A. C. Upton	New Developments in Radiation Protection and Recovery	In Progress in Nuclear Energy, Medical Science, Vol. 2 (ed. by J. C. Bugher, J. Coursaget, and J. F. Loutit), Pergamon Press, London, 1959, p 139–150 and In Proc. Second U. N. Intern. Conf. on the Peaceful Uses of Atomic Energy, Geneva, 1958, Vol. 23, United Nations, New York, 1958, p 3–9
Kimball, A. W.	Disease Incidence Estimation in Populations Subject to Multiple Causes of Death	Intern. Statist. Bull. 36, Part 3, 193–204 (1958)
Kimball, R. F.	D1 Le Vacuome de la Cellule Vegetale-Morphologia; D2 Le Vacuome Animal; D3a Contractile Vacuoles of Protozoa; D3b Food Vacuoles, In Protoplasmatologica. Handbuch der Protoplasmaforschung. Vol. III (ed. by L. V. Heilbrunn and F. Weber). Springer-Verlag, Vienna, 1956 (Book Review)	Quart. Rev. Biol. 34, 87–88 (1959)
	The International Congress of Radiation Research at Burlington	Intern. J. Radiation Biol. 1, 91–94 (1959)
Kimball, R. F., and T. Barka	Quantitative Cytochemical Studies on *Paramecium aurelia*. II. Feulgen Microspectrophotometry of the Macronucleus During Exponential Growth	Exptl. Cell Research 17, 173–183 (1959)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Kimball, R. F., T. Caspersson, G. Svensson, and L. Carlson	Quantitative Cytochemical Studies on *Paramecium aurelia*. I. Growth in Total Dry Weight Measured by the Scanning Interference Microscope and X-Ray Absorption Methods	Exptl. Cell Research 17, 160–172 (1959)
Kimball, R. F., N. Gaither, and S. M. Wilson	Recovery in Stationary-Phase Paramecia from Radiation Effects Leading to Mutation	Proc. Natl. Acad. Sci. U.S. 45, 833–839 (1959)
	Reduction of Mutation by Postirradiation Treatment After Ultraviolet and Various Kinds of Ionizing Radiations	Radiation Research 10, 490–497 (1959)
Krieg, D. R.	A Study of Gene Action in Ultraviolet-Irradiated Bacteriophage T4	Virology 8, 80–98 (1959)
Kroeger, H.	Determination Mosaics from Combined Implanted Imaginal Discs in *Ephestia kuehniella* Zeller (in German)	Wilhelm Roux' Arch. Entwicklungsmech. Organ. 151, 113–135 (1959)
Lindsley, D. L., and E. H. Grell	The Genetic Extent of Dp(3;1)0–5	Drosophila Info. Serv. 32, 136 (1958)
Lindsley, D. L., and E. Novitski	Compound Chromosomes Involving the X and Y Chromosomes of *Drosophila melanogaster*	Genetics 44, 187–196 (1959)
	Localization of the Genetic Factors Responsible for the Kinetic Activity of X Chromosomes of *Drosophila melanogaster*	Genetics 43, 790–798 (1958)
McGrath, R. A.	A Chambered Microscope Slide	Microscope 12, 57–59 (1958)
McGrath, R. A., and C. C. Congdon	X-Ray-Induced Abnormal Differentiation of the Epithelium of the Small Intestine in the Mouse	Intern. J. Radiation Biol. 1, 80–85 (1959)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Mota, M.	Karyokinesis Without Cytokinesis in the Grasshopper	Exptl. Cell Research 17, 76–83 (1959)
Nichol, J. C.	Electrophoretic Methods for Desalting Amino Acids	Science 129, 1549–1550 (1959)
Nystrom, C. W., N. E. Tolbert, and S. H. Wender	Formation of β-Phenylglucoside in Plant Leaves	Plant Physiol. 34, 142–143 (1959)
Odell, T. T., Jr., and B. Anderson	Production and Life Span of Platelets	In The Kinetics of Cellular Proliferation (ed. by F. Stohlman, Jr.), Grune & Stratton, New York, 1959, p 278–280
Owen, R. D.	Genetic Aspects of Tissue Transplantation and Tolerance	J. Med. Educ. 34, 366–383 (1959)
	Immunogenetics	Proc. Tenth Intern. Congr. Genetics 1, 364–374 (1959)
	Immunology of Tissue Transplantation and Tolerance	In A Symposium on Immunity and Virus Infection (ed. by V. A. Najjar), John Wiley & Sons, New York, 1959, p 31–37
Peck, H. D., Jr.	The ATP-Dependent Reduction of Sulfate with Hydrogen in Extracts of *Desulfovibrio desulfuricans*	Proc. Natl. Acad. Sci. U.S. 45, 701–708 (1959)
Rogers, P., and G. D. Novelli	Formation of Ornithine Transcarbamylase in Cells and Protoplasts of *Escherichia coli*	Biochim. et Biophys. Acta 33, 423–436 (1959)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Russell, W. L.	Radiation-Induced Genetic Damage in Mammals	In Genetics, Radiobiology, and Radiology Proceedings, Midwestern Conference. Chairmen, W. G. Scott and T. C. Evans. Charles C Thomas, Springfield, Ill., 1959, p 41–55
Russell, W. L., and L. B. Russell	The Genetic and Phenotypic Characteristics of Radiation-Induced Mutations in Mice	Radiation Research, Suppl. 1, 296–305 (1959); Proc. Intern. Congr. Radiation Research (ed. by D. E. Smith)
	Radiation-Induced Genetic Damage in Mice	In Progress in Nuclear Energy, Biological Sciences, Vol. 2 (ed. by J. C. Bugher, J. Coursaget, and J. F. Loutit), Pergamon Press, London, 1959, p 179–188 and In Proc. Second Intern. Conf. on the Peaceful Uses of Atomic Energy, Geneva, 1958, United Nations, New York, 1958.
Russell, W. L., L. B. Russell, and J. S. Gower	Exceptional Inheritance of a Sex-Linked Gene in the Mouse Explained on the Basis that the X/O Sex-Chromosome Constitution Is Female	Proc. Natl. Acad. Sci. U.S. 45, 554–560 (1959)
St. Amand, W., and L. H. Smith	A Method for the Quantitative Estimation of Rat Erythrocytes in the Mouse-Rat Chimera	J. Appl. Physiol. 14, 445–447 (1959)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Schwartz, D.	Further Studies on Breakage-Fusion-Bridge Cycles in Maize Endosperm	Maize Genet. Coop. News Letter 33, 88–89 (1959)
de Serres, F. J.	Recombination and Interference in the ad-3 Region of *Neurospora crassa*	Cold Spring Harbor Symposia Quant. Biol. 23, 111–118 (1958)
Shekarchi, I. C., and T. Makinodan	Persistence of Heterologous Bone Marrow in Mice as a Function of X-Ray Dose	Proc. Soc. Exptl. Biol. Med. 100, 414–417 (1959)
Sheppard, C. W.	An Electromathematical Theory of Circulatory Mixing Transients	In Proc. First Natl. Biophys. Conf. (ed. by H. Quastler and H. J. Morowitz), Yale Univ. Press, New Haven, 1959, p 476–493
Tolbert, N. E.	Secretion of Glycolic Acid by Chloroplasts	In Brookhaven Symposia in Biology, No. 11: The Photochemical Apparatus, Its Structure and Function. Brookhaven Natl. Lab., 1958, p 271–275
Upton, A. C., D. G. Doherty, and G. S. Melville, Jr.	Chemical Protection of the Mouse Against Leukemia Induction by Roentgen Rays	Acta Radiol. 51, 379–384 (1959)
Urso, I. S., C. C. Congdon, and R. D. Owen	Effect of Foreign Fetal and Newborn Blood-Forming Tissues on Survival of Lethally Irradiated Mice	Proc. Soc. Exptl. Biol. Med. 100, 395–399 (1959)
Weimberg, R.	L-2-Keto-4,5-dihydroxyvaleric Acid: An Intermediate in the Oxidation of L-Arabinose by *Pseudomonas saccharophila*	J. Biol. Chem. 234, 727–732 (1959)

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Welshons, W. J.	The Analysis of a Pseudoallelic Recessive Lethal System at the Notch Locus of *Drosophila melanogaster*	Cold Spring Harbor Symposia Quant. Biol. 23, 171–176 (1958)
Welshons, W. J., and L. B. Russell	The Y Chromosome as the Bearer of Male Determining Factors in the Mouse	Proc. Natl. Acad. Sci. U.S. 45, 560–566 (1959)
Wolff, S.	Interpretation of Induced Chromosome Breakage and Rejoining	Radiation Research, Suppl. 1, 453–462 (1959); Proc. Intern. Congr. Radiation Research (ed. by D. E. Smith)
(Members of the Biology Division)	Summary of Reports of Conferences Organized by Members of the Biology Division, ORNL	Reprint No. 1325

Scientific Society Lectures and Traveling Seminars. – Forty-four of the lectures given by Division personnel were before professional society groups, including speeches at several international meetings and symposia.

AUTHOR(S)	TITLE	PLACE PRESENTED
Adler, H. I.	Physiology and Radiation Behavior of a Catalase-Negative, Cytochrome-Negative Bacterium	Institut Pasteur, Service de Biochimie Cellulaire, Paris, France
	As above	Instituta di Microbiologie, Université, Citta Universitaria, Rome, Italy
	Radiation Behavior of a Catalase-Negative, Cytochrome-Negative *Escherichia coli*	British Empire Cancer Campaign, Research Unit in Radiobiology, Mount Vernon Hospital, Northwood, England

AUTHOR(S)	TITLE	PLACE PRESENTED
Adler, H. I.	As above	Medical Research Council Experimental Radiopathology Unit, Hammersmith Hospital, London, England
	Response of a Catalase-Negative Bacterium to Ionizing Radiation	Symposium on Immediate and Low-Level Effects of Ionizing Radiation, Venice, Italy
	Synthesis of Respiratory Enzymes	The Eldridge Reeves Johnson Foundation for Medical Physics, University of Pennsylvania, Philadelphia
Anderson, N. G.	Cell Physiology at the Molecular Level	Meharry Medical College, Nashville, Tenn.
Arnold, W. A.	Changes in Science	Sigma Xi, University of Georgia, Athens
	Chloroplasts as Semiconductors	Southeastern Section, Am. Phys. Soc., New Orleans, La.
Bender, M. A.	Effects of Ionizing Radiation on Mammalian Chromosomes *in vivo* and *in vitro*	Symposium on Immediate and Low-Level Effects of Ionizing Radiation, Venice, Italy
Bollum, F. J.	Observations on DNA Primers	Dept. of Biochemistry, University of Wisconsin, Madison
von Borstel, R. C.	The Nature of Radiation-Induced Death in *Habrobracon* and *Drosophila* Gametes	Dept. of Zoology, University of North Carolina, Chapel Hill

AUTHOR(S)	TITLE	PLACE PRESENTED
van Borstel, R. C.	Radiation Inactivation of Habrobracon and Drosophila Gametes	Donner Laboratory, University of California, Berkeley
	As above	Dept. of Zoology, California Institute of Technology, Pasadena
Brassington, N., and A. H. Haber	Nonrespiratory Gas Released from Seeds During Moistening	Assoc. Southeastern Biologists, Knoxville, Tenn.
Carson, S. F. (Paper by E. F. Phares and S. F. Carson)	Bacterial Succinic Acid Decarboxylase System	Soc. Am. Bacteriol., St. Louis, Mo.
Cohn, W. E.	An Unusual Constituent of Ribonucleic Acids	Dept. of Microbiology, Vanderbilt University, Nashville, Tenn.
	An Unusual Nucleotide from Ribonucleic Acids	Rockefeller Institute, New York, N. Y.
Congdon, C. C.	Immunologic Problems in Tissue Transplantation for Recovery from Radiation Injury	XXI Intern. Congr. Physiol. Sci., Buenos Aires, Argentina
Congdon, C. C., and D. Duda	Prevention of Bone Marrow Heterografting in Irradiated Mice by Isologous Thymus	Radiation Research Soc., Pittsburgh, Pa.
Cosgrove, G. E.	Therapy for Radiation Damage	Conf. on Radioactive Isotopes in Agriculture, Stillwater, Okla.
Countryman, J. L.	Nucleic Acid Metabolism and Ribonucleic Acid Heterogeneity in Normal and Phage-Infected Bacteria	Genetics Dept., University of Texas, M. D. Anderson Hospital, Houston
Doherty, D. G.	Chemical Protection Against Ionizing Radiation	Dept. of Biochemistry, Tulane University, New Orleans, La.

AUTHOR(S)	TITLE	PLACE PRESENTED
Doherty, D. G.	As above	Dept. of Biochemistry, Loyola University, New Orleans, La.
	As above	Am. Chem. Soc., Lafayette, La.
	As above	Sigma Xi, Lafayette, La.
Fisher, W. D.	The Effect of X Rays on Deoxyribonucleoprotein from Rat Thymus	Radiation Research Soc., Pittsburgh, Pa.
Gengozian, N.	Antibody Production in Mice as a Function of Age and Antigen	Biology Dept., University of Houston, Houston, Tex.
	As above	M. D. Anderson Hospital and Tumor Institute, University of Texas, Austin
	As above	Dept. of Bacteriology, University of Kentucky, Lexington
	Bone Marrow Transplantation After Irradiation	Biology Dept., Baylor University, Waco, Tex.
	Radiation Protection Studies in Young Mice	Radiation Research Soc., Pittsburgh, Pa.
Haber, A. H.	Seed Germination	Botany Dept., University of Tennessee, Knoxville
Hollaender, A.	Studies on the Mechanism of Protection and Recovery at the Biology Division of the Oak Ridge National Laboratory	Radiobiological Study Group, University of Oslo, Oslo, Norway
Hollcroft, J., and C. C. Congdon	Bone Marrow Protection of Guinea Pigs After Termination of Chronic Irradiation	Radiation Research Soc., Pittsburgh, Pa.

AUTHOR(S)	TITLE	PLACE PRESENTED
Kimball, R. F.	Modern Concepts of Mutation	Beta Beta Beta, Biological Fraternity, George Peabody College for Teachers, Nashville, Tenn.
	Recent Advances in the Study of Mutation and the Hazards of Radiation	Radiation Biology Institute, Tuskegee Institute, Tuskegee, Ala.
Kimball, R. F., N. Gaither, and S. M. Wilson	Recovery of Nongrowing Paramecia from Mutation-Producing X-Ray Damage	Radiation Research Soc., Pittsburgh, Pa.
Kirby-Smith, J. S.	Electron Spin Resonance in DNA and Protein	Radiation Research Soc., Pittsburgh, Pa.
	Production and Lifetimes of Radiation-Induced Free Radicals in Some Molecules of Biological Importance	Symposium on Immediate and Low-Level Effects of Ionizing Radiation, Venice, Italy
Kølmark, G.	Chemical Protection Against Radiation Effects in *Neurospora crassa*	Radiation Research Soc., Pittsburgh, Pa.
Krieg, D. R.	(Informal Report)	Conf. on Bacteriophage Research, Cold Spring Harbor, N. Y.
Lindsley, D. L.	Y-Suppressed Sex-Linked Recessive Lethals in *Drosophila melanogaster*	Zoology Dept., University of California, Los Angeles
Luippold, H. E.	Effect of Three Different Types of Radiation on Barley Seeds	Botany Dept., University of Tennessee, Knoxville
McGrath, R. A.	Abnormal Cell Enlargement in the Mouse Small Intestine After Total-Body X Irradiation	Radiation Research Soc., Pittsburgh, Pa.
Maisin, J., J. Moutschen, G. D. Novelli, and D. G. Doherty	Chemical Protection of the Intestine Against Radiation Damage	Radiation Research Soc., Pittsburgh, Pa.

AUTHOR(S)	TITLE	PLACE PRESENTED
Makinodan, T., and N. Gengozian	X-Ray Depression of the Recognition Mechanism of Antibody-Forming Cells	Symposium on The Mechanism of Antibody Formation, Liblice, Prague, Czechoslovakia
Makinodan, T., E. H. Perkins, I. C. Shekarchi, and N. Gengozian	Use of Lethally Irradiated Isologous Mice as *in vivo* Tissue Cultures of Antibody-Forming Cells	Symposium on The Mechanism of Antibody Formation, Liblice, Prague, Czechoslovakia
Oakberg, E. F.	Effects of Radiation on the Gonads of Mice	Conf. on Radioactive Isotopes in Agriculture, Stillwater, Okla.
	Irradiation Damage to Animals and Its Effect on Their Reproductive Capacity	Fourth Biennial Symposium on Animal Reproduction, University of Illinois, Urbana
Odell, T. T., Jr.	Platelet Production and Life Span	Zoology Seminar, Dept. of Zoology and Entomology, University of Tennessee, Knoxville
	Tissue Transplantation in Mammals After Treatment with Radiation and Chemical Agents	Lynchos Society, Longwood College, Farmville, Va.
Owen, R. D.	Erythrocyte Repopulation After Transplantation of Homologous Erythropoietic Tissues into Irradiated Mice	Colloquium on Biological Problems of Grafting, Liège, Belgium
Peck, H. D.	Hydrogenase	Microbiology Dept., Tulane Medical School, New Orleans, La.
Perkins, E. H., and T. Makinodan	Lethally X-Irradiated Isologous Mice as *in vivo* Tissue Cultures for Quantitative Study of Antibody Production	Radiation Research Soc., Pittsburgh, Pa.
Popp, R. A., and G. E. Cosgrove	Solubility of Hemoglobin as a Red Cell Marker	Radiation Research Soc., Pittsburgh, Pa.

AUTHOR(S)	TITLE	PLACE PRESENTED
Randolph, M. L.	Magnetic Resonance Studies on Amino Acids	Dept. of Physics, Washington University, St. Louis, Mo.
Randolph, M. L., and D. L. Parrish	Long-Term Decay Patterns of Radiation-Induced Electron Spin Resonances in Crystalline Amino Acids	Radiation Research Soc., Pittsburgh, Pa.
Royal, G. W., C. C. Congdon, and G. C. Royal	A Comparison of 5-Hydroxytryptamine (Serotonin) Levels in Tissues from Lethally Irradiated Mice After Isologous or Heterologous Bone Marrow Treatment	Radiation Research Soc., Pittsburgh, Pa.
Russell, L. B.	Radiation Hazards During Embryonic Development	Ninth International Congress of Radiology, Munich, Germany
	Radiation Hazards in Obstetrics and Gynecology	Annual Clinical Meeting, American College of Obstetricians and Gynecologists, Atlantic City, N. J.
Russell, L. B., S. K. Badgett, and C. L. Saylors	Comparison of the Effects of Acute, Continuous, and Fractionated Irradiation During Embryonic Development	Symposium on Immediate and Low-Level Effects of Ionizing Radiation, Venice, Italy
Russell, W. L.	Mammalian Radiation Genetics	Dept. of Human Genetics, University of Michigan Medical School, Ann Arbor
	As above	Biophysics Seminar, Sloan-Kettering Institute for Cancer Research, New York, N. Y.

AUTHOR(S)	TITLE	PLACE PRESENTED
Russell, W. L.	Recent Advances in Genetics in Mice	Symposium on Molecular Genetics and Human Disease, State University of New York, Upstate Medical Center, Syracuse
	A Survey of Mammalian Radiation Genetics	Ninth International Congress of Radiology, Munich, Germany
Russell, W. L., L. B. Russell, and E. M. Kelly	Dependence of Mutation Rate on Radiation Intensity	Symposium on Immediate and Low-Level Effects of Ionizing Radiation, Venice, Italy
Russell, W. L., L. B. Russell, M. H. Steele, and E. L. Phipps	Extreme Sensitivity of an Immature Stage of the Mouse Ovary to Sterilization by Irradiation [Science 129, 1288 (1959)]	Natl. Acad. Sci., Washington, D. C.
Schwartz, D.	Genetic Studies on Enzymes in Maize Endosperm [Science 129, 1287 (1959)]	Natl. Acad. Sci., Washington, D. C.
Smith, L. H.	Effects of Radiation on Mammals	Division of Natural Sciences, Tuskegee Institute, Tuskegee, Ala.
	In vitro Protection of Irradiated Bone Marrow Cells	Radiation Research Soc., Pittsburgh, Pa.
	Modification of Radiation Injury in Mammals	Pre-Medical Club, Maryville College, Maryville, Tenn.
	Treatment of Radiation Injury	Medical College of Virginia, Richmond
Snider, P. J.	Genetic Control of Nuclear Ratios in *Schizophyllum*	Botany Dept., University of Texas, Austin

AUTHOR(S)	TITLE	PLACE PRESENTED
Snider, P. J.	Translational Movements of Nuclei in Fungi, as Related to Development and Influenced by Genetics	Dept. of Health, State of New York, Division of Laboratories and Research, Albany
Sproul, J. A., Jr., K. W. Christenberry, M. L. Randolph, and A. C. Upton	Relative Biological Effectiveness of 14-Mev Neutrons and Co^{60} Gamma Rays for Cataract Induction in Mice	Radiation Research Soc., Pittsburgh, Pa.
Stevens, B. J., and M. E. Gaulden	The Effects of Radiation on Mitotic Rate of Individual Stages of Mitosis in the Grasshopper Neuroblast	Assoc. Southeastern Biologists, Knoxville, Tenn.
Stuy, J. H.	The Behavior and Biological Activity of Deoxyribonucleic Acid in Irradiated and Unirradiated Bacteria	M. D. Anderson Hospital, University of Texas, Houston
Upton, A. C.	Dose Response Relations in Radiation Carcinogenesis	Symposium on Radiation and Cancer, Am. Assoc. Cancer Research, Atlantic City, N. J.
	Inhibition of Foreign Spleen Reaction by "Immunological Paralysis" of Donor Spleen Cells Before Implantation	Radiation Research Soc., Pittsburgh, Pa.
	Radiation and Cancer	Rotary Club, Oak Ridge, Tenn.
Vos, O., J. W. Goodman, and I. S. Urso	Treatment of Irradiated Mice with Chimera Bone Marrow Cells	Radiation Research Soc., Pittsburgh, Pa.
Wells, C.	Some Aspects of Radiation Biology	Biology Dept., Agnes Scott College, Atlanta, Ga.
Welshons, W. J.	Nature of Basic Research and the Life of a Researcher (7 lectures)	AIBS Visiting Lecturer Program, Stetson University, DeLand, Fla.

AUTHOR(S)	TITLE	PLACE PRESENTED
Wolff, S.	Radiation Studies on the Nature of Chromosome Breakage	Radiation Research Soc., Pittsburgh, Pa.
Wood, N. P.	A Purified Diaphorase from Streptococcus faecalis	Soc. Am. Bacteriol., St. Louis, Mo.

Visiting Lecturers. – During the period covered by this report, the Biology Seminar program has included the following scientists from research organizations, universities, and laboratories located in the United States and abroad.

LECTURER	AFFILIATION	TOPIC
Raymond Latarjet	Laboratoire Pasteur de l'Institut du Radium, Paris, France	1. Recent Experimental Work on Lymphoid Leukemia of the AK Mouse 2. Yugoslavian Radiation Accident
Cyril Darlington	Department of Botany, Oxford University, Oxford, England	Chromosome Breakage: The Theory of What Happens Between Breakage and Observation
Helmut Pauly	University of Pennsylvania, Philadelphia	Effects of X Rays on Enzymes and Adaptive Enzyme Synthesis Within Bacteria
U. Westphal	Fort Knox, Ky.	Steroid-Protein Interactions
Alexander R. Todd	The Chemical Laboratory, Cambridge University, Cambridge, England	Some Aspects of Phosphorylation
Irwin Tessman	Massachusetts Institute of Technology, Cambridge	Structure and Function of the Very Small Phages
Lola S. Kelly	Donner Laboratory, University of California, Berkeley	Autoradiographic Studies of Leukocyte Formation
Georges Mathé	Fondation Curie, Paris, France	Experimental and Clinical Data on Bone Marrow Transplantation
Norman H. Giles, Jr.	Yale University, New Haven, Conn.	Complementation Maps – Biochemical and Genetical Implications
Frank Davis	Agricultural Experiment Station, Rutgers – The State University, New Brunswick, N. J.	Nucleotides of Minor Occurrence

LECTURER	AFFILIATION	TOPIC
David Bishop	Carnegie Institution of Washington, Baltimore, Md.	Extracted Sperm Cells as "Micromuscular" Systems
A. N. J. Heyn	School of Textiles, Clemson Agricultural College, Clemson, S. C.	Interference Microscopy
Robert W. Merriam	Zoology Laboratory, University of Pennsylvania, Philadelphia	Some Physical Aspects of Nucleocytoplasmic Relationships in Cells
Waclaw Szybalski	Institute of Microbiology, Rutgers — The State University, New Brunswick, N. J.	Dispersive Mode of DNA Replication in Unimolecular Phage ΦX-174
John P. Hummel	Biochemistry Department, State University of Iowa, Iowa City	The Binding of Polyxenylphosphate to Tumor Tissue
C. C. Lushbaugh	Biomedical Research Division, Los Alamos Scientific Laboratory, Los Alamos, N. Mex.	Application of Liquid Scintillators to Clinical Medicine
B. A. Newton	Department of Biochemistry, University of Cambridge, Cambridge, England	Nucleic Acid Synthesis in a Parasitic Flagellate
William A. Jensen	Department of Botany, University of California, Berkeley	Cell Division and Cellular Differentiation in Roots
Eichi Yamada	Kurume University, Japan, and the Rockefeller Institute, New York	The Fine Structure of the Pigment Epithelium of the Frog
Shlomo Hestrin	National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.	Control of Fermentation of Carbohydrates in Yeasts by Genes and Inducers (two lectures)
Lowell Hager	Chemistry Department, Harvard University, Cambridge, Mass.	Enzymatic Studies on the Biosynthesis of Chlorine-Containing Natural Products
P. Starlinger	Botanisches Institut der Universität Koln, Koln-Lindenthal, Germany	Synthesis of Galactokinase in Cells of *E. coli* Infected with Transducing Phage Lambda
H. G. Hers	Laboratoire de Chimie Physiologique, Université de Louvain, Louvain, Belgium	Glycogen Storage Disease

LECTURER	AFFILIATION	TOPIC
Mortimer M. Elkind	Radiation Branch, National Cancer Institute, Bethesda, Md.	X-Ray Damage and Repair in Mammalian Cells
Christine Jardetsky	Biological Laboratory, Harvard University, Cambridge, Mass.	The Structure of Ribose in Ribosides and Ribotides as Determined by Proton Magnetic Resonance
Oleg Jardetsky	Department of Pharmacology, Harvard Medical School, Boston, Mass.	Proton Magnetic Resonance Study of the Conformation of Chloramphenicol

Speakers at Professional Meetings. – During the period between August 15 and the end of autumn, members of the Biology Division will present papers at the following professional meetings:

Symposium on Hematology and Hemophilia (Buenos Aires, Argentina) – C. C. Congdon (2 papers, one coauthored by O. Vos)

Ninth International Botanical Congress (Montreal, Canada) – D. Davidson, A. H. Haber (coauthor, H. J. Luippold), R. Rabson, and S. Wolff

American Institute of Biological Sciences – R. E. Canning (with N. G. Anderson), R. F. Grell, R. F. Kimball (coauthors, N. T. Gaither and S. M. Wilson), G. Y. Kikudome, B. Nicoletti (coauthor, D. L. Lindsley), L. B. Russell (coauthor, J. W. Bangham), O. C. Wells (2 papers), and S. Wolff

Faraday Society Symposium on Aspects of Behavior and Metabolism of the Cell Nucleus (Cambridge, England) – N. G. Anderson (coauthor, W. D. Fisher), F. J. Bollum, M. E. Gaulden, and D. Schwartz

American Chemical Society – J. X. Khym (with W. E. Cohn) and E. F. Phares (coauthor, S. F. Carson)

Symposium on Comparative Biochemistry of Photoreactor Pigments – F. T. Wolf

New York Academy of Sciences – P. Mazur

Brookhaven Symposium on Bioenergetics – J. Jagger

Foreign Travel. – Dr. Takashi Makinodan was invited to present two papers at the Symposium on the Mechanism of Antibody Formation sponsored by the Czech Academy of Science in Liblice, near Prague.

In June of this year, the Symposium on Immediate and Low-Level Effects of Ionizing Radiation in Venice, Italy, included five papers from the Biology Division. Alexander Hollaender served as a chairman, and the papers were given by H. I. Adler, M. A. Bender, J. S. Kirby-Smith, L. B. Russell, and W. L. Russell.

While on the European trip, W. L. Russell and L. B. Russell also attended the Ninth International Congress of Radiology in Munich. Each presented a paper at the Congress.

A. C. Upton attended the Ciba Foundation Third Annual Lecture on Aging Research, in London, England, and gave an invited paper.

Soon after the beginning of the period of this report, Alexander Hollaender made a trip to several South American countries to assist in organizing programs in basic research in biology, especially genetics. This trip was sponsored by the USAEC Division of International Affairs.

During the summer, C. C. Congdon attended the XXI International Congress of Physiological Sciences in Buenos Aires, where he presented a paper. While in South America, Dr. Congdon also participated in the Symposium on Hematology and Hemophilia by giving two papers. This Symposium was sponsored by the National Academy of Medicine in Buenos Aires.

Alexander Hollaender also attended the Physiological Sciences Congress and served as chairman of the Symposium on Radiation Protection and Recovery. Dr. Hollaender was United States representative to the Committee on International Relations in Biophysics to the Congress for the National Academy of Sciences-National Research Council. The trips included further discussions at several research institutions on South American programs in basic and radiation biology.

All Division members participating in the foreign travel listed visited many research laboratories, taking part in informal discussions and giving lectures.

Late August will see four Division members taking part in a Faraday Society Symposium on Aspects of Behavior and Metabolism of the Cell Nucleus in Cambridge, England. Each of the following will present a paper and participate in the discussions: N. G. Anderson, F. J. Bollum, M. E. Gaulden, and D. Schwartz.

1959 Biology Symposium. Enzyme Reaction Mechanisms. — The proceedings of the Twelfth Annual Biology Symposium held in Gatlinburg in April are being published as a supplement to the Journal of Cellular and Comparative Physiology. The table of contents of the supplement (which will

be a December 1959 publication) follows:

Alexander Hollaender. Introduction

Alexander R. Todd. Introduction to the Symposium on Enzyme Reaction Mechanisms

H. Gobind Khorana. Synthesis and Structural Analysis of Polynucleotides. Seven figures

Mildred Cohn. Mechanisms of Enzymic Cleavage of Some Organic Phosphates. Five figures

Feodor Lynen. Participation of Acyl-CoA in Carbon Chain Biosynthesis. Twenty-five figures

Melvin Calvin and Ning G. Pon. Carboxylations and Decarboxylations. Thirty figures

Fritz Lipmann, W. C. Hülsmann, G. Hartmann, Hans G. Boman, and George Acs. Amino Acid Activation and Protein Synthesis. Seven figures

Bernard L. Horecker. Aldol and Ketol Condensations. Twenty-two figures

F. M. Huennekens, H. R. Whiteley, and M. J. Osborn. Mechanisms of Formylation and Hydroxymethylation Reactions. Eighteen figures

Shlomo Hestrin. Substrate Specificity of Chain Propagation Steps in Saccharide Synthesis. Two figures

John M. Buchanan, Standish C. Hartman, Robert L. Herrmann, and Richard A. Day. Reactions Involving the Carbon-Nitrogen Bond: Heterocyclic Compounds. Sixteen figures

Esmond E. Snell and W. Terry Jenkins. The Mechanism of the Transamination Reaction. Twelve figures

Hans Neurath and Brian S. Hartley. The Hydrolysis of Peptide and Ester Bonds by Proteolytic Enzymes. Eleven figures

Brian S. Hartley. The Chemical Structure of Chymotrypsin. One figure

Frederic M. Richards. Comments on the Modification of Enzymes, with Special Reference to Ribonuclease

Christian B. Anfinsen. Some Approaches to the Study of Active Centers. Three figures

Max Brenner. The Aminoacyl Insertion Reaction. Nine figures

J. A. Cohen, R. A. Oosterbaan, H. S. Jansz, and F. Berends. The Active Site of Esterases. Eight figures

Daniel E. Koshland, Jr. Enzyme Flexibility and Enzyme Action. Nine figures

Philip Handler. Summarizing remarks

Thirteenth Annual Research Conference. — The 1960 Research Conference sponsored by the Biology Division of the Oak Ridge National Laboratory will be held on April 4–7 in Gatlinburg, Tennessee, at the Mountain View Hotel. This conference, supported by the U.S. Atomic Energy Commission, will consist of invited papers and open discussions on "Mammalian Genetics and Reproduction." Biology Division members comprising the organizing committee for this conference are W. L. Russell, Liane B. Russell, E. F. Oakberg, and W. J. Welshons.

Bone Marrow Conferences. — The spring meeting in the series of informal conferences on bone marrow transplantation and chemical protection was held immediately preceding the Federation in Atlantic City. About 150 people interested in the experimental and clinical aspects of the problems attended the meeting. An account of the proceedings was written by Otto Vos of this Laboratory and has been submitted to Blood, The Journal of Hematology.

The fall conference will be held September 18 and 19 at the National Cancer Institute at Bethesda, Maryland. Dr. Gordon Zubrod is in charge of this meeting.

Education. — The Biology Student Trainee program was carried on for the second summer in cooperation with the Oak Ridge Institute of Nuclear Studies, and the following students participated in this program from the schools indicated:

Charles L. Byrd	Centenary College of Louisiana, Shreveport
Verna M. Cook	Alabama College, Montevallo
Robert M. Flora	Bridgewater College, Bridgewater, Virginia
Marvin N. Goldstein	Western Maryland College, Westminster

David I. Hirsch (National Science Foundation Fellow)	Reed College, Portland, Oregon
Nancy N. Lanier	Randolph-Macon Woman's College, Lynchburg, Virginia
Sally A. Sapp	Winthrop College, Rock Hill, South Carolina
Linda E. Smith (National Science Foundation Fellow)	Reed College, Portland, Oregon
Sondra E. Wagner	Maryville College, Maryville, Tennessee
Joe D. White	Mississippi Southern College, Hattiesburg
Joanne L. Yundt	The Woman's College of the University of North Carolina, Greensboro

This program was set up primarily to give students from small Southern schools who have completed at least three years of college work the opportunity to work in a laboratory devoted to full-time basic research. Trainees are selected on the basis of scholastic records and interest in scientific research, and work in the Division for approximately ten weeks during the summer months. They work on a specific problem in one of the Division groups where research is being done in a field most closely related to the student's interest. They also visit and observe the work going on in other groups, attend Division seminars, and have frequent informal discussions with research scientists.

Since we have established close cooperation with Reed College, Portland, Oregon, one student from there joined the program in 1958 on a National Science Foundation fellowship, and in 1959 there were two students from that school on NSF fellowships. In 1959, there was also an additional student sponsored by the Division: Marcus T. Cohn, The College of Wooster, Wooster, Ohio.

In addition to students coming to the Division, during the past half year several members of the Division have visited other institutions and given short courses in various phases of radiation biology.

C. C. Congdon presented a course consisting of five lectures entitled "Tissue Transplantation" at the Wadley Research Institute in Dallas, Texas. G. E. Cosgrove also gave a five-lecture series at the Summer Institute of

Radiation Biology, University of Kansas, Lawrence. His subject was "Tissue Damage," with particular reference to both early and delayed pathological effects. J. Jagger and T. T. Odell, Jr., participated in the NSF Summer Institute for High School Teachers at Columbia College, Columbia, South Carolina. Dr. Jagger's subject was "Biological Effects of Radiation," Dr. Odell's was "Radiation Effects and Modifications in Multicellular Animals, Especially Man." In addition, Dr. Odell lectured on blood platelets to the summer students of the college. A. C. Upton, serving on the lecture program for the ORINS Traveling Teachers, gave a course entitled "Radiation Effects on Somatic Tissue."

CYTOLOGY AND GENETICS

CYTOGENETIC EFFECTS OF RADIATION

R. F. Kimball
R. T. Brumfield[1]
D. Davidson[2]
A. P. Davis[1]
D. M. Green[3]
G. Y. Kikudome
H. G. Kølmark[2]
D. R. Krieg
M. Moutschen-Dahmen[2]
D. Schwartz
F. J. de Serres
P. J. Snider, Jr.[3]
O. C. Wells[3]
D. B. Williams[4]
S. Wolff
I. R. Cox
N. T. Gaither
M. N. Goldstein[5]
D. I. Hirsh[5]
H. E. Luippold
K. H. McGrath
M. E. Picinich[1]
L. E. Smith[5]
A. P. Teasley
S. M. Wilson

Further Studies on the Postirradiation Period in X-Ray Mutagenesis in *Paramecium aurelia*

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

Introduction. – Previous work[6,7] with recessive lethal and slow growth mutations in *Paramecium aurelia* has shown that mutation can be decreased by various posttreatments, and the hypothesis has been proposed that posttreatments act by increasing the time available for loss of premutational damage, a loss that would occur even in the absence of treatment. A further analysis of this situation requires investigation of the effect of agents not only on the time available for loss but on the rate of loss, and beginning investigations along these lines were described in the last report.[8] The present report considers this problem on the basis of newly acquired evidence.

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[1]Consultant.

[2]Visiting investigator from abroad.

[3]Research Associate.

[4]Research Participant.

[5]Biology Student Trainee.

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

[7]R. F. Kimball, N. Gaither, and S. M. Wilson, Proc. Natl. Acad. Sci. U. S. 45, 833–39 (1959).

[8]R. F. Kimball, N. Gaither, and S. M. Wilson, Biol. Semiann. Prog. Rep. Feb. 15, 1959, ORNL-2702, p 28–30.

Results. — In the previous report[8] the basic experiment for measuring the rate of loss of premutational damage was described. Dividing paramecia were selected and put into a sublethal concentration of the agent to be tested shortly after division. Different groups were irradiated in the agent with 4.5 kr of 250-kvp X rays at two or more times after division and left in the agent for 24 hr, by which time they had divided once or twice. Recessive lethal and deleterious mutations were measured as previously described.

Data derived in this way were given in the previous report but are now more extensive, and an example is shown in Fig. 1. A straight line gives a good fit to the more numerous control data, and the slope of this line measures the rate of loss of premutational damage on the assumption that the later the radiation is given, the less the time for loss.

Previous evidence[6,7] has shown that mutation can no longer be modified by posttreatment after about the midpoint (2.5 hr) of the normal interdivision interval. This time (T) seems to be associated with a large and abrupt drop in the amount of mutation (Fig. 1). The reason for this association is unknown, but it can be used as a way to estimate T under various circumstances.[8] Thus T is about 2.5 hr after division for unirradiated controls (data from earlier experiments) while it is about 4.5 hr after division in paramecia growing in 0.2 mg/ml of streptomycin (Fig. 1).

Data are now available from experiments of this kind for four agents. The slopes in M per hour of the linear fit to the data for the first 2 hr are: controls, 0.14±0.03 and 0.18±0.03 for experiments done in different years; 0.06 mg/ml chloramphenicol, 0.033±0.043; 0.2 mg/ml streptomycin, 0.064±0.032; 0.3 mg/ml caffeine, 0.021±0.034; 0.2 mg/ml iodoacetic acid, 0.029±0.049. All the agents significantly decrease the slope below that of the control; but only streptomycin gives a slope significantly different from zero. The estimated T for both streptomycin and chloramphenicol is 4.5 hr. No estimates are available for the other two agents though both delay division and probably T.

The low rate of loss of premutational damage might find another explanation, if a lower limit of unmodifiable mutation were being approached in these experiments. There was no evidence for nonlinearity in earlier

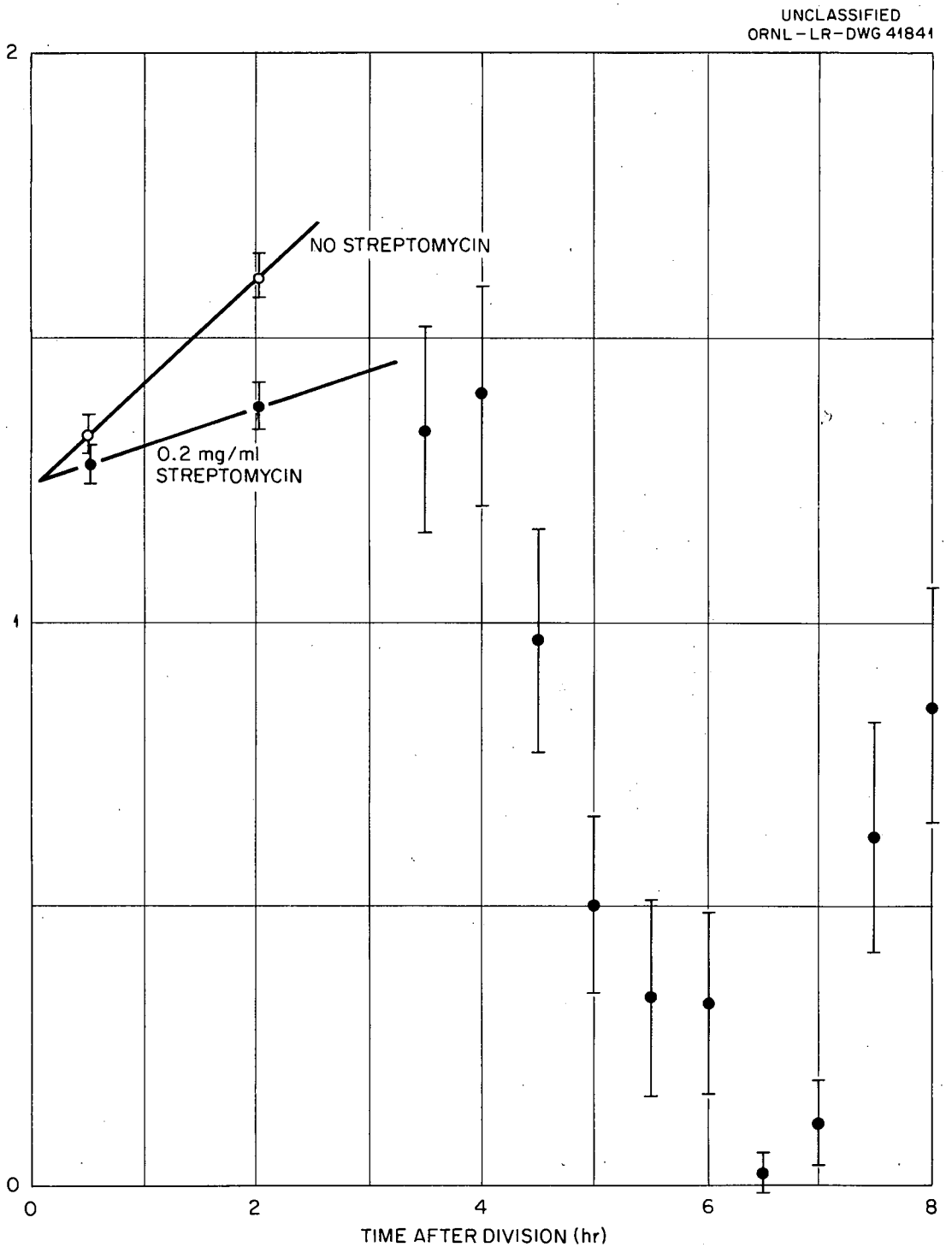

Fig. 1. Amount of Mutation in Arbitrary Units (M) as a Function of the Time of Irradiation in Hours After Division. Log-phase paramecia growing in culture fluid with or without the addition of 0.2 mg/ml of streptomycin. Some of the paramecia in the 7.5- and 8-hr groups were dividing.

control data for which there were several points, and the intercept at zero time was nearly the same (1.2–1.3 M) in all cases. Moreover, work[7] with stationary-phase paramecia has suggested a lower limit of about 0.6 M for 4.5 kr of X rays, which is well below any values in the present data. It seemed desirable, however, to show that lower levels than about 1.2 M could be obtained with log-phase paramecia. Therefore, recently divided paramecia were put into various concentrations of streptomycin, irradiated with 4.5 kr shortly thereafter, and left in the antibiotic overnight. At the highest concentration (1.5 mg/ml) giving 24-hr survival, a value of 0.92±0.06 M was obtained. The data gave a good fit to a straight line with an intercept of 1.30±0.02 and a slope of -0.26±0.04 M per mg/ml. Therefore, an approach to a lower limit of unmodifiable mutation could not have been responsible for the low slopes, and it seems quite probable that the lower limit for log-phase paramecia is at least as low as 0.6 M, the estimate from the stationary phase.

Conclusions. – On the hypothesis on which the present experiments were based, all four agents decrease the rate of loss of premutational damage. Streptomycin and chloramphenicol, which are effective posttreatment agents, must delay T somewhat more than they decrease the rate of loss, whereas caffeine and iodoacetate, which have little if any effect as posttreatments, must delay T and decrease the rate of loss to nearly the same extent. The fact that point estimates of the slopes for caffeine and iodoacetate are somewhat lower than those for streptomycin and chloramphenicol might be taken as favoring this view, but the statistical uncertainty in the slopes is too high to rely on this relation. The corollary to these conclusions is that the effectiveness of an agent as a posttreatment is not a good measure of its action in the mutational process.

Unfortunately, all the data can also be explained by another hypothesis with opposite conclusions. The slopes of the curves in Fig. 1 could be interpreted as a progressive increase in the sensitivity to initial damage with no postirradiation loss of premutational damage in the controls. The decreased slopes in the presence of a metabolic inhibitor would be a consequence of a slowing of this increase in sensitivity. Only the effective posttreatment agents need be assumed to influence the rate of loss of premutational damage, and they would increase it above the zero rate in the controls.

The main reason for preferring the first hypothesis to the second is that originally given,[6] it predicts the increase in mutation with time of irradiation in the controls. This finding is unpredictable from but compatible with the second hypothesis. The two hypotheses have nearly identical consequences in most feasible experiments. Nevertheless, there are a few situations in which a certain outcome would be possible on only one of the hypotheses, though others would be possible on both. Three of these situations have been tested so far, but with one of the ambiguous outcomes in each instance. We prefer the first hypothesis because it predicts the high control slope and for some other, though less compelling ones;[7] but a clear-cut experimental distinction, though extremely important, seems very hard to obtain.

Further Studies on the Cause of Different Survival After X Irradiation of Two Clones of *Tetrahymena pyriformis*

O. C. Wells

Two clones (II and III) of variety 6, *Tetrahymena pyriformis*, are about three times as sensitive to the killing effects of high levels of X radiation as another clone (I) of the same variety.[9] Biochemical and cytological studies of these clones are in progress in efforts to find differences that may be correlated with the different sensitivities.

Methods. – DNA and RNA content of gross cultures (10^7 animals per culture) of each clone were measured by total phosphorus analyses of suitable extracts.[10]

The mitotic divisions occurring at 24 hr after 100 and 200 kr were examined in clones I and III. Clones were given a posttreatment of 1 g/liter of antibiotic during the 24 hr. Slides were prepared of the entire irradiated population; Gomori's hematoxylin technique[11] was used.

After irradiation, single cell isolates were routinely exposed to a treatment of SRD antibiotic. This antibiotic, a mixture of penicillin,

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

[10] B. L. Griswold, F. L. Humoller, and A. R. McIntyre, Anal. Chem. *23*, 192–94 (1951).

[11] Y. Melander and K. G. Wingstrand, Stain Technol. *28*, 217-23 (1953).

dihydrostreptomycin, and streptomycin, was added to the culture medium in order to maintain axenic conditions. Concentrations of 1, 2, and 3 g/liter of antibiotic were used as posttreatment to determine the effect, if any, of antibiotic concentration on final survival.

Results. – Biochemistry. – The DNA and RNA content of all three clones were essentially the same. The data are expressed as units per 10^7 organisms:

	Clone I	Clone II	Clone III
RNA	131	127	124
DNA	7.5	6.9	6.7

Attempts are being made to measure free -SH content of the clones.

Cytology. – Figure 2A is an illustration of a control mitotic telophase of the micronucleus of both clones I and III. Twenty-four hours

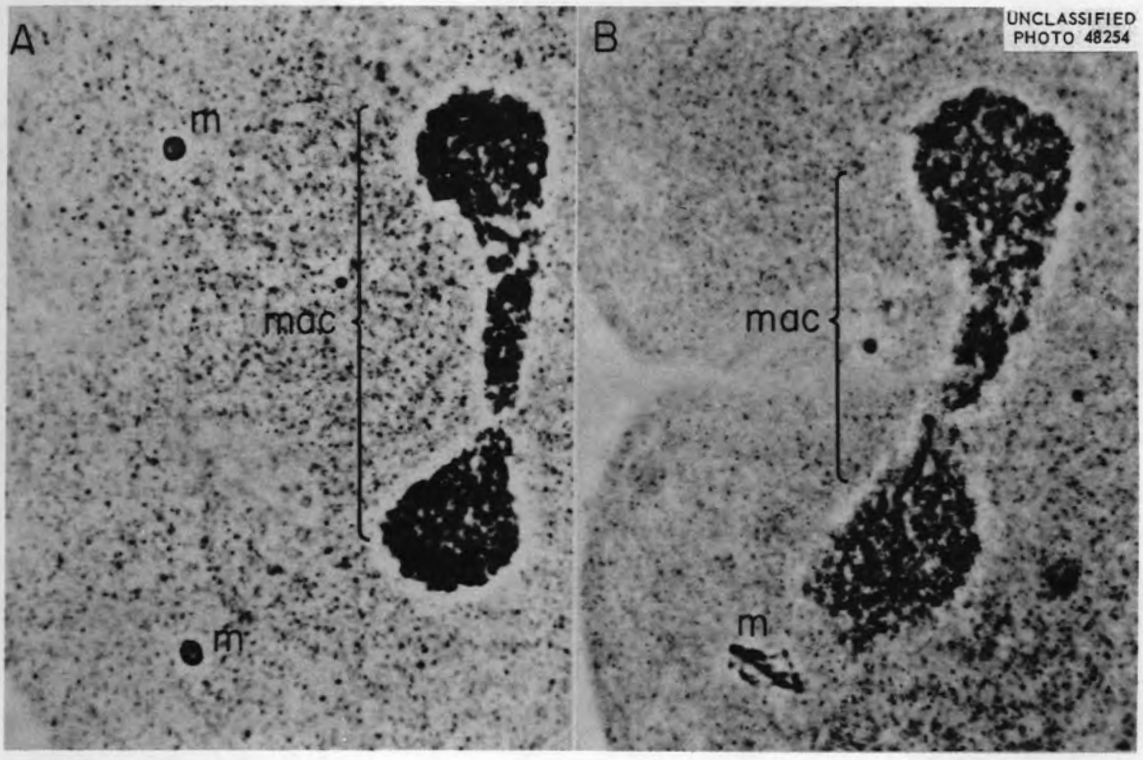

Fig. 2. (A) A Control Organism of Clone III, T. pyriformis. The micronucleus is in telophase. m = micronucleus; mac = macronucleus. (B) Disorganized Micronucleus of Clone III 24 hr After 200 kr of X Ray. The micronucleus (m) has failed to divide and the spindle is disoriented.

after the administration of 100 kr of X rays, slides made of either clone show little micronuclear abnormality. Survival in both clones from this dose is not significantly different from controls. Some mitotic divisions occurring 24 hr after 200 kr, however, are abnormal. Figure 2B illustrates a typical micronuclear disorganization which is observed in both clones after this dose. Lagging chromosomes, bridges, and amicronucleates are observed. Clone I (70% survival at 200 kr) has appreciably less mitotic aberrations and more normal division figures than clone III (25% survival at 200 kr).

Posttreatment. – The concentration of SRD antibiotic used as a posttreatment to irradiated cells has a definite effect on the final survival of the clone. The effect is illustrated by the survival data accumulated for the 200-kr level:

	1 g/liter	2 g/liter	3 g/liter
Clone I	70%	67%	30%
Clone III	25%	22%	14%

Controls in all instances gave 90–95% survival.

Discussion. – Cytological studies of clones I and III indicate a relation between survival after radiation and micronuclear damage. Many amicronucleate organisms are found in the sensitive clone and few in the resistant one after 200 kr. Since the Tetrahymena have passed through two or three fissions at 24 hr, disorganization of the mitotic micronucleus (Fig. 2B) probably brings about the formation of amicronucleate animals. Although viable amicronucleate clones of Tetrahymena are known, we have never recovered them after irradiation. The observation that there are more aberrant micronuclei of all kinds in III than in I suggests that the disorganization of the micronucleus is related to the inability of the organism to establish a clone, possibly through production of amicronucleate types.

Cytological studies have not been made on organisms that have been subjected to posttreatments other than 1 g/liter of antibiotic. The fission rate of these organisms decreases with increase in antibiotic concentration. It is not known whether the slowing of fission rate or

some interaction of radiation with antibiotic causes the decrease in survival. At any rate, it is evident that the different survival of clones I and III to X rays cannot be accounted for by antibiotic posttreatment, and that the survival of T. pyriformis after X irradiation alone is probably higher than earlier results indicate.[9]

Effects of X Rays on Trophic and Also Dry Encysted Spathidium spathula

D. B. Williams

Introduction. – Spathidium spathula was chosen as an experimental form, for this ciliate was known to be quite sensitive to X radiation. Doses in the range of 3–10 kr are sufficient to cause significant depression in the growth of trophic forms.[12] Generally, ciliates can withstand doses at least ten times as great without showing growth depression.[13]

We irradiated logarithmically reproducing spathidia with doses up to 25 kr and studied growth rate (daily divisions) of isolation cultures. We also irradiated dry cysts, allowed the animals to excyst, and recorded growth of isolation cultures. Finally, we investigated incidence of recessive lethal mutations after X irradiation by allowing descendants of irradiated forms to undergo conjugation (selfing), after which exconjugants were isolated and their viability recorded.

Results and Discussion. – Trophic animals exposed to 5, 10, or 15 kr react in one of two ways. They may experience a slight depression in growth (1–3 daily divisions), which lasts about one day, but recover (4–6 daily divisions) by the end of the second day (a few lines did not even show a slight depression). Other lines, however, show a depression (0–3 daily divisions) from which no recovery occurred during the period of investigation (in this case seven days). Death can occur in such lines at any time. The frequency of lines with good growth decreases with dose (6/15 at 5 kr, 14/15 at 15 kr).

The failure of lines with poor growth to recover was checked in a 25-kr group. No recovery was found after 15 fissions. Then encystment

[12] D. B. Williams, J. Protozool. 5, 25 (1958).

[13] R. F. Kimball, Ann. Rev. Microbiol. 11, 199–220 (1957).

was allowed to occur; and the lines were still poor after excystment, four weeks after irradiation.

Observation of animals from lines that showed poor growth revealed a high incidence of abnormalities in form and size. The most striking abnormality consisted of unusually large animals, some of which possessed two to five (instead of one) headlike cytosomes. It was also observed that the normal transverse cleavage was sometimes disturbed.

Peroxide produced in the medium can, in part, account for effects of high doses of radiation on ciliates.[13] The question arises whether it could also account for the effects of low doses on Spathidium. Animals irradiated with 2, 4, 6, 8, and 10 kr of X rays in sterile medium showed the same effect on growth as those irradiated in bacterized (Aerobacter aerogenes) medium. These bacteria have been shown[13] to protect Paramecium against peroxide. Therefore, peroxide produced in the medium does not contribute detectably to the growth disturbances induced in Spathidium.

One of the reasons for using Spathidium was that, unlike many ciliates it encysts and excysts readily, and the cysts remain viable after drying. Cysts dried in vacuo for one day were exposed to X radiation and then allowed to excyst. The excysted forms were then grown in isolation cultures for one day and their growth compared to trophic forms. Figure 3 shows that the dried cysts are more resistant to X rays than trophic animals, although the shape of the curves indicates that the manner of reaction is similar. Even the dried cysts, however, are much more sensitive than trophic forms of other ciliates.

Efforts are being made to develop a method for studying X-ray-induced deleterious and recessive lethal mutations, making use of the occurrence of selfing conjugation in Spathidium. Preliminary results indicate that a method can be developed, but it is too early to report these findings in detail.

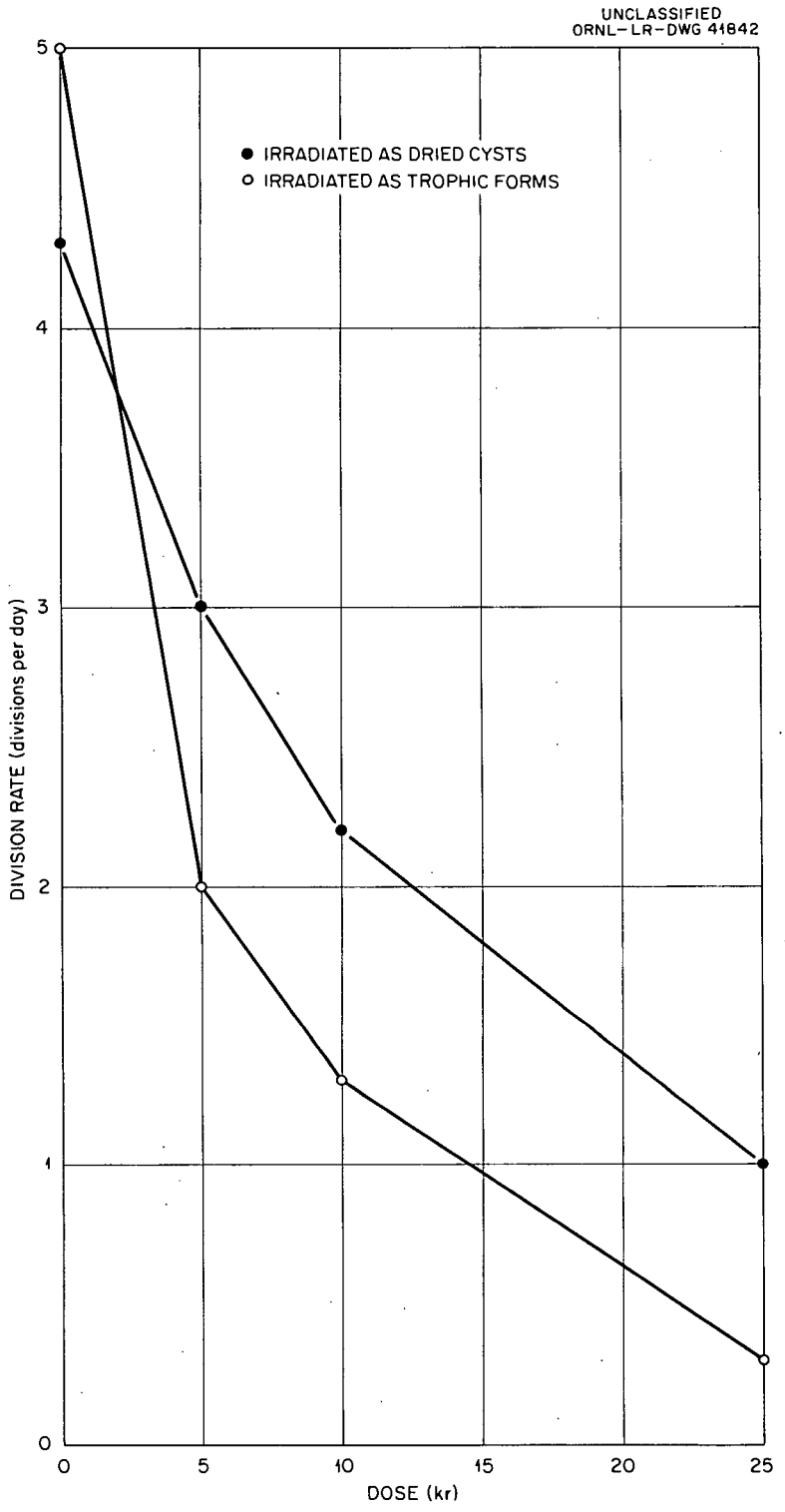

Fig. 3. Number of Divisions During the First 24 hr After Irradiation in Trophic Forms and During the First 24 hr After Excystment (12 hr After Irradiation) in Cysts.

Further Studies on Factors Influencing the Frequency of Recombination Between Markers on the Right Arm of Linkage Group I in *Neurospora crassa*

F. J. de Serres

Introduction. — Previous studies[14] on crosses of *ad-3A* and *ad-3B* mutants have shown a marked correlation between the origin of the mutants used in the cross and the recombination frequency. In general, less recombination was observed in the *ad-3A*-*ad-3B* region in crosses of mutants derived from the same wild-type strain than from crosses of mutants derived from different wild-type strains. These observations led to the development of the hypothesis that the rate, or type, of exchange in a given region is influenced by the structure of the homologous chromosomes in that region so that less recombination occurs between two markers when the intervening region on homologous chromosomes is structurally homozygous than heterozygous. Support[15] for this hypothesis was obtained from crosses of *ad-3A* mutants with linked markers (induced in wild-type strain 74A) at the *ragged* and *hist-3* loci. Less recombination was observed in the *ragged*-*hist-3* and *hist-3*-*ad-3A* regions in crosses with *ad-3A* mutants induced in 74A than with *ad-3A* mutants induced in the Lindegren wild-type strains. In the present experiments, the effect of changes in homology in a given region of homologous chromosomes on the frequency of recombination in that region has been studied. The approach used was as follows: the structure of homologous chromosomes in the *hist-3*-*ad-3* region was kept constant as a control, and the extent of structural heterozygosity in the *ad-3*-*nic-2* region has been decreased systematically by selection of recombinants resulting from exchanges in that region in successive crosses.

Results. — A cross of the mating type *A* *ad-3A(2)* strain to a mating type *a* strain carrying the *nic-2* marker gave 2.99±0.22 crossover units for the *ad-3A*-*nic-2* region.[16] An *A* *ad-3A* *nic-2* recombinant from this cross was crossed to an *a* *ragged* *hist-3* strain, and eight *a* *ragged* *hist-3* *nic-2* recombinants, which result from an exchange in the *ad-3A*-*nic-2* region, were

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[14]F. J. de Serres, Biol. Semiann. Prog. Rep. Aug. 15, 1957, ORNL-2390, p 26–27.

[15]F. J. de Serres, Biol. Semiann. Prog. Rep. Aug. 15, 1958, ORNL-2593, p 25–27.

[16]F. J. de Serres, Biol. Semiann. Prog. Rep. Aug. 15, 1956, ORNL-2155, p 19.

selected for further study. Since all markers, except the nic-2 marker (which was induced in the Abbott wild-type strains[17]), were induced in wild-type 74A, the ragged-hist-3-ad-3A region in both parental strains was derived from 74A, and homologous chromosomes in this region are thus structurally homozygous. The chromosome material in the eight recombinants obtained from this cross, then, is derived from 74A from some point to the left of ragged in the sex-ragged region up to the point of exchange in the ad-3A-nic-2 region. Since we expect a random distribution of exchanges in this region, recombinants should have varying amounts of the Abbott chromosome material brought in with the nic-2 marker, but always either the same or less than was present in the A ad-3A(2) nic-2 parent. The eight a ragged hist-3 nic-2 strains were then crossed to ad-3 mutants ad-3A(2) and ad-3B(2) to determine the effect of such structural alterations on crossing over in the hist-3-ad-3 and ad-3-nic-2 regions. The data from the analysis of these 16 crosses are given in Table 1.

[17]G. W. Beadle and E. L. Tatum, Am. J. Botany 32, 678–86 (1945).

Table 1. Estimates of the Frequency of Recombination in the hist-3-ad-3 and ad-3-nic-2 Regions in Crosses of A ad-3 Mutant Strains ad-3A(2) and ad-3B(2) to Eight a ragged hist-3 nic-2 Strains

95% confidence intervals are given for each estimate

Class	Parental Strains				
	a ragged hist-3 nic-2 Parent	A ad-3 Parent			
		ad-3A(2)		ad-3B(2)	
		hist-3-ad-3A	ad-3A-nic-2	hist-3-ad-3B	ad-3B-nic-2
I	53-OR6-3a	0.44±0.06	2.11±0.14	0.57±0.08	2.10±0.16
	53-OR6-7a	0.57±0.07	1.82±0.13	0.53±0.07	1.92±0.13
II	53-OR6-1a	0.45±0.07	1.39±0.13	0.52±0.07	1.43±0.11
	53-OR6-4a	0.50±0.08	1.52±0.14	0.48±0.08	1.52±0.14
	53-OR6-5a	0.55±0.06	1.40±0.10	0.49±0.07	1.46±0.13
	53-OR6-8a	0.51±0.07	1.56±0.12	0.53±0.07	1.43±0.11
III	53-OR6-6a	0.63±0.08	1.09±0.10	0.50±0.07	0.93±0.10
IV	53-OR6-2a	0.33±0.06	0.66±0.09	0.31±0.06	0.45±0.07

Discussion. — On the basis of the hypothesis, since the homologous chromosomes in these crosses are structurally homozygous in the hist-3-ad-3 region, no difference in the frequency of recombination in this region is expected. However, since repeated exchange in the ad-3-nic-2 region in successive crosses decreased the structural heterozygosity in this region, we expect to get variation in the frequency of recombination and either the same or less recombination than was observed in this region in previous crosses. On the basis of the differences observed in parallel crosses with mutants ad-3A(2) and ad-3B(2) the eight a ragged hist-3 nic-2 recombinants can be grouped into at least four classes. With the exception of crosses involving strain 53-OR6-2a, the recombination frequency in the hist-3-ad-3 shows no significant variation. Furthermore, there is considerable variation in the ad-3-nic-2 region, and all estimates were considerably lower than the value of 2.99±0.22 obtained in the cross of A ad-3A(2) × a nic-2.

The decrease in recombination in both regions observed in the crosses of 53-OR6-2a probably result from segregation of some other factor with a more generalized effect on the frequency of recombination in this region of Linkage Group I. Since only one of the eight strains shows such a change in the hist-3-ad-3 region, segregation of a linked modifier either on the right arm distal to nic-2 or on the left arm distal to sex could account for the decreases in recombination observed. Preliminary analyses of crosses of the eight a ragged hist-3 nic-2 strains with a strain carrying an albino (al-2) marker located distal to nic-2 show no change in recombination limited to the cross of 53-OR6-2a. Such data suggest that such a modifier, if linked, is probably on the left arm distal to sex.

Evidence for Nonallelic Complementation in Common-A Heterokaryons with Controlled Nuclear Ratios in *Schizophyllum*

P. J. Snider, Jr.

Introduction. — The common-A heterokaryon of *Schizophyllum*, discovered by Papazian,[18] is a sterile heterokaryon resulting from mating haploid individuals homozygous for the A incompatibility locus and heterozygous for the B incompatibility locus. The fertile, or dikaryotic, heterokaryons

[18] H. P. Papazian, Botan. Gaz. 112, 143–63 (1950).

are heterozygous for both the A and B loci. Raper and San Antonio[19] have described in detail about 12 differences between common-A and dikaryotic heterokaryons. In addition to the ability to fruit, there are differences[19] in mycelial and hyphal morphology, in growth as dry weight, in expression of mutant sectors, in nuclear ratios, and in nonallelic complementation. The common-A heterokaryon in most instances either partially or totally lacks a capacity exhibited by the dikaryotic heterokaryon. As a part of a long-range study of the incompatibility mechanism in Schizophyllum, the differences above are being examined systematically to determine which are actually the direct effects of the incompatibility reaction in the common-A heterokaryon.

The reported lack of nonallelic complementation[19] for nutritional deficiencies in the common-A heterokaryon is of interest in two respects: (1) it appears to be an exception to the general rule that nonallelic complementation is characteristic of the heterokaryons of fungi, such as Neurospora, Aspergillus, Pencillium, Streptomyces, or dikaryons of Schizophyllum, and (2) it might be used to assay the intensity of the incompatibility reaction, if it were actually a direct effect of the reaction. The original evidence[19] was derived from a procedure consisting in inoculating agar plate cultures of minimal medium with common-A heterokaryons composed of nuclei with nonallelic deficiencies in various combinations and observing that no growth had occurred after seven days at 22°C; similarly composed dikaryotic heterokaryons, serving as controls, grew as wild-type. The authors state[19] that, inasmuch as they did not use controlled or known nuclear ratios, they could not decide from their evidence whether the failure of growth was a direct inhibition of genetic complementation by the incompatibility reaction or merely the inability of the minority type nuclei to complement when the nuclear ratio has a disparity of several orders of magnitude.

Results. — A common-A heterokaryon with an input nuclear ratio of 1:1 for nuclei of A^2B^1 uracilless and A^2B^{41} niacinless-2 was incubated in liquid shake cultures of minimal medium at 22°C, and the dikaryotic heterokaryons $A^2B^1u \times A^{41}B^{41}$ nic-2 and A^2B^{41} nic-2 $\times A^4B^4u$ were used as controls.

[19] J. R. Raper and J. P. San Antonio, Am. J. Botany 41, 69–86 (1954).

Other controls also included will not be discussed here. (The direct comparison of a common-A heterokaryon on minimal and minimal plus varying amounts of supplement would probably be invalid because the inoculum unavoidably contains a significant proportion of homokaryotic cells.) The common-A heterokaryon grew well on minimal medium (Fig. 4), indicating the occurrence of nonallelic complementation, and although the growth was

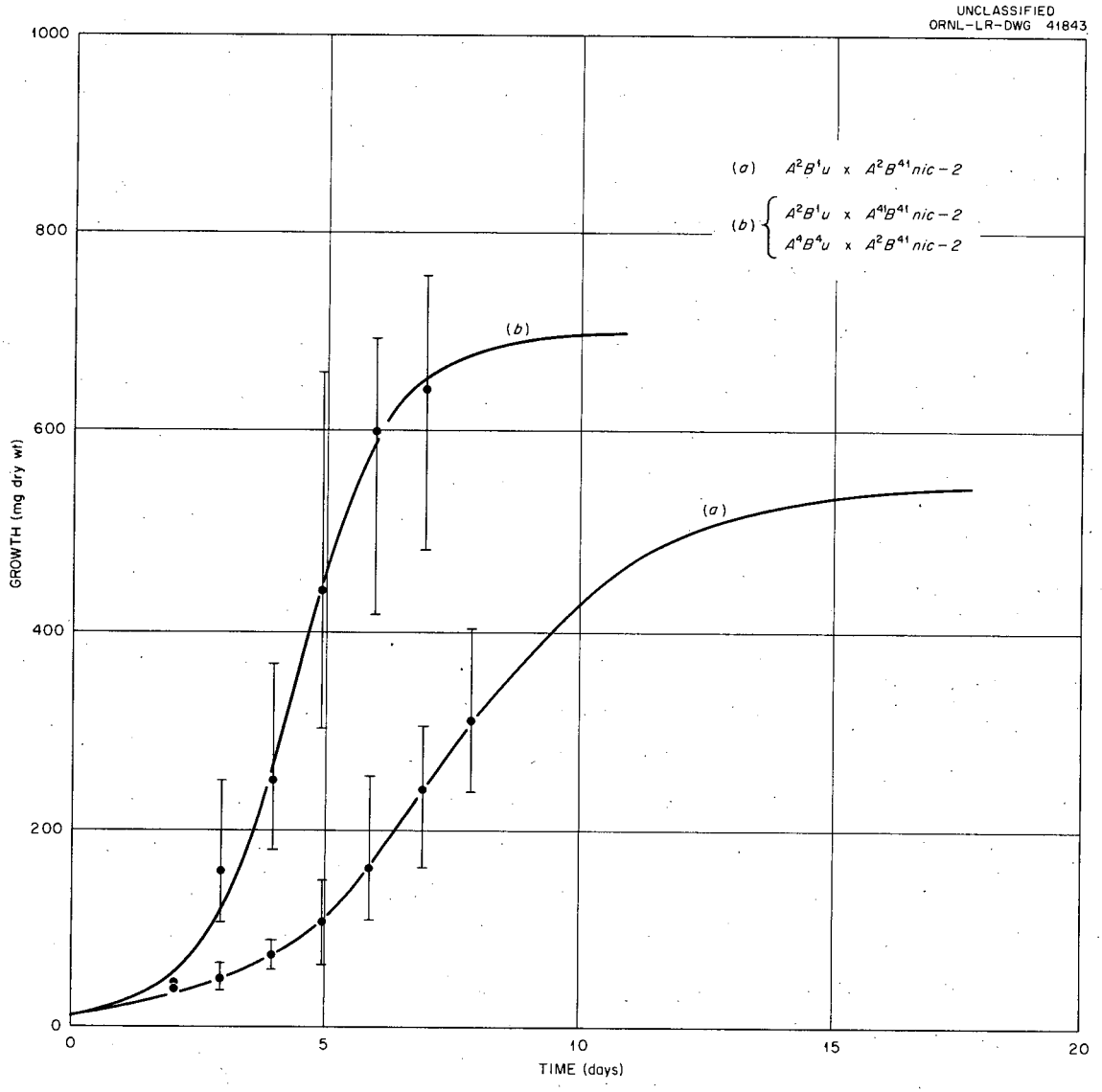

Fig. 4. Growth on Minimal Medium of Nutritionally Deficient Heterokaryons (Schizophyllum) with Nuclear Ratios near 1:1. (a) A common-A heterokaryon; (b) two dikaryotic heterokaryons as controls (pooled data). In the presence of a favorable nuclear ratio nonallelic complementation of nutritional deficiencies does occur in common-A heterokaryons of Schizophyllum. (See text for fuller explanation.)

significantly less than that of the two dikaryotic controls, this does not necessarily mean complementation was partially impaired; the depression is no more than occurs in wild-type common-A heterokaryons grown on complete medium.[19] Each datum (Fig. 4) is the mean dry weight of five cultures, except that the data for the two dikaryotic heterokaryons, which gave practically identical growth, have been pooled for simplicity; the limits are for the total range of measurements, not for the standard error of the mean or the standard deviation. The actual curves represent an attempt to calculate the most probable upper asymptotes, or end points, of growth, and although there appears to be no acceptable way for setting probability levels in confidence statements about the end points, the extrapolations hardly need to be more than rough approximations for the principal purpose of the experiment.

Conclusion. – The original experiments of Raper and San Antonio[19] were repeated and their observations were shown to be reproducible; inocula with randomly determined input nuclear ratios often fail to grow and thus fail to show evidence for nonallelic complementation of nutritional deficiencies on minimal medium, even when incubated many weeks longer than in their experiments. When nuclear ratios are kept near 1:1, however, such complementation is normal, as growth of such common-A heterokaryons is essentially equal to that of wild-type ones on complete medium. The difference between the growth of dikaryotic and common-A heterokaryons that exists whether or not there is a necessity for complementation of nutritional deficiencies indicates a direct effect of the incompatibility reaction on growth, an effect independent of and not mediated through an impairment of nonallelic complementation of nutritional deficiencies. It is concluded that lack of nonallelic complementation of nutritional deficiencies is not characteristic of common-A heterokaryons of Schizophyllum and is not directly related to the incompatibility mechanism. All the evidence for complete failure of complementation can probably be attributed solely to disparate nuclear ratios; thus common-A heterokaryons of Schizophyllum are not exceptional among the heterokaryons of fungi so far as nonallelic complementation of nutritional deficiencies is concerned.

Other Problems in Advanced Stages

P. J. Snider, Jr.

Mutagenesis of the A Incompatibility Locus. — Attempts to demonstrate induced mutation of the A locus have failed (Snider and Raper, unpublished) even at doses of gamma radiation up to 2×10^5 r from Co^{60} (2% survival). In a systematic examination of these negative results, reconstruction experiments have indicated that failure caused by simple plating inefficiency has been ruled out. Individuals representing mutant types have been recovered quantitatively in concentrations as low as 10^{-6} survivors. Therefore, the selective mechanism works as predicted. Other possible causes of failure are now being examined.

Membrane-Nucleus Relations and the Incompatibility Mechanism. — Specific information about the relation of endoplasmic membranes to the nuclei may possibly aid the study of the incompatibility mechanism in *Schizophyllum*. Examination of 200-Å-thick sections of hyphae in a Siemens Elmiskope 1b electron microscope has established that the plasma membrane is continuous with the nuclear membrane via an endoplasmic connecting membrane. There is also some evidence of internuclear membrane connections between the pairs of nuclei in dikaryotic cells. The details of these relationships are being established currently in collaboration with Dr. J. H. McAlear of the New York State Department of Health Laboratory at Albany.

Efficient Isolation of Fungal Spores. — A device has been developed allowing the isolation of single spores at a rate of 300–500 per hour with a low fatigue factor. The method has been thoroughly proved. An ultrarapid method allowing automatic isolation of single spores at a rate one order of magnitude higher than the previous method is currently under development. The ultrarapid method has been proved sound in principle, but some operational details remain to be developed. These methods are intended to assist in the efficient study of nonselective genes by random spore analysis but, in principle, should also be adaptable to certain special applications, such as the rapid isolation of spores for tetrad analysis.

Electrophoretic and Immunogenetic Studies with Endosperm Mutants of Maize[20]

D. Schwartz K. H. McGrath

In the last report,[21] we discussed strain differences in the migration rate of the four slowest moving protein bands on starch gel electrophoresis. These are the protein bands whose enzymatic activity in digesting starch can be observed directly by staining the gel with iodine after a period of incubation. The lines used in these studies were not isogenic and it was therefore not established whether these differences were caused by the sugary gene itself or some other unrecognized factors. Tests with the sugary mutant gene in other genetic backgrounds point to some other factor or factors as responsible for the differences observed in the migration rates. Linkage studies are under way in an attempt to locate these factors.

The Sh_1 gene on chromosome 9 was found to control the synthesis of a protein that appears as a fast moving band and assumes a forward position in the starch gel after electrophoresis. This protein band does not change the color of the iodine-stained starch gel from blue to red and as yet has not been characterized as to activity.

Ten different genetic strains of maize, all homozygous for the dominant allele Sh_1, have been tested. This protein band is present in all of these strains and shows the same rate of migration. This band is however absent in the three $sh_1/sh_1/sh_1$ mutant lines tested, and in its place a new protein band appears which shows a somewhat faster rate of migration. Heterozygous endosperms carrying both the Sh_1 and sh_1 alleles show both protein bands. These data indicate that the sh_1 mutant alters this protein in such a fashion as to result in an increased migration rate on starch gel electrophoresis.

Antibodies against the proteins in the maize endosperm were obtained by injecting rabbits with the same extracts as are used in the electrophoretic studies. When the Ouchterlony double diffusion technique is

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[20]Cooperative study with G. D. Novelli (Enzymology) and T. Makinodan (Radiation Immunology).

[21]D. Schwartz *et al.*, Biol. Semiann. Prog. Rep. Feb. 15, 1959, ORNL-2702, p 46.

used, a number of precipitation lines appear when the extract is tested against the antiserum. By testing eluates from different regions of the starch gel after electrophoresis, we have been able to identify the precipitation line formed by the protein controlled by the $\underline{Sh_1}$ gene. This precipitation line is completely missing when extracts from the $\underline{sh_1}$ mutant are tested against the $\underline{Sh_1}$ antiserum. Thus the alteration in the protein induced by the $\underline{sh_1}$ mutant gene is so drastic as to prevent a cross-reaction with the $\underline{Sh_1}$ antibody. To date it has not been possible to find an antibody in the $\underline{sh_1}$ antiserum that reacts with the $\underline{sh_1}$ antigen but not with that of $\underline{Sh_1}$. Either the altered protein is not antigenic or antibodies against it can cross-react with the $\underline{Sh_1}$ antigen.

UV-Induced Mutations of Bacteriophage

D. R. Krieg

When extracellular bacteriophage T4 particles are irradiated with ultraviolet light (UV) and allowed to reproduce in unirradiated bacteria, more mutants are found among their progeny than among the progeny of control infections with unirradiated phage. Studies of the reversion of $\underline{r}_{II}$ mutants to $\underline{r}^+$ have demonstrated this to be a phenomenon of induced mutation.[22] Some of the parameters influencing the frequency of induced reversions have been described in previous progress reports; this work has now been extended to induced "forward mutations": $\underline{r}_{II}$ mutants arising from irradiated $\underline{r}^+$. Since such experiments are not facilitated by the selective technique previously employed, many more plaques must be examined and apparent mutants are picked and confirmed. It is not necessary to produce so high a titer of progeny phage, however, and a wider range of experimental conditions (e.g., higher doses and lower multiplicities of irradiated phage per bacterium) can be investigated. The results of present experiments support some of the conclusions drawn from studies of induced reversions and have also revealed some additional phenomena.

The "dose-response curve" has been extended beyond that previously described for induced reversions, and the results with this "*in vitro*

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[22]D. R. Krieg, manuscript accepted by Virology.

system" now resemble those described by others from irradiation of bacteria and fungi. The frequency of r mutations first increases with dose, and then irradiations exceeding an "optimum dose" do not increase, and may decrease, mutant frequency. The average multiplicity of phage adsorbed per bacterium apparently influences not only the number of induced mutants detected, but also the magnitude of the "optimum dose." When irradiated phage are adsorbed at a mean of much less than one per bacterium, so as to reduce the occurrence of multiplicity reactivation relative to the reproduction of "survivors," some induced mutants are still found. This suggests that the reactivation phenomenon is not essential for the induced mutations to occur. These observations are based on experiments in which the frequency of induced mutants did not exceed five times control values. Tens of thousands of progeny phage were scored, but the scarcity of the mutants makes the magnitude of these effects difficult to assess, and the above observations may not yet be stated as firm conclusions.

Characterization of Bacteriophage Mutants Induced by Ethyl Methane Sulfonate

D. R. Krieg D. M. Green M. N. Goldstein

One approach to the understanding of the action of a mutagen is to study a set of mutants induced by that mutagen. T4r_{II} mutants induced by a variety of treatments have been investigated for their spontaneous and induced revertability and the distribution of their mutant sites within the linkage map.[23–25] A similar study has been begun here with r_{II} mutants induced by the action of ethyl methane sulfonate (EMS) on extracellular r^+ phage.

One hundred and six independent r mutants were recovered after an EMS treatment in which the frequency of mutant plaques was 40 times the control frequency. The mutants have been individually tested for phenotype, and about a third of them are of the r_{II} type. Stocks of these mutants

[23] S. Benzer and E. Freese, Proc. Natl. Acad. Sci. U. S. 44, 112–19 (1958).

[24] S. Brenner, S. Benzer, and L. Barnett, Nature 182, 983–85 (1958).

[25] E. Freese, Proc. Natl. Acad. Sci. U. S. 45, 622–33 (1959).

have been prepared and tested for the occurrence of spontaneous revertants to r^+. The frequency of revertants relative to total phage titer in the stock ranges from 1 per 10^8 to 4 per 10^6. Since a much larger range of revertant frequencies is generally found among stocks of spontaneous r_{II} mutants, this indicates that EMS-induced mutants are relatively homogeneous in this respect. The induced mutants have been tested for complementation with r_{II} mutants of the A and B cistrons (complementation groups, or loci). A more-detailed analysis of the distribution of the mutant sites within the linkage map can be achieved in two steps: (1) crosses of the mutants with a set of "deletions" (kindly provided by Dr. S. Benzer) to classify them into ten subgroups (segments); and (2) all possible pairwise intercrosses among mutants in the same subgroup. When complete, the data will show whether the mutants all occurred at different sites or whether some are the result of independent mutations at the same site. This can indicate the total number of sites within the phage genetic material at which the mutagen can cause a change recognizable as an r_{II} mutant, and whether some sites are particularly prone to such mutation. To date, step 1 has been completed for 22 mutants, and the results are shown in Table 2. Segments 1–6 are in the A cistron, and segments 7–11 are in the B cistron.

Table 2

Segment	Number of Mutants
1	7
2a	2
2b	2
3	1
4	1
5	3
6	3
7, 9, and 11	1
8	2
10	0

This work is being continued, and it is planned to cross the EMS-induced mutants with other induced and spontaneous mutants to see if any sites mutable by EMS are also mutable by other treatments. It has been suggested that some mutagens have a specificity of action at the nucleotide

level of DNA, and this work should contribute to the revealing and interpretation of such specificity.

The Induction of r^+ Revertants in Bacteriophage T4r by Ethyl Methane Sulfonate

D. M. Green

The mode of production of T4 bacteriophage mutants induced by ethyl methane sulfonate has been further investigated. A previous study has shown for the clone size distribution of the mutation from r^+ to r an equal number of clones in each generation size class.[26] An examination of the reverse mutation has confirmed this mode of production for the r to r^+ reversion.

In a manner analogous to the previous "single-burst" experiments, ethyl methane sulfonate-treated r phage were adsorbed to Escherichia coli strain B at a low (0.01) multiplicity and distributed to tubes such that, on the average, only one tube in ten contained a bacterium capable of yielding r^+ mutant phage. The tubes were incubated to allow lysis of the bacteria and liberation of the phage. Subsequently, the contents of the tubes were plated individually on K-12 (λ). K-12 (λ) shows the ability to selectively plate only T4r^+ phage so that direct scoring of the plates for mutant clones only was possible. A similar distribution was also made to determine the mode of appearance of spontaneous mutations in the experiment.

Assuming that these mutant bursts arise by clonal growth with a normal rate of division once division has begun, it is possible to present these data as to the number of divisions required to produce each clone of mutants. This is presented in Table 3 for the earlier ethyl methane sulfonate-induced r^+ to r mutation, the ethyl methane sulfonate-induced r to r^+ mutation, and the spontaneous r to r^+ mutation. In the analysis of the r to r^+ induced mutation, the eighth generation has been excluded since the mean burst size of 256 in the experiment limits the number of bursts in that class. Chi-square test of the numbers of clones in each generation from 0 to 7 shows a variation from the mean number significant

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[26]D. M. Green, Biol. Semiann. Prog. Rep. Feb. 15, 1959, ORNL-2702, p 50–51.

Table 3. Size Distribution of Mutant Clones

Generation	Clone Size	EMS Induced		Spontaneous
		$\underline{r}^+ \rightarrow \underline{r}$	$\underline{r} \rightarrow \underline{r}^+$	$\underline{r} \rightarrow \underline{r}^+$
0	1	15	23	15
1	2–3	10	11	2
2	4–7	7	5	2
3	8–15	11	11	
4	16–31	13	10	
5	32–63	8	12	
6	64–127	7	10	
7	128–255	1	5	
8	256 –	0	1	

at the 0.01 to 0.001 level. Consideration of the clones in the generations from 1 to 7 shows no significant variation from the mean.

The excess of clones in the zeroth generation may be attributed to several factors. First, approximately four clones of spontaneous origin are to be expected in the distribution. Analysis of the clone size distribution of spontaneous mutants suggests that three of the four would be expected to be of size 1. Second, it is believed that random maturation of stabilized mutants occurs during the reproduction of phage. The effect of this process would be to add those mutants that are matured before reproduction to the zero generation class. Third, it is possible that the zero generation class is composed of heterozygous as well as homozygous mutant phage. This is to be expected if the replication of phage genetic material follows semiconservative behavior. In the initial formation of the mutation, it would be formed as a new strand paired with a preexisting nonmutant strand to form a heterozygote. In the next reproductive cycle, the new strand would reproduce and be paired with a copy of itself and would now be a homozygous mutant. The products of each of these reproductions would be expected to plate as single plaques on K-12 (λ), thus increasing the number of clones in the zero generation twofold.

The failure to observe a significant excess of clones of size 1 in the previous r^+ to r distribution may be attributable to the inefficiency of distinguishing mottled (heterozygous) plaques from r^+ plaques.[26]

The similarity between the distributions suggests that the mutagen acts on all mutable sites in the same manner since the distribution in the forward direction is based on mutations at a great number of mutable sites in the r_{II} cistrons as well as in other loci of the phage genome, whereas the reverse mutation distribution is based on the mutations that occur at a specific mutation site.

The Effect of Knob Size on Preferential Segregation and Recombination in Maize

G. Y. Kikudome

Earlier studies[27] on chromosome 9 have revealed that the extent to which preferential segregation and the frequency of recombination are affected by the knob occupying the terminal position of the short arm of chromosome 9 is dependent on the size of that knob in knobbed-knobless (K/k) heterozygotes. Thus, in such plants heterozygous for the abnormal chromosome 10 (K10), the degree of preferential segregation observed is greater when the size of the terminal knob is larger. Furthermore, when both homologues of chromosome 9 are knobbed but with one being larger than the other, it is the homologue with the larger of the two knobs that is preferentially recovered over the smaller knob in the functional megaspores. As for the frequency of recombination in the three types of knobbed-knobless heterozygotes studied, the recombination values are essentially the same when the abnormal chromosome 10 is present in the complement in the heterozygous condition. However, in the absence of this chromosome 10, the values observed in each type of knobbed-knobless heterozygote are different. Although the three sets of recombination values were all lower than those found in the class heterozygous for the abnormal chromosome 10, the recombination value was lowest in the largest knobbed-knobless control and highest in the control class of the smallest knobbed-

[27]G. Y. Kikudome, "Studies on the Phenomenon of Preferential Segregation in Maize," Genetics (in press).

knobless group. It seems, then, that in the absence of the abnormal chromosome 10, larger knobs have a greater suppressive effect on recombination than do the smaller ones.

The data presented in Table 4 represent further analysis on the effect of knob size. In this particular case, comparison is made between sibs of the following constitutions: (1) K^M 9 + + + +/ K^S 9 $\underline{yg_2}$ $\underline{c}$ $\underline{sh_1}$ $\underline{wx}$ K10/k10 and (2) K^S 9 + + + +/ K^S 9 $\underline{yg_2}$ $\underline{c}$ $\underline{sh_1}$ $\underline{wx}$ K10/k10. It is evident from the

Table 4. Results of Backcrosses of (1) K^M 9 + + + +/ K^S 9 $\underline{yg_2}$ $\underline{c}$ $\underline{sh_1}$ $\underline{wx}$ K10/k10 and (2) K^S 9 + + + +/ K^S 9 $\underline{yg_2}$ $\underline{c}$ $\underline{sh_1}$ $\underline{wx}$ K10/k10 Sibs

Constitution	Preferential Segregation (%)				Total Population
	$\underline{Yg_2}$	$\underline{C}$	$\underline{Sh_1}$	$\underline{Wx}$	
1	69.3	64.4	63.2	54.0	5244
2	52.7	51.9	51.8	50.0	4953

Constitution	Recombination Frequency (%)						
	$\underline{yg_2}$-$\underline{c}$	$\underline{c}$-$\underline{sh_1}$	$\underline{sh_1}$-$\underline{wx}$	$\underline{yg_2}$-$\underline{sh_1}$	$\underline{c}$-$\underline{wx}$	$\underline{yg_2}$-$\underline{wx}$	Double Crossover
1	12.1	3.0	20.6	15.3	23.6	34.9	0.5
2	18.2	4.3	18.1	22.4	22.3	39.9	0.4

data that preferential segregation is realized only when the chromosome 9 bivalents are heteromorphic for the knobs. This finding is in agreement with the observation of Rhoades and Dempsey[28] that preferential segregation for chromosome 3 bivalents occurred only when they were heterozygous for the knob and either heterozygous or homozygous for the abnormal chromosome 10. Also the present data show that, irrespective of the knob situation on chromosome 9, the recombination values of the two classes are essentially of the same magnitude. In the earlier studies[27] when K^M 9 + + +/ K^S 9 $\underline{c}$ $\underline{sh_1}$ $\underline{wx}$ K10/k10 and K^S 9 $\underline{c}$ $\underline{sh_1}$ $\underline{wx}$/ k 9 + + + K10/k10 sibs were

[28]M. M. Rhoades and E. Dempsey, Maize Genet. Coop. News Letter 33, 57–58 (1959).

compared, it was found that both sets of sibs had similar values of recombination. In the 1957 study of Rhoades and Dempsey,[29] the recombination values in K3/K3 K10/k10 and k3/k3 K10/k10 sibs were found to be similar and higher than the values found for sibs homozygous for the normal chromosome 10. Insofar as recombination is concerned, it seems that knob size on either chromosome 9 or 3 has little or no effect if the abnormal chromosome 10 is present. In the absence of this chromosome 10, there is considerable difference in the recombination values.

Future experiments dealing with the effect on chromosome 9 bivalents of knobs of varying sizes in the homomorphic condition and both in the presence and absence of the abnormal chromosome 10 should present some clue as to how knobs effect changes in recombination frequencies.

Far-Red-Induced Delay of Mitosis and the Apparent Synergistic Effect of Far Red and X Rays on Chromatid Aberration Production

S. Wolff H. E. Luippold

Introduction. – One of the anomalous observations in radiation biology has been that treatment of *Drosophila*,[30] *Tradescantia*,[31] or *Vicia*[32] chromosomes with far-red light increases the yield of chromatid aberrations induced by X rays, although the far red does not induce aberrations by itself. The enhancing effect of far-red light has been variously attributed to an increase in the numbers of breaks induced in the chromatids[30,31] or to a decrease in the amount of restitution and a concurrent increase in reunion of breaks.[31,32] In an attempt to differentiate between these two hypotheses, we obtained results that indicate that in *Vicia faba* roots neither is the cause of the apparent increase.

Results. – 1. Soaked seeds. – Seeds of *Vicia faba* were soaked for 18 hr, peeled, and irradiated with either 300 or 600 r of 250-kvp X rays. For either 3 hr before or after X irradiation or between two doses of

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[29] *Ibid.* 31, 77–78 (1957).

[30] B. P. Kaufmann, A. Hollaender, and H. Gay, Genetics 31, 349–67 (1946).

[31] C. P. Swanson and A. Hollaender, Proc. Natl. Acad. Sci. U. S. 32, 295–302 (1946).

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

X rays, the beans were exposed to far-red radiation at a radiance of 37,000 $\mu w/cm^2$. When the first divisions were occurring in the primary root tips, slides were made and 300 cells scored in each treatment for chromosome aberrations (cells irradiated in interphase), which were the only types induced. From 23 separate experiments it was concluded that there was no enhancing effect of far red on X-ray-induced chromosome aberrations. (In only two experiments was a difference on the border line of statistical significance noted, $p = \sim 0.05$.)

2. Growing lateral roots. – Lateral roots of Vicia faba were grown in aerated distilled water at about 24°C and then given 150 r of 250-kvp X rays. After this, they were treated for 4 hr with far-red light. The plants were then returned to the growth vessels and kept under constant aeration. At the end of 24 hr, six of the roots were fixed and scored for chromatid aberrations (cells irradiated after chromosome duplication) that appear at this time. At the end of 48 hr, the remaining roots were fixed and scored for chromosome aberrations (cells irradiated in interphase) that were now apparent. The results of one typical experiment are presented in Table 5. They indicate that the far-red treatment after the X irradiation is able to increase the yield of chromatid breakage observed at 24 hr after X irradiation but not the yield of chromosome aberrations observed 48 hr after. Furthermore, when roots are grown at 24°C under complete aeration, at 24 hr there are not just chromatid aberrations present but also a few cells containing chromosome aberrations. The far-red treatment decreases the proportion of these latter cells that appear. Similarly, at 48 hr most of the cells contain only chromosome aberrations, but a few cells are present with chromatid aberrations. The far red increases the proportion of these.

Discussion. – Because far-red radiation has no effect on the aberrations induced in interphase in either lateral roots or first division of primary roots and also because it has no effect between two doses of radiation, we do not believe that far red either increases the numbers of X-ray-induced breaks or affects the restitution of breaks. However, if the far red, which has many inhibitory effects (e.g., seed germination and

Table 5. Effect of Far Red on Radiation-Induced Aberrations

Type	150 r		150 r + Far Red	
	Aberrations		Aberrations	
	Number	Per Cent	Number	Per Cent
	24-hr Fixation			
Chromatid deletion	34	10.2	24	8.7
Isochromatid	38	11.4	25	9.1
Chromatid exchange	10	3.0	31	11.3
Total chromatid breaks	92	27.5	111	40.4
Cells with chromosome aberrations	48	14.4	26	9.5
Total cells	334		275	
	48-hr Fixation			
Dicentrics and rings	31	10.3	22	7.3
Interstitial deletions (dots)	19	6.3	17	5.7
Terminal deletions (rods)	8	2.7	7	2.3
Cells with chromatid aberrations	1	0.3	19	6.3
Total cells	300		300	

flowering[33]), inhibits mitotic rates, we would expect an effect on aberrations induced after the chromosomes react as though double, at which times the cells do not exhibit a uniform sensitivity to X-ray-induced breakage. The result of inhibiting cell division with far-red light at any given time after radiation is that the cells scored were relatively closer to

[33]R. B. Withrow and A. Withrow (eds.), Photoperiodism and Related Phenomena in Plants and Animals, Am. Assoc. Advance. Sci., Washington, D.C. (in press).

metaphase at the time of X irradiation than cells not exposed to far-red light. Since, after chromosome duplication, the sensitivity of cells increases as metaphase is approached, this sample of cells selected after X irradiation plus far red would have more aberrations than would those that had just received X rays. This would not be true for cells irradiated in interphase, which exhibit a uniform sensitivity to X radiation. Also, if such an inhibition of cell division occurred, then at a time when most of the cells present contain chromatid aberrations and only a few contained chromosome aberrations, the delay would result in a greater proportion of the cells containing chromatid aberrations. Similarly, the result observed at 48 hr that the proportion of the cells containing chromatid aberrations increased after far-red light is consistent with the interpretation of a delay in mitosis causing the apparent synergistic effect of X rays and far red.

Studies on Chromosome Breakage

D. Davidson

Introduction. – The study[34] of the survival of cells with aberrant chromosomes in irradiated primary roots of *Vicia faba* has been continued. Stable chromosome changes act as cell markers, and they have now been studied in roots fixed three weeks after irradiation.

The recovery of irradiated roots begins in the period of slow growth that follows irradiation. Little is known, however, of the cell behavior underlying recovering. By using chromosome markers it has already been shown[35] that some cells divide repeatedly in a slow growing root. In the present study they have been used to estimate the number of cells from which root regeneration occurs.

Further experiments were carried out on the effects of azaserine on roots of *Vicia faba*. Combined treatments with X rays and azaserine were also used in order to test the hypothesis[34] that azaserine delays cells during interphase.

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[34]D. Davidson, Biol. Semiann. Prog. Rep. Feb. 15, 1959, ORNL-2702, p 54–56.

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

Results. – The technique used in the previous study[36] of the survival of aberrant chromosomes in irradiated roots of Vicia differed in two respects from the technique I have used: (1) the dose of X rays (300 r) was half the dose used in the present study (600 r), and (2) the results described here and previously[34] were obtained from squash preparations and not from sections.[36] These differences in technique must be borne in mind when considering the differences between the two sets of results. Certain types of aberrations, for example, deficiencies, are more readily identified in squashes than in sections.

The number of different types of chromosome aberrations that occurs in the roots has been used to estimate the minimum of cells from which the irradiated root was derived. Present results indicate that at least nine cells (Table 6, roots 4 and 25; eight cells with aberrant and one with normal chromosomes) contributed to the growth of the roots; it had been estimated[36] that roots of Vicia had three or four initial cells.

If the relative proportion of cells with normal and changed chromosomes is used to estimate the number of initial cells, a number much higher than nine is obtained. Such values are quite consistent with recent evidence on the structure of normal root meristems and the number of initial cells that are present.

Roots of Vicia treated with solutions of azaserine showed B" + SR, B' + R', but no B" + R", after 48 hr recovery; those irradiated with 100 r showed B" + R" and little B" + SR or B' + R'. Roots given combined treatments, azaserine and X rays, showed B" + R", and B" + SR and B' + R', but in different cells. This result indicates that azaserine acts in the period of interphase that follows chromosome splitting, and that the cells affected by azaserine are taking 48 hr to pass from late interphase to metaphase, a period that lasts less than 24 hr in normal or irradiated roots.

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[36]R. T. Brumfield, Am. J. Botany 30, 101 (1943).

Table 6. Effect of 600 r of X Rays on *Vicia faba* Primary Roots

Root	Root Length (cm)	Cells		No. of Chromosome Changes	Percentage Changed Cells	Total Cells
		Normal	Changed			
1	10.5	108	46	6	23.3	154
14	17.5	84	26	7	23.6	110
19	9.0	245	72	7	22.7	317
11	9.6	63	20	2	24.1	83
4	9.0	212	111	8	34.4	323
23[a]-1	10.0[b]	84	34	1	29.9	118
23 -2	7.5[b]	79				79
10	12.4	71	13	3	15.5	84
20[c]-1	1.5[b]	9	48	1	84.2	57
20 -2	2.1[b]	47	14	1	22.9	61
20 -3	1.4[b]	35				35
20 -4	1.9[b]	30				30
25	9.1	75	62	8	22.1	137
31	16.7	108	17	3	13.6	125
		1250	463		27.03	1713

[a]The root was 3.4 cm long before it split.

[b]Measured from the point of split.

[c]The root was 1.5 cm long before it split.

The Control of Growth in Timothy Roots

R. T. Brumfield M. E. Picinich A. P. Davis

The cells in the distal ~300 μ of the timothy root elongate slowly and undergo division; proximal to these and extending to ~1500 μ from the tip the cells elongate rapidly but do not divide. Unilateral exposure to ultraviolet light (2537 Å; 8 ergs/mm^2/sec; 4 min) induces curvatures toward the source (inhibition) in the elongating cells and curvatures away from the source (stimulation) in cells in and near the region of division. Roots were grown in air and, beginning with the tip, successive regions

were exposed to a UV beam 150 μ wide. Whereas curvatures toward the source were regularly induced throughout the elongation zone, the response of the distal 300 μ was irregular, some roots exhibiting stimulation, some inhibition, and some no response.

In roots grown in moist air, the root cap and meristematic zone are covered by a viscous transparent substance that swells when the root is immersed in water. Since individual roots vary widely in the amount of this substance, it is thought to be responsible for the variation in response when the distal portion of the root is irradiated. It may also account for differences observed in the effects of radiations of limited penetration (UV; alpha particles) and X rays.

When the tip of an irradiated root is placed in contact with a nonirradiated root at a point ~300 μ from its tip and some of the material transferred, the nonirradiated root bends away from the contact side. A nonirradiated root induces a curvature toward the contact side of a nonirradiated root. Thus the radiation-induced stimulation can be transferred from one root to another.

The substance is dispersed by RNase (50 mg/liter) in ~75 min (24°C) with some undissolved solids remaining. Chymotrypsin and papain also disperse the substance but more slowly than RNase. The presence of 2,4,6-trichlorophenoxyacetic acid (30 mg/liter), a compound inhibiting the UV response and geotropism, shortens the dispersal time in RNase whereas indole-3-acetic acid (1 mg/liter), ineffective in the UV response and geotropism, inhibits the dispersal in RNase.

The nature of the substance is being studied by spectrophotometric and chromatographic methods.

INSECT CYTOLOGY AND GENETICS

M. E. Gaulden
R. C. von Borstel
J. G. Carlson[37]
A. M. Clark[37]
H. Kroeger[38]
A. R. Whiting[37]
P. A. Smith
B. J. Stevens
M. A. Ashcraft[37]
V. J. Cristofalo[37]
R. C. Griggs[37]
M. A. Rubin[37]

Effects of Microspot Irradiation of the Nucleolus with 2650 Å and 2804 Å Ultraviolet Radiation on Mitotic Activity of Grasshopper Neuroblasts

M. E. Gaulden J. G. Carlson J. Jagger

Observations by Gaulden and Perry[39] have indicated that the nucleolus of the grasshopper neuroblast is directly involved in the mitotic process from late telophase to the middle of midprophase, beyond which stage the nucleolus quickly loses its influence on mitosis. With a high-intensity, polychromatic ultraviolet microspot 3 μ in diameter, they found that irradiation of only one of the two nucleoli for 3 sec ($\sim$0.01 erg$\cdot\mu^{-2}\cdot$sec^{-1}; total dose: 0.2 erg) results almost immediately in permanent cessation of mitotic progress of neuroblasts treated at stages from late telophase to the middle of midprophase. About half the cells nucleolar-irradiated in the latter part of midprophase divide, and all those irradiated in late and very late prophase divide. Irradiation of a non-nucleolar portion of the nucleus at any stage does not usually prevent division but does cause some retardation in mitotic rate.

Although these observations implicate the nucleolus as an important structure in mitosis, they do not reveal its specific contributions. Biochemical analyses of isolated nucleoli from other organisms have shown that the nucleolus is composed of approximately 3–5% RNA and 95–97% protein. In view of evidence for high turnover of these substances in the nucleolus and their importance in cell activities, it was of interest to try to determine whether one or both of them are responsible for the permanent blockage of mitosis that results from irradiation of a neuroblast

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[37] Consultant.

[38] Visiting investigator from abroad.

[39] M. E. Gaulden and R. P. Perry, Proc. Natl. Acad. Sci. U.S. 44, 553–59 (1958).

nucleolus. The present study was undertaken to examine the effects of microspot irradiation of the nucleolus with two wavelengths of ultraviolet that are close to the absorption maxima of nucleic acids and proteins, namely, 2650 Å and 2804 Å, respectively.

Materials and Methods. — A quartz monochromator was placed between the source (General Electric AH-6 high-pressure mercury arc) and the primary aperture of the microbeam apparatus.[40] At 2650 Å the band width was about 70 Å and at 2804 it was about 84 Å.

Since apparatus was not available at the time of the experiments for measuring directly the amount of radiation in the microspot, a UV-sensitive phototube was used to determine the intensity of radiation incident on the primary aperture. After these experiments were completed apparatus was developed for direct measurement of the energy in the microbeam. A small six-stage photomultiplier tube (DuMont 6365) not sensitive to far UV was fitted with a uranium glass window, which converts far UV to fluorescent near UV and visible light that the photomultiplier can detect. This photomultiplier was inserted in the opening of the stage of the microscope of the microbeam apparatus where it intercepted the entire microbeam. A series of ratios of phototube measurements at the primary aperture to photomultiplier tube measurements at the microscope stage was used to estimate the amount of radiation in the microspot. Both tubes were compared with a thermopile that was calibrated with a National Bureau of Standards standard tungsten lamp. The complexity and difficulty of these measurements make it unlikely that estimates of absolute dose are closer than a factor of 2 to the true dose. The estimates of relative dose, however, should be much more precise.

The diameter of the microspot was 3 μ (area: 7.07 μ^2). The dose rate of 2650 Å ultraviolet was varied between 0.0001 and 0.0004 $erg \cdot \mu^{-2} \cdot sec^{-1}$ and that of 2804 Å between 0.001 and 0.003 $erg \cdot \mu^{-2} \cdot sec^{-1}$. Time of exposure to 2650 Å varied from 0.01 sec to 40 sec and to 2804 Å from 0.01 sec to 180 sec.

The previous study[39] utilized cultures of isolated neuroblasts. By adjustment of the substage condenser of the microbeam apparatus, it was

[40] R. B. Uretz and R. P. Perry, Rev. Sci. Instr. 28, 861–66 (1957).

later found that neuroblasts could be viewed in situ. This made possible the use of embryo cultures. Although the over-all dimensions of the cell and nucleus are the same in both types of culture, a nucleolus is about 4–5 μ in diameter in neuroblasts in situ but only about 3 μ in diameter in isolated neuroblasts. Thus embryo cultures were preferred to isolated cells.

Sixteen-day-old embryos were made into hanging-drop culture preparations as previously described[41] except that Shaw's medium[42] and quartz cover glasses were used. In the initial experiments, only those neuroblasts were selected for irradiation in which one nucleolus lay less than 7.5 μ beneath the cover glass; this figure was later changed to 5 μ to reduce the amount of absorption of radiation from the microbeam by overlying materials.

One nucleolus was microspot-irradiated in cells at the six stages from middle telophase to the middle of midprophase. A wide range of doses was used for each stage. Irradiated and unirradiated cells were observed until they reached prometaphase (nuclear membrane breakdown). This end point was chosen because it is easily determined and because cells reaching prometaphase usually complete division. Irradiation and observation of cells were carried out at room temperature (26 ± 2°C); those wavelengths of visible light effective in photoreactivation were filtered from the microscope lights and the cultures were shielded from room lights.

Results and Discussion. – A large number of cells at each of the six mitotic stages were studied to give a total of 660 cells. The effect of nucleolar irradiation on mitotic rate was designated for each irradiated cell as a ratio of the median time in which the unirradiated cells reached prometaphase to the time in which the irradiated cell reached the same point. A least squares regression analysis[43] of these

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

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

[43] The authors are indebted to A. W. Kimball of the Mathematics Panel for this analysis.

ratios was made for each stage to determine the dose which retarded mitotic rate to 0.667 of the control rate; in other words, the dose that increased by 1.5 the time between irradiation and prometaphase.

The efficiencies of 2650 and 2804 Å microspot irradiation of one nucleolus in retarding mitosis rate are given in Fig. 5 as the reciprocal

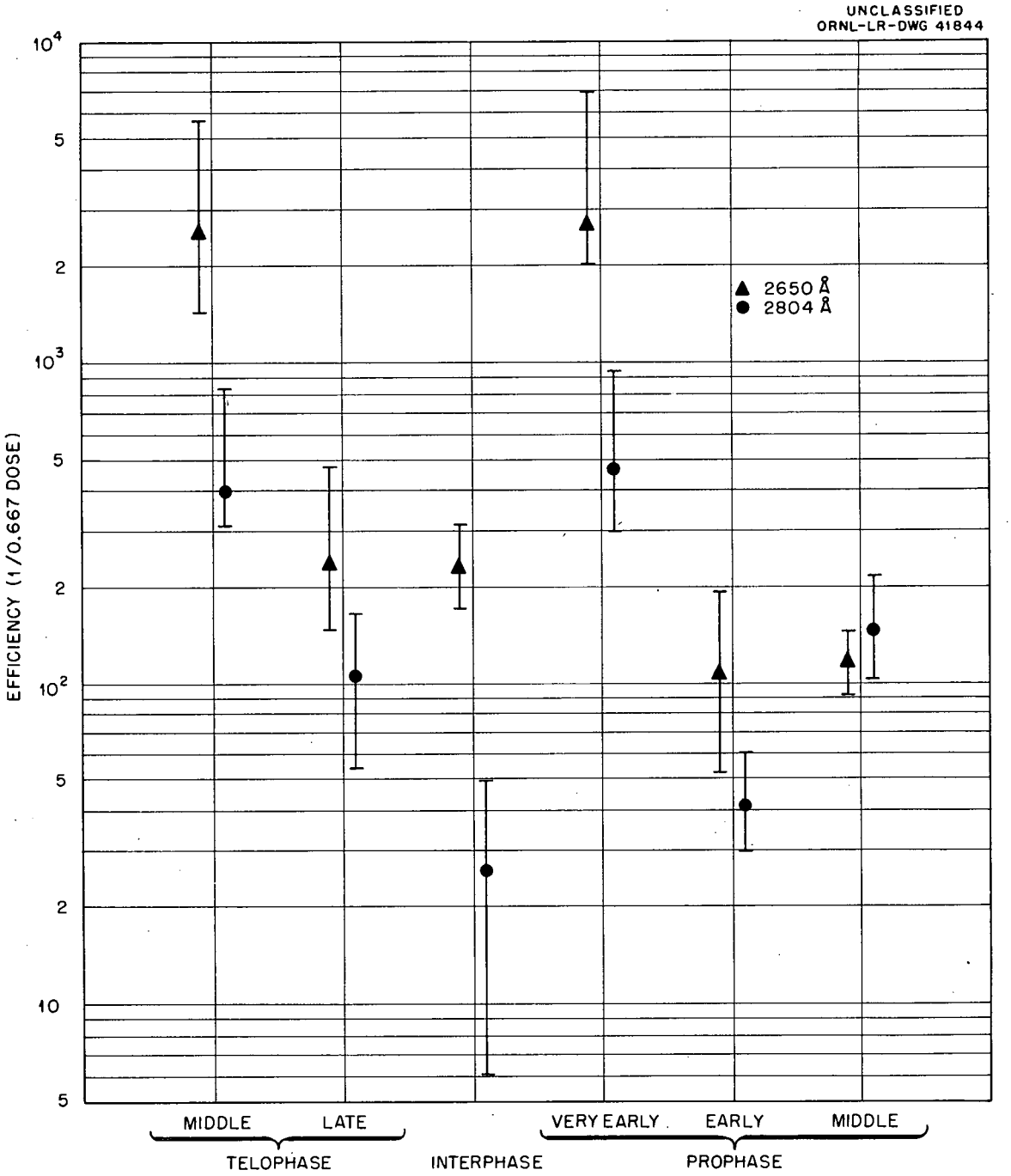

Fig. 5. Efficiency of 2650 and 2804 Å Ultraviolet Radiation in Retarding Mitotic Rate of Nucleolar Irradiated Neuroblasts to 0.667 of the Rate of Unirradiated Cells. The 70% confidence limits are indicated for each point.

of the dose that reduces the rate to 0.667 of control. The 70% confidence limits are indicated for each point. The nucleic acid absorbing wavelength (2650 Å) was more effective in retarding mitosis than the protein absorbing one (2804) at five consecutive stages: middle and late telophase, interphase, very early and early prophase. At midprophase, both wavelengths are about equally effective.

The neuroblast is most susceptible to microspot irradiation of a nucleolus at middle telophase and very early prophase, two stages characterized by the beginning of marked mitotic changes. In middle telophase, there is a considerable amount of nuclear growth — the nucleoli form and increase in size; the nuclear region, not yet completely surrounded by a membrane, begins to increase in size; and the synthesis of DNA begins.[44] At very early prophase development of the chromosomes begins, as evidenced by the appearance of extremely fine threads in the living nucleus.

Radiation-Induced Dominant Lethality in *Habrobracon* Oogonial Cells

R. C. von Borstel P. A. Smith

Introduction. — It generally has been assumed that dominant lethal mutations induced by radiation in gonial cells are cell lethals and thus will not pass through meiosis to cause death of an embryo. This assumption is consistent with the observation that frequency of embryonic deaths is not appreciably increased after X irradiation of spermatogonia of mice or *Drosophila*. Recently, La Chance (personal communication) has observed that dominant lethals can be induced in gonial cells of *Habrobracon* females, where embryo hatchability is the criterion. Since precise methods for studying the nature of cell death and dominant lethality induced by radiation have been developed,[45,46] oogonial dominant lethality

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[44] M. E. Gaulden, in Proc. First Pan-Am. Cancer Cytol. Congr. (in press).

[45] R. C. von Borstel, p 175–200 in *The Beginnings of Embryonic Development*, ed. by A. Tyler, R. C. von Borstel, and C. B. Metz, Am. Assoc. Advance. Sci., Washington, D.C., 1957.

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

was investigated to see if the induced lethality could be related to the types induced in mature oocytes or sperm.

Results. — In the parasitic wasp Habrobracon, fertilized eggs become diploid females and unfertilized eggs become normal haploid males. Forty No. 33 virgin females were X-irradiated with 3000 r and half of the batch were mated with No. $17o^{i}$ males. Grosch and Sullivan[47] have shown that 3000 r is about the maximum dose permissible to insure survival of oogonial cells. The females were then transferred daily to new dishes to lay eggs on caterpillars of the host, Ephestia. The eggs were counted and hatchability was recorded.

The first eggs laid were in the first meiotic metaphase when irradiated and all these were killed by the radiation. The next batch and succeeding batches until day 4 were in the first meiotic prophase when irradiated, and hatchability was about 90% for days 2 and 3 and dropped to 70% on day 4, whereas control hatchability remained at 97% during this period.

At days 5 through 8, the irradiated females went through a sterile period and only 1.2 eggs per female per day were laid compared to 14.4 eggs per female per day for the controls. Hatchability of the few irradiated eggs was 52.2 for the mated females and 57.5 for the unmated females during this period compared to 89.7% for the controls.

After the sterile period, eggs were laid that had been oogonial cells when irradiated. Egg production of the irradiated females for day 10, a representative sample from near the beginning of the poststerile period, was 5.5 eggs per female and 23.7 eggs per female for the controls. Hatchability was 39.5% for the irradiated mated females, 43.6% for the irradiated virgin females, and 90.1% for the controls. It is clear that both egg production and embryo hatchability are reduced in oocytes developing from irradiated oogonia.

The majority of dead embryos from irradiated gonial cells at the beginning of the poststerile period progress in development to a point where ureate cells form, a time shortly before the embryo would normally hatch.

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[47] D. S. Grosch and R. L. Sullivan, Radiation Research 1, 294 (1954).

The gut usually ruptures or breaks in these embryos. Fertilization of these eggs does not delay the time of death.

Discussion. — We have confirmed the observation that dominant lethality can be induced by X radiation in oogonia of Habrobracon. Significantly, the embryos usually die at a different time of oogenesis from that characterized by radiation-induced chromosome imbalance or mitotic inhibition.[46] The embryos appear to die under conditions resembling those seen when lethal doses of ultraviolet radiation are delivered to the cytoplasm of a mature egg. For this type of lethality, radiation may not interfere with the ability of an oogonial cell to reproduce itself, either immediately or eventually, but does upset the ability of the cell to manufacture the proper type of cytoplasm during egg maturation. This explanation is consistent with the observation that embryo dominant lethality is not measurably induced in irradiated spermatogonia since deformed sperm would probably be nonfunctional.

Effects of X Rays on Longevity in Habrobracon

A. M. Clark M. A. Rubin M. A. Ashcraft

Introduction. — The wasp Habrobracon varies during its life cycle in its sensitivity to X rays from 100 r for embryos in the cleavage stage to 100,000 r for older pupae.[48] The criterion of injury used for irradiated embryos, larvae, and pupae has been the ability to continue development and to emerge as adults from cocoons. Life span as a measure of radiation damage has been used for irradiated adults and 25,000 r has been shown to affect survival.[49] Embryos that have been injured by X rays must compensate for this injury (or perish) as they develop and differentiate to the adult stage. To what extent do these injuries persist among surviving adults and is longevity a useful measure of injury? In Habrobracon, the existence of haploid males, diploid males, and diploid females makes it possible to determine whether decreased longevity is related to genetic injury.

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[48] A. M. Clark, Am. Naturalist 91, 111–19 (1957).

[49] D. S. Grosch, J. Econ. Entomol. 49, 629–31 (1956).

In the experiments reported here, Habrobracon were X-irradiated as larvae-in-cocoons and as white pupae, and the emergence ratios and longevity of adults were obtained. Data on the longevity of irradiated adults were also obtained. Only data from females are presented.

Results and Discussion. — Habrobracon females were irradiated as white pupae with doses of X rays ranging from 5000 to 30,000 r. The adult emergence and longevity of the surviving adults were obtained (Table 7). The females were fed upon paralyzed Ephestia larvae to make it easy for them to feed. Comparison of adult emergence with longevity,

Table 7. Emergence and Adult Longevity of Habrobracon Females After Exposure as White Pupae to X Rays

X-Ray Dose (r)	Adult Emergence		Median Life Span (days)
	Ratio	Per Cent	
0	288/306	95	29.0
5,000	220/242	91	21.5
10,000	249/286	86	12.0
15,000	168/199	84	10.5
20,000	176/203	87	9.0
30,000	64/122	53	7.5

as measured by median life span, shows that a considerable amount of injury induced in pupae persists among surviving adults. In fact, longevity is a good differentiator of injury from radiation damage. Doses of radiation that show little effect on the ability of the pupae to develop to the adult stage show much greater effects when longevity is used as the criterion of injury. Comparable data were obtained for irradiated larvae-in-cocoons where doses of 1000–3000 r were used. Irradiation of adult females with 25,000 r yielded a median life span of 12.0 days in contrast to the control group, which showed a median life span of 26.0 days.

The above results show that longevity is a useful and sensitive criterion of radiation damage for Habrobracon and probably for other insects.

Oxygen Poisoning and X-Radiation Damage in Insects

A. M. Clark V. J. Cristofalo

Introduction. – The toxic effects of oxygen at increased pressures are well known.[50] Recent findings that agents such as cysteine and β-mercaptoethylamine (which afford some measure of protection against radiation damage) will also protect against oxygen poisoning have indicated that there may be a mechanism in common.[51] Insects present a unique system for this type of study since they show a well-defined stage sensitivity to both oxygen and X rays.[52] The present report is concerned with a comparison of the effects of high oxygen pressures and X radiation on the larval and pupal stages of the following insects: Tenebrio molitor, Prodenia eridania, Anagasta kühniella, Galleria mellonella, and Habrobracon juglandis.

Results. – Although both radiation and high oxygen pressures cause a cessation of development and abnormalities among the adult survivors, some striking differences have been found. The first effect of high oxygen pressure is an immediate reduction in oxygen uptake. Whenever this occurs the insects fail to develop to the adult stage. This may or may not be accompanied by a paralysis. Doses of X radiation that cause a cessation of development, however, do not result in a reduction of oxygen uptake or paralysis. Treatment of pupae at low temperatures effectively protects against oxygen damage. Larvae, however, which have a higher rate of respiration than pupae, are much more resistant to high oxygen pressures than are pupae. In contrast to this, larvae are much more sensitive to radiation than pupae. For example, pupae of Tenebrio molitor fail to

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[50] J. W. Bean, Physiol. Rev. 25, 1 (1945).

[51] R. Gerschman et al., Project Report, February 1955, Air Univ. 21-1201-0013, p 12.

[52] A. M. Clark, Ann. Entomol. Soc. Am. (1959).

develop after treatment with 60 psi of oxygen but the larvae are resistant to 135 psi of oxygen. With X rays, however, 60,000 r are required to inhibit the development of the pupae whereas only 5000 r will stop the development of the larvae.

Discussion. — These data clearly show that some aspects of the mechanisms of damage are different in each case. The protection with sulfhydryl reagents would seem to be best interpreted as a protection of some basic cellular mechanisms that ultimately reflect injury of many types.

Modification of Longevity and Succinic Dehydrogenase Activity by Temperature in *Habrobracon*

R. C. Griggs

Introduction. — Previous work on the wasp *Habrobracon* showed that males have a much greater succinic dehydrogenase activity than females and that this activity declines with age in both. It has been suggested that there is an inverse relation between metabolic rate and speed of growth and longevity in invertebrates.[53] The higher activity of the male, coupled with the fact that males do not live so long as females, may indicate therefore a possible correlation between the level of succinic dehydrogenase activity and longevity. Furthermore, since the activity declines with age, a relation between this decline and aging is suggested.

Present work deals with the modification of longevity by temperature change and investigation of concomitant effects on enzyme activity.

Results and Discussion. — *Habrobracon* kept at 25°C during development and adulthood live longer than comparable ones kept at 30°C.[54] In the present study, unfed adults that had been reared from eggs at 25°C also live longer when placed at 30° than unfed adults reared from eggs at 30°C and kept at 30°. There was, however, no succinic dehydrogenase activity difference between the two groups for newly emerged adults on

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[53] F. Bouvlière, Symposia Inst. Biol. 6, 27–34 (1957).

[54] D. S. Grosch, Biol. Bull. 99, 65–73 (1953).

a per organism basis (determined spectrophotometrically, with triphenyltetrazolium chloride as an indicator). However, organisms raised at 25°C are somewhat larger than those raised at 30°C, the males in particular being 10–20% heavier, so that on a weight basis a difference may be indicated. If the difference between the temperatures at which the organisms were reared was larger, perhaps there would be a more noticeable difference in activity.

Unfed Habrobracon males and females raised at 30°C were divided into two groups. One group was kept at 10°C, the other at 30° for three days. The succinic dehydrogenase activity decreased less in the 10°C organisms than in the 30° ones. This would seem to indicate that lower temperature not only increases longevity but also curbs the decrease in succinic dehydrogenase activity.

The Induction of a New Puffing Pattern by Transplantation of Drosophila Salivary Gland Nuclei into Egg Cytoplasm

H. Kroeger

Introduction. – The detection of a functional differentiation of specific sections in the giant chromosomes in salivary gland cells of Diptera – visible as the appearance of swellings ("puffs") of certain bands during development – has provided strong support for the notion that somatic differentiation of cells is a reflection of the functioning of different arrays of genes in the different cell types.[55] The development of a method that allows for an exposure of salivary gland nuclei to cytoplasm other than that of salivary gland cells allows now for an induction of new puffing patterns and for a study of the factors determining activation or suppression of gene action.

Results. – Incubation of salivary gland nuclei (from Drosophila busckii larvae shortly before pupation) in cytoplasm from eggs before blastoderm formation (0–2 hr) of D. melanogaster resulted in the disappearance of puffs normally present in donor chromosomes of this age

[55] W. Beermann, in Developmental Cytology, Ronald Press Co., New York, 1959.

and in the induction of a number of new puffs. Three regions were selected for detailed analysis. In region IIR(B)17-25 (approximate locations according to the chromosome maps of Sirotina[56]), a very large puff appears at approximately 22-23, which exceeds in size any puff seen in normal development; a smaller puff appears in 17. A small puff appears in region IIIL(C)4-7 and another one in X5-7. In approximately 35 nuclei in which region IIR(B)22-23 could be identified the puff at this point was observed. The other regions are more difficult to recognize, but the puffs were usually observed in them when they could be positively identified. No puffs were found in these regions in the nuclei *in situ* during the entire last instar.

Discussion. — From these results it seems that the factors determining activities of specific chromosome regions reside in the cytoplasm. The stimulating factor may be either a substrate for the reaction mediated by the gene or its products or it may be RNA or protein, the only cell constituents that contain enough specificity to single out genes from the whole array present in the genome. Experiments to decide between these possibilities are in progress.

DROSOPHILA CYTOLOGY AND GENETICS

D. L. Lindsley
G. E. Brosseau, Jr.[57]
E. H. Grell[57]
B. Nicoletti[58]
M. Warters[59]
R. F. Grell
M. S. Moore
M. L. P. Rekemeyer
M. A. Robinson
R. D. Wilkerson

Elimination of the Nucleolus Organizer and the Centromere as Targets for X-Ray Inactivation of Spermatozoa

D. L. Lindsley M. S. Moore R. D. Wilkerson

Introduction. — In a previous report,[60] we have developed a model that estimates differences in radiation sensitivity of spermatozoa of

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[56] M. I. Sirotina, Mem. Genet. Acad. Sci. Ukr. S.S.R. 2, 61–90.

[57] Research Associate.

[58] Visiting investigator from abroad.

[59] Research Participant.

[60] D. L. Lindsley, Biol. Semiann. Prog. Rep. Feb. 15, 1959, ORNL-2702, p 66–68.

different genotypes. These estimates are based on the relation between X-ray dose and the relative frequencies with which the genotypes are represented in the progeny of treated males. If two genotypes, one of which has a chromosomal element that the other lacks, are compared, it is possible to estimate the sensitivity that can be attributed to the element in question. The sensitivity measured is to damage interfering with imago formation any time between treatment of the sperm and eclosion. From a combination of sex ratio and hatchability determinations, it has been possible to infer that X-ray-induced sex ratio shift represents elimination of gametes from the fertilizing pool and that the target for this phenomenon is chromosomal, whereas the reduction in hatchability is the consequence of irradiation of a nonchromosomal target. The chromosomal targets responsible for sex ratio shift are associated with the X and the Y chromosomes; the following experiments represent an attempt to define more clearly these chromosomal targets.

Results. — The question arises as to whether the target is chromosomal material *per se* or whether some chromosomal organelle or locus may be implicated. One organelle common to both X and Y is the nucleolus organizer; by utilizing combinations of inversions it is possible to construct chromosomes with nucleolus organizers carried both distally and proximally (DP), a distal nucleolus organizer (Dp), a proximal nucleolus organizer (dP), or no nucleolus organizer (dp). Manipulation of the nucleolus involves the simultaneous manipulation of a segment of heterochromatin equivalent to about 3/8 of the total length of the X chromosome, such that lengthwise DP:dP:Dp:dp = 1.375:1.000:1.000:0.625. By comparing the sensitivity of each of these chromosomes to the same Y chromosome, it has been demonstrated that DP = Dp > dP = dp in sensitivity; thus any differences in sensitivity are attributable neither to the nucleolus organizer nor to its associated heterochromatin. Having ruled out the nucleolus organizer and heterochromatin in general as the target in question, the remaining candidates, at least for the Y chromosome, seem to be specific heterochromatic regions or the centromere. To check the sensitivity of the centromere, the survivals of sperm with four vs five and three vs four centromeres were compared. Again, because the

elements of the X are easier to manipulate than those of the Y, the fifth centromere was derived from the X. The source of this extra centromere was an extremely small free X chromosome duplication marked with the normal allele of yellow and consisting of little more than the right arm of the X and the centromere; it was kindly supplied by Drs. K. W. Cooper and J. Krivshenko. Males of constitution XY/Dp produce four types of sperm: XY, Dp, XY/Dp, and O. Their relative sensitivities are XY/Dp = XY > Dp = O. Thus it can be seen that XY bearing sperm are more sensitive than nullo-XY sperm, but the presence of the duplication has no effect on sensitivity.

Discussion. — In the previous report,[60] it was shown that both the X and the Y chromosomes provide targets for radiation inactivation of sperm. The experiments described above seem to rule out the nucleolus organizer of the X chromosome and a sizable portion of its associated heterochromatin as well as the X centromere and short arm as contributing appreciably to the target provided by the X. It is inferred that the Y centromere, the Y nucleolus, and its associated heterochromatin similarly do not contribute to the Y-linked target. It is concluded that the sensitivity of the X and the Y chromosomes reside in specific regions that, in the Y at least, are heterochromatic.

Further Data on the Reciprocal Translocations Between the X and the Y Chromosome in *Drosophila melanogaster*

B. Nicoletti D. L. Lindsley

In the previous report,[61] the technique to obtain this kind of translocations and data concerning the first nine of them were discussed. Using the same technique (irradiation with 4000 r of sperms carrying the X and a marked Y chromosome), we obtained a sufficient sample of X-Y translocations for further observations. From a total of 6548 tested sperms, 87 translocations were recovered with an average of 1.3% for the indicated dose. Among them 22 were between the X and the sc^8.Y (marked with y^+) and 65 were between the X and the Y.B^S (marked with B). The frequency for the

[61] B. Nicoletti and D. L. Lindsley, Biol. Semiann. Prog. Rep. Feb. 15, 1959, ORNL-2702, p 71.

X-Y translocations involving the two different Y's did not differ significantly (1.33 and 1.32%, respectively).

In Table 8 there are data concerning the viability and the fertility for the males carrying the described translocations; in Fig. 6 there is the distribution of the break points along the X chromosome according to the frequency with which the two arms of the Y chromosome are involved.

Table 8. Genetic and Cytological Analyses of X-Y Translocations

	Fertile Males	Sterile Males	Lethal Males	Total	Cytologically Analyzed	Y Broken	
						Long Arm	Short Arm
$sc^8.Y$	8	7	7	22	17	10	7
$Y.B^S$	33	17	15	65	56	29	27
Total	41	24	22	87	73	39	34

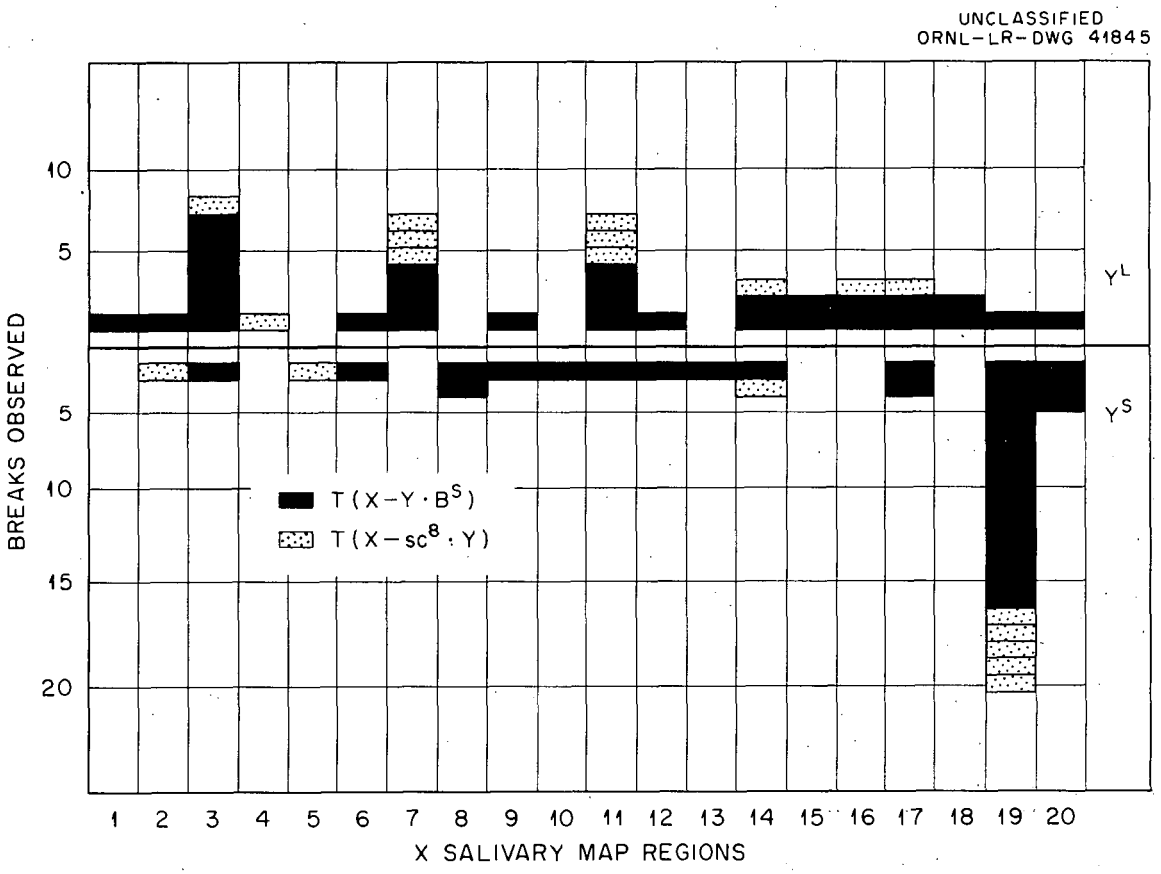

Fig. 6. Distribution of X Chromosome Break Points in X;Y Translocations.

The short arm of the Y appears to be involved with greater frequency than expected: 34 times as compared with 39 (expected ratio according to the metaphase length 1:3). In 21 cases, however, the short arm was involved in the translocations when the X was broken in the proximal heterochromatic part (region 19-20). Since the Y was marked only on the long arm and the euchromatic part of the X transferred to the Y centromere can yield males viable and fertile, we may assume that many translocations involving the long arm and the base of the X were undetected. In this case, it is impossible to recognize these as translocations, owing to the lack of the linkage between the X and the marked fragment of the Y put on the X centromere.

All the 41 male fertile translocations were cytologically analyzed by the salivary technique and all were found to be simple reciprocal translocations. On the other end, the salivary analysis showed more-complex arrangements involving the autosomes as well as the X and the Y for 18 of 23 sterile male translocations. This is in agreement with Lindsley's findings[62] that most of the X-autosome translocations are male sterile and offers the possibility for further observations on the male sterility not covered by an extra normal Y chromosome.

V-Type Position Effect at the Bar Locus in *Drosophila melanogaster*

G. E. Brosseau, Jr.

The mutant Bar (*B*) is thought to be the consequence of breakage near salivary chromosome band 16A1-2 and juxtaposition of this region to foreign euchromatin. The resulting phenotype does not show variegation and is not subject to modification by additional heterochromatin and is therefore considered to be a stable (s-type) position effect. Several of the *B* mutants are tandem duplications for several salivary bands including 16A1-2. In the case of one particularly strong *B* allele (very narrow eyes), Bar-Stone, the two components of the duplication were separated by means of an induced translocation with the fourth chromosome. It was

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[62] D. L. Lindsley *et al.*, *Biol. Semiann. Prog. Rep. Feb. 15, 1958*, ORNL-2481.

found that the narrow eye phenotype was associated with the right portion of the B region, which remained attached to the base of the X and carried the tip of the fourth chromosome. By use of the Bar-Stone translocation (B^S), a Y chromosome that carried the B^S region at the end of the long arm was constructed. This dominantly marked Y chromosome (YB^S) was the source of the chromosome fragments described in this report. These fragments were recovered by B. Nicoletti in the progeny of irradiated males that carried YB^S in addition to a normal X and another marked Y, sc^8.Y.

Results. – Five YB^S fragments (designated $YB^{SN-1 \cdots 5}$) were recovered. The conditions under which the fragments were recovered suggested that they consisted of less than a whole Y chromosome. In the course of tests to assess the amount of the Y chromosome present in the fragments, it was discovered that the B phenotype was strongly modified by the amount of heterochromatin in the nucleus. For example, when YB^{SN-1} is present in an attached XY male the phenotype is strong Bar; when it is with a normal X, the eyes are almost wild type. When the X has only one Y arm attached, the phenotype is intermediate. Within each category, the phenotype is quite variable although individual flies are fairly symmetrical. The eye does not show mottling of Bar and wild-type regions but is simply wider or narrower depending on the amount of heterochromatin present. The phenotypes of the B^S translocation and YB^S are not influenced by changes in the number of Y chromosomes present. The results of a genetic analysis of the YB^{SN} chromosomes are given in Table 9. YB^{SN-1} and YB^{SN-4} carry bb^+ and KS (the Y short fertility complex) but not KL (the Y long complex); these are probably deleted Y chromosomes. The remaining fragments have neither KS or KL and are probably one-half of an XY translocation where the tip of Y^L (including B^S) has been attached to the base of the X. Hyperploids for these fragments are viable and fertile, and hence this fragment has probably become separated from the rest of the translocation.

Discussion. – The YB^{SN} fragments represent the first cases of v-type position effect for a genetic factor that is itself presumed to be the consequence of an s-type position effect. The most interesting feature of these position effects is the direction of the response to extra heterochromatin. Usually, the consequence of adding heterochromatin to a v-type position effect is to make the phenotype more like the wild type.

Table 9. Results of Genetic Analysis of the YB^S Fragments

Fragment Number	Variegation	Factors Tested For*			Probable Rearrangement
		KS	KL	bb	
N-1	Yes	+	-	+	Deleted Y
N-2	No	-	-	+	1/2 T (X:Y)
N-3	Yes	-	-	-	1/2 T (X:Y)
N-4	Yes	+	-	+	Deleted Y
N-5	Yes	-	-	+	1/2 T (X:Y)

*Symbols: + indicates that the factor is present on the fragment; - indicates that the factor is absent on the fragment.

In this case, removing heterochromatin makes the phenotype more normal. One possibility is that these are exceptional cases of position effect that respond to heterochromatin in a way opposite to the usual response. An alternative is that, since the genetic effect of Bar is dominant, the B phenotype should be considered as the "normal" type for these flies and the restoration of the wild type by removing heterochromatin is really an enhancement of the position effect. The latter explanation leads to an interesting line of speculation. If the B phenotype is the consequence of s-type position effect brought about by foreign heterochromatin next to band 16A1-2, then perhaps placing this euchromatin next to heterochromatin inactivates it and restores normal activity to band 16A1-2. The v-type position effect neutralizes the s-type position effect. Suppressing the v-type position effect by the Y restores the s-type position effect and the B phenotype. Further genetic and cytological studies should shed light on these phenomena.

Production of Tetra-4 Adults in *Drosophila*

E. H. Grell

Drosophila with one less or one more fourth chromosome than the normal pair have been known since Bridges[63] described such flies in 1921.

[63] C. B. Bridges, Proc. Natl. Acad. Sci. U.S. 7, 186–92 (1921).

The progeny of triplo-4 parents should include a portion of tetra-4 animals, but there has been no evidence published that animals with four fourth chromosomes survive into adulthood. Li[64] reported that most tetra-4's die in the embryonic stage and the remainder during larval life. Bridges[65] found one female pupa with four fourth chromosomes in oogonia. Schultz[66] states that he probably saw a tetra-4 fly carrying four doses of the mutant sv. He gives no description of the tetra-4 other than that it had extra bristles and offers no evidence that it was indeed tetra-4.

The difficulty in proving the tetra-4 constitution by genetic means is caused by the lack of usable genetic markers on chromosome four. To circumvent this difficulty, translocations of the fourth chromosome with other chromosomes can be used. The markers on the other member of the translocation indicate the presence of the fourth chromosome.

Adult flies with the material of the fourth chromosome in quadruplicate have been produced by the translocation method. Adults of the following genotypes were observed: T(3;4)88B, Ubx/T(3;4)86D, $bx^{34e}/ci^{D}/ey^{D}$, $T(1;4)w^{m5}$ v $T(1;4)B^{S}$ (homozygous) and $T(1;4)w^{m5}$ T(1;4)9A (homozygous). Flies of these genotypes eclose quite rarely and are short lived. They have a very characteristic phenotype. The body is narrower and the abdomen more pointed than normal. The wings are pointed and are often held out from the body. Fertility in both sexes is very low.

Genetic Studies on the Behavior of Chromosomal Elements Involved in Nonhomologous Associations

R. F. Grell E. H. Grell

Introduction. — It has been shown that very high frequencies of nonrandom assortments between nonhomologues are induced in *Drosophila melanogaster* females by the presence in the genome of two or more unpaired, nonhomologous chromosomes or chromosomal elements.[67,68] That these nonrandom

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[64] J. C. Li, Genetics 12, 1–58 (1927).

[65] C. B. Bridges, Trudy po Dinamike Razvitiia 10, 463–74 (1935).

[66] J. Schultz, Am. Naturalist 69, 30–54 (1935).

[67] R. F. Grell, Genetics 42, 374 (1957).

[68] R. F. Grell, Genetics 44 (1959), in press.

assortments are a consequence of meiotic associations between the nonhomologues is established by the equality of the reciprocal classes that result. Studies have been undertaken in an attempt to define the conduct of the elements involved, with the purpose of establishing a set of rules that govern nonhomologous associations. Previous experiments have determined that competition occurs among unpaired elements for nonhomologous associations.[68]

Results and Discussion. — A comparison has been made of the associations of three elements, a Y chromosome, a fourth chromosome, and a T_4 chromosomal element (a fragment of a 3:4 translocation that carries the centromere and proximal heterochromatin of four plus the distal euchromatin of 3R) under different conditions of competition. The frequencies of association of the three sets, the Y and the fourth chromosome, the Y and the T_4, and the partial homologues, the fourth chromosome and the T_4, have been measured in the absence and in the presence of a third element. They have also been measured when T_4 is predominantly paired with its homologous region in a normal third chromosome and when T_4 is free owing to the introduction of an inversion into its homologous region in 3R.

The frequencies of associations which were obtained in these experiments are given in Table 10.

Table 10. Percentage Frequency of Associations Between Y and 4, Y and T_4, 4 and T_4

Values in parentheses are inferred from data

Extra Element ⟶	T_4	4	Y	Y,4
Association ⟶	Y and 4	Y and T_4	4 and T_4	T_4 and 3
Extra element absent				
1. Normal 3	84.2	26.2	32.9	(64.7)
2. Inverted 3	--	--	97.6	(0)
Extra element present				
3. Normal 3	53.2	30.2	0	(64.7)
4. Inverted 3	5.8	96.6	0	(0)

A study of the shifts in pairing frequencies under the different conditions permits several new conclusions to be drawn concerning nonhomologous associations.

Any association between three elements may be described on the basis of a bivalent-univalent model or a trivalent model. In a bivalent-univalent model a given element (A) can associate with but one of the other two elements (B and C) at a time; consequently, the sum of the frequencies with which it associates with the two cannot exceed 100% (A-C + A-B $\leq$ 100%). In the trivalent model, however, it may pair with both other elements simultaneously, allowing a maximum association with each of 100% and thus a maximum total association of 200%. According to the former model, if A is associated 100%, a reciprocal relation must exist between the frequencies of A-B and A-C associations when an increase occurs in one. A consideration of the values in Table 10 shows that in no case is an element associated with a frequency significantly in excess of 100%. Furthermore, a comparison of lines 3 and 4 shows that the increase in Y,T_4 association of 66.4% is compensated for on the one hand by a decrease in Y,4 association of 47.4% and on the other hand by a decrease in T_4,3R association of 64.7%. Hence:

The nonhomologous associations of the Y and T_4 and the Y and 4 appear to fit a bivalent-univalent model.

The frequencies of associations between the Y and the fourth chromosome and between the Y and T_4 in line 4 of Table 10 show that the Y pairs with the T_4 some 17 times as frequently as it pairs with the fourth chromosome. Under the same conditions but in the absence of a Y (Table 10, line 2), the fourth chromosome and the T_4 pair 97.6%, indicating that both are available with this frequency. Clearly the Y shows a preference for the T_4. Hence the following generalization may be made:

Nonhomologues possess different degrees of attraction for each other.

The fourth chromosome carries all of the proximal heterochromatin present in T_4. Conversely, the euchromatic composition of the fourth chromosome is entirely different from T_4. The preference shown by the Y

for the T_4 must be attributed to the difference in the euchromatic composition of the fourth chromosome and T_4. Hence:

Nonhomologous associations are not entirely dependent on proximal heterochromatic regions but are influenced by euchromatic regions as well.

X Autosomal Translocations of *Drosophila melanogaster*

M. Warters

Introduction. — Many translocations involving the X chromosome of *Drosophila melanogaster* are associated with either recessive lethality or male sterility. Since most translocations are picked up from F_1 males of irradiated parents, this class of X autosome translocations is automatically screened out. This results in the recovery of about 15% of the expected number of X chromosome translocations based on chromosome length. It would be of interest to know just what the difference is between those translocations that are male sterile and those that are fertile. This requires recovering the translocation in F_1 females rather than in F_1 males. To do this, all chromosomes must be sufficiently tied up with inversion heterozygosis to assure complete linkage between the break point and any marker that is used.

Results. — Special stocks have been designed for both parental genotypes. Yellow shaven males (y/Y; sv^n/sv^n) were irradiated with 4000 r and mated in mass cultures with FM1 or FM6/In(1)AM.y^2; Cy; Ubx 130, e/T (2-3)bw^V; +/+ females. FM; Cy; Ubx daughters were selected and mated individually with In(1)sc^7AM/Y; +/+; e/e; sv^n/sv^n males. The progeny of these matings were scored for translocations. Individuals from translocation-bearing vials were outcrossed to determine fertility and for salivary analyses. It is hoped that a comparison of break points in the fertile and sterile groups will provide some correlation between break points and male sterility in X autosomal translocations.

A pilot experiment has demonstrated the effectiveness of this method in recovering translocations induced in the X chromosome. A repetition of the experiment on a larger scale is now in progress.

MAMMALIAN GENETICS AND DEVELOPMENT

GENETIC AND DEVELOPMENTAL EFFECTS OF RADIATION IN MICE

W. L. Russell, Section Chief

E. F. Oakberg
L. B. Russell
J. S. Gower[1]
W. J. Welshons
L. Wickham
J. R. Inman
R. A. Goff[2]
J. Moutschen[3]
S. K. Badgett
J. W. Bangham
E. B. Blalock
M. B. Cupp
D. L. Hartman[4]
P. R. Hunsicker
E. M. Kelly
M. M. Larsen
S. C. Maddux
E. S. Padgett
E. L. Phipps
C. L. Saylors
J. A. Smith
M. H. Steele
K. F. Stelzner
E. S. Von Halle[1]
M. C. Bahner

Dependence of Mutation Frequency on Radiation Intensity

W. L. Russell L. B. Russell E. M. Kelly

In a series of recent papers we have presented evidence that, contrary to the view that had long been accepted, radiation induction of mutations is dependent on radiation intensity, that is, dose rate. New data on this subject were presented by us at the Symposium on Immediate and Low-Level Effects of Ionizing Radiation, June 22–26, 1959, at Venice, and will be published soon. A brief summary of the findings is presented here.

1. The new data strongly support the earlier results showing a lower mutation frequency with chronic gamma irradiation than with acute X rays.

2. The possibility, rather remote to begin with, that the results could be explained in terms of radiation quality rather than radiation intensity now seems to be ruled out by additional data obtained from experiments comparing mutation rates induced by acute gamma irradiation with those induced by chronic gamma irradiation. These additional data were

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[1]Consultant.

[2]Research Participant.

[3]Visiting investigator from abroad.

[4]Summer employee.

obtained with the genetic criterion (specific locus mutations) and cell stage (spermatogonia) most cogent for the comparison.

3. The view that the difference in mutation rates is due to an effect of radiation intensity on the mutation process rather than on cell selection was originally supported primarily by the results obtained with oocytes. The additional data obtained with spermatogonia now also provide some evidence on this point:

a. Where a probable effect of cell selection on mutation rate was found, it worked in the direction opposite to that required to account for the above difference in mutation rates.

b. The mutation rates at 86 and 300 r, for chronic gamma irradiation of spermatogonia, are not higher than a linear interpolation between the control points and points at higher doses. Thus, there is no evidence of a distortion of mutation rates by cell selection.

4. We have reexamined the possibility that cell selection might account for the intensity effect in oocytes:

a. Even when the comparison between the results from chronic and acute irradiation is restricted to follicle stages in which oocyte killing is infrequent, an intensity effect on mutation rate is still observed.

b. Chronic irradiation was found to cause less killing of oocytes than acute irradiation. Thus, any effect of cell selection would work in the wrong direction to that required to account for the intensity effect, unless one imagined a negative correlation between sensitivity to killing and mutational sensitivity.

5. We also reexamined the possibility that the difference in mutation rates obtained with chronic and acute irradiation of oocytes could be due to differences in oocyte stage. Since intensity involves a time factor, it is, of course, impossible to have the oocyte stages identically the same in the comparison. However, when the comparison was restricted to oocyte stages that are closely similar, an intensity effect on mutation rate was still found.

In general, the new data strongly support the earlier conclusion that mutation frequency in spermatogonia and oocytes is markedly dependent on radiation intensity.

Comparison of the Effects of Acute, Continuous, and Fractionated Irradiation During Embryonic Development

L. B. Russell S. K. Badgett C. L. Saylors

The effects of chronic irradiation accumulated during the first two weeks of prenatal development were compared, on the one hand, with the effects of the same dose of acute irradiation given only once during the same two weeks (at one of seven stages) and, on the other hand, with the effects of daily fractions of acute irradiation given during the same interval and accumulated to the same total dose. The dose used was close to 200 r. Characters studied were mortality before, at, and after birth; total life span; weight at birth and at 36 days; fertility; and morphology. A total of 731 animals irradiated or sham-irradiated *in utero* (132 pregnant females treated) were studied. Of these, 156 were used in the fertility tests and had a total (to date) of 8151 offspring.

The various results, which are being published in detail elsewhere,[5] are summarized in Table 11. This summary shows, at a glance, the striking contrast between chronic irradiation during the first two weeks of prenatal development, which is virtually without effect; and acute irradiation at various stages during the same period, which has a multiplicity of effects, depending on stage.

The data for acute irradiation extend earlier findings, which showed a strict stage specificity of effects produced.[6–9] The earlier studies had been terminated at birth. The new results show clearly that stage of irradiation is as important in determining postnatal differences between animals as it is for characters expressed by the time of birth. It should be noted that the new data also extend the earlier finding of an all-or-none effect of irradiation during the preimplantation period: although

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[5] L. B. Russell, S. K. Badgett, and C. L. Saylors, Intern. J. Radiation Biol., suppl. (1959) (in press).

[6] L. B. Russell, J. Exptl. Zool. 114, 545–602 (1950).

[7] L. B. Russell, p 861–918 in Radiation Biology, vol. 1 (ed. by Alexander Hollaender), McGraw-Hill Book Co., Inc., New York, 1954.

[8] L. B. Russell and W. L. Russell, J. Cellular Comp. Physiol. 43, suppl. 1, 103–49 (1954).

[9] L. B. Russell, J. Exptl. Zool. 131, 329–95 (1956).

Table 11. Summary of the Effects of Radiation Received in the First Two Weeks of Prenatal Development: Continuous Irradiation, or Acute Irradiation at Various Stages

Effect	Type of Radiation and Stage Irradiated					
	Continuous, Days $\frac{1}{2}$–$14\frac{1}{2}$	Acute, Preimplant[a]	Acute, Day $7\frac{1}{2}$	Acute, Day $9\frac{1}{2}$	Acute, Day $11\frac{1}{2}$	Acute, Day $13\frac{1}{2}$
Mortality						
Prenatal	0	++[b]	+++	0	0	0
Neonatal	0	0	++	+++	+	0
2–6 days	0	0	+	++	+	0
16–36 days	0	0	0	(+)	+++	0
>36 days	0	0	++	0	++	+
Weight						
At birth	0	0	+	+++	+++	++
At 36 days	0	0	+	++	+++	++
Fertility						
♂♂	0?	0?	++	+	+++[c]	++
♀♀	++	0?	++	++	+++	+++
Morphology	0[d]	0	+++	+++	+++	0

[a]Combined results for groups irradiated days $\frac{1}{2}$, $1\frac{1}{2}$, and $4\frac{1}{2}$.

[b]Prenatal death occurred only in groups irradiated days $\frac{1}{2}$ or $1\frac{1}{2}$.

[c]All males completely sterile.

[d]Results of series 2; the other entries in this table are based on the results of series 1.

some of the animals die shortly after irradiation, the survivors are normal, not only at birth, but in postnatal development, in survival (at least to the present age of 486 days), and in fertility.

Chronic irradiation during the first two weeks postfertilization appeared to have no marked ill effects other than a shortening of the breeding period of females. Daily acute fractions (results not contained in Table 11), in a rather limited series, were found to have no greater effect than continuous exposure.

It is postulated that the total frequency of cell killing from chronic irradiation is smaller than from acute; and/or that, with chronic irradiation, the total dose received during the short period in the development of any given character when radiation damage can be caused is too small to affect more than the threshold number of cells required to produce developmental effects. This point of view finds support in the fact that a daily acute exposure (dose rate 315 times as great as for chronic) produced no greater effect than continuous exposure to the same total dose in the same interval.

The two factors proposed to account for lower total damage from chronic irradiation must presumably more than cancel out another factor working in the opposite direction. Thus, if there is any cyclical activity with a recurrent sensitive phase, for example, a stage in the mitotic cycle which is considerably more sensitive than the rest, then continuous or fractionated radiation would be more damaging than acute. This has been shown to be the case in a specific situation by Auerbach.[10]

It is interesting to note that not even weight was detectably affected by chronic irradiation. Since it seems reasonable to assume that weight is a reflection of total cell number, thresholds, such as may exist for abnormalities, not being involved, it may be concluded either that there is compensatory cell division following cell killing, or else that the frequency of cell killing is, indeed, extremely low following low-intensity irradiation. In view of these considerations, it is all the more striking that chronic irradiation during the first two weeks postfertilization markedly affects the fertility of females. This may indicate either that the cells involved are peculiarly sensitive to chronic irradiation, or that there is less replacement of these cells than of cells elsewhere in the embryo. More work on the fertility effects is now in progress.

X-Ray Sensitivity of Primary Spermatocytes of the Mouse

E. F. Oakberg E. B. Blalock

The following experiments were designed to estimate the sensitivity of different meiotic prophase stages of primary spermatocytes to cell

[10] R. Auerbach, Nature 177, 574 (1956).

killing and to chromosome breakage. An estimate of the loss of spermatocytes from degeneration during meiotic division was obtained by comparing the number of stage II spermatids[11] in irradiated and control mice; frequency of chromosome breakage was estimated by scoring at anaphase I and anaphase II. On the basis of the duration of spermatogenesis reported earlier,[12] mice were killed at intervals selected to measure sensitivity of stages from preleptotene to metaphase I.

Data in Table 12 indicate that preleptotene was the most sensitive to cell killing, with an LD_{50} (205 r) which is significantly lower than for all other stages, and diakinesis-metaphase I was the most resistant. Late pachytene appears to be more resistant than early or midpachytene, and diakinesis-metaphase I was more resistant than zygotene, early, and midpachytene.

[11]E. F. Oakberg, Am. J. Anat. 99, 391–414 (1956).
[12]E. F. Oakberg, Am. J. Anat. 99, 507–16 (1956).

Table 12. X-Ray Sensitivity of Primary Spermatocytes of the Mouse

Stage Irradiated	Spermatocyte Survival			Chromosome Abnormalities	
	LD_{50} (r)	95% Confidence Interval		Dose (r) Required to Produce 50% Abnormal Anaphases	
		Lower	Upper	Anaphase I	Anaphase II
Preleptotene	205	175	233	612 ± 93	2770 ± 757
Leptotene	492	273	719	1443 ± 182	2052 ± 324
Zygotene	520	413	637	411	630
Pachytene (early)	404	329	470	242	448
Pachytene (mid)	382	301	452	265	507
Pachytene (late)	664	601	733	321	450
Diplotene	564	365	771	275	343
Diakinesis-metaphase I	837	679	1161		
Diakinesis				204	
Metaphase I					138 ± 6

Sensitivity to chromosome breakage (Table 12) shows a tendency to be inversely related to cell killing, being lowest for preleptotene and leptotene, and highest for diakinesis to metaphase I. The general pattern of changes in sensitivity is similar to that known for other organisms, with metaphase I more sensitive than propnase stages. For stages from preleptotene through diplotene, consistently fewer abnormalities were observed at anaphase II. (Metaphase I is an exception in that anaphase II represents the first postirradiation division.) This suggests that most aberrations are of a type detectable at the first division, but since degeneration is a concurrent process, the possibility that anaphase II represents a selected population cannot be excluded.

These data demonstrate that doses in the 200–500 r range significantly reduce the number of spermatids formed by irradiated spermatocytes. Furthermore, the frequency of abnormal anaphases indicates that a high proportion of surviving spermatids will show various degrees of aneuploidy and heteroploidy, a proportion of which should be sufficient to result in nonfunctional gametes. Thus while we have been able to estimate the loss of spermatocytes, it probably is an underestimate of the total damage in terms of production of viable gametes.

Recombination Between the Sex-Linked Mutants Tabby and Scurfy

W. J. Welshons

Tabby (Ta) is a sex-linked dominant; hemizygous males and homozygous females are viable and fertile. The mutant scurfy[13] (sf) is completely recessive in heterozygous females, while hemizygous males are characterized by scaliness of the tail and other parts of the body, "tight" skin, and late opening of the eyelids. Affected males can be classified as to phenotype from about 11–15 days of age, but such males generally die before weaning or shortly thereafter. Both of the mutants Tabby and scurfy

[13]W. L. Russell, L. B. Russell, and J. S. Gower, Proc. Natl. Acad. Sci. U.S. 45, 554–60 (1959).

have played prominent roles in the detection and production of the hemizygous X/O chromosomal constitution, and the proof that this constitution is a female one.[13,14]

In order to measure the linkage between the two mutants, heterozygous females of the constitution Ta +/+ sf were mated to normal males, and the F_1 male progeny were utilized for the calculation of the recombination value, since both parental nonrecombinant chromosomes and the two recombinant chromosomes could be recognized phenotypically in the hemizygous males. The progeny were examined every day and tentatively classified as to phenotype, then reexamined daily until identification was certain. Of 302 progeny from the above cross, only 15 were dead at birth or died shortly after birth; thus a total of 287 animals were classified, of which 147 were males and 140 were females, including two X/O females which are not incorporated into Table 13.

Table 13. Classification of Progeny from the Cross
Ta+/+sf × ++/Y

Classification	Number
Tabby	69
Non-Tabby	69
Ta+/Y	40
+sf/Y	44
++/Y	31
Ta sf/Y	32

The data of Table 13 yield a value of 63/147 or 43% recombination between Tabby and scurfy; thus scurfy is loosely linked to the mutant Tabby, which occupies a position relatively near the center of the known linkage group. Consequently, with Tabby near the center, one would expect scurfy to be more closely linked to either Bent (Bn) or jimpy (jp), since these two mutants are at opposite ends of the same linkage group. Additional experiments will yield a more precise localization of the mutant scurfy.

[14]W. J. Welshons and L. B. Russell, Proc. Natl. Acad. Sci. U.S. 45, 560–66 (1959).

A Study of X-Ray-Induced Abnormalities in the Posterior Limb of the Chick Embryo

R. A. Goff

The induction of abnormalities in the chick embryo by means of X radiation has never been reported in detail[15–17] although this approach to a better understanding of normal embryonic development is being actively pursued with mammalian embryos.[18–24] The advantage the chick embryo offers in such a study is that the stage of development at the time of irradiation can be accurately determined, thereby providing a closer correlation with the responding morphogenetic process.

Embryos were irradiated at intervals throughout the period in which the limbs develop (stage 8–35 in the normal series).[25] Particular attention was given to the determination of the developmental status of the embryos at the time they were irradiated. Observations were made on embryos which had been irradiated in each of the described stages of development in the early period of limb formation (stage 8–21), and in the later period at least every other stage was represented. In terms of incubation time, the period of limb formation studied began early on the second day and ended on about the eighth day of incubation.

A gross analysis of the response of the leg to radiation in different periods of development is shown in Fig. 7. The responses followed X-ray

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[15]E. G. Butler, p 389–410 in Biological Effects of Radiation (ed. by B. M. Duggar), McGraw-Hill Book Co., Inc., New York, 1936.

[16]J. P. O'Brien, Ann. Rev. Nuclear Sci. 6, 423–51 (1956).

[17]R. Rugh, Ann. Rev. Nuclear Sci. 3, 271–302 (1953).

[18]L. B. Russell, J. Exptl. Zool. 114, 545–602 (1950).

[19]L. B. Russell, p 861–918 in Radiation Biology, vol 1 (ed. by Alexander Hollaender), McGraw-Hill Book Co., Inc., New York, 1954.

[20]L. B. Russell, J. Exptl. Zool. 131, 329–95 (1956).

[21]L. B. Russell and W. L. Russell, J. Cellular Comp. Physiol. 43, suppl. 1, 103–49 (1954).

[22]J. G. Wilson and J. W. Karr, Am. J. Anat. 88, 1–34 (1951).

[23]J. G. Wilson, H. C. Jordan, and R. L. Brent, Am. J. Anat. 92, 153–88 (1953).

[24]S. P. Hicks, Am. J. Roentgenol., Radium Therapy Nuclear Med. 69, 272–93 (1953).

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

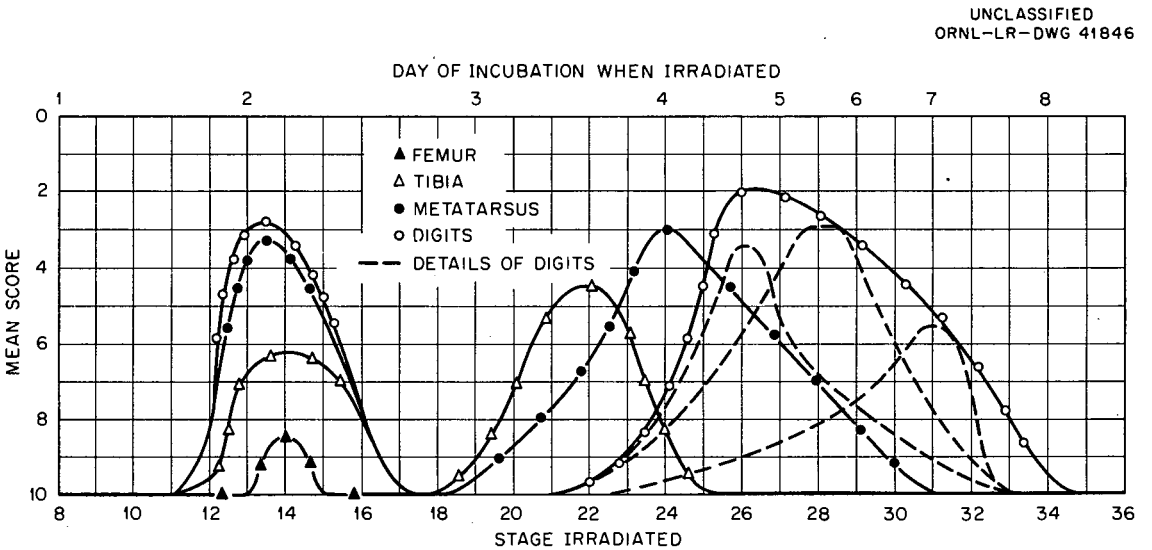

Fig. 7. Periods of Sensitivity to X Radiation by Region in the Development of the Leg.

doses which were approximately equal in their lethal effect by 12 days of incubation. The doses ranged in decreasing order from 800 r at the beginning of the period to about 600 r at the end. The effect on each leg was evaluated according to region (femur, tibia, metatarsus, digits) by a method of scoring from 0 (extreme effect or failure of the part to form) to 10 (normal part). Recorded in the curves are the mean scores for each stage irradiated.

The effect on stage 12–16 embryos was to eliminate parts of the appendage. Parts were deleted in a disto-proximal direction as the most sensitive period was approached. This sequence was also observed within a homogeneous group of embryos which always demonstrated a degree of variation in sensitivity. The evidence available also indicated that increasing the X-ray dose caused the elimination of progressively more proximal parts. Equal killing doses before stage 12 had no effect upon the limb, and after stage 16 the response was different. In this period there was no morphological evidence of the limb. When it first appeared as a bud at stage 17–18, it was resistant to X radiation, but after it had elongated to the size typical of stage 19 embryos the tibial region began to show differential sensitivity. This was followed, in succession,

by specifically sensitive periods in the metatarsal and toe regions. The curves show again, a disto-proximal sequence in severity of response, but this may be an artifact here, since there was more subjectivity in the scoring of effects in this period.

The proximo-distal sequence of specific sensitivities after stage 18 is consistent with the present knowledge of limb development, that is, more-proximal regions form first from growth at the distal end of the limb bud.[26,27] As the limb regions are laid down behind the growing tip, different morphogenetic processes become involved, leading to the enlargement of the mesenchymal model of the part and subsequent chondrification. The peaks in sensitivity shown might have been due either to the effect of irradiation on the growing tip or to subsequent processes, or both. In each sensitive period there were a variety of effects, but in the case of the toes particularly, three different effects showed a temporal sequence in frequency of occurrence as shown in the broken line curves. The early effect upon toes was the absence of one or more of them, the intermediate effect was the absence of phalanges but not whole digits, and the latest was the fusion of phalanges. The early effect was reminiscent of the response observed in the limb after irradiation at stage 14, and it may be inferred that similar processes were taking place at the two sites. The subsequent effects were then apparently caused by the disruption of morphogenetic processes in the early blastema and late blastema or precartilage phases of toe formation.

The sequence of sensitive periods observed is consistent with the morphological description of development, but the results of the irradiation experiments have provided a more precise knowledge of stages in development where critical events for the formation of parts are taking place. How X radiation produced the effects observed was not revealed in this investigation, but it is apparent that there was no direct interference with the organizing influence, since whenever any vestige of a structure was present it had recognizable characteristics of a limb component located in proper sequence.

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[26] A. Hampe, Compt. rend. 243, 970–73 (1956).

[27] J. W. Saunders, Jr., J. Exptl. Zool. 108, 363–404 (1948).

RADIATION PROTECTION — LIVING CELLS

Alexander Hollaender

G. E. Stapleton
H. I. Adler
M. S. Engel
A. M. McCarthy
A. K. Bruce[1]
J. H. Stuy[2]
A. S. Angel
N. N. Lanier[3]

Effect of Growth Conditions on X-Ray Sensitivity of *Escherichia coli*

G. E. Stapleton M. S. Engel

Introduction. — Hollaender *et al.*[4] showed several years ago that anaerobiosis, and presence of glucose in the culture medium, increased the radioresistance of *E. coli* B/r over broth-grown cells. This finding has been of considerable interest to theoretical radiobiologists[5,6] since the resistance is shown by a dramatic change in the shape of the survival curve rather than a change in the slope. This suggests a remarkable increase in the number of targets required for inactivation of the anaerobic or glucose-grown cells.

The simplest explanations for such a change in sensitivity are that clumping of cells occurs, or that multicellular forms result from such cultural conditions, or, less likely, that the cells are multinucleate. The purpose of this paper is to show that none of these most obvious suggestions explain the radioresistance of such cells.

Material and Methods. — The following strains of *E. coli* have been used: B, B/r, and the heminless H_7 strain. Cells were cultured in 1% peptone with varying concentrations of glucose and phosphate buffer. The cultures were incubated at 37°C either with aeration or under anaerobiosis

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[1] Research Participant.

[2] Visiting investigator from abroad.

[3] Biology Student Trainee.

[4] A. Hollaender, G. E. Stapleton, and F. L. Martin, Nature 167, 103 (1951).

[5] P. Howard-Flanders and D. Moore, Radiation Research 9, 422–37 (1958).

[6] T. Sargeant, UCRL-8513, p 61 (1958).

by continuous bubbling with 95% N_2–5% CO_2. Cultures grown under various conditions produced final viable or total cell titers ranging from 3×10^9 to 2×10^{10} cells per milliliter. The final pH of cultures under these various conditions ranged from 4.8 to 8.6. All data to be reported are on stationary-phase cultures.

Irradiations were carried out at room temperature on cell suspensions having about 2×10^7 cells per milliliter. This was accomplished by simply diluting a full-grown culture 100-fold or more into M/15 phosphate buffer at room temperature. The diluted suspensions were irradiated in glass-stoppered 2-ml volumetric flasks at 14 cm from the target of a General Electric Maxitron 250. Two millimeters of aluminum filters were interposed between the port and sample (hvl = 0.34 mm of copper). Six samples placed in a rotating ring-type Lucite holder could be uniformly irradiated at 4400 r/min, as determined by a Victoreen thimble chamber or chemical dosimetry, or both.

Irradiated suspensions and their irradiated controls were appropriately diluted in M/15 phosphate buffer and plated by surface spreading of 0.05 ml of the diluted suspension on solid medium composed of 1% peptone–0.1% glucose–M/15 phosphate buffer, 1.5% agar.

Results and Discussion. – The previous observation[4] of the phenomenon under consideration suggested that anaerobiosis plays a role in development of the resistance of *E. coli* B/r. More-recent experiments indicate that cells grown in aerated culture (peptone-glucose) with limiting buffering capacity are probably equally as resistant as are anaerobic cultures from the same medium. It appears that the final pH attained by the culture in stationary phase is better correlated with resistance than any other parameter investigated. Table 14 shows the percentage survival of *E. coli* B/r at a constant dose of 30 kr as a function of the final pH of the growth medium.

Similar data have been obtained for *E. coli* B and H_7. The several strains tested show a rather remarkable increase in resistance if they attain a final pH below neutrality.

The nature of the effect is clearly demonstrated in Fig. 8, which compares the most and least resistant cell populations (from cultures whose final pH's are 4.8 and 8.0, respectively).

Table 14. Survival of Stationary-Phase *E. coli* B/r at 30 kr as a Function of Final pH

Final pH of Culture	Surviving Fraction ($\times 10^{-3}$)
8.6	4.0
8.0	8.0
7.0	12.0
6.0	50.0
5.6	100.0
5.0	180.0

It is important that cultures grown at pH 8.0 show no variation in sensitivity if irradiated in buffers adjusted to the various hydrogen-ion concentrations. Also, no reduction in sensitivity is noted for cells grown at the high pH and incubated for 1–2 hr at the various pH's. The resistance is not an effect of pH *per se*, but seems to be a result of the metabolic activity during growth of the cells at the various pH's. Preliminary evidence has been obtained indicating that the resistance is not obtained by cultures until they proceed into stationary phase. It is tempting to postulate that cells grown under conditions of maximum resistance (<pH 5.0) produce a metabolic product that serves as a protective agent. We have to consider the possibility, on the other hand, that some radiosensitive system is replicated many times in resistant cells. Either possibility is consistent with the nature of the survival curves obtained.

Chemical analyses and macroscopic observation and cell counting yield no evidence that clumping, multicellularity, or multinucleate condition of cells might explain the resistance of these cells. Table 15 represents data from a typical experiment.

The cultures that have a final pH of 5 or below have a definite tendency to clump, and changes in ionic strength of suspending media will produce some clumping. Nevertheless, no clumping has been observed in fresh cultures in the medium in which they have grown, in cultures diluted in *M*/15 phosphate buffer, nor in diluted irradiated suspensions.

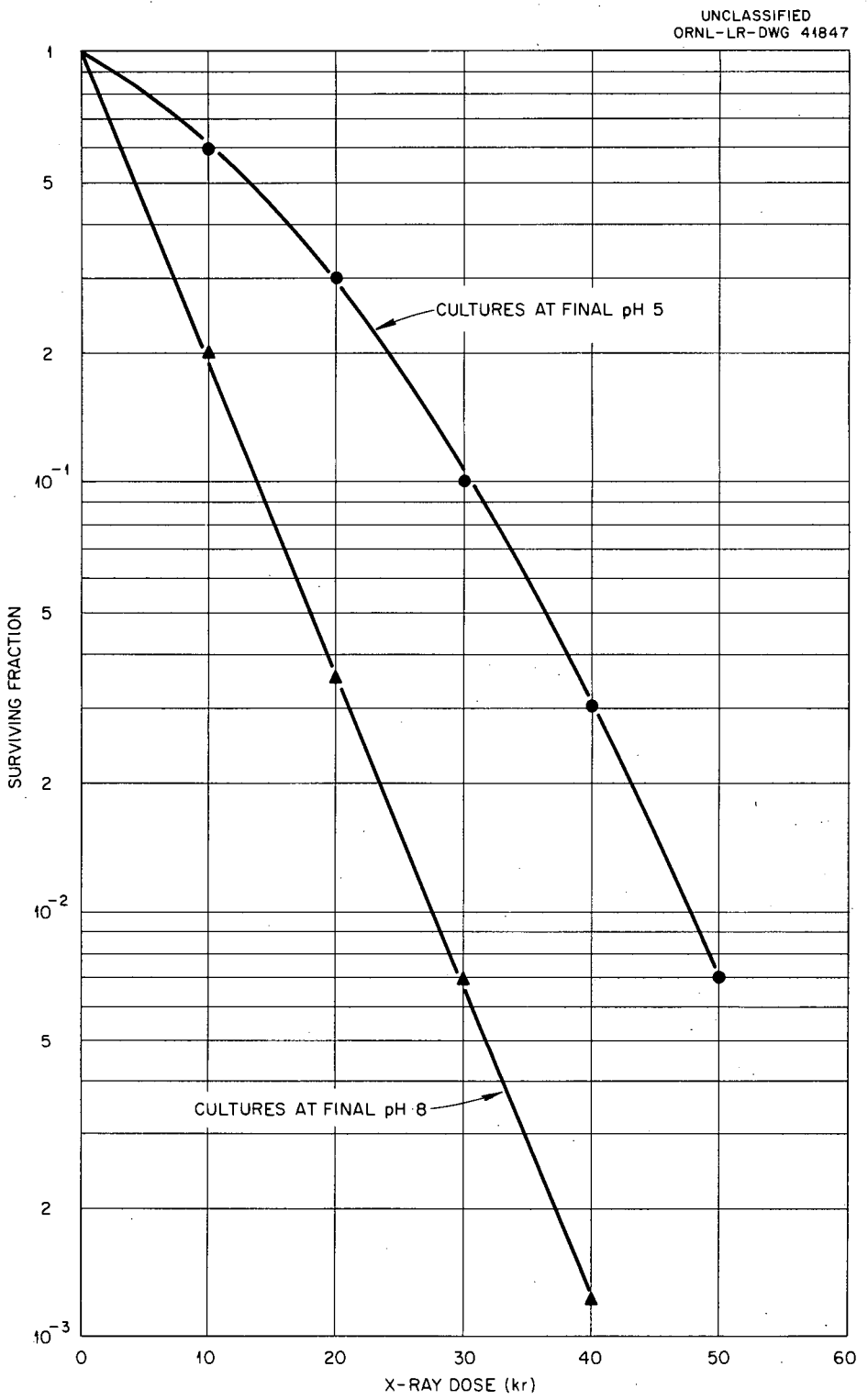

Fig. 8. Survival of E. coli B/r Grown at Various Hydrogen-Ion Concentrations.

Table 15. Comparison of Resistant and Sensitive Cells

	Resistant	Sensitive
Total cell count, cells $\times 10^9$ per ml	10.0	6.5
Viable cell count, cells $\times 10^9$ per ml	5.6	3.5
Percentage viable cells	56.0	54.0
Packed cell volume, μl per 10^9 cells	0.6	0.4
DNA, μg per 10^9 cells	1.4	1.5
RNA, μg per 10^9 cells	23.2	23.0

The resistant cells are larger cells, as indicated by the relative packed cell volumes and also by microscopic observation.

The nature of the resistance of such cells is currently being investigated on the basis of a "built-in" protective agent and from the point of view of an altered physiological state.

Physiology and Radiation Behaviors of a Hemin-Dependent Mutant of *Escherichia coli*

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

Several new observations on the physiology of this organism (H_7) have been made that may contribute to our understanding of its response to ionizing radiations.

Under growth conditions in which glucose is limiting, an increase in final titer may be measured at hemin levels below 0.05 μg/ml. Half-maximal growth is obtained in approximately 0.3 μg of hemin per milliliter. In view of the sensitivity of the response to hemin, several compounds known to be intermediates in hemin synthesis were tested for their ability to substitute for hemin. δ-Aminolevulinic acid, porphobilinogen, and protoporphyrin were without any effect. These results suggest that the genetic block may be at a locus controlling the mechanism concerned with the insertion of iron into the protoporphyrin ring.

The presence of cytochromes in H_7 cells grown with hemin has previously been inferred from circumstantial evidence. Several attempts to find cytochromes by the use of spectrophotometric techniques available in our laboratory have been unsuccessful. These attempts have involved the use of whole cells, cell extracts, and partially purified preparations. Through the cooperation of Dr. Britton Chance of the University of Pennsylvania, we were able to make use of very sensitive spectrophotometric equipment available at the Eldridge Reeves Johnson Foundation of the University of Pennsylvania. These experiments have established that H_7 cells grown with hemin do have a cytochrome spectrum typical for the genus Escherichia. Prominent absorption peaks are at 528, 556.5, 557, and 625.6 mμ. This knowledge is particularly valuable for a study of photoreactivation in H_7 after ultraviolet light being carried out with J. Jagger of the Biophysics group.

Further experiments on the X-ray-induced sensitization to hydrogen peroxide described previously[7] have been performed. One possible explanation for the increased sensitivity to H_2O_2 observed after X irradiation of catalase-negative cells is that radiation increases the permeability of the cells to H_2O_2. To test this possibility, H_7 was grown with hemin and the catalase-containing cells were assayed for their rate of reaction with hydrogen peroxide. A small, but significant, permeability barrier was detected by treating the cells with toluene, a procedure known to destroy cell membranes. However, X-radiation doses four times those routinely used for studying the sensitization phenomenon did not influence permeability significantly. We have concluded that increase in permeability is probably not the mechanism by which X radiation causes increased sensitivity to H_2O_2. Currently, we are exploring the possibility that sensitization may be an alteration in nucleoprotein components of the cell. Preliminary experiments done by irradiating with 2637-Å radiation indicate that this wavelength is effective in causing sensitization to hydrogen peroxide.

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

Sensitivity of *Haemophilus influenzae* to X Rays

J. H. Stuy

In an earlier study,[8] the effects of ultraviolet radiation on growing cells of *Haemophilus influenzae* were reported. The purpose of the present work is to perform similar investigations with X radiation to obtain answers to the following questions: (1) Do X rays damage DNA *in vivo*? (2) Are irradiated cells able to repair such damage? (3) Is the DNA formed by irradiated cells normal DNA?

Logarithmic-phase cells of *H. influenzae* strain Rd 4(3M) were exposed to 250-kvp X rays in broth at ice-bath temperature. The dose rate was about 4.8 kr/min. The following immediate, dose-dependent effects were found: (1) The cells showed exponential inactivation; (2) the 99% inactivation dose was about 24 kr; (3) a dose of 48 kr degraded about 10% of the DNA, as indicated by a decrease in the fraction of DNA precipitable by 1 *N* perchloric acid; and (4) the activity of three markers was reduced exponentially, about 30% reduced by a dose of 48 kr.

Incubation of the irradiated cultures showed the following dose-dependent phenomena: (1) Growth, as measured turbidimetrically, was inhibited and was essentially halted after about 60 min. (2) RNA synthesis was inhibited, and ceased after about 60 min. (3) DNA was rapidly degraded for about 20 min, after which DNA formation resumed. The X-ray dose required to inactivate 99% of the cells reduced the DNA content per milliliter of cell suspension to about 30%. Resumption of DNA synthesis ceased after 60–90 min, at which time the DNA had increased by a factor of 2. (4) The curve relating the activity of the three markers to time of incubation has the same shape as the curve for DNA. In fact, they are identical after about 10 min of incubation, indicating that the DNA synthesized is normal. (5) The survivors of this X-ray case showed no lag in cell division.

Similar experiments performed with other bacteria showed the same effects. Breakdown of DNA was observed in all cases but to a lesser extent than that found in *H. influenzae*. Of interest are the results of

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[8] J. H. Stuy, J. Bacteriol. 78, 49 (1959).

experiments with E. coli, strains B and B/r. The postirradiation growth phenomena were identical for both strains at the same dose of X rays. The survival was much less for B than for B/r at this dose.

It is tentatively concluded from these observations that DNA breakdown as measured in log-phase cells is not the primary cause of cell inactivation but may be a secondary effect.

The in vivo DNA is resistant to X rays as measured by transforming activity; and upon postirradiation incubation, therefore, intracellular repair of damage inflicted on the DNA is difficult to study.

Studies on a Radiation-Resistant Bacterium

A. K. Bruce

Introduction. — The response of the highly resistant pigmented bacterium, Micrococcus rubens, which grows in tetrads, was studied in order to characterize factors contributing to its remarkable radioresistance. How such an organism is able to withstand massive amounts of radiation is of obvious significance to our understanding of fundamental radiobiology. One interesting possibility is that this organism produces its own protective compound, which confers upon it the observed radioresistance. The identification of such a naturally produced material, if it exists, and its relation to known protective agents is the purpose of this investigation.

A culture of the resistant organism, designated U_1, was obtained from W. D. Bellamy (General Electric Research Laboratory). It was cultured at 25°C in a medium containing 1% yeast extract, 1% hydrolyzed casein, 0.1% glucose, 0.5% K_2HPO_4, and distilled water, as was used by Kilburn et al.,[9] in flasks shaken for good aeration. Plate assays of viability were carried out in the same medium containing 2% agar incubated at 37°C for two days.

This organism shows a long lag phase with a logarithmic doubling time of approximately 3 hr, reaching a maximum titer of about 5×10^8 cells/ml after one day, with viability dropping off rapidly thereafter.

[9] R. E. Kilburn, W. D. Bellamy, and S. A. Terni, Radiation Research 9, 207–15 (1958).

Cells were usually taken from late log phase, as determined turbidimetrically. They were centrifuged and diluted to a concentration of 5×10^7 cells/ml in 1% sodium chloride, and X-irradiated in a closed tube through which either air or nitrogen was bubbled. The X-radiation source was a General Electric Maxitron 250 operated at 250 kvp and 30 ma (details elsewhere) at a dose rate of about 10,000 r/min. Ultraviolet irradiation was carried out with a G-E germicidal lamp as a source of 2537-Å radiations. Here the cell concentration was adjusted to about 5×10^6 per milliliter in M/15 phosphate buffer. After exposure to radiation, the cells were immediately diluted with M/15 phosphate buffer and spread on plates.

Results and Discussion. – Results obtained from X irradiation are in general agreement with those reported by Kilburn *et al.*,[9] being highly sigmoidal and with a sensitivity of approximately the same. The LD_{50} was about 400,000 r under aerobic conditions and 500,000 r anaerobically. Cells taken from early log phase had a greater sensitivity.

The protective action of cysteamine was also tested in this organism. A test of toxicity showed that this bacterium can withstand a 5% solution (650 μmoles/ml) with no loss of viability. Irradiation was carried out at 325 μmoles/ml of cysteamine with the sample bubbled with nitrogen to prevent oxidation of the compound. Survival remains at 100% to 1,000,000 r, dropping to 50% at 1,400,000 r. This amounts to three times the dose required under nitrogen for the same effect on survival.

The ultraviolet resistance was also great, as reported by Anderson *et al.*[10] A highly sigmoid curve was found, as is also for *E. coli* B/r, but exhibits a resistance 20 times as great.

Mutagenicity of Sublethal X-Ray Doses in *Aspergillus terreus*

Alexander Hollaender A. M. McCarthy A. W. Kimball

Saline suspensions of *Aspergillus terreus* spores do not show logarithmic inactivation with 250-kvp X rays. The curve relating surviving fraction to dose is S-shaped. The present study was undertaken in an

[10] A. W. Anderson *et al.*, Bacteriol. Proc., p 20 (1959).

attempt to answer the following questions:

1. Does the survival curve show a true threshold and, if so, can this threshold be extended by irradiating in the presence of cysteamine?
2. Does a threshold exist for mutation production or do mutations increase in a linear way with dose?
3. If mutations are produced in the absence of killing, is it possible to reduce their number by the use of cysteamine?

Results and Discussion. — The spores were subjected to X-ray doses that varied from 1 to 40 kr. Cysteamine, when used, was at a 0.12 M concentration.

A χ^2 method described by Cochran[11] was used to test for the X-ray dose at which killing begins. The results of these tests are shown in Table 16. From the table, conservative estimates indicate that when

[11] W. G. Cochran, Biometrics 10, 417–51 (1954).

Table 16. Significance Levels (%) for the χ^2 Test to Detect the Dose at Which Killing Begins

X-Ray Dose (kr)	Cysteamine Absent					Cysteamine Present				
	Experiment No.					Experiment No.				
	1	2	3	4	5	1	2	3	4	5
1	>99	>99	>99	>99	-	>99	>99	>99	>99	-
3	>99	-	-	>99	-	>99	-	-	>99	-
4	-	>99	>99	-	-	-	>99	>99	-	-
5	7	-	-	>99	-	>99	-	-	96	-
7	>99	>99	>99	>99	-	>99	>99	>99	>99	-
9	>99	-	-	9.7	-	>99	-	-	>99	-
10	-	>99	<0.1	-	-	-	>99	>99	-	-
11	83	-	-	8.5	>99	92	-	-	>99	-
14	1	-	<0.1	-	>99	>99	-	76	-	-
15	-	0.7	-	-	-	-	>99	-	-	9
19	<0.1	-	<0.1	-	-	>99	-	1	-	27
20	-	<0.1	-	-	-	-	3.6	-	-	-
23	-	-	-	-	-	-	-	-	-	<1
26	<0.1	-	<0.1	-	-	<0.1	-	<0.1	-	-
32	<0.1	-	<0.1	-	-	<0.1	-	<0.1	-	-
40	<0.1	-	<0.1	-	-	<0.1	-	<0.1	-	-

cysteamine is absent, killing starts at about 9–10 kr, and when cysteamine is present it starts at about 15–20 kr. Thus a true threshold exists in the survival curve, and this may be extended by the addition of cysteamine.

In contrast, a threshold does not exist for mutation production, and mutations were shown to increase in a linear way as a function of X-ray dose.

In order to answer the third question, straight lines were fitted to the mutation data by the method of weighted least squares. The slopes for the treated and untreated cultures were then tested for the significance of their difference separately for each of four experiments. Finally, the four independent tests were combined into one test by the method described by Fisher.[12] The results shown in Table 17 indicate that, even in the absence of killing, cysteamine causes a significant reduction in mutation rate of the order of 30–60%.

[12] R. A. Fisher, Statistical Methods for Research Workers, 10th ed., p 99–101, Oliver and Boyd, 1946.

Table 17. Comparison of Mutation Rates for Treated and Untreated Cultures

Experiment No.	Mutation Rate (% per kr)		Significance Level for Difference (%)	Reduction (%)
	Cysteamine Absent	Cysteamine Present		
1	0.40145	0.20818	3.2	48
2	0.59199	0.34373	1.0	42
3	0.33518	0.24283	6.0	28
4	0.61853	0.25949	0.08	58

Combined Test of Significance

$$\chi^2 = 40.588$$

$$\text{d.f.} = 8$$

$$P < 0.01\%$$

MAMMALIAN RECOVERY

M. A. Bender
R. R. Bigelow[1]
C. C. Congdon
P. G. Delker
D. B. Duda
L. E. Fox[1]
P. C. Gooch
J. W. Goodman
A. P. Henley
K. S. Leppert
J. Maisin[2]
R. A. McGrath
T. T. Phan[2]
S. Rogers[1]
L. H. Smith
D. C. Swartzendruber
P. Urso
O. Vos[2]
S. E. Wagner[3]

Chromosome Studies on the Callithricidae and *Callimico*

M. A. Bender

Introduction. – The infra-order Platyrrhina is generally agreed to consist of two large families, the Cebidae and the Callithricidae or marmosets, plus the anomalous genus *Callimico*, which various authors have placed in both families. In a previous study,[4] the karyotypes of four genera of Cebidae were determined. Not only did all four have different chromosome numbers, but there was a correlation between reduced chromosome number and degree of specialization. There was also a correlation between chromosome number and the number of telocentric or acrocentric chromosomes in the karyotype, which suggests centric fusion. The present study considers two genera of Callithricidae and the genus *Callimico*, in an attempt to further elucidate chromosome evolution in the order Primates.

Materials, Methods, and Results. – Eight animals were investigated; three *Callithrix chrysoleucos*, four *Leontocebus illigeri*, and one *Callimico goeldii*.[5] Chromosome counts and karyotypes were made from slides prepared from tissue cultures of kidney and spleen. Karyotypes derived from such material are exactly the same as those found in somatic cells *in vivo*.[4,6]

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[1]Consultant.

[2]Visiting investigator from abroad.

[3]Biology Student Trainee.

[4]M. A. Bender and L. E. Mettler, Science *128*, 186–90 (1958).

[5]The purchase of the specimens and the culture work were carried out under grants from the National Science Foundation (G-1760 and G-3272).

[6]M. A. Bender, in *Symposium on the Immediate and Low-Level Effects of Ionizing Radiation*, Venice, June 1959, in press.

The chromosome counts and karyotype analyses obtained are listed in Table 18. The karyotypes of Callithrix and Leontocebus are identical, while that of Callimico is quite different, resembling in fact some of the previously obtained cebid karyotypes more closely than that of the marmosets studied.

Table 18. Somatic Chromosome Numbers and Classifications of Two Genera of Marmosets and of Callimico

M, metacentric; S, subterminal; T, telocentric or acrocentric

Species	Common Name	Sex	Chromosomes					
			2N	Autosomes			X	Y
				M	S	T		
Callithrix chrysoleucos	Golden or silky marmoset	♂	46	4	30	10	S	M
		♀	46	4	30	10	S	
		♀	46	4	30	10	S	
Leontocebus illigeri	Red-mantled tamarin	♂	46	4	30	10	S	M
		♂	46	4	30	10	S	M
		♀	46	4	30	10	S	
		♀	46	4	30	10	S	
Callimico goeldii	Goeldi's tamarin	♀	48	2	30	16		

Discussion. — The fact that both Leontocebus illigeri and Callithrix chrysoleucos have a diploid chromosome number of 46 suggests that these genera may be closely related. Failure by even a careful inspection of their karyotypes to find any difference between them strengthens this conclusion. Even though the chromosome numbers differ only by 2, the marked difference in karyotype between Callimico and the marmosets suggests that the former is not so closely related to the marmosets as has been previously supposed. Of the genera of Cebidae examined previously, the Callimico karyotype most closely resembles that of Callicebus. Comparison of all the Platyrrhina examined to date suggests that Callimico should be included in the Cebidae, rather than the Callithricidae or in a separate family Callimiconidae, as has sometimes been done. The additional material presented

here does not contradict the hypothesis that chromosome evolution in the Platyrrhina has followed a pattern of chromosome number reduction with increasing specialization, and that this reduction has been accomplished, at least in part, by the mechanism of centric fusion.

Prevention of Bone Marrow Heterografting in Irradiated Mice by Isologous Thymus

C. C. Congdon D. B. Duda

Introduction. — Van Bekkum and Vos[7] demonstrated that isologous thymus cells would prevent the therapeutic action of rat bone marrow in lethally irradiated mice. We have confirmed this finding and, in addition, have made serial histologic studies on mice killed at daily intervals after 950 r plus 108×10^6 rat marrow cells and 107×10^6 isologous thymus cells intravenously.

Results. — Transplantation of rat bone marrow was well established in the animal examined on day 4. By day 7 it had disappeared, and the bone marrow remained aplastic from then on. No special changes were seen in the thymus except those caused by irradiation. From the animal examined on day 3 to the last animal examined on day 10, unusual cellular changes took place in the white pulp of the spleen and lymph node cortex that resulted from the injection of isologous thymus cells. Many new cells resembling antibody-forming elements appeared, and collections of regenerating germinal-center cells were present as early as day 5.

Discussion. — Many other isologous tissues have been tested for their ability to prevent bone marrow heterografting. In large amounts nearly any tissue except muscle was effective. In small amounts lymph nodes, spleen, thymus, peripheral blood, and bone marrow were most effective. Moderate doses of isologous liver prevented bone marrow heterografting and in addition caused some stimulation of blood-forming cell production in the spleen. This interesting finding was then further studied and some survival of lethally irradiated mice was obtained by injecting adult isologous liver cells alone (see next report, by P. G. Delker).

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[7]D. W. van Bekkum and O. Vos, J. Cellular Comp. Physiol. 50, suppl. 1, 139–56 (1957).

Treatment of Lethal Irradiation Injury by Adult Isologous Liver Cells

P. G. Delker

Introduction. – Preliminary studies by Congdon and Duda (see preceding report) showed histologic evidence of blood cell formation in lethally irradiated mice (950 r) given massive doses of adult isologous liver cells. In a series of experiments, mice were exposed to 950 r and given 100 mg of adult isologous liver cells intravenously on days 0, 1, and 2 after irradiation. Mortality was recorded.

Results. – Preparing large amounts of a satisfactory liver cell suspension was difficult. Table 19 shows the data from several experiments. Mortality patterns were quite erratic, but some mice have lived after 950 r with this treatment. In addition there is some histologic evidence to indicate that adult liver cells are having an effect.

Table 19. Effect of Adult Liver on Radiation Mortality

Treatment	Number Dead/Number Treated on		
	Day 14	Day 30	Day 60
950 r + isologous liver	15/23	19/23	22/23
950 r + isologous liver	3/10	4/10	
950 r + isologous liver	20/20		
950 r only	20/20		
950 r + bone marrow and isologous liver	2/9	2/9	

Discussion. – The hematopoietic organs have been considered the only tissues capable of causing recovery from the acute radiation syndrome. Adult liver was reported to be ineffective. Formerly, large doses of liver could not be successfully administered intravenously. The techniques of liver cell preparation and intravenous injection, developed by D. B. Duda, have made the present experiments feasible.

Jacobson *et al.*[8] obtained a slight effect on survival of lethally irradiated mice by shielding the liver during exposure; and Lorenz and

[8] L. O. Jacobson *et al.*, J. Lab. Clin. Med. *37*, 683–97 (1951).

Congdon[9] found that an intravenous injection of a liver reticulum cell sarcoma into irradiated mice promoted some survival. It is of interest to reconsider these observations in view of the results of the present experiments.

Growth of Hematopoietic Cells in Tissue Culture

L. E. Fox

Introduction. — Studies on survival and growth of suspensions of hematopoietic cells in tissue culture were made with different types of tissue culture flasks and several media.

Methods and Results. — Suspensions of cells multiplied and matured more rapidly in spinner flasks than in vaccine vials or petri dishes. Cells grew better when supplied with 5% CO_2 in air than with 5% CO_2 in pure O_2. Cells survived better in Special Mouse B.S.S. than in Hank's B.S.S. as tested by the eosin absorption test, and better in mixture 199 plus 10% horse serum than in Eagle's basal medium. Beef embryo extract, ultrafiltrate of chick embryo extract, and chick embryo extract proved toxic to the cells. Additions of N.C.T.C. 109 to mixture 199 were not toxic but did not support maturation or multiplication of cells. Addition of ultrafiltrate of beef embryo extract promoted limited multiplication of cells and maturation. The best medium found to date consists of 63.4% mixture 199, 8.5% Special Mouse B.S.S., 7.0% horse serum, and 21.1% ultrafiltrate of beef embryo extract.

Multiplication of bone marrow cells in this medium is limited, and microscopic examination revealed alterations in the matured cells stained with Wright-Giemsa. Immature cells and those of the granulocytic and erythrocytic series may be identified, however.

Since it was thought that hematopoietic cells from fetal liver might grow better than femoral bone marrow cells, the livers of a group of 18-day-old fetuses of strain 101 were cultured in the above medium, and also the livers of the newborn (less than one day old). These hematopoietic

[9]E. Lorenz and C. C. Congdon, p 192–211 in Proc. 4th Intern. Congr. Intern. Soc. Hematol., Grune and Stratton, New York, 1954.

cells multiplied freely in culture. The cells of the 18-day-old fetal livers multiplied more rapidly, as determined by cell counts of the cultures, than those from newborn liver or from adult bone marrow. After two weeks in culture, hematopoietic cells of both mature and immature forms were identified. Fetal liver cultures seem to have a larger percentage of immature hematopoietic cells than bone marrow cultures. After five weeks, only immature hematopoietic cells were identified. Many altered cells were present.

Bone marrow cells from mice injected with estrogens mature more rapidly. Addition of thyroid, testosterone, and isologous embryo extract to the medium had a similar effect.

Life Span of Rat and Mouse Erythrocytes in the Rat-Mouse Chimera

J. W. Goodman L. H. Smith

Introduction. — The study of erythrocyte life span in chimeras has continued[10] to determine whether immunologically competent cells in the chimera originate from the donor, the host, or a combination of both. Erythrocyte life span studies might be expected to throw light on whether this secondary reaction, insofar as it is reflected in red cell survival, is of host or of donor origin.

Results and Discussion. — Nine (C57BL × 101)F_1 female mice that survived 60 days after 900 r of X rays (LD_{100}/20 days) and an injection of Sprague-Dawley rat bone marrow (55×10^6 nucleated cells per mouse) on day zero, were transfused intravenously with 0.2 ml of a 45% suspension of Cr^{51}-labeled red blood cells in saline. Four mice were given labeled isologous erythrocytes and five were given labeled rat erythrocytes; blood was pooled from normal donors for each group. The Cr^{51} activity in circulating blood measured at 24 hr was taken as 100%, and measurements were made at intervals for seven weeks. Twenty-five days after the transfusion of labeled cells, these animals had essentially 100% rat erythrocytes by the mannitol test.[11]

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[10] J. W. Goodman, Biol. Semiann. Prog. Rep. Feb. 15, 1959, ORNL-2702.

[11] W. St. Amand and L. H. Smith, J. Appl. Physiol. 14, 445–47 (1959).

The level of Cr^{51} activity is plotted against time in Fig. 9, and it can be seen that rat cells disappeared more rapidly in the chimera than did mouse cells. The latter followed a normal life span curve for this strain. The curve for rat erythrocytes is intermediate between those of Smith and Tohá[12] for rat cells in the normal rat (maximum life span: 60 days) and that for rat cells in the normal mouse (maximum life span: 4 days). It was found by Smith and Tohá[12] that rat cells from a rat-mouse chimera survived in normal recipients like cells from a normal rat.

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[12]L. H. Smith and J. Tohá, Proc. Soc. Exptl. Biol. Med. 98, 125–28 (1958).

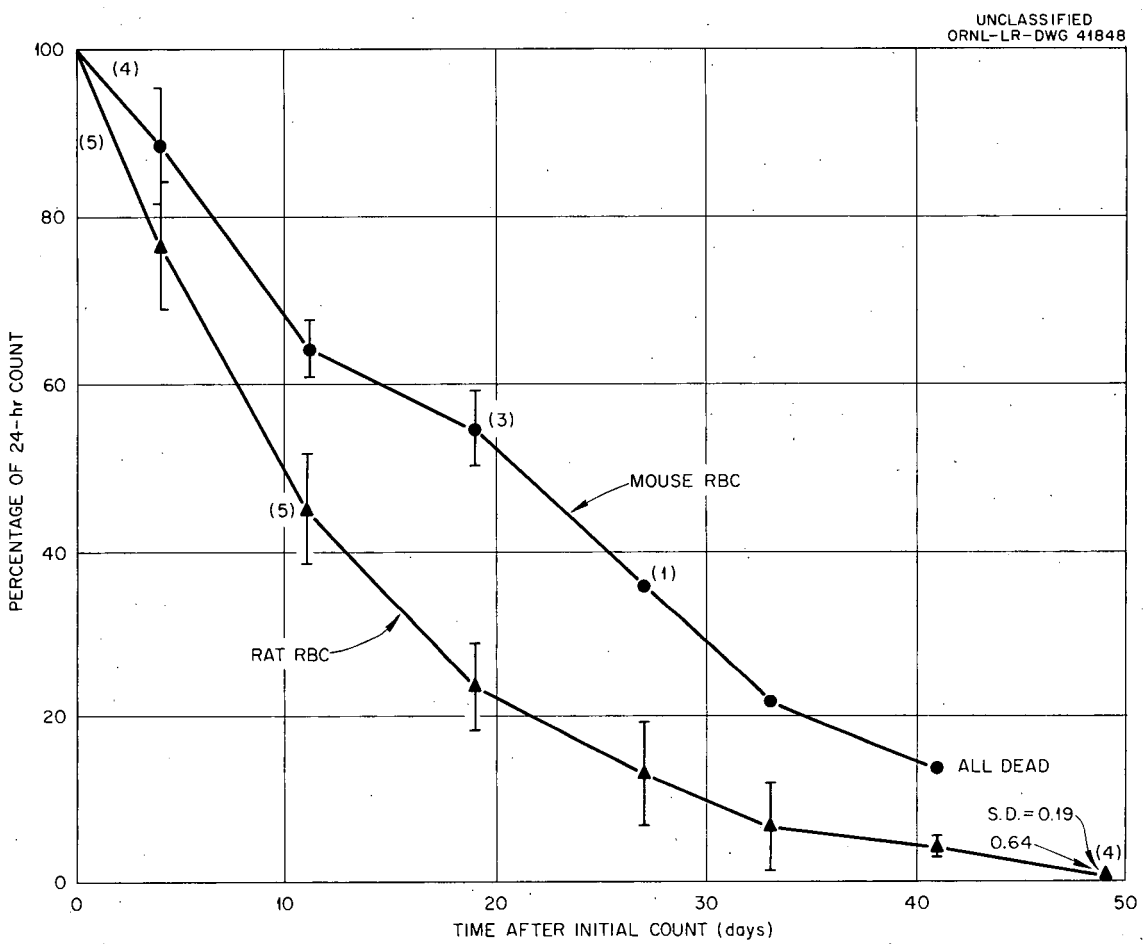

Fig. 9. Disappearance of Cr^{51} Activity from the Circulating Blood of Rat-Mouse Chimeras Transfused on Day Zero Minus One with Either Mouse or Rat Labeled Erythrocytes. Number of surviving animals in each group is shown in parentheses at three time points on the curves. Standard deviations are indicated by vertical bars at each point plotted.

It is clear from these preliminary data that the long-term heterologous chimera, although 100% of its circulating erythrocytes are of rat origin, provides an imperfect environment for rat red blood cells. On the other hand, the presence of active rat hematopoietic tissues in such a host does not influence the survival of transfused mouse erythrocytes. This study therefore provides no evidence of an active rat immune system.

AET Protection Against Radiation Damage to the Small Intestine of Mice

J. Maisin G. D. Novelli[13]

Introduction. — The effect of AET (8.8 mg intraperitoneally and 16.0 mg orally) protection in the small intestine of A-1 mice exposed to total-body irradiation was determined by measuring mitotic index, synthesis of DNA, RNA, and protein, and potassium and sodium levels.

Results and Discussion. — In mice given AET and exposed to 2000 r, the histologic recovery and mitotic index of the intestine were essentially the same as in unprotected animals exposed to 1100 r. With exposures as high as 2500 r of X rays in AET-protected mice, we found that an upper limit of protection was being reached. Doubling the dose of AET (oral administration) did not give twice the protection to the intestine. At relatively low exposures (225 r), it is difficult to show a large effect of AET protection on mitotic inhibition. This finding, together with other data, indicates that there is an optimal radiation dose for maximal chemical protection of the mitotic activity in the intestine.

The results of measuring DNA and RNA synthesis by P^{32} incorporation after 1500 r showed a marked increase of DNA in the protected group on day 3 and an increase of RNA on day 4. Protein synthesis, measured by incorporation of C^{14}-glycine, showed a marked increase on day 4 in the protected mice. In the nonprotected mice these increases were not seen.

After 1500 r there was an increase in the mitotic index on day 4 and in the intestinal weight on day 5. We attribute the overshoot of DNA, RNA, and protein synthesis to the synchronization of the cell population during recovery.

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[13]Enzymology Group.

These findings, together with the evidence that at maximal DNA depression there is an abortive rise in the mitotic count, suggest separate mechanisms of radiation damage to DNA synthesis and mitotic activity.

Potassium and sodium levels in the intestine of 1500-r AET-protected mice remained near normal, but nonprotected mice showed a great loss of potassium and a great rise of sodium (Fig. 10).

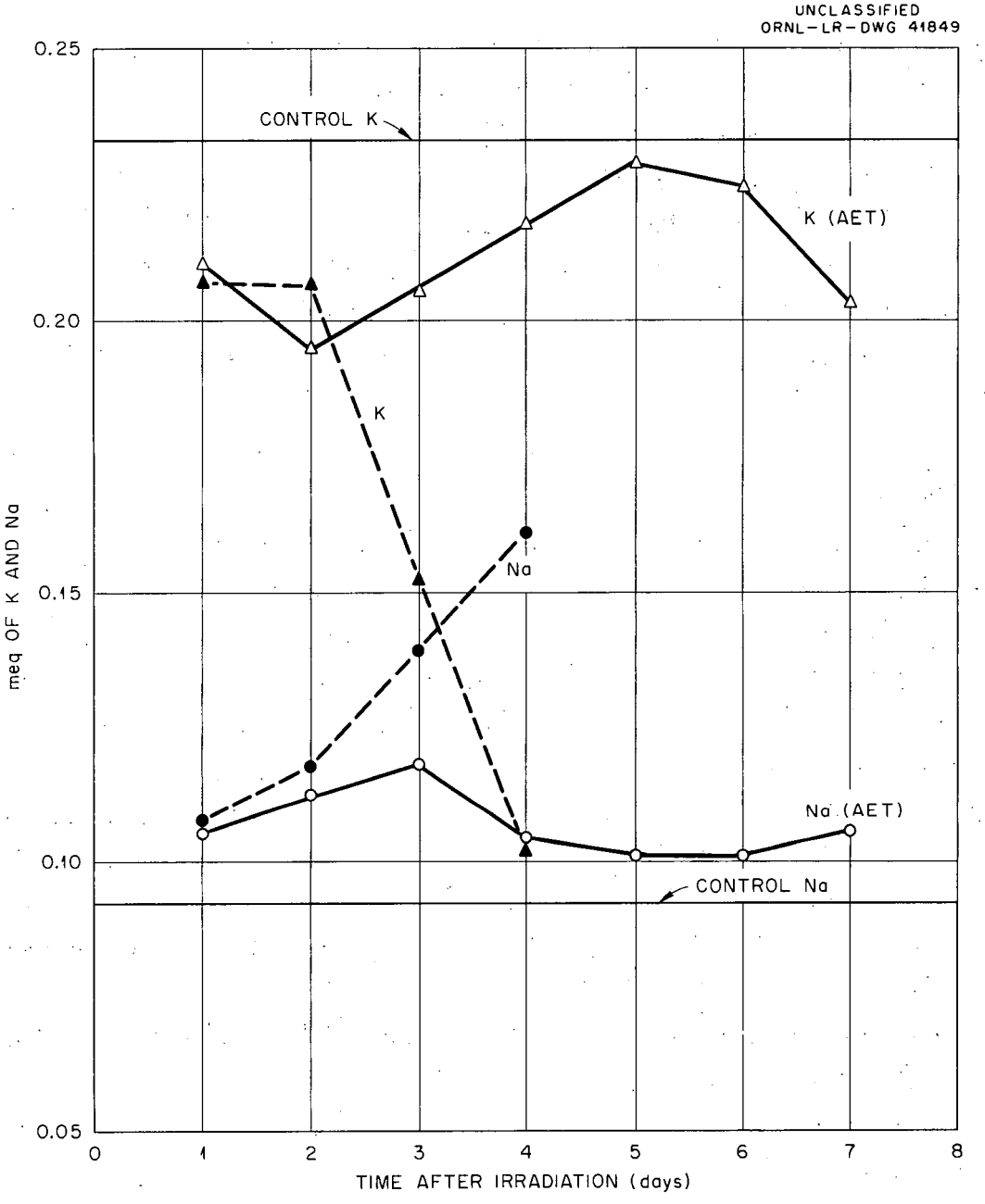

Fig. 10. Milliequivalents of K and Na per 350 mg (Wet Weight) of Small Intestine of Normal and X-Irradiated (1500 r) A-1 Mice.

Conclusion. – These results show that intraperitoneally administered AET affords excellent protection to the small intestine of X-irradiated mice.

Cell Enlargement and Migration in the Small Intestine of Irradiated Mice

R. A. McGrath

Introduction. – Abnormal hypertrophy of intestinal crypt cells after 2500 r of X rays to the mouse has been reported by McGrath and Congdon.[14] The present study was undertaken to (1) determine the relation of dose response to enlargement, (2) follow cell migration by a population analysis, and (3) determine whether incorporation of tritium-labeled thymidine was different between enlarged nuclei and unirradiated control nuclei.

Methods. – Approximately equal groups of $(C57L \times A)F_1$ mice were exposed to different total-body X-ray doses of 500–10,000 r. Fixed and stained slides and autoradiographs of duodenal cross sections were studied.

Results. – Results of the study of the dose-response relation (Fig. 11) indicate that enlargement is greatest 48–60 hr after all doses and is a function of X-ray dose, being greater with increasing dose. A cell population analysis shows that cell loss from the crypt region by death and by migration onto the villus is accompanied by abnormal crypt cell enlargement and probably migration of the villus core stroma into the crypt region. Experiments with tritiated thymidine suggest that total accumulation of labeled compound is not increased in abnormally enlarged cells.

Greatest enlargement 48–60 hr after all doses is interpreted as the cessation of growth that results from migration of the enlarging cells into an environment that does not support growth.

Abnormal crypt cell enlargement is characterized by a dose-dependent increase in enlargement rate during the first 48–60 hr after the X-ray dose levels studied. Whether enlargement is virtually "unbalanced growth" cannot be determined from the results of this study.

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

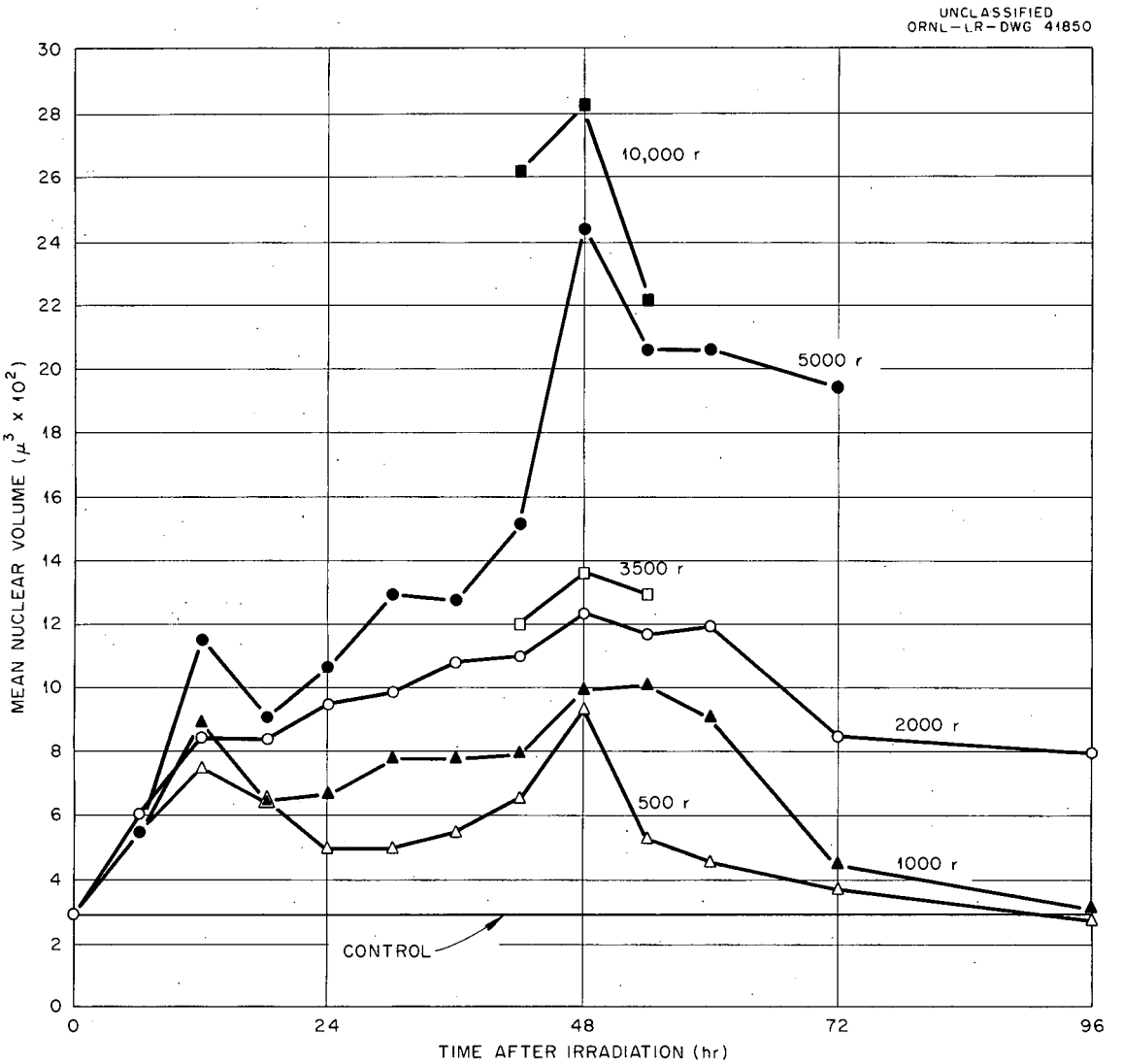

Fig. 11. The Relation Between Mean Nuclear Volume and Time After Irradiation.

The Effect of Additives on the Ability of Bone Marrow Frozen to -196°C to Promote Survival of Lethally Irradiated Mice

T. T. Phan M. A. Bender

Introduction. — It has been known for some time that spermatozoa could be frozen in glycerin mixtures and stored at low temperatures for long periods without losing their ability to fertilize ova after thawing. Barnes and Loutit[15] froze infant spleens in glycerin and showed that they still protected lethally irradiated mice after 83 days storage at -79°C.

[15]D. W. H. Barnes and J. F. Loutit, J. Natl. Cancer Inst. 15, 901 (1955).

Schwartz et al.[16] and Ferrebee et al.[17] have shown that mouse bone marrow is protective after freezing in 15% glycerin and storage at -79°C for eight days and six weeks, respectively. Preservation of bone marrow for infusion after irradiation is of obvious clinical value and would be even more valuable if an additive better than glycerin could be found. The lethally irradiated mouse—mouse bone marrow system constitutes an excellent method for measuring the viability of mammalian cells after freezing, and therefore a valuable tool for the investigation of the freezing phenomenon itself, as well as for the search for better additives.

Methods. — The effectiveness of freezing techniques was assayed by determining the 30-day survival of groups of (C57BL × 101)F_1 mice given 900 r plus the material being tested. Bone marrow suspensions were made in the solution to be tested and slow-frozen in a device that lowers the temperature at the rate of 1°C per minute down to -20°C, and then more rapidly to -79°C. The frozen material was then placed in liquid nitrogen (-196°C) for 0.5—2 hr. All samples were fast-thawed by placing them directly into a 37°C water bath. Thawed suspensions were counted, diluted to 4×10^6 cells/ml with the additive solution, and diluted further to 2×10^6 cells/ml with Tyrode's solution. One-half ml (10^6 cells) was then injected into the tail vein of each irradiated animal. All additive solutions were made up in Tyrode's solution. Thus the material injected always had one-half the concentration of additive in which the cells had been frozen.

Results and Discussion. — Results of experiments with various additives are summarized in Table 20. Figures for control groups represent the sum of individual controls for all experiments. Only figures for the optimum concentration of each compound tested are shown in the table. Additional experiments show that most of the compounds are effective at other concentrations; many, like glycerin and sorbitol, are effective over a wide range of concentrations. In many cases the additive solutions were not viscous. In these solutions cell clumping became a problem. Some experiments were therefore performed in the presence of isologous mouse serum

[16] I. R. Schwartz et al., J. Appl. Physiol. 11, 22 (1957).

[17] J. W. Ferrebee et al., Blood 12, 1096 (1957).

or polyvinylpyrrolidone, which have no protective effect by themselves. It was noted, however, that in experiments where mouse serum was used the 30-day survival was consistently lower than in experiments with the same compound alone or with polyvinylpyrrolidone. Although the 30-day survival

Table 20. 30-Day Survival of Lethally Irradiated (C57BL × 101)F_1 Mice Given 10^6 Isologous Bone Marrow Cells That Had Been Frozen in Various Additives

	Bone Marrow	Additive	Total Number of Mice	30-Day Survival (%)	Number of Experiments
Controls	None	None	403	0.3*	31
	Fresh	None	381	75.1	31
	Fresh	15% glycerin	23	52.2	2
Negative	Frozen	None	21	0.0	2
	Frozen	100% isologous mouse serum	7	0.0	1
	Frozen	7% PVP	22	0.0	2
	Frozen	0.3 M methanol	16	0.0	2
	Frozen	0.5 and 1 M glucose	20	0.0	2
Positive	Frozen	7% dextran	21	23.8	2
	Frozen	15% bovine serum albumin	21	19.0	2
	Frozen	0.3 M ethylene glycol	9	44.5	1
	Frozen	15% glycerin	49	87.8	5
	Frozen	0.3 M i-erythritol	29	41.4	3
	Frozen	0.4 M ribitol	21	42.8	2
	Frozen	0.7 M mannitol	10	80.0	1
	Frozen	0.2–0.8 M sorbitol	60	73.4	6
	Frozen	0.1 M inositol	30	60.0	3
	Frozen	0.2 M sucrose	10	70.0	1
	Frozen	0.5 M lactose	10	70.0	1
	Frozen	0.05 M choline Cl	20	75.0	2

*One animal.

was consistently higher when marrow frozen in 15% glycerin was used than it was for the control animals that received fresh marrow in Tyrode's solution, the control animals that received fresh bone marrow suspended in 15% glycerin showed a considerably poorer survival.

Table 20 shows that a great number of compounds may be successfully used as additives. One common objection to glycerin is its mild toxicity. Some of the other compounds are not toxic, however, and could be used for clinical procedures, especially since they are effective at very low concentrations. One commonly held theory states that mechanical injury by ice crystal growth is the major cause of cell death during freezing and this may be prevented only by vitrification. Our experiments make this theory untenable, since many of the solutions listed in Table 20 will not vitrify.

Intermediate Steps in Antibody Production

O. Vos

Introduction. – In studies on antibody production usually the end result of the process, that is, the amount or concentration of circulating antibodies, is investigated. Little is known about the steps that lie between the first contact of the tissue with the antigen and the ultimate production of antibodies. It is not known for instance whether the cells that phagocytize the antigen (or their descendents) produce the antibodies or if these cells stimulate other cells to this production.

This study is an attempt to investigate the last problem. Irradiated mice were used since, in these mice, the first steps of the process that normally leads to antibody production still proceed,[18] whereas the ultimate antibody production is much suppressed or completely inhibited.

Methods. – Lethally irradiated (C57BL × 101)F_1 mice were injected with 1 ml of 1% rat RBC within a few hours after irradiation. A few days afterward, the animals were killed and a spleen or lymph node cell suspension was prepared. This cell suspension was injected into nonirradiated mice (1) immediately after preparation or (2) after the cells were

[18] C. C. Congdon, Radiation Research 9, 102 (1958).

killed by freezing and thawing. Antibody production in the latter group was measured by agglutination tests on rat RBC.

Results. – Suspensions of mouse spleen, prepared eight days after injection of rat RBC into lethally irradiated mice, caused a production of antibodies (titer 1/64–1/256) after transplantation into nonirradiated mice. No antibody production was found if the cells were killed by freezing and thawing immediately before transplantation. The same results, but lower titers, were obtained in another experiment, when the spleen cells were transplanted two, four, six, or eight days after injection of rat RBC into the irradiated mice.

Discussion. – Before we can conclude that spleen cells, which do not contain antibody to a measurable amount, are able to stimulate the nonirradiated host to antibody production and before we can postulate that at least two different cells are involved in the chain of events leading to antibody production, some control experiments have to be done. These experiments include (1) the injection of spleen cells into lethally irradiated mice to verify their own inability to produce antibody and (2) the effect of freezing and thawing on the antigen and its ability to stimulate antibody production in the mouse.

RADIATION IMMUNOLOGY

T. Makinodan
N. Gengozian
R. D. Owen[1]
E. H. Perkins
I. C. Shekarchi[1]
M. G. Tolbert
V. M. Cook[2]
P. F. Lincoln[1]
W. J. Peterson

Secondary Antibody Response During the Maximum Depression Period After Varying Doses of X Radiation

T. Makinodan M. G. Tolbert

Introduction. – The following studies were completed in this laboratory, with (C3H × 101)F_1 mice: (1) establishment of the optimum age, antigen (foreign red blood cells) concentration, and route of injection to achieve maximum agglutinin response;[3,4] (2) the temporal relation between primary antigen injection and X-ray exposure on antibody response;[5] (3) primary antibody response to distantly and closely related heterologous antigens during the maximum depression period after varying X-ray doses;[4,6] and (4) the relation of secondary antigen injection to time of irradiation on antibody response.[7] This report describes the secondary sheep agglutinin response in mice as affected by varying doses of X radiation.

At six weeks of age all mice were given 1 ml of 0.2% sheep red blood cells (RBC) intraperitoneally. Six weeks later a second antigen injection of 1 ml of 1.0% sheep RBC was administered intraperitoneally within 1 hr after 0, 100, 300, 500, 710, 800, 850, 900, or 950 r. Mice receiving 800 r or more received intravenously 12 × 10^6 nucleated isologous bone marrow cells within 4 hr after irradiation. This treatment not only permits mice to live longer than two weeks but also does not alter the recipient's ability to respond to the antigen injection.[3–7] At 3, 6, 12, 18, 24, 30,

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[1]Consultant.

[2]Biology Student Trainee.

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

[4]T. Makinodan and N. Gengozian, J. Immunol. 81, 150 (1958).

[5]N. Gengozian and T. Makinodan, J. Immunol. 80, 189 (1958).

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

[7]T. Makinodan et al., J. Immunol., in press.

40, and 50 days after the secondary injection, ten mice from each group were bled by decapitation and serum samples were collected. This yielded 80 samples per group for antibody titration and provided data for the determination of mean total titer. For comparative purposes, a portion of results of our previous study on primary sheep agglutinin response after various X-ray doses[6] are shown here, together with our present findings.

Results. – The results, expressed as (mean total titer exptl.)/(mean total titer normal) × 100 as a function of X-ray dose, are summarized in Fig. 12. The above-normal secondary antibody response after 100 and 300 r can be caused either by the "release" of antibodies into the circulation by storage cells under stress, by hemoconcentration, or both. It would seem that below 800 r the secondary responders were more radioresistant than the primary responders, but above 800 r both responders were equally radiosensitive. It can be seen further that the SD_{50} (i.e., X-ray dose necessary to suppress the mean total antibody titer of the normal less the residual antibody titer by 50%) is ~800 r for the secondary responders and ~625 r for the primary responders, respectively.

Discussion. – It would seem valid to conclude that the secondary antibody response is more radioresistant than the primary antibody response in mice. Implicit in this conclusion is that the number of potential antibody-forming cells among the primary and secondary antibody responders to a given antigen is comparable. However, our results on *in vivo* tissue culture of spleen cells suggest that there is a tremendous increase in the number of potential antibody-forming cells after primary sensitization.[8] Further studies are being carried out in an attempt to resolve this problem.

Transplantation of Antibody-Producing Cells in X-Irradiated Recipient Mice

N. Gengozian T. Makinodan I. C. Shekarchi

Introduction. – Previous studies have indicated that transplantation of isologous, homologous, or heterologous bone marrow cells in lethally irradiated mice makes no significant contribution to the host's immune

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

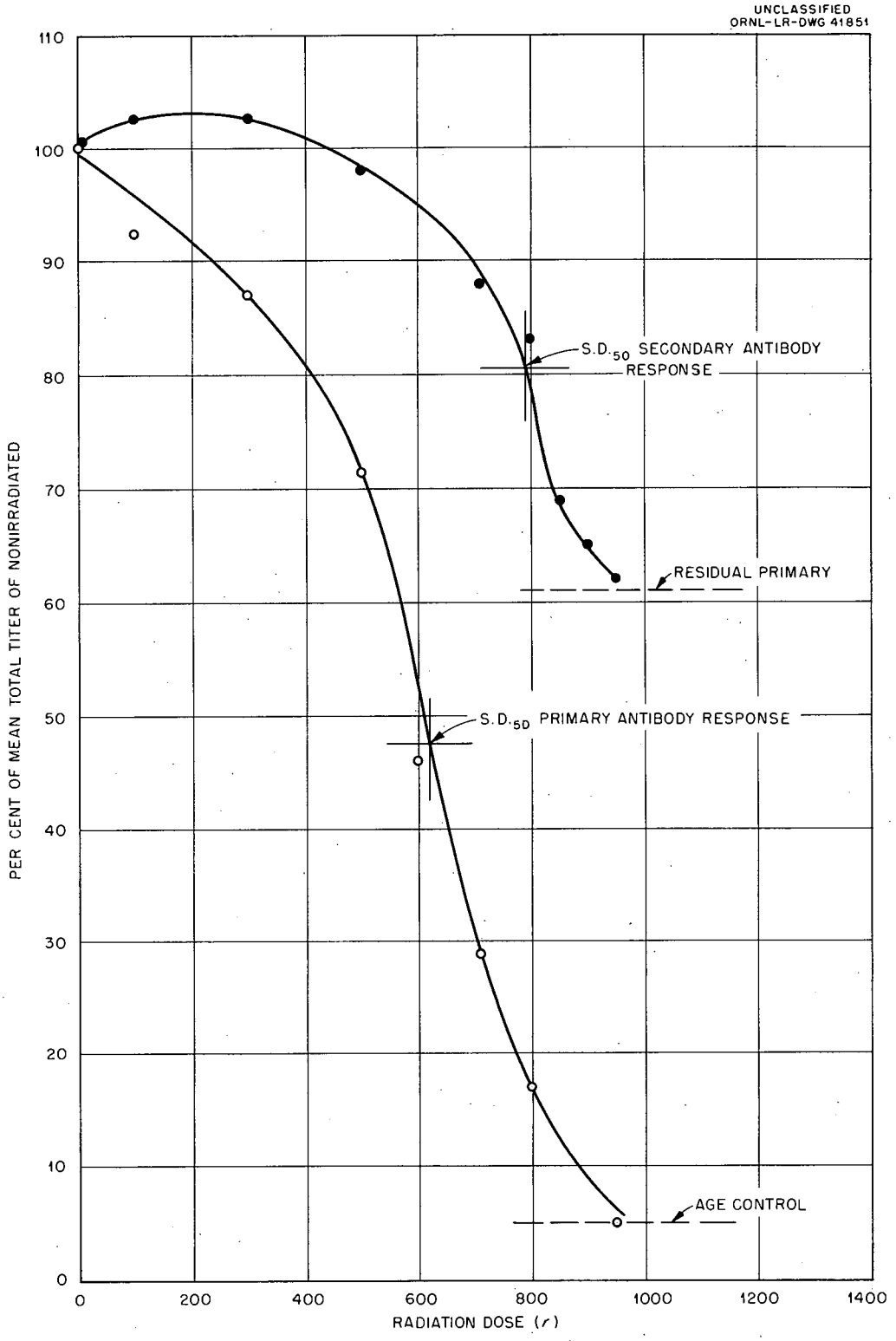

Fig. 12. Determination of the SD_{50} for Primary and Secondary Antibody Response.

mechanism. In a subsequent comparison of the antibody-forming potentialities of splenic and bone marrow tissue, agglutinin production was demonstrated in lethally irradiated mice treated with isologous spleen and an RBC antigen but not in irradiated mice treated with isologous bone marrow and the antigen. The present report is a comprehensive study of the agglutinin response to rat RBC antigens in lethally irradiated mice that had received isologous spleen cells or bone marrow cells from normal or preimmunized donors.

Results. — Male and female (101 × C3H)F_1 mice 12 weeks old were used both as lethally irradiated recipients and also as donors of splenic and bone marrow tissue. Donors were preimmunized at six weeks of age by injecting them with 1 ml of 0.1% rat RBC suspension intraperitoneally. Six groups of 12-week-old mice were exposed to 950 r of X rays and then injected intravenously with bone marrow or splenic tissue from normal or preimmunized donor mice. Each animal received 12×10^6 nucleated cells. Immediately after this treatment, four of the groups also received an intraperitoneal injection of the antigen (1 ml of 1.0% suspension). Mice from each group were then bled at intervals of 1–50 days after treatment and the serum samples titrated individually for agglutinating antibodies. As shown in Table 21, the data are expressed in terms of the mean total agglutinin titer ($\log_2$) and the groups ranked by statistical analysis of differences at the 5% level. The data show the superiority of the splenic tissue over bone marrow in agglutinin production. The inability of normal bone marrow to contribute antibody-producing cells is quite apparent, but marrow from presensitized donors does show definite antibody-producing capabilities in animals receiving the marrow and the RBC injection. In the two groups receiving either presensitized spleen or bone marrow but not the antigen, agglutinin production was observed only with transplantation of presensitized spleen cells, not with presensitized bone marrow cells. The most interesting observation was in the group of mice that had received presensitized spleen cells and the RBC antigen. The level of agglutinin production attained here was greater than that observed in the primary antibody response in normal nonirradiated mice and comparable to a secondary response in normal animals.

Table 21. Antibody Production in Lethally Irradiated Mice Treated with 12 × 10^6 Isologous Spleen or Bone Marrow Cells

Group (rank)	Treatment	Mean Total Agglutinin Titer ($\log_2$)
I	950 r + IBM[a]	0
	950 r + IBM + RBC	0.22
II	950 r + ISp[a,b]	0.61
III	950 r + ISp + RBC	2.11
IV	950 r + IBM[a] + RBC	3.23
V	Normal primary	7.71
VI	Normal secondary	8.47
	950 r + ISp[a] + RBC	9.27

[a]Presensitized.
[b]Isologous spleen.

In agreement with our previous work, the present study also indicates that normal bone marrow contributes little, if anything, to the immune system of the irradiated host. On the basis of the present positive observation with preimmunized bone marrow, we hypothesize that: during this preimmunization period, there is a shift in cell type population in the marrow in that antibody-producing cells are now present, resulting from a migration through the circulatory system from antibody-producing sites such as the spleen and lymph nodes and a subsequent localization in the marrow. Such a shift in cell type population could then account for the agglutinin production observed in mice injected with preimmunized bone marrow and the RBC antigen. A biological test of this hypothesis was invoked. We reasoned that, if upon some preimmunization procedure such a shift in cell type population occurred in either the spleen or bone marrow (i.e., a shift in more antibody-producing cells), there would be a relative decrease in those cells capable of protecting mice from radiation death. As a result,

the capacity of cells from these tissues to protect lethally irradiated mice should be lower because of this shift in cell type population. Preimmunized donors to be used for protection studies were sensitized with homologous tissues. With a cell dose of 1×10^6 injected into 950-r animals, the 30-day mortalities obtained with normal spleen and bone marrow were 37 and 10%, respectively. With cells from the preimmunized donors, the 30-day mortalities of 950-r mice treated with spleen and bone marrow were 97 and 35%, respectively.

Discussion. – Using isologous spleen cell transfer, we have been able to show that as few as 12×10^6 cells in 950-r mice are capable of producing antibodies in amounts comparable to those obtained among normal animals eliciting a secondary immune response. Also, during the presensitization period, there is a definite shift in cell type population in the marrow and spleen in favor of antibody-producing cells, with a relative decrease in cells capable of protecting mice from radiation death.

Lethally X-Irradiated Mice as an *in vivo* Tissue Culture for Quantitative Study of Antibody Production

E. H. Perkins T. Makinodan

Introduction. – Since 1899 when Deutsch[9] reported the appearance of antityphoid agglutinins in the circulation of normal guinea pig recipients injected with spleen cells from preimmunized guinea pigs, much has been done in transplantation of antibody-forming cells. Similarly, since Benjamin and Sluka[10] first described the radiosensitive nature of the antibody response, the number of reports confirming and extending their observations are legion. Information acquired from these two areas of investigation has offered a rational approach to the demonstration that a most important factor in antibody production and its quantitation is the number of cells participating in the "immune response." With lethally irradiated mice used as *in vivo* tissue cultures, it has been possible to study in detail the relation of antibody response to cell number by transplanting isologous spleen cells from normal and preimmunized donors.

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[9]L. Deutsch, Ann. inst. Pasteur 13, 689–727 (1899).

[10]E. Benjamin and E. Sluka, Wien. klin. Wochschr. 21, 311 (1908).

Results. – Intravenous injection of 1.5, 3, 6, 12, 24, or 48 million normal (nonimmunized) or preimmunized spleen cells into lethally irradiated isologous recipients, which immediately thereafter received an intraperitoneal antigen injection, resulted in hemagglutinin titers that were a function of the number of spleen cells injected. Indeed, a linear $\log_2$ relation between cell number and antibody titer was clearly evident. In subsequent experiments, regardless of (1) the strain of mice, (2) the immunization procedure (i.e., time of primary antigen injection or concentration of antigen), (3) the time of bleeding after cell transfer and antigen injection, and (4) whether the cells were obtained from normal or preimmunized donors, a linear "$\log_2$ antibody titer–$\log_2$ nucleated cell relation" was obtained. The slopes of the lines representing the "immune response," however, were dependent on the time of bleeding but independent of the immunization procedure at a given time. The absolute amount of titratable antibody per unit of nucleated spleen cells varied, however, with the immunizing procedure.

We have tried to quantitate more precisely the antibody response by using Anderson's continuous density gradient centrifugation technique[11] in attempts to separate "antibody-forming cells" from nonantibody-producing hematopoietic cells of the spleen before injection into the isologous recipient. Crude spleen cell suspensions were layered over a Tyrode's-BSA (bovine serum albumin) continuous density gradient column and centrifuged. Distinct layers and fractions thereof were then washed and resuspended in Tyrode's solution for injection. Although, by these methods, we could not clearly separate distinct cell types, we were able to concentrate antibody-forming cells, relative to the crude spleen cell suspension. Fractions showed no antibody-forming activity, a twofold reduction of activity, or a fourfold increase in the absolute amount of titratable antibody per unit of nucleated spleen cells.

Discussion. – The use of lethally X-irradiated isologous mice as an *in vivo* tissue culture has provided an effective system for quantitative assay of the antibody response because homologous tissue reaction and the

[11] N. G. Anderson, T. Makinodan, and I. C. Shekarchi, "Continuous Density Gradient Centrifugation of Bone Marrow Cells," to be published.

response of host cells to the test antigen are eliminated. Using this system, we can now critically study (1) the relative rate of antibody synthesis, (2) the concentration of antigen necessary for optimum response, (3) time relations with regard to antigen injection in antibody synthesis, and (4) the number of functional antibody-producing cells within an organ or tissue, as well as other challenging problems dealing with the cellular mechanisms involved in the inductive and productive phases of antibody formation.

The Effect of 6-Mercaptopurine on Antibody Reproduction in Mice

M. G. Tolbert T. Makinodan

Introduction. – Recently Schwartz and Dameshek[12] reported that 6-mercaptopurine (6-MP) could be used to eliminate antibody response to radioiodinated human serum albumin (HSA) in rabbits. At the beginning of the experiments, rabbits were given a single intravenous injection of HSA and 6 mg of 6-MP per kg of animal weight intramuscularly daily for two weeks, beginning at the time of the antigen injection. This eliminated the immune response to that and subsequent injections of HSA. They proposed that the 6-MP interferes with the information-storing mechanism and consequently the antigen is no longer recognized as a foreign substance. Work has been carried out in this laboratory to determine the effect of 6-MP on antibody responses in mice.

Results. – Three groups of 12-week-old (C3H × 101)F_1 mice were given daily intraperitoneal injections of 3, 15, and 30 mg of 6-MP per kg of body weight per dose, respectively, for five days before antigen injection and for the duration of the experiment. A fourth group of mice received 30 mg of 6-MP per kg of body weight per dose for ten days before antigen injection and for the duration of the experiment. In all four groups, 1 ml of a 1% suspension of rat RBC was used as antigen. Five mice from each group were bled on days 3, 6, and 9 after antigen injection. Agglutinin titers of these sera were not significantly different from results reported in normal mice[3] (Fig. 13).

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[12]R. Schwartz and W. Dameshek, Nature 183, 1682–83 (1959).

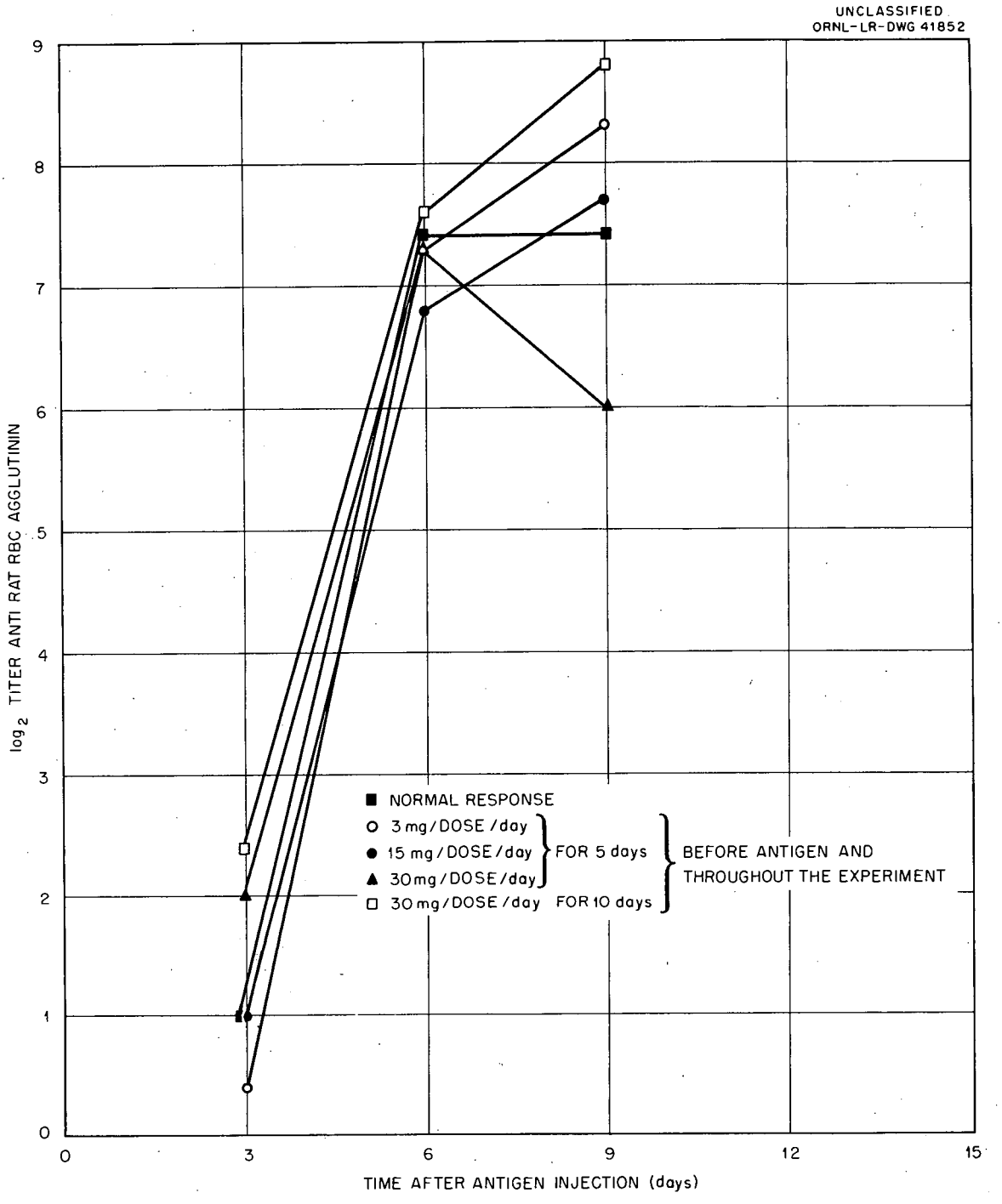

Fig. 13. Rat Agglutinin Response of Mice Treated with 6-Mercaptopurine.

Another type of antibody response is that seen in irradiated mice treated with homologous or heterologous bone marrow. The secondary weight loss and death observed in these animals are attributed to an *in vivo* antigen-antibody reaction.[13]

To test the effect of 6-MP on this reaction, two groups of 12-week-old $(C3H \times 101)F_1$ mice were given 30 mg of 6-MP per kg of body weight per dose for seven consecutive days before and also on the day of irradiation and bone marrow treatment. Group I received lethal doses (900 r) of X rays and then intravenous injection of 140×10^6 nucleated rat bone marrow cells; group II received the same X-ray dose and $25–30 \times 10^6$ cells of $(L \times A)F_1$ bone marrow given intravenously. Controls were mice given the same treatment as the experimental groups but without 6-MP. Animals were weighed weekly and deaths recorded. The 6-MP did not prevent secondary weight loss and, furthermore, death patterns were similar for each experimental group and its respective control group. These results would suggest that the 6-MP failed to induce tolerance to foreign bone marrow.

Discussion. — In these experiments there was no apparent effect of 6-MP on antibody response, even though the mice in some cases received as much as 450 mg of 6-MP per kg of body weight over a 15-day period, compared to a dose of 84 mg/kg over a period of 14 days in rabbits. The results reported here as compared to results in rabbits could be attributable to a difference in the effect of 6-MP on the antibody-producing mechanism in rabbits and mice, or it may be caused by a difference in antigens used — the one in rabbits being a soluble antigen, and that in the mice a particulate antigen.

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

PATHOLOGY AND PHYSIOLOGY

A. C. Upton, Section Chief

T. T. Odell, Jr.	E. S. Ledford
W. H. Benedict[1]	B. C. Caldwell
K. W. Christenberry[1]	E. P. Sniffen
J. A. Sproul, Jr.[2]	T. P. McDonald
W. D. Gude	D. E. Reynolds
G. E. Cosgrove	T. Mack
E. B. Darden, Jr.	M. A. Bradley
R. A. Popp	T. W. McKinley, Jr.
W. Friedberg	S. A. Sapp[5]
R. L. DeHart[3]	V. K. Jenkins
E. D. Morris[4]	M. S. Gude
B. Anderson	

Comparison of the Effects of Whole-Body and Part-Body Irradiation on Longevity and Neoplasia

G. E. Cosgrove E. S. Ledford

Introduction. — The present study was undertaken to extend previous observations on the protection afforded by partial shielding against the induction of delayed effects of radiation.

Results. — Shielding two-thirds of the body significantly reduced the life-shortening and oncogenic effectiveness of 250-kvp X radiation in female mice of the RF strain, irrespective of the region shielded (Table 22).

Discussion. — Although the effects of whole-body irradiation were not reproduced by irradiation of any one third of the body, induction of certain effects, such as cataracts and ovarian tumors, did not appear to be inhibited unless the involved organ itself was shielded. From these data, it would appear that the life-shortening and oncogenic effects of ionizing radiation result from a complex interaction of direct and indirect influences on diverse tissues of the body and that the elucidation of these effects will require further comparison of systemic and localized exposures.

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[1]Consultant.
[2]U.S. Air Force.
[3]Temporary employee.
[4]Research Participant.
[5]Biology Student Trainee.

Table 22. Comparative Effects of Whole-Body and Part-Body X Irradiation on Life Span and Neoplasm Incidence in RF Female Mice*

Part of Body Exposed	X-Ray Dose (r)	Mean Life Span (months)	Leukemia Incidence (%)		Tumor Incidence (%)	
			Myeloid	Thymic Lymphoma	Ovary	Lung
None	0	19.4	6	18	14	11
Whole	100	17.7	5	25	63	6
Whole	300	15.2	19	31	56	10
Upper 1/3	300	19.6	3	11	12	9
Middle 1/3	300	18.1	3	14	41	10
Lower 1/3	300	18.5	4	18	25	6

*Mice were irradiated when ten weeks of age at a dose rate of 80–100 rads/min; 97–100 animals per treatment group.

Effects of X Radiation on Intestinal Esterases

R. A. Popp J. Maisin[6]

Introduction. – The esterase activity in the intestine of control, X-irradiated, and AET-protected–X-irradiated mice was determined for comparison with histologic effects on the intestine.

Methods and Results. – Twelve- to 16-week-old strain A-1/Jax mice were used. A clear extract was prepared from the duodenum and upper jejunum for colorimetric assay of enzyme,[7] electrophoresis,[8] and protein determination,[9] and sections were taken for histochemical study. The tissue was homogenized with 2 ml of distilled water per gram, the homogenate was then frozen overnight at -15°C, and cellular debris was finally removed by centrifugation at 1500 × g for 20 min. Specific activities of extracts are shown in Fig. 14. When quantitative determinations were made of single esterases in extracts after electrophoresis,[8] the relative quantities of some esterases in unirradiated controls were significantly different from

[6]Mammalian Recovery Group.

[7]G. Gomori, J. Lab. Clin. Med. 42, 445–53 (1953).

[8]C. Markert and R. Hunter, J. Histochem. and Cytochem. 7, 42–49 (1959).

[9]T. Weichselbaum, Am. J. Clin. Pathol. 16, 40–49 (1946).

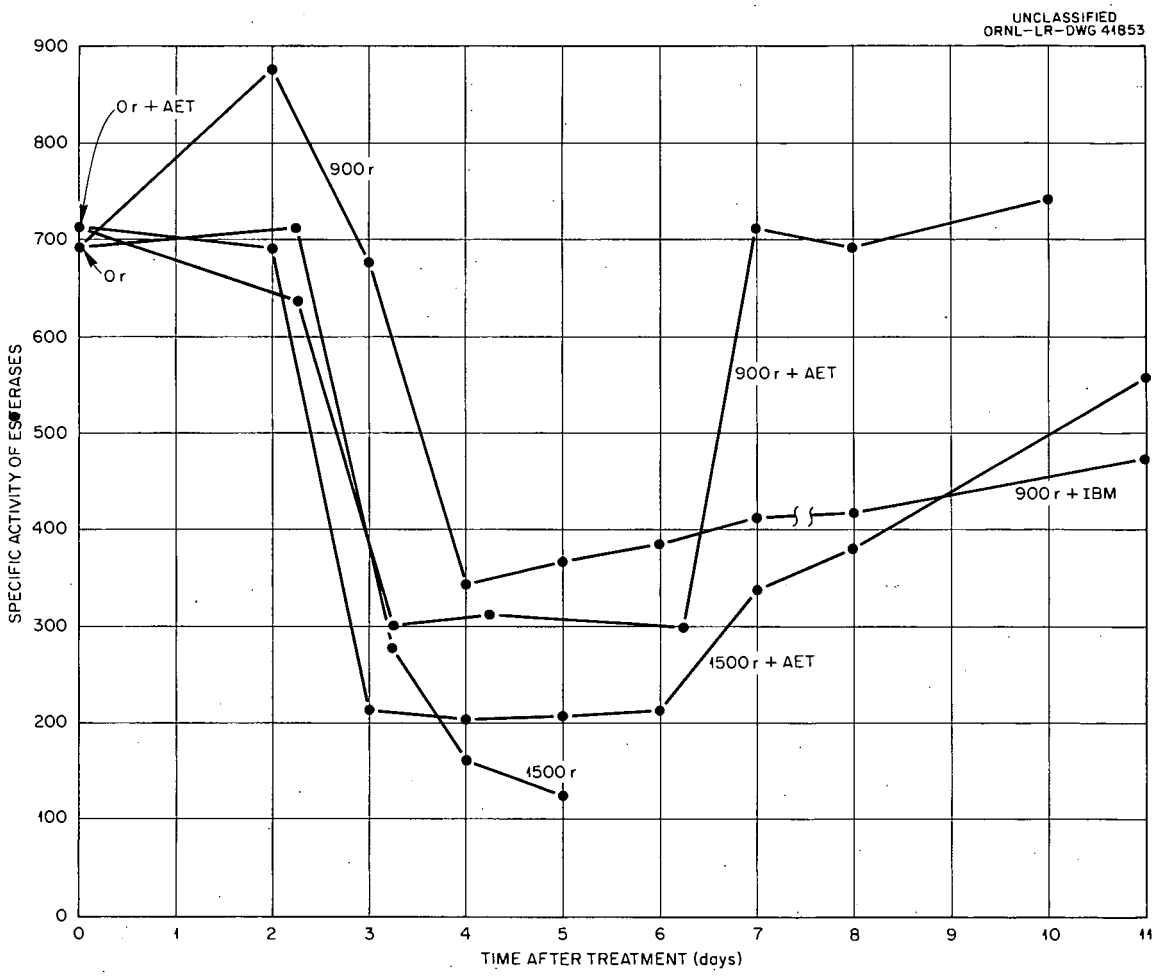

Fig. 14. Comparative Effects of 900 and 1500 r of X Rays and Protective Effects of AET on Intestinal Esterase Activity. Data plotted refer to specific activities of esterases in intestinal extracts. One unit of enzymic activity equals 1 mg of α-naphthol, liberated from α-naphthyl acetate substrate, per milligram of protein per hour, at 37°C, pH 6.8. AET-protected mice were injected intraperitoneally with 8.8 mg of AET per 25 g. Values plotted are averages based on 2–10 mice of each sex per point.

those in mice in the recovery phase after AET protection and 1500 r of X rays.

Discussion. – Since esterase is not directly affected by irradiation,[10] the reduced quantity of detectable enzyme in the intestine probably reflects lethal radiation injury of cells that synthesize esterase. This also seems likely since intestinal epithelium is readily destroyed by X

[10]S. Ludewig and A. Chanutin, Arch. Biochem. 29, 441–45 (1950).

irradiation[11] and since histochemical stains reveal that most of the esterase activity is in the epithelium.[12] Data from the chemical assay of the enzyme also agree with histologic and histochemical findings, if we assume that there is a direct correlation between the number of epithelial cells and enzymic activity. Changes in the relative quantities of esterase during the recovery phase suggest that the biological half life of esterases may differ, their rates of synthesis or catabolism may be differentially affected by irradiation, or the populations of cells that synthesize different esterases may be changed after irradiation.

Effects of Antigen-Inactivated Spleen Cells on the Lymphoid Tissues of Irradiated Recipients

R. L. DeHart G. E. Cosgrove

Introduction. — Earlier experiments disclosed that exposure *in vitro* to recipient antigen before implantation inhibited the lethal reaction of parental spleen cells against irradiated F_1 hybrid recipients.[13] This study concerns the effects of such "inactivated" spleen cells on the lymphoid tissues of the recipients.

Methods and Results. — Ten- to 12-week-old (C57BL × 101)F_1 mice were exposed to 600 r of whole-body X radiation and injected thereafter with C57BL spleen cells "inactivated" by incubation with (C57BL × 101)F_1 liver, according to the method outlined previously.[13] Prompt and lasting regeneration of lymphoid and other blood elements occurred in such recipients, in contrast to the progressive secondary atrophy of these elements in recipients of noninactivated spleen cells. Histologically, mice receiving "inactivated" spleen cells also showed little or none of the granulomatous reaction in lymphoid tissues that was conspicuous in mice receiving noninactivated spleen cells.

Discussion. — The observed results are consistent with the interpretation that exposure of the donor spleen cells to recipient antigen *in*

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[11] R. Conard, Radiation Research 5, 167–88 (1956).

[12] G. Gomori, Intern. Rev. Cytol. 1, 323–35 (1952).

[13] G. E. Cosgrove *et al.*, Biol. Semiann. Prog. Rep. Feb. 15, 1959, ORNL-2702, p 116.

vitro interferes with their subsequent immunologic reaction against the recipient after implantation. The nature of this effect and the identity of the cells affected cannot be specified without further investigation.

Protein Metabolism in the Rat-Mouse Chimera

W. Friedberg D. E. Reynolds

Introduction. – The increased fractional rate of protein loss in irradiated mice bearing transplanted rat bone marrow[14] has been investigated further in an effort to elucidate the basis of this disturbance.

Results. – The fractional rate of protein loss (Table 23) in mice injected with rat bone marrow after exposure to 950 r of X rays (950 r-RBM mice) is increased significantly more than in similarly irradiated mice with autologous bone marrow protection (leg shielding). The loss of protein cannot be ascribed to leakage into the urine, since HSA-I^{131}-injected control and 950 r-RBM mice had no detectable nondialyzable radioactivity in their urine. The possibility of leakage into the gastrointestinal tract, however, is under investigation. In the 950 r-RBM mice, there was also a decrease in the serum albumin pool (Table 24). This conclusion is based on the 20-min distribution of intravenously injected HSA-I^{131} (iodinated human serum albumin). Recipient blood was obtained by heart puncture; serum albumin was separated by paper electrophoresis, stained with bromphenol blue, and scanned with a Spinco model RB Analytrol. Radioactivity was measured in a well-type scintillation counter.

Discussion. – According to available evidence[15,16] the fractional rate of catabolism of plasma protein is not affected by its pool size; change in rate may, however, affect the pool size. The decrease in the serum albumin pool in the rat-mouse chimeras is, therefore, considered the result rather than the cause of an increased fractional rate of protein catabolism or protein leakage, or both, into the gastrointestinal

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[14]W. Friedberg and D. Reynolds, Biol. Semiann. Prog. Rep. Feb. 15, 1959, ORNL-2702, p 123.

[15]D. Gitlin, Ann. N.Y. Acad. Sci. 70, 130 (1957).

[16]A. S. McFarlane, p 155 in Progress in Biophysics, ed. by J. A. V. Butler and B. Katz, Pergamon Press, New York, 1957.

Table 23. Fractional Rate of Loss of HSA-I^{131} as Influenced by Irradiation and Bone Marrow Treatment

Experiment I		
Time after irradiation, days[a] ⟶	21	32
Fractional loss per hour		
A. Control mice	0.0278 ± 0.0020 (8)[b]	0.0270 ± 0.0018 (8)
B. 950 r-RBM mice	0.0437 ± 0.0019 (9)	0.0408 ± 0.0019 (7)
Difference (P)[c]		
A vs B	0.001	0.001
Experiment II		
Time after irradiation, days ⟶	4	31
Fractional loss per hour		
A. Control mice	0.0267 ± 0.0008 (4)	0.0239 ± 0.0003 (5)
B. Leg-shielded-950-r mice	0.0312 ± 0.0006 (9)	0.0284 ± 0.0005 (11)
C. AET-950-r mice	0.0314 ± 0.0006 (8)	0.0271 ± 0.0005 (6)
Difference (P)		
A vs B	0.001	0.0023
A vs C	0.001	0.04
B vs C	0.50	0.28

[a]Day of HSA-I^{131} injection.

[b]Number of mice shown in parentheses.

[c]P denotes significance level for "Student" t-test.

tract. Although the data do not conclusively demonstrate an increase in albumin volume (P = 0.084), this is suggested by the decrease in albumin concentration relative to the decrease in pool size. Since an increase in plasma volume has previously been observed in irradiated rats without bone marrow treatment,[17] the increased albumin volume in 950 r-RBM mice may not be related to chimerism.

[17]H. Supplee, J. D. Hauschildt, and C. Entenmann, Am. J. Physiol. 169, 483–90 (1952).

Table 24. Comparison of Serum Albumin and Body Weight in 950 r-RBM Mice and Controls*

	Percentage of Controls
Mouse weight, g	82.3 ± 5.8
Albumin pool**	80.8 ± 7.8
Albumin pool per gram of mouse weight	98.2 ± 5.6
Albumin concentration	72.8 ± 6.3

*Six control mice compared with six 950 r-RBM mice (25 days after irradiation). The 950 r-RBM mice had 43% (33–51%) circulating RBC of rat type.

**The serum albumin pool was computed from the following formula:

$$\frac{\text{counts/min, injected}}{\text{counts/min, albumin sample}} \times \text{Analytrol reading, albumin sample.}$$

Hemoglobin as a Red Cell Marker in Irradiated Mouse Chimeras

R. A. Popp
G. E. Cosgrove
D. E. Reynolds
S. A. Sapp

Introduction. – The usefulness of hemoglobin solubility as a red cell marker in irradiated mouse chimeras, previously reported,[18] has been extended by development of more-quantitative methods for determining the proportions of erythrocytes with different hemoglobins present in various mixtures.

Results. – Mixtures of different carbomonoxyhemoglobin (HbCO) types gave optical density readings intermediate between those of the unmixed HbCO samples; that is, increased or decreased, depending on the relative proportions of the two HbCO types in the mixture (Fig. 15). On salting out, HbCO of homozygous-diffuse types formed an amorphous precipitate; that of single types formed large, hexagonal crystals; and that of heterozygous-diffuse types formed a mixture of amorphous precipitate and crystals.

[18]R. A. Popp, G. E. Cosgrove, and D. Reynolds, Biol. Semiann. Prog. Rep. Feb. 15, 1959, ORNL-2702, p 118–20.

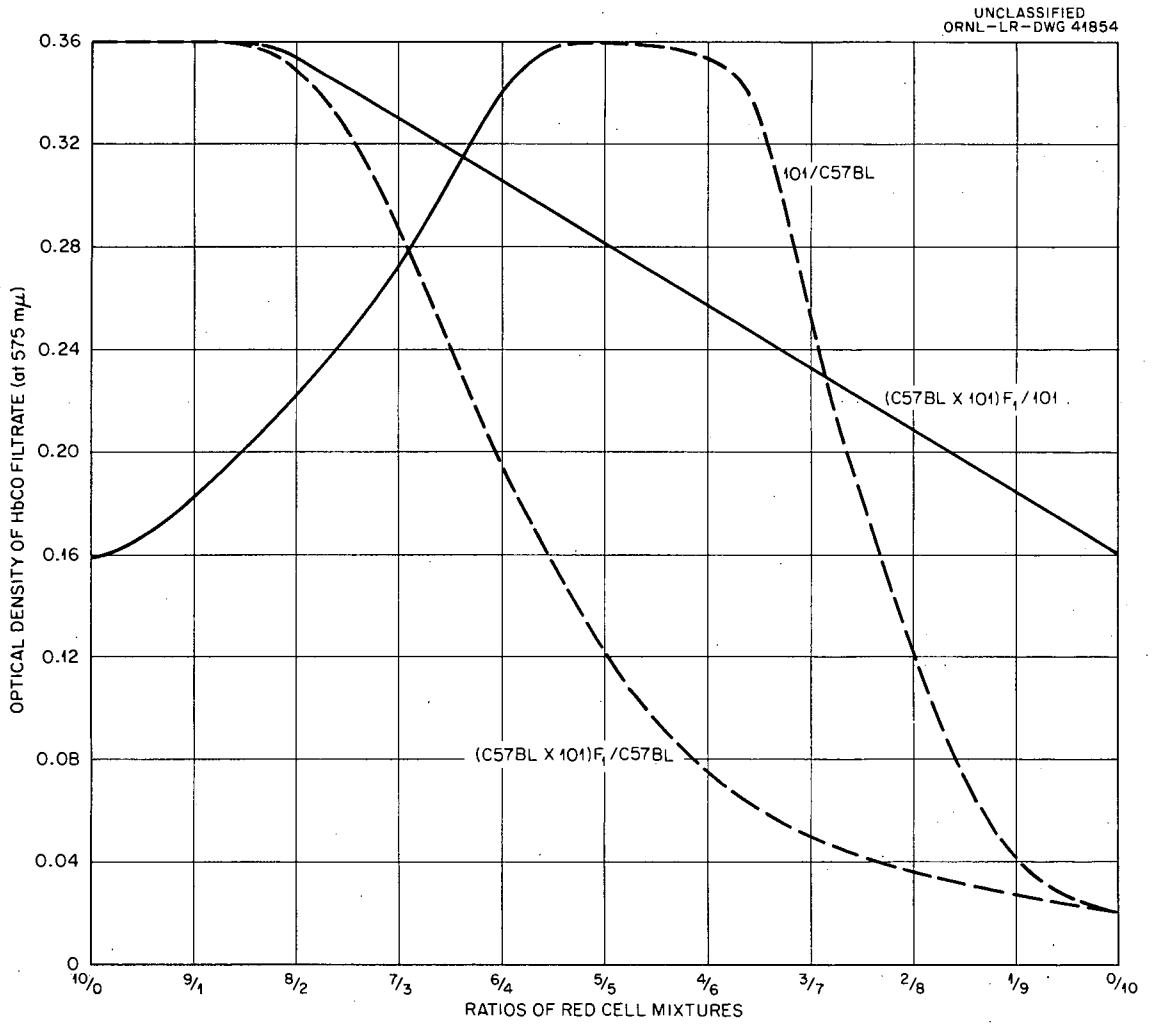

Fig. 15. Solubility of Mixtures of Mouse HbCO. Conditions were 3.08 M K_2HPO_4-KH_2PO_4, pH 6.7, at 24°C, for 21 hr. Points where HbCO crystals formed are designated by broken lines, those where amorphous precipitates formed by solid lines.

The optical density of the HbCO filtrates decreased with increased salt concentration, with increased pH (range 6.7–7.9), with increased temperature (range 4–37°C), and with increased time. Optimal conditions for distinguishing most mouse hemoglobins by salting-out procedures were 3.08 M K_2HPO_4-KH_2PO_4, pH 6.7, at 30°C, for 21 hr.

Discussion. – The percentage of donor-type erythrocytes in irradiated mouse chimeras may be easily determined from the optical density of the HbCO filtrate and from the HbCO precipitate formed. Conditions for typing each new combination of donor and recipient strains should be standardized,

however, since, for example, the single-type hemoglobins of SeC/R1 and C57BL/S mice have very different solubilities in phosphate buffer although their electrophoretic mobilities are similar to one another. Whether such variations are controlled by different hemoglobin alleles or are caused by interactions of the hemoglobin locus and other loci remains to be established.

Effects of Aging and Irradiation on Mucopolysaccharides of Rat Cartilage

B. Anderson T. T. Odell, Jr.

Introduction. – Several investigators have shown that the mucopolysaccharide (MPS) composition of human and guinea pig cartilage changes with age. The purpose of this study was to extend these observations to the rat and, since radiation hastens the appearance of various changes associated with senescence, to investigate the effect of X radiation on changes in costal cartilage that might occur naturally with age.

Method and Results. – Male Sprague-Dawley rats, about 65 days old, were exposed to 100–900 r of X rays and killed ~6, 9, or 12 months later along with age controls. The costal cartilage was removed, freed of other tissue, finely chopped, dried in a vacuum oven at 58°C, and ground to pass through a 60-mesh screen. Weighed samples were then reacted with trypsin, and since this treatment released only part of the MPS, the residue was further reacted with papain. Both digests were treated with chloroform–amyl alcohol reagent to free MPS from protein. The hexuronic acid content of one aliquot of each of the MPS fractions was determined by the Dische procedure, and another aliquot was hydrolyzed and analyzed for hexosamine by a modification of the Elson-Morgan procedure. With age, there was a decrease in the content of uronic acid and of hexosamine in the trypsin digests of costal cartilage (Fig. 16), which was not apparent in the papain digests from ages 100–624 days. Within the limits of the techniques used, no increase in rate of change of these components of cartilage was apparent as a result of whole-body irradiation.

Discussion. – Although irradiation seems to hasten certain aging changes in mammals, the rate of decrease of uronic acid and hexosamine

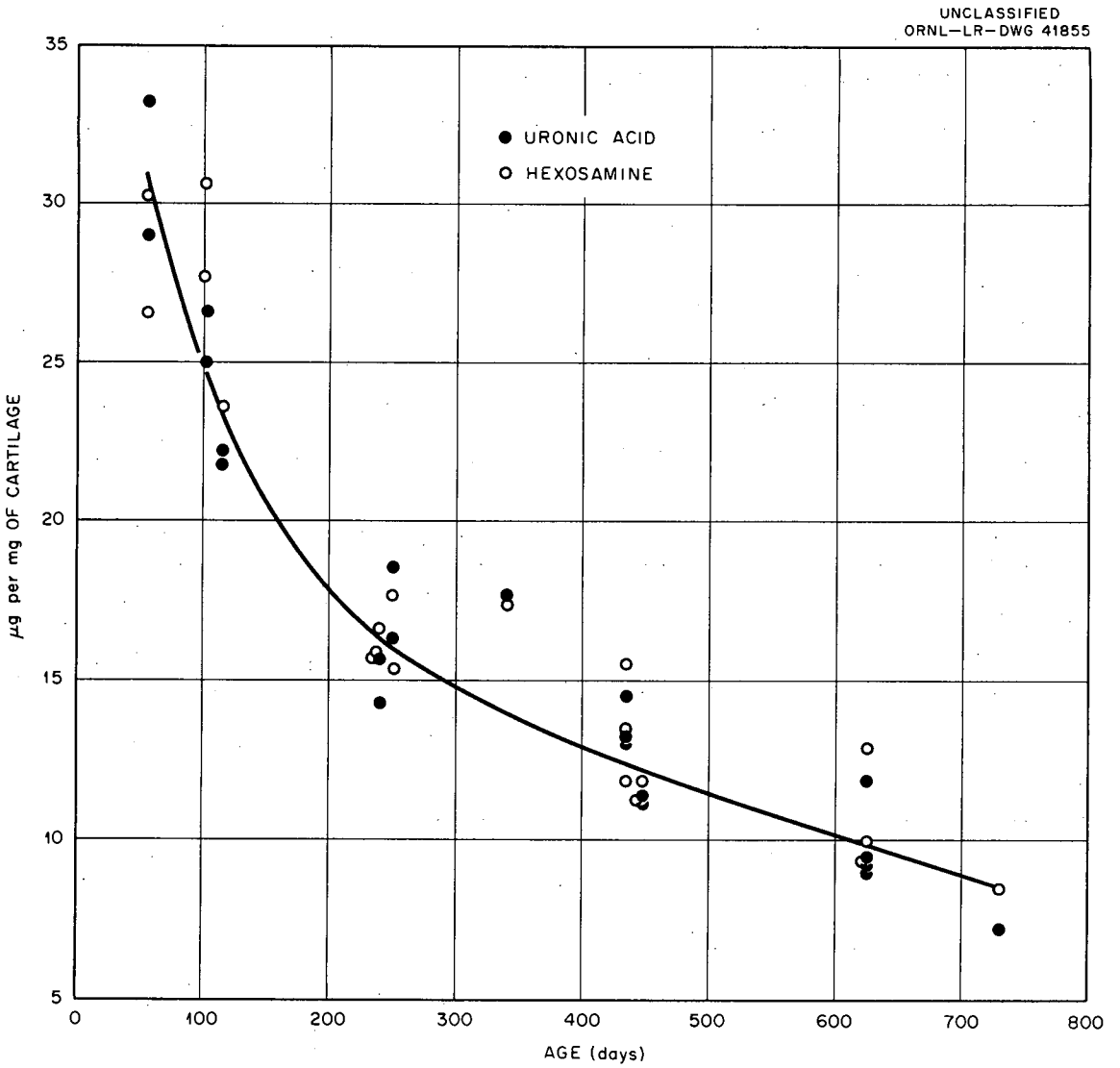

Fig. 16. Uronic Acid and Hexosamine Content of Rat Cartilage in Relation to Age.

in a MPS fraction of cartilage was not affected in these experiments. It should be noted, however, that the changes in cartilage examined in this study occurred most rapidly relatively early in life and are therefore not typically senescent changes.

Age Changes in Mouse Collagen Fibers

E. B. Darden, Jr. M. B. Bradley

Introduction. — Of various physical and chemical changes that collagen fibers undergo with age,[19] one is being investigated in an attempt to assess the effects of irradiation on senescence.

[19] F. Varzar, Gerontologia 1, 363 (1956).

Results. – Tail tendon fibers from mice of the RF strain were heated in a saline bath, and thermal contraction of the fibers was continuously registered by a strain gauge recorder. The temperature at which maximum contraction (TMC) occurred increased fairly smoothly with the age of the animal (Fig. 17). The TMC's for several fibers from the same tail generally agreed within 3° and often with 1° of one another. The TMC's for fibers from a few animals of different strains showed approximately the same behavior with increasing age. Fibers from a few animals receiving whole-body X radiation (450 r) several months previously did not show any changes strikingly different from those of the controls. Fibers from

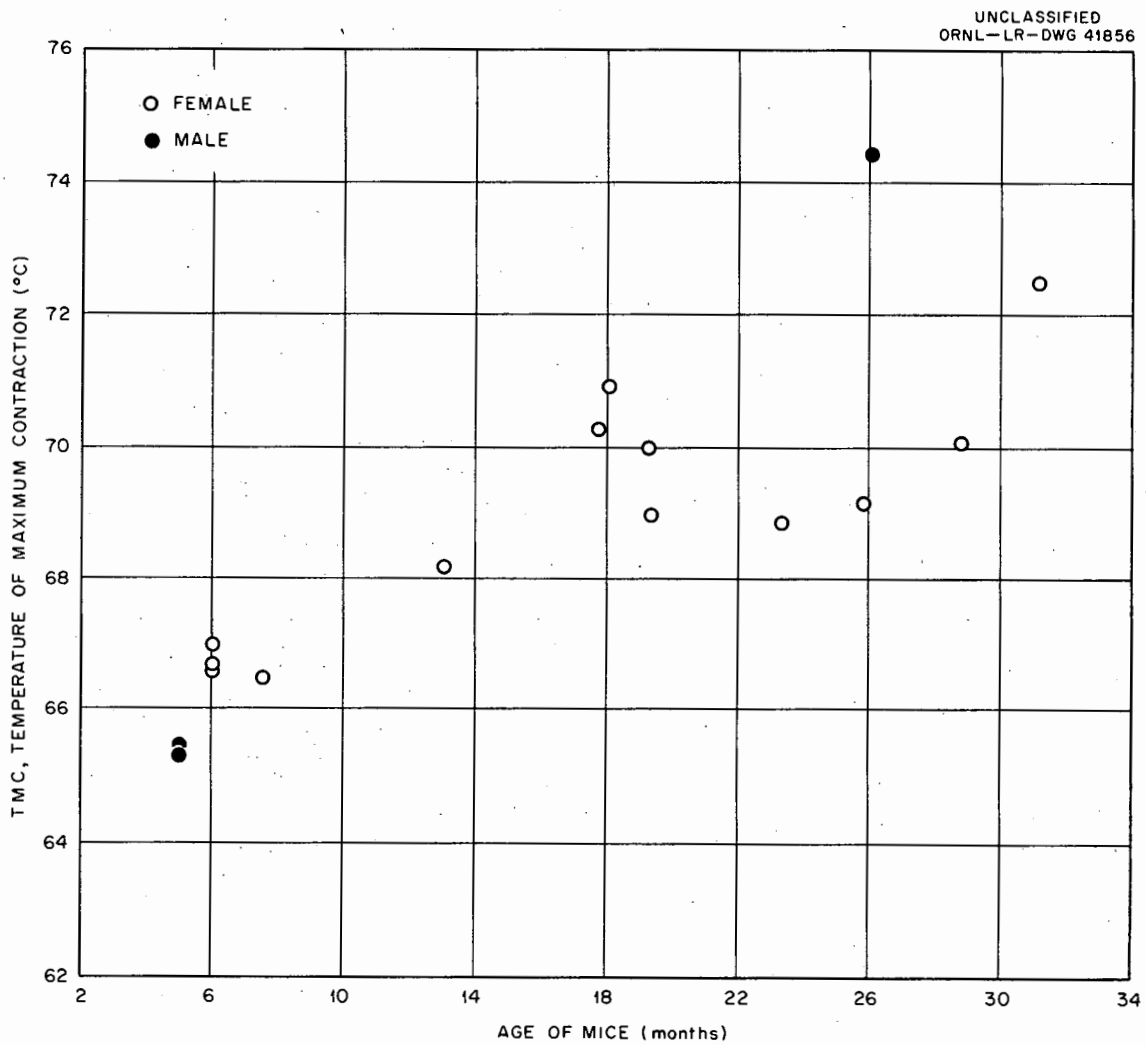

Fig. 17. Thermal Contraction as a Function of Age in Tail Tendon Fibers of RF Mice. Each point is the average of 2–3 fibers from one animal.

animals in the breeding colony, however, showed a much wider variation in TMC than those from nonbreeders of similar ages.

Discussion. — The method seems to be satisfactory for studying effects of aging and other factors on mouse tail tendon collagen. Although the data are as yet inconclusive, preliminary results do not indicate any striking effect of whole-body radiation. The results do suggest, however, that the reaction of the collagen to aging in breeding mice differs from that in nonbreeders, possibly because of the influence of hormonal or environmental differences.

Spontaneous and Radiation-Induced Nephrosclerosis in RF Mice

W. D. Gude M. B. Bradley T. Mack

Introduction. — Nephrosclerosis, a common complication of irradiation, occurs spontaneously in mice of the RF strain.

Results. — Although the kidneys of RF mice are normal early in life, nephrosclerosis begins to appear as early as six months after birth and is moderately severe by the twentieth month (Table 25). Preliminary data indicate that the lesion is advanced in onset and severity in mice exposed at 5–6 weeks of age to 450 r of whole-body X radiation.

Table 25. Nephrosclerosis in Relation to Age in RF Mice

Age (months)	Number of Mice*	Average Severity of Nephrosclerosis**
1–5	26	0
6–10	33	± to +
11–15	23	+
16 –	20	+ to ++

*Males and females in approximately equal numbers.

**Grades are as follows: 0, no lesion; ±, lesion barely detectable; +, slight but definite lesion; ++, lesion of moderate to advanced severity.

Discussion. — Although spontaneous and radiation-induced nephrosclerosis are morphologically similar, further detailed comparison of the two diseases is in progress to determine whether differences between them can be found. The common occurrence of nephrosclerosis as a spontaneous manifestation of senescence in RF mice contrasts with the situation in most other strains. For this reason, its spontaneous occurrence is noteworthy in relation to the interpretation that radiation-induced nephrosclerosis may be a premature senescent change.

CELL PHYSIOLOGY

N. G. Anderson
A. A. Barber[1]
C. J. Brokaw[2]
C. L. Burger[3]
W. D. Fisher[4]
E. C. Horn[1]
C. B. Norris
H. D. Swanson[5]
M. L. Anderson[1]
C. L. Byrd[6]
R. E. Canning
G. B. Cline[1]
J. A. Levinson
M. L. Barber[1]

Physical Properties of Rat Liver Nucleoli

N. G. Anderson C. B. Norris

Introduction. – Although the nucleolus seems to play a central role in the synthesis of RNA (and possibly also protein), this organelle has not previously been readily available to direct biochemical attack. A study of the physical properties of nucleoli in isolated nuclei was therefore undertaken to facilitate the isolation of these organelles to be attacked in a rational manner. If the nucleoli are to be isolated by the use of high shearing fields, factors that condense them into resistant masses are of value. Once isolated, conditions that will bring them into solution are required.

Results. – Nucleoli in isolated rat liver nuclei seem to be much more sensitive to low concentrations of mono- and multivalent cations than does chromatin. In very dilute solutions of diamines (0.001 M cadaverines), the nucleoli appeared as aggregates of small granules, which became more well defined as the concentration increased. With spermine and spermidine, a nucleolar condensing effect was observed at all concentrations studied (0.001–0.5 M) even where marked nuclear swelling was produced by charge reversal (0.2 M).

Nucleoli were found to dissolve in distilled water after condensation with simple mono- or diamines but not after treatment with agmatine, betaine

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[1] Consultant.
[2] Research Associate.
[3] Physiological Society Fellow.
[4] Research Participant.
[5] ORINS Fellow.
[6] Biology Student Trainee.

(carboxymethyltrimethylammonium hydroxide anhydride), spermidine, or spermine. Polyvalent anions have no condensing effect.

Discussion. – Isolation of nucleolar proteins and nucleic acids is now feasible by density gradient centrifugation, DNase digestion in the presence of sufficient condensing agent to keep the nuclei intact, followed by shredding of the nuclear envelope in a shearing field to yield a suspension of nucleoli or direct extraction with distilled water.

Microsomal Polysaccharides

N. G. Anderson

Introduction. – Previous collaborative work with the Immunology Group[7] showed that transplantation antigens are present in considerable amount in liver microsomes. Enzymatic studies suggested that the antigen was probably a polysaccharide. This work has been continued with an examination of polysaccharides split from microsomes by hot 1 M NaCl.

Experimental. – Batches of microsomes (100–250 g wet) from livers rapidly perfused with cold Locke's solution were extracted with 1 M ammonium formate and frozen until use. Polysaccharides and RNA were extracted by heating in 1 M NaCl, precipitated with acetone, and dialyzed. Contaminating glycogen was removed with salivary amylase, and RNA by either ribonuclease treatment or alkaline digestion at 37°C. Continuous curtain electrophoresis yielded four fractions, including a rapidly moving polysaccharide peak, two intervening peaks consisting almost entirely of protein, and a neutral polysaccharide peak. None of the fractions gave a metachromatic reaction with azure A. Assay for transplantation antigen activity in mice has been delayed because of a transient infection in the mouse colony. Isolation of similar materials from mouse liver is under way.

Discussion. – A method has been devised for isolating two polysaccharide fractions from rat liver microsomes.

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[7] T. Makinodan, N. Gengozian, and J. F. Albright, Ann. N.Y. Acad. Sci. 73, 757 (1958).

The Effect of Amines on the Structure of Isolated Nuclei

N. G. Anderson C. B. Norris

Introduction. — Studies on the condensation of chromatin by organic substances were stimulated by attempts to formulate the properties of the chromosomal condensant active during cell division. Several lines of evidence suggest that the condensant is a positively charged substance. The relation of nuclear volume to the concentration of a variety of amines has therefore been studied to determine (1) the effects of increasing the number of charges on the condensant, (2) the effects of intercharge distance, and (3) the reversibility of the volume changes when distilled water or a nuclear isolation medium is used.

Results. — n-Propylamine, taken as an example of an aliphatic monoamine, produced swelling in low concentrations (0.001–0.01 M) but little volume change in the range 0.025–0.36 M, closely paralleling the results previously obtained with NaCl and KCl. Diamines (diaminopropane, -butane, -pentane, -hexane, -heptane, and -decane) produced shrinkage and granulation in ~0.025 M solutions and less shrinkage as the concentration of the diamines was increased. The effects of diamines resemble those of magnesium and calcium. Condensation was readily reversed by either distilled water or the isolation medium.

Spermidine, with three positive charges, and spermine, with four, produce marked condensation at very low (0.001 M) concentrations, with marked swelling attributable to charge reversal at ~0.2 M. Condensation was not reversed by distilled water and was only slowly reversed by the phosphate-containing isolation medium. Chromosome-like structures were observed in a number of solutions but accurate counts were not possible. In a comparison of *Amphiuma* erythrocyte nuclei and rat liver nuclei, relatively few granules were seen in the former (2N = 24) whereas many were seen in liver nuclei (4N = 84), suggesting that the structures observed are chromosomal in origin.

Discussion. — The results obtained suggest that the prophase chromosome-condensing agent contains more than two charges if condensation is caused only by charge effects.

Studies on Isolated Flagella

C. J. Brokaw

Introduction. – Two new approaches to the problem of understanding the movements of flagella and cilia have recently been introduced. "Sperm models" have been prepared from many types of spermatozoa by extraction at 0°C in glycerol solutions. These models can be stored for several weeks at -20°C and can be partially reactivated when placed in solutions containing ATP. Other workers have studied the chemical composition and enzymatic activity of isolated sperm tail preparations and of flagellar preparations from algae. The biochemical similarities between flagella and muscles revealed by these studies have underlain recent speculations about the mechanism of flagellar movement.

Results. – The present work has aimed at combining these approaches by obtaining preparations of isolated flagella suitable both for biochemical analysis and study of the movement resulting after addition of ATP. The orange biflagellate alga, *Polytoma uvella*, was used as a source of flagella. The cells are grown in a liquid nutrient medium, harvested by centrifugation, washed, and suspended at 1°C in 3–4 volumes of a solution containing 70% v/v glycerol, 0.01 M $MgCl_2$, and 0.02 M Tris-thioglycolate buffer, pH 7.8. The mixture is rapidly cooled to -20°C, stirred vigorously, the cell bodies removed, and the flagella collected by centrifugation near -20°C. This procedure differs from earlier procedures used for spermatozoa and for muscles in that it involves a low ionic strength, does not eliminate divalent cations, and does not incorporate an extraction period at 0°C.

When diluted with a solution containing 0.02 M Tris-thioglycolate buffer (pH 7.8), 0.05 M KCl, 0.004 M $MgCl_2$, 5×10^{-4} M ATP, and 3% polyvinylpyrrolidone and observed with dark-field illumination at 18°C, a large proportion of the isolated flagella swim vigorously through the medium at speeds of 20–30 μ/sec, which is about one-sixth their normal speed.

ATPase activity of the isolated flagella was measured in the same medium used for observation of movement, except that the polyvinylpyrrolidone was eliminated. The isolated flagella continue to dephosphorylate ATP at the rate required to supply energy for normal movement,

even though only a small part of this energy is used in moving the flagella against the viscous resistance of the medium.

Although the rate of flagellar beating depends on the ATP concentration in the range 10^{-5} to 10^{-3} M, this is independent of the rate of dephosphorylation of ATP, since the K_m of the flagellar ATPase is near 10^{-5} M, as in muscle.

With ADP, the flagella beat, and liberate inorganic phosphate at the same rate as with ATP, because the flagella possess an adenylate kinase. Although other nucleoside triphosphates are dephosphorylated at about one-fifth the rate observed with ATP, they do not induce movement, nor do the corresponding diphosphates. The nucleoside requirement for flagellar movement is therefore more specific than that for muscular movement, presumably because of the more complicated nature of the cycle of flagellar contraction and relaxation. The adenylate kinase of the flagella might be involved in the relaxation phase of this cycle.

In the near future, the adaptability of these techniques to the green alga, *Chlamydomonas moewusii*, will be examined, to investigate the differences between isolated flagella from motile and nonmotile mutants of this organism.

The Antioxidant Activity of Vertebrate Blood

A. A. Barber

Introduction. – The aerobic incubation of rat brain or rat liver homogenates results in the formation of lipid peroxides.[8] Mammalian intestinal mucosa, ascitic fluid, blood, and bone marrow inhibit this oxidation. Whole-body X irradiation with 800 r destroys the antioxidant activity of the intestinal mucosa but has no effect on the activity of the other tissues examined,[8] indicating a difference in the mechanism of tissue antioxidant activity. Work on characterizing the inhibitors in both the intestinal mucosa[9] and blood has been started. This report describes preliminary results on the antioxidant activity of vertebrate bloods.

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[8] A. A. Barber and K. M. Wilbur, Radiation Research 10, 167 (1959).

[9] A. Ottolenghi and F. Bernheim (work in progress at Duke University).

Results. — All types of vertebrate blood plasma examined inhibited peroxide formation in rat brain homogenates. Rat, turtle, and frog plasma inhibited at slightly lower concentrations than did the plasma of the domestic fowl. The activity was nondialyzable in all plasma types. Separation of a number of plasma proteins on continuous-flow paper electrophoresis resulted in the following characterization of activity: The antioxidant activity of rat plasma is localized in a single fraction. The component involved appears to be a globulin protein, presumably a β-globulin. With turtle plasma, two main peaks were obtained in the electrophoretic separation, and antioxidant activity was present in both. Work is in progress on the characterization of the activity in fowl and frog plasma by this technique.

Rat plasma inhibits peroxide formation by an iron-binding mechanism. This is indicated by the following two lines of evidence: (1) small quantities of iron reverse the inhibitory effect and (2) plasma from rats injected with iron (1–6 hr previously) failed to inhibit peroxide formation. Presumably, other vertebrate bloods act by a similar mechanism. This assumption is currently being investigated by determining the iron reversibility of each of the plasma types, as well as the active fractions from these samples.

Proteins of Rat Liver Microsomes

E. C. Horn M. L. Barber

Introduction. — The protein components of the rat liver microsome include constituents of the membranous part of the endoplasmic reticulum, structural protein of the ribonucleoprotein particles, proteins in various stages of synthesis, and enzymes that may or may not participate in structure formation. Previous studies in this laboratory have shown that dialyzable peptides and nondialyzable protein could be extracted from microsomes in 1 M NaCl or ammonium formate. A systematic examination of proteins extracted from microsomes has been undertaken to determine whether (1) structural components could be isolated and (2) large intermediates in protein synthesis could be found.

Results. – Microsomes were isolated from sucrose homogenates of rat liver and extracted with 0.14 M NaCl, deoxycholate, 1 M NaCl, and dilute NaOH. The products of these extracts that are soluble at or below pH 9.5 were analyzed electrophoretically and ultracentrifugally. Each extraction medium seems to release relatively few components and, in most instances, one protein seems to be released in greater quantities than the rest, this major component varying with the type of extraction medium.

Parallel attempts to isolate and to extract microsomes from the Ehrlich ascites tumor cells of mice have shown considerable differences in the two types of microsomes. The isolation is more difficult to sediment in the same media used for liver products, the yields are lower, and some of the extractions are accompanied by the release of relatively large amounts of material that produces electrophoretic anomalies suggestive of lipoprotein.

A Comparison of the Effect of X Rays, Gamma Rays, and Neutrons on Rat Thymus Nucleoprotein

W. D. Fisher C. L. Burger

Introduction. – A previous study of the effect of X rays on the viscosity of nucleoprotein extracts[10,11] has been extended to include a comparison of the effects of different radiations.

Results. – Dose curves for nucleoprotein in 1 M NaCl–Na phosphate buffer, pH 6.82, were similar in shape to those obtained previously. The dose required to reduce the apparent viscosity of the extracts by 50% was 85 rads for 250-kv X rays, 120 rads for Co^{60} gamma rays, and 250 rads for 14-Mev neutrons.

Discussion. – The relative effectiveness of neutrons compared to X rays was ~0.3, a low figure when compared to the RBE's for the two radiations in most biological materials. The higher RBE for neutrons has been attributed to the disruption of organized cellular structures by densely

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[10] W. D. Fisher, N. G. Anderson, and K. M. Wilbur, Exptl. Cell Research (1959), in press.

[11] W. D. Fisher, Biol. Semiann. Prog. Rep. Feb. 15, 1957, ORNL-2267, p 61.

ionizing tracks. The results obtained support the conclusion that the extraction procedure disperses the multistranded chromosomal material into a solution of strands consisting of DNA linked end to end by protein.

Zone Electrophoresis of Soluble Tissue Proteins

H. D. Swanson

Introduction. – Zone electrophoresis is being used to isolate for study some of the fractions shown by analytical electrophoresis of the supernatant proteins of rat liver, kidney, brain, and testis.

Methods and Results. – Investigation of several techniques indicated that the most satisfactory method of zone electrophoresis is that in which cotton powder is used as a stabilizer. The protein solution is first passed through a column of this material, without electrophoresis. Approximately 4% of the protein of the rat liver supernatant is adsorbed on the column and can be removed by alkali. This fraction moves as one peak in moving boundary electrophoresis, at pH 7.5, with a mobility of 4×10^{-5}.

Further studies of its electrophoretic and ultracentrifugal properties are being made.

The protein not adsorbed onto the cotton powder column is placed as a narrow zone in a column electrophoresis apparatus and subjected to electrophoresis. On elution, three major fractions and several minor ones can be demonstrated by determining absorption at 280 mμ. Physical and chemical properties of these fractions are being studied.

Conclusion. – Electrophoresis in a column of cotton powder is proving a satisfactory method for isolating the electrophoretic fractions of tissue proteins.

Effect of Heat on Nucleoprotein in 1 M NaCl

W. D. Fisher C. L. Burger

Introduction. – The effect of heat on aggregates of protein and DNA previously shown to exist in crude 1 M NaCl extracts of thymus nuclei[12]

[12] W. D. Fisher, N. G. Anderson, and K. M. Wilbur, Exptl. Cell Research (1959), in press.

may provide an indication of the nature of the protein-DNA linkages. Changes in viscosity as a function of temperature, time, and concentration of nucleoprotein were therefore examined in detail and an equation derived that describes the effects observed.

Results. – Heating at temperatures from 65 to 95°C causes a decrease in the viscosity of crude fresh nucleoprotein extracts. The extracts were prepared by treating thymus homogenates for ~12 hr at room temperature (~25°C) with 1 M NaCl–Na phosphate buffered at pH 6.82. Viscosity was measured with a top loading capillary viscometer. Figure 18 shows some typical data.

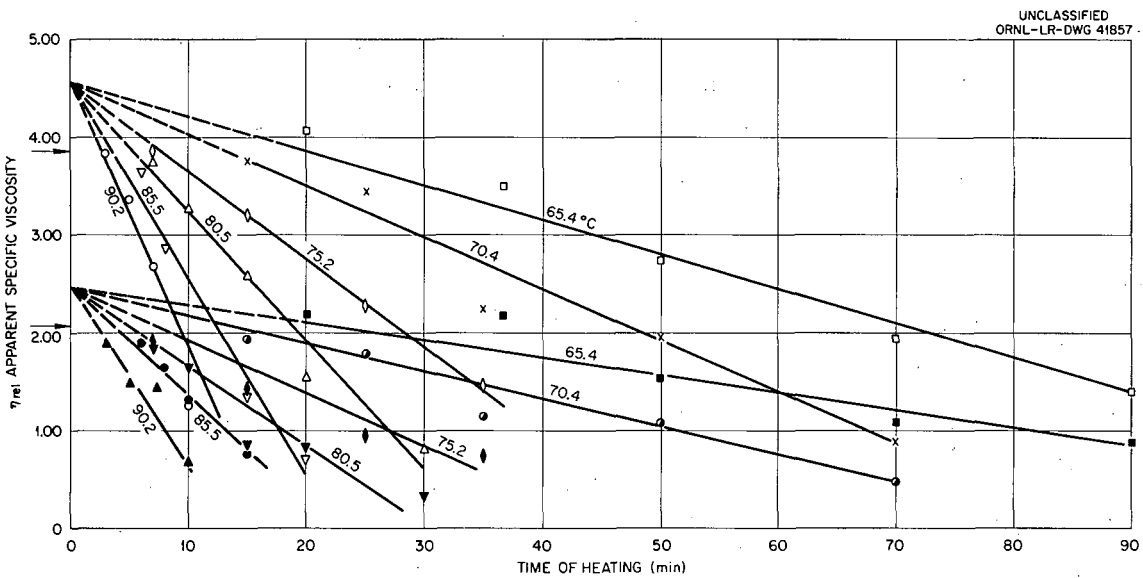

Fig. 18. Apparent Specific Viscosity as a Function of Time of Heating at Various Temperatures. The upper set of curves is for a preparation containing 335 μg of DNA per ml; the lower set for a preparation with 23 μg of DNA per ml. Arrows indicate viscosity of unheated preparation.

The rate of change of viscosity with time at a given temperature increased with concentration. However, the temperature dependence of rate was constant from ~19 to 60 μg of DNA per ml. The data for temperatures between 65 and 95°C can be described by the equation

$$\frac{V}{C^{1.7}} = \alpha e \exp\left(-\frac{\Delta E}{RT}\right) ,$$

where V is the rate, α is a constant, R is the gas constant, and T is the

absolute temperature, C is the DNA concentration, and ΔE is the activation energy and equals 23,700 cal. For T = 288°K, $V/C^{1.7} = 0.2$.

Discussion. — The results differ in two respects from published results on "thermal denaturation" of DNA. The activation energy is considerably smaller, 23,700 cal vs 60,000–90,000 for DNA. Also, the rates for thermal denaturation in DNA are not measurable before about 80°C. In the nucleoprotein extracts, a decrease in viscosity occurs at 65°C. Thus the decrease in viscosity observed in the extracts does not appear to be associated with denaturation of the DNA. The results suggest that either the protein or DNA-protein linkages are involved.

The large concentration dependence of rate would suggest an extremely rapid process at concentrations present in the nucleus. This of course assumes that the relationship seen here in 1 M NaCl and over a limited concentration range still holds in the nucleus. In agreement with this idea, nuclei heated at 70°C or above for as little as 2 min yield extracts of very low viscosity.

Rat Serum Macroglobulins

R. E. Canning T. Makinodan[13] N. G. Anderson

Introduction. — The recent discovery in this laboratory that serum macroglobulins coprecipitate with the antigen-antibody complex (see Immunology Section) prompted a search for better methods for isolating and characterizing these substances.

Results. — The macroglobulins of rat serum, when separated by repeated ultracentrifugal sedimentation, show three main peaks with sedimentation coefficients of 35, 26, and 19 at 20°C. Since this method of preparation is very time consuming and does not yield a pure preparation, other techniques have been investigated. With a combination of ammonium sulfate precipitation and paper electrophoresis, a macroglobulin having an $S^0_{20,w}$ value of 20.15 and a molecular weight of 800,000 has been isolated with only a few per cent contamination by more slowly sedimenting proteins.

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[13] Radiation Immunology Group.

Discussion. — The isolation of a serum macroglobulin in a high state of purity enables us to determine whether or not the recently described tissue macroglobulins[14] are identical with those obtained from serum, and whether they also coprecipitate with the antigen-antibody complex. If the latter is the case, tissue macroglobulins may be causally related to cellular serological responses.

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[14] N. G. Anderson and R. E. Canning, Exptl. Cell Research 17, 465 (1959).

NUCLEIC ACID CHEMISTRY

W. E. Cohn
F. J. Bollum
J. X. Khym
T. Iwamura[1]
A. J. Bandy
G. E. Houts

Pseudouridine

W. E. Cohn A. J. Bandy

The investigation of the exact structure of pseudouridine and the substances produced from it by simple chemical treatment has continued. The results of nuclear magnetic resonance examination of the four nucleoside forms described in the previous report and termed A_F, A_S, B, and C (C being the natural ribofuranosyl form) indicate a C6 proton but no C5 proton in all forms. From this alone it may be concluded that the changes that convert the C form into the others are limited to the ribosyl part of the molecule. The nuclear magnetic resonance spectra bear out this conclusion, for those sections of the spectra derived from the sugar portion show definite changes from those of pseudouridine C or of uridine. The last two, however, show identity among the protons at all positions in the sugar except at C1'. A difference here was anticipated, inasmuch as uridine has a nitrogen atom attached to C1' whereas pseudouridine has a carbon atom.

Work on the nucleotide forms has progressed in the direction of preparing and identifying each possible combination of nucleoside and phosphate. Inasmuch as acid treatment of the normal a and b pseudouridylic acids would be expected to give many substances (the above four nucleosides with phosphate in every possible position), we have resorted to treatment with alkali. This should, and indeed appears to avoid phosphate migration; the changes seem limited to the sugar moiety. Many of these forms have now been separated by a combination of ion exchange and paper chromatography (neither is sufficient alone) to yield what appears to be a minimum of three phosphate isomers of the A_S nucleoside and that many or more phosphate isomers of the B nucleoside. At least one nucleotide that can be dephosphorylated

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[1]Visiting investigator from abroad.

to A_F has been found, thus indicating that this nucleoside also may occur in nucleotide form and may be created without loss of phosphorus.

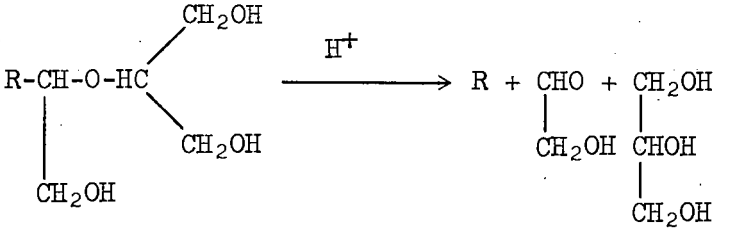
With adequate amounts of each of these forms of pseudouridylic acid in hand, we may proceed to precise identification of each one.

Reactions of Periodate-Oxidized Nucleosides

J. X. Khym

Structural studies on pseudouridine have led us to characterize the dialdehydes that are formed when ribonucleosides react with periodate. The use of phenylhydrazine to degrade and identify the component parts of uridine and pseudouridine dialdehyde has been reported.[2] Since then it has been shown that (1) the dialdehydes are more stable in alkali than has been generally thought; (2) they react essentially quantitatively with $NaBH_4$ to form dialcohols; (3) hydrolysis of the dialcohols with mineral acids yields two- and three-carbon residues as well as the free bases; (4) these carbohydrate residues can be identified by the uptake of periodate with release of CH_2O, by the action of phenylhydrazine, and by paper chromatography.

Solutions of uridine and pseudouridine, after oxidation with periodate and reduction with $NaBH_4$, form the corresponding dialcohols in good yield. These alcohols give less than 10 mole % of free glycerol after treatment with alkaline $NaBH_4$, indicating the intact structure, move slower in butanol-water than the corresponding dialdehydes (e.g., the dialcohol of uracil moves four times slower than its dialdehyde), are stable to phenylhydrazine, and are hydrolyzed by mineral acid in accordance with Eq. (1).



(1)

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

Hydrolysis appears complete in 6 N H_2SO_4 at 120°C for both uridine and pseudouridine, as indicated by the quantitative yield of glycerol (which identifies the three-carbon fragment of the dialcohols) obtained from both compounds. This procedure destroys the two-carbon fragment from uridine but gives complete recovery of uracil. The base fraction from pseudouridine has not as yet been identified; we presume that it remains attached to the two-carbon fragment. In 1 N H_2SO_4 (2 hr flux) the dialcohol of uridine hydrolyzes only partially, and all the products of Eq. (1) have been identified.

Similar tests on pseudouridine are planned. Preliminary experiments indicate that, if the reductions of the dialdehydes are carried out at pH 4–5, a selective reduction of the distal aldehyde group occurs, leaving the proximal group reduced. The characteristics of these semialcohols are now under investigation.

DNA Synthesis by Mammalian Enzymes

F. J. Bollum

A rather disturbing observation made early in the fractionation of calf thymus polymerase was that a "native" DNA from calf thymus would not prime the reaction, whereas a DNA preparation of rather low viscosity from salmon sperm was very effective. Lehman[3] reported that heating calf thymus DNA for 10 min at 100°C doubles the rate of DNA synthesis by *Escherichia coli* polymerase. Since the polymerase from *E. coli* readily utilizes unheated, presumably native DNA preparations, it was not possible to make a clear-cut distinction of what the heating step had done to the native DNA.

The primer requirement of a deoxynucleoside triphosphate-polymerizing enzyme (polymerase) isolated from calf thymus gland has now been studied in greater detail. Heat denaturation of such preparations can bring about the conversion of primer. Lack of priming by native (two-stranded) DNA's and the thermal conversion to primer has been found in T1, T2, T4, T6, *Diplococcus pneumoniae*, and *Hemophilus influenzae* DNA as well as calf thymus DNA. Single-stranded DNA from bacteriophage ΦX-174 (courtesy of R. L. Sinsheimer) is active as primer without heating.

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[3] I. R. Lehman, Ann. N.Y. Acad. Sci., in press.

From these results, two conclusions can be drawn. First, calf thymus polymerase (although far from pure and considerably less active than the E. coli polymerase) does not exhibit enzyme activity capable of converting nonpriming DNA to primer DNA. This enzyme therefore provides an adequate test for the priming function of a DNA preparation. The second conclusion is that primer DNA may be formed by thermal activation of "native" DNA preparations.

It is suggested that priming activity may be a significant biochemical test for what is currently termed "denatured DNA." A question of semantics is also raised, since at least one DNA that is unquestionably native – that from ΦX-174 coliphage – has the priming activity of denatured DNA and does not require thermal activation to give maximum priming with E. coli polymerase or our thymus polymerase. An answer to the question of what is really "native" and what is really "denatured" is not obvious, unless one is willing to consider the enzymatic synthesis of polydeoxynucleotide material in vitro as an unnatural phenomenon.

If primer DNA is actually single-stranded DNA and if the synthetic reaction produces double-stranded (native) molecules, then the idealized reaction should cease when the primer DNA has been doubled. DNA increases greater than twofold would be possible only with enzyme systems containing nucleases capable of converting native (two-stranded, nonpriming) DNA to denatured (single-stranded, priming) DNA. Since calf thymus polymerase alone does not activate nonpriming DNA, it will be of considerable interest to see if such a reaction limit is demonstrable after further purification.

Among the many experiments possible in a system that distinguishes so clearly between primer and nonprimer DNA preparations is the correlation of primer activity with the physical properties of such preparations, as reported by Zamenhof et al.[4] and Rice and Doty.[5] Other possible lines of experimentation include resynthesis of transforming activity with heat-inactivated transforming DNA as primer.

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[4] S. Zamenhof, N. E. Alexander, and G. Leidy, J. Exptl. Med. 98, 373 (1953).

[5] S. A. Rice and P. Doty, J. Am. Chem. Soc. 79, 3937 (1957).

Nucleic Acid Metabolism of *Chlorella* Cells

T. Iwamura

Introduction. — By a synchronous culture technique, nucleic acid metabolism of *Chlorella* cells was investigated. Both RNA and DNA change not only in their content in the cell but also in their base compositions during the life cycle. The results indicated existence of heterogeneity of nucleic acids in the cell and changes of the species in their amounts with the changes of cellular metabolism.

Since those results were obtained on chemical fractions of the whole cells, the current study has been directed toward physical fractionation of *Chlorella* cells into the cellular components and isolation of high-molecular-weight nucleic acids in each fraction to see how nucleic acids are distributed within the cells.

Results. — Over 90% of *Chlorella* cells (*Chlorella ellipsoidea*) can be disrupted with the French pressure cell in a salt solution (0.07 *M* KCl, 2×10^{-3} *M* Ca^{++}, 5×10^{-3} *M* Mg^{++}). The homogenates are subjected to a differential centrifugation, and three fractions are obtained after the whole cells and cell walls are eliminated. The three fractions are: P1, dark green pellet by centrifugation at 20,000 × *g* for 20 min; P2, green-tinted pellet by centrifugation at 105,000 × *g* for 90 min; and S, clear, slightly brownish supernatant. P1 consists mainly of chloroplasts, but it may probably contain mitochondria-like particles. P2 consists exclusively of microsomal or ribonucleoprotein particles.

Nucleic acids can be extracted from each fraction by a modified Kay and Dounce method in a yield ~70 to 90%.

RNA is found in all the three fractions, but DNA is found in P1 and S fractions. The RNA in the S fraction contains more cytidylic acid and less adenylic acid than that in the P2 fraction. Also, DNA's in the two fractions show some differences. It seems that the nucleic acids in the three fractions not only differ in their base compositions but also their relative amounts vary during the life cycle.

The nucleic acid pattern of synchronized *Chlorella* cells harvested at various stages of its life cycle is now being examined.

NUCLEIC ACID ENZYMOLOGY

E. Volkin J. L. Countryman[1]
L. Astrachan M. H. Jones
K. B. Hudgins

Growth and Nucleic Acid Metabolism of Glucose-Starved *Escherichia coli*

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

In studies previously described, we investigated some features of the nucleic acid metabolism of bacterial cultures in which growth is just beginning. These cultures were obtained by diluting, in fresh medium, cell suspensions from 24-hr cultures. Such old cultures are not satisfactory because they represent a heterogeneous population in which the cells are in various physiological states and many are no longer viable. We decided that a more homogeneous system might be obtained from a culture in which the exhaustion of a nutrient such as glucose has resulted in an abrupt cessation of logarithmic growth. The present report describes some observations of *Escherichia coli* B under conditions of glucose starvation and of the behavior of starved cells upon their introduction into medium in which they can grow.

An overnight culture of *E. coli* B in synthetic medium containing 4 mg of glucose per ml is centrifuged and the cells resuspended in synthetic medium containing 0.5 mg of glucose per ml to give an optical density of 0.025 (Beckman colorimeter, green filter). Incubation is at 37°C with aeration. For 3.5–4 hr, growth as measured by optical density is logarithmic; it then slows, and after 4.5–5 hr, there is no further change in the optical density for as long as 24 hr. The numbers of viable bacteria, as determined by colony counts, increase logarithmically for 2–3 hr, then appear to decrease for about 1.5 hr, and then increase again rapidly for another 2 hr up to a titer of $4–5 \times 10^8$ cells/ml. This remains quite constant for 8–10 hr more and then begins to decline. The apparent loss in viability at 2–3 hr may indicate an increased fragility as the

[1] Research Associate.

cells begin to starve, a state from which they later recover. Direct microscopic counts are now being carried out to check these results.

The RNA and DNA contents of these cultures both increase normally for about 4 hr. During the following 2 hr, however, the DNA at least doubles while the amount of RNA decreases 10–12%. For at least 12 more hours, the DNA content remains about constant, but the RNA continues to decrease. This results in a rapid decline in the RNA/DNA ratio during the period when the cells are apparently just exhausting the available glucose and a lower, but continual, decline thereafter.

Subcellular fractions were obtained as previously by alumina grinding and subsequent differential centrifugation to yield P_1, pellet at 20,000 × g for 20 min; P_2, pellet at 100,000 × g for 90 min; and S, final supernatant fluid. The distribution of RNA associated with the fractions appears to change after the cells have exhausted the glucose. Whereas less than 5% of the total RNA is found in the P_1 fraction from a growing culture (Table 26, 2.5 hr), 35–40% of it is found there in the starving cells (Table 26, 4 and 5.5 hr).

Table 26. Distribution of RNA in Subcellular Fractions Obtained from a Culture Grown with Limited Glucose Supply

Fraction	Percentage of Total RNA		
	Time		
	2.5 hr	4 hr	5.5 hr
P_1	5	38	35
P_2	30	26	26
S	65	36	39

Phosphorus-32 was incorporated normally by the cells at 2.5 hr but very little (in a 5-min incorporation period) by those at 4 and 5.5 hr. The metabolic activity of the RNA fractions in the starving cells could therefore not be investigated by use of the label.

Cells from the starved cultures proved more satisfactory as inocula for fresh medium than those used before in that nucleic acid synthesis

was more extensive and results more reproducible. When cultures grown as described are centrifuged after 16 hr and the cells diluted 1:5 in fresh medium containing 4 mg of glucose per ml, the RNA content begins to increase immediately and P^{32} incorporation into it is rapid. An increase in DNA begins only after 25–30 min, with a corresponding extent of P^{32} incorporation. The amount of DNA doubles within ~1 hr after it begins to increase.

In one set of experiments, cultures were allowed to grow in fresh medium for 15, 25, and 35 min, allowed to incorporate P^{32} for 5 min, ground with alumina, and separated into P_1, P_2, and S fractions. The relative specific activities of the RNA contained in the fractions were as follows: P_1:P_2:S at 15 min, 2.4:1.0:2.7; at 25 min, 2.4:1.0:3.1; and at 35 min, 3.8:1.0:2.3. This suggests that the metabolic activities of the RNA fractions are not alike at all times during the early period of growth under the present conditions. Further investigation may permit more distinct separation of these activities and their function(s).

Composition of Metabolically Active RNA from Phage-Infected Bacteria

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

Data from our investigations on the metabolism of RNA in bacteriophage-infected *E. coli* have consistently indicated that, shortly after infection, a new RNA is synthesized whose composition bears a striking resemblance to the DNA of the infecting bacteriophage. In experiments where subcellular particulates were isolated, we found rapid metabolic activity in only a small part of the infected cell's RNA. In other experiments, with cells inhibited by chloramphenicol, the new RNA had to be synthesized before the infected cell could make DNA destined for incorporation into bacteriophage particles.

In all these experiments, cells were isotopically labeled by adding P^{32} orthophosphate to the medium, and the evidence for the new RNA was derived from data obtained from alkaline hydrolysis of isolated radioactive RNA and chromatographic separation of the resultant 2',3' mononucleotides. When RNA from uninfected cells was used, the four mononucleotides had equal specific radioactivities. The RNA from infected

cells, however, yielded mononucleotides with specific radioactivities that varied exactly in proportion to the composition of the DNA of the infecting bacteriophage.

We recognized that RNA is probably synthesized from 5' mononucleotides and not 2',3' mononucleotides. Therefore, when RNA is hydrolyzed by alkali, the phosphorus that entered RNA attached to one mononucleotide is recovered on a neighboring nucleotide. This raises the question whether the different specific radioactivities truly reflect the composition of the RNA synthesized after infection. If they did, however, the same relative specific radioactivities should be obtained if the RNA is hydrolyzed to 5' mononucleotides. This report presents the results obtained when radioactive RNA isolated from uninfected, T2 bacteriophage-infected, or T7 bacteriophage-infected cells was hydrolyzed by snake venom diesterase to yield 5' mononucleotides.

Log-phase E. coli was grown and infected in nutrient broth or synthetic medium. Two to three minutes after infection, P^{32}-labeled orthophosphate was added to the medium and samples were taken at various intervals for isolation and hydrolysis of RNA. In all experiments the isolated RNA was divided; half was hydrolyzed to 2',3' mononucleotides and half to 5' mononucleotides. Regardless of the interval between adding P^{32} and taking the sample, the 2',3' mononucleotides always maintained their characteristic relative specific radioactivities. This was not always the case with the 5' mononucleotides. If samples were taken 2–3 min after P^{32} addition, the ratio between the specific radioactivities of adenylic and guanylic acids was that seen with 2',3' mononucleotides, but the uridylic to cytidylic ratio was higher than that obtained with 2',3' mononucleotides. With longer periods of incubation with P^{32}, however, the ratios of specific radioactivities of 5' mononucleotides duplicated the ratios obtained with 2',3' mononucleotides.

Aside from the results observed after short incubations with P^{32}, the data obtained with 5' mononucleotides duplicate the data with 2',3' mononucleotides. The most probable explanation for the high uridylic and low cytidylic specific activities observed in short incubation periods is that the precursors to RNA are not equally labeled. This must be verified by

investigation of the precursor material, but we expect that uridylic acid, which for some enzyme systems is a precursor to cytidylic acid, will be labeled with P^{32} more rapidly. With longer incubations with P^{32}, the RNA precursors would have equal specific radioactivities, so that the counts associated with each of the four 5' mononucleotides would reflect the composition of the newly synthesized RNA. The 2',3' mononucleotides from RNA do immediately present the specific activity ratios characteristic of the particular RNA used. This suggests that the differences in specific activities of precursor material are smoothed out by alkaline hydrolysis distributing the high and low specific activity phosphorus to neighboring nucleotides.

In summary, the data obtained support the conclusion that a new RNA is synthesized after infection and that the composition of this RNA may be inferred by comparison of the ratios of specific radioactivities of the RNA mononucleotides.

CHEMICAL PROTECTION AND PROTEOLYTIC ENZYME STUDIES

D. G. Doherty C. K. Myers
J. D. Bales, Jr. M. T. Cohn[1]

Structure and Activity in Radiation-Protective Sulfhydryl Compounds

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

Introduction. — Interest in chemical protective agents has prompted a direct comparison of the two most active ones — mercaptoethylamine (MEA) and S,2-aminoethylisothiourea (AET) — in the same strain of mice under the same conditions. Both compounds were administered either orally or intraperitoneally to (101 × C3H)F_1 mice ($LD_{50/30}$, 710 r) and compared at various radiation and compound dose levels. In addition, preliminary studies were made on the recovery of bone marrow in MEA-treated, X-irradiated mice.

Results and Discussion. — The dose-response curves for both AET and MEA are presented in Fig. 19. AET was rearranged to mercaptoethylguanidine (MEG) with NaOH, and the concentrations in Fig. 19 are the amounts of MEG injected into the mice. MEA at the maximum dose, 240 mg/kg, gave an $LD_{50/30}$ of 1325 ± 25 r. At this compound dose level, there were approximately 15% deaths caused by toxicity within the first day. MEG at the maximum dose, 152 mg/kg (or 360 mg/kg as AET), yielded an $LD_{50/30}$ of 1425 ± 25 r with less than 5% deaths attributable to toxicity of the compound. Comparison of the two lines shows that, on a weight basis, MEG is about 2.5 times as effective as MEA whereas, on a molar basis, it is a little over three times as effective, the molecular weights being in the ratio of 77 for MEA to 119 for MEG. MEA given orally 30 min before irradiation (400 mg/kg) yielded an $LD_{50/30}$ of 925 r; under the same conditions, MEG (270 mg/kg — 640 mg/kg AET) gave the same 1425 r LD_{50}. MEG remained fully effective for 1 hr after oral administration.[2] Initial results in the bone marrow system indicate that the relative effectiveness is in about the same ratio as the $LD_{50/30}$, 2.5 times as much MEA being required to match the recovery rate for MEG.

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[1]Biology Student Trainee.

[2]D. G. Doherty *et al.*, *Biol. Semiann. Prog. Rep. Aug. 15, 1957*, ORNL-2390, p 78.

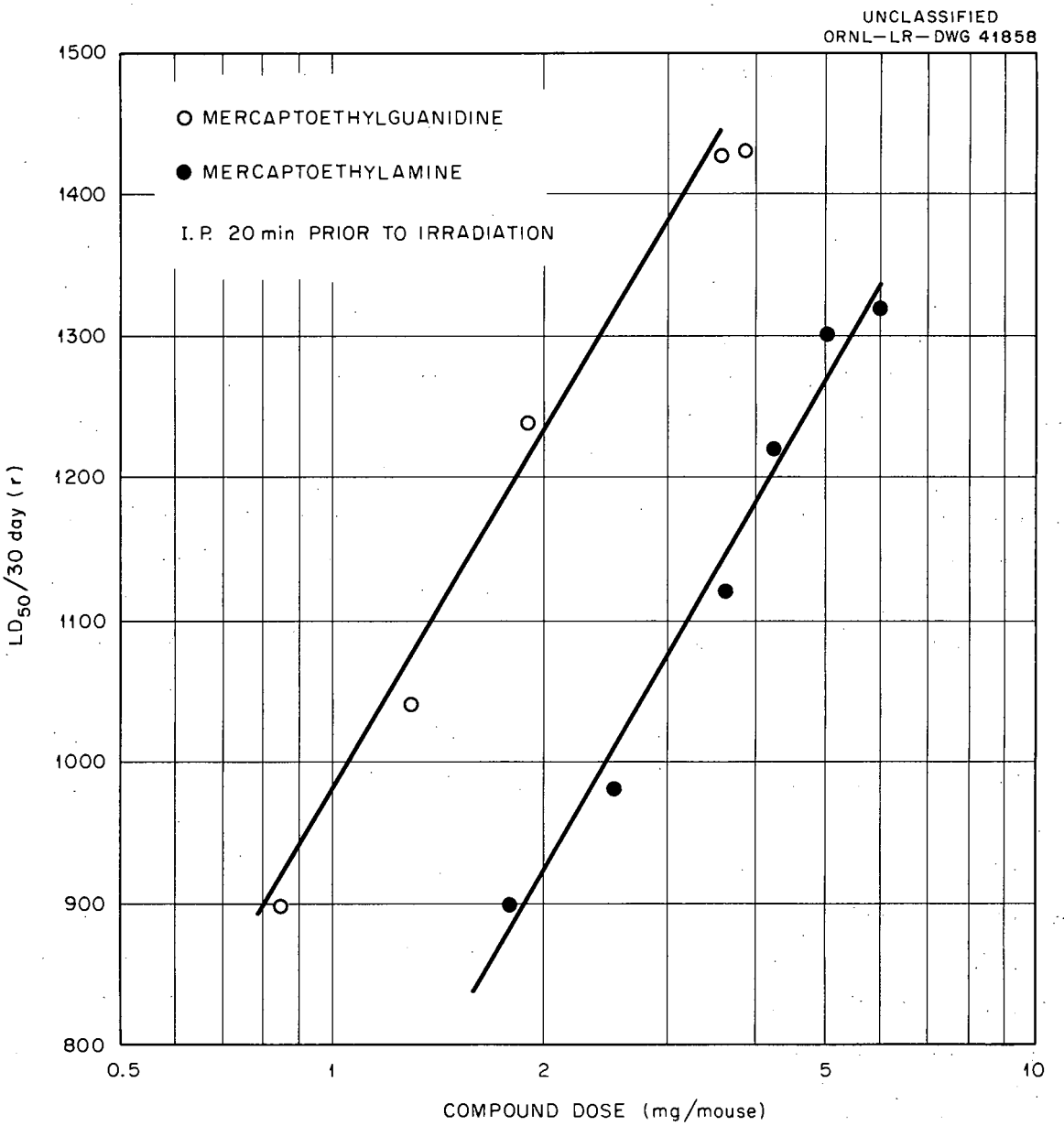

Fig. 19. Dose-Response Curves for AET and MEA.

ENZYMOLOGY

G. D. Novelli
F. T. Kenney
H. D. Peck
G. D. Duda
M. P. Stulberg
K. B. Jacobson
P. Rogers[1]
R. W. Bernlohr[2]
T. Kameyama[3]
K. Tonomura[3]
O. J. Koeppe[4]
R. Rabson[4]
W. E. Jefferson[4]
A. N. Best
P. F. Lincoln
J. L. Yundt[5]
R. M. Flora[5]
J. Davis, Jr.

Synthesis of Ornithine Transcarbamylase by Disrupted Protoplasts

P. Rogers, Jr.

Introduction. – In order to study the *de novo* synthesis of a complete protein, we have investigated the formation of ornithine transcarbamylase (OTC) in *Escherichia coli*. Studies of synthesis of this enzyme in cells and protoplasts[6,7] and in concentrated cell suspensions[8] have been reported previously.

We have now extended these studies to protoplasts mechanically disrupted in the Hughes press. Other workers, using water-lysed protoplasts, have demonstrated an ATP requirement for enzyme synthesis[9] and C^{14} amino acid incorporation into protein.[10] Our experiments demonstrate new requirements for OTC synthesis in disrupted protoplasts not found in cells or protoplasts. In addition, preliminary fractionation experiments indicate that both a particulate and a soluble fraction are necessary for OTC synthesis in these disrupted protoplasts.

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[1]Fellow of The American Cancer Society.

[2]U.S. Public Health Service Fellow.

[3]Visiting investigator from abroad.

[4]Research Participant.

[5]Biology Student Trainee.

[6]P. Rogers and G. D. Novelli, Biochim. et Biophys. Acta 33, 423 (1959).

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

[8]P. Rogers, Jr., Biol. Semiann. Prog. Rep. Feb. 15, 1959, ORNL-2702.

[9]E. M. Roth and B. Magasanik, Abstr. 136th Natl. Meeting Am. Chem. Soc., 1959.

[10]B. Nisman, Biochim. et Biophys. Acta 32, 18 (1959).

Results. – Cells grown to log phase in the presence of arginine[6] are treated with lysozyme and Versene to produce protoplasts and then disrupted in the Hughes press block at liquid nitrogen temperature in the presence of 0.33 M sucrose. Examination with phase-contrast microscope shows that this treatment converts the protoplasts to large pieces and ghosts. The disrupted material is suspended in 2.0 ml at about 5 mg of protein per ml and incubated at 37°C for from 60 to 90 min. OTC activity increases up to twofold during this period and then begins to decrease with further incubation. This increase in OTC activity occurs only in the presence of amino acids, ATP, Mg^{++}, and glucose. Additional OTC is synthesized when polyvinylsulfate is added. Polyvinylsulfate inhibits the intracellular RNase of *E. coli* W at the concentrations needed to stimulate OTC synthesis. Also, addition of pancreatic RNase inhibited formation of OTC during incubation for 90 min.

Optimal concentration for ATP is 5×10^{-3} M in this system; but, in the presence of phosphoenolpyruvate and pyruvate kinase as an ATP-generating system, OTC formation is optimal with 10^{-4} M ATP. Other sources of high-energy phosphate, such as creatine phosphate together with creatine transphosphorylase or hexosediphosphate or phosphoglyceric acid, have not duplicated the ATP requirement in our experiments. In some experiments, addition of the triphosphates of uridine, cytidine, and guanosine together with ATP has significantly increased the OTC formed.

Mn^{++} did not stimulate enzyme formation, as Nisman[10] reported for water-lysed protoplasts. Addition of a large number of cofactors and vitamins to the disrupted preparation has not further augmented our system. The OTC formation observed is, however, completely inhibited by addition of chloramphenicol and incubation at 0°C.

After two preliminary centrifugations of the disrupted protoplasts at 6000 × g, the supernatant is centrifuged at 105,000 × g. The 105,000 × g supernatant, when combined with the washed 105,000 × g particulate fraction, will form OTC, whereas either particles or supernatant alone will not. The supernatant, precipitated at pH 5 with acid, resuspended in 0.33 M sucrose, and incubated with the particles, still supports OTC formation.

Protein Biosynthesis in Cell-Free Systems from Alcaligenes faecalis

O. J. Koeppe

Introduction. – Beljanski and Ochoa[11,12] have reported that particles prepared by sonic disintegration of Alcaligenes faecalis (ATCC 8750) cells would incorporate amino acids into particulate protein. They have further shown that the incorporation into washed particles was stimulated by a preparation purified from the soluble portion of the cells. This purified fraction, which they termed "incorporating enzyme" (IE), seems to activate amino acids by a mechanism other than the conventional one involving amino acid adenylate intermediates. This idea is based on the enzyme's failure to catalyze an amino acid-dependent exchange between ATP and inorganic pyrophosphate. The enzyme, however, does catalyze an exchange between ATP and ADP. The purpose of the present investigation was to reproduce the system of Beljanski and Ochoa and to study further the mechanism of amino acid activation in this system.

Results and Discussion. – Particles were prepared from Alcaligenes faecalis, which consistently catalyze considerable incorporation of glycine-C^{14} into protein (about 100 mμmoles per mg of protein per hour). The IE was purified and found to stimulate the incorporation of glycine into washed particles. The IE did not catalyze an amino acid-dependent exchange of P^{32} between ATP and inorganic pyrophosphate. Contrary to Beljanski and Ochoa's report, we observed an amino acid-dependent PP-ATP exchange with well-washed particles. Attempts have been made to show a stimulation by IE of the incorporation of leucine-C^{14} into rat liver microsomes when this enzyme was used in place of the "pH 5 enzyme" in the system of Keller and Zamecnik.[13] As yet no stimulation has been demonstrated.

Rendi and Hultin[14] have reported evidence indicating that the so-called "S-protein" of Sachs,[15] purified from rat liver, has certain properties

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[11] M. Beljanski and S. Ochoa, Proc. Natl. Acad. Sci. U.S. 44, 494 (1958).

[12] M. Beljanski and S. Ochoa, Proc. Natl. Acad. Sci. U.S. 44, 1157 (1958).

[13] E. B. Keller and P. C. Zamecnik, J. Biol. Chem. 221, 45 (1956).

[14] R. Rendi and T. Hultin, Exptl. Cell Research 17, 540 (1959).

[15] H. Sachs, J. Biol. Chem. 228, 23 (1957).

similar to those of the IE. This "S-protein" will catalyze the incorporation of amino acids into "S-RNA." Experiments to be carried out will test whether the IE will catalyze a similar reaction. The use of either "S-protein" or IE in the presence of "S-RNA" and hydroxylamine should provide a system in which there is sufficient net reaction of amino acids to warrant the use of O^{18}-labeled amino acids. These experiments should shed further light on the mode of amino acid activation in these systems.

Biosynthesis of Bacitracin

R. W. Bernlohr J. L. Yundt

Introduction. — *Bacillus licheniformis* produces the polypeptide antibiotic, bacitracin, during the period in its life cycle between log-phase growth and spore formation.[16] Attempts have been made to correlate the production of this antibiotic with the metabolic activity of the sporulating cell. In addition, the mechanism of activation of several D amino acids has been investigated.

Results and Discussion. — Growth experiments performed were designed to increase the efficiency of sporulation of *B. licheniformis*. Increasing the concentration of several minerals and decreasing the concentration of glucose in the medium decreased the time required for the formation of spores from 72 to 30 hr with at least 80% of the cells sporulating. At the same time, bacitracin production was also stimulated, and the antibiotic could then be found in the media after 12 hr.

Sporulation of *B. licheniformis* is completely inhibited by ethyl malonate but growth is unaffected. This ester also inhibits the production of bacitracin at least 80%. Inhibition of both sporulation and bacitracin production can be reversed simply by removing the inhibitor. Because of these and many other such experiments, we have proposed a hypothesis that bacitracin production is a function of the metabolism of the differentiated cell.

Because of the presence of D amino acids in the bacitracin molecule, we decided to determine the mechanism of activation of the D amino acids.

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[16] R. W. Bernlohr, Biol. Semiann. Prog. Rep. Feb. 15, 1959, ORNL-2702, p 148.

D-Glutamic acid, D-aspartic acid, and D-alanine are reported to be present in the cell walls of Bacillus sp.[17] D-Phenylalanine and D-ornithine are also found in the bacitracin molecule. Extracts of stationary-phase B. licheniformis will catalyze an ATP-PP exchange stimulated by each of these five D amino acids. It therefore appears that the activation of these D amino acids is similar to that reported for the L amino acids.[18]

Induced Enzyme Formation in Cell-Free Preparations of Rat Liver

F. T. Kenney

Introduction. — The tyrosine-α-ketoglutarate transaminase activity of rat liver can be increased five- to tenfold in vivo by parenteral administration of L-tyrosine;[19] an analogous, though larger (up to 100-fold) increase occurs naturally during the first 12 hr after birth in this species.[20] Under certain conditions it can be demonstrated that the latter change is sensitive to analogues of amino acids,[21] suggesting that these changes in activity represent de novo enzyme synthesis. Since the enzyme activity can be rapidly and specifically increased, a system is provided that is admirably suited to the study of the mechanism of synthesis of proteins in animal tissues. The present experiments were initiated in an attempt to define experimental conditions under which induced changes may be observed in the activity of this enzyme in broken cell preparations.

Results. — Liver homogenates from rats injected with large doses of tyrosine 2–3 hr before sacrifice demonstrate a tyrosine-α-ketoglutarate transaminase level 3–6 times as high as that of noninjected controls. With "induced" homogenates, supplemented with ATP and appropriate systems for maintaining ATP in phosphorylated form, a small, variable increase in transaminase activity is observed during the first few minutes of incubation at

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[17] R. E. Strange, Bacteriol. Rev. 23, 1 (1959).

[18] J. A. DeMoss and G. D. Novelli, Biochim. et Biophys. Acta 22, 49 (1956).

[19] E. C. C. Lin and W. E. Knox, Biochim. et Biophys. Acta 26, 86 (1957).

[20] F. Serini, F. T. Kenney, and N. Kretchmer, J. Biol. Chem. 234, 609 (1959).

[21] F. T. Kenney and N. Kretchmer (in preparation).

37°C. The activity then slowly declines during further incubation. It does not increase when ATP is omitted; homogenates of liver from noninduced controls, whether or not ATP is added, show only the decline in activity. There is, then, a small increase in the amount of transaminase, which can be observed only with homogenates from induced animals and is apparently energy dependent. After centrifugation at 10,000 × g, the supernatant retains the activity of the homogenate. Further centrifugal fractionation has yielded equivocal results. Attempts to increase the magnitude of the increase observed by addition of amino acids, nucleotides, and other materials that may be of significance in the formation of proteins have not yet been successful.

Discussion. — The slight extent of the *in vitro* increase in activity observed thus far is insufficient to support further experimentation. Accordingly, the major effort in experiments planned and under way is toward increasing this change. Purification of the enzyme from rat liver by techniques previously described[22] is also under way. It may be possible to measure enzyme formation immunochemically as well as enzymically, and thus to correlate increase in activity with increase of enzyme protein.

Amino Acid Incorporation into the Protein of Heart Mitochondria

M. P. Stulberg

Introduction. — Several reports[23,24] have indicated that amino acid is incorporated into protein in soluble systems prepared from calf heart mitochondria. Such systems would be of particular value for investigating the mechanism of protein synthesis, since they would allow for fractionation and isolation of the component reactions of the system.

Results. — Mitochondria from the heart tissue of young rats, pigeons, and calves were isolated and studied for their ability to catalyze the incorporation of amino acids into mitochondrial protein. The most active

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[22] F. T. Kenney, J. Biol. Chem. (in press).

[23] H. M. Bates, G. F. Kalf, and M. V. Simpson, Federation Proc. 18, 187 (1959).

[24] G. F. Kalf, H. M. Bates, and M. V. Simpson, J. Histochem. and Cytochem. 7, 245 (1959).

systems seem to be obtainable from young animals, especially calves. Methods have been devised for isolating active preparations in large quantities. A method of preserving the amino acid-incorporating activity of these large quantities of mitochondria has been designed, in cooperation with N. G. Anderson, involving extremely rapid freezing in liquid nitrogen and storage in liquid nitrogen. No loss of activity has been observed after one month of storage. These frozen particles, when thawed and assayed for activity, display energy requirements different from those obtained with fresh mitochondria.[25,26] No added source of energy is necessary and a nitrogen atmosphere is inhibitory in both the presence and absence of an energy-generating system.

Discussion. — The procedures and results mentioned have now provided sufficient amounts of starting material to make attempts at solubilization of amino acid incorporation activity feasible. If these attempts are successful, we foresee that isolation and examination of component reactions of protein synthesis with the use of O^{18} and other methods will be initiated.

Reduction of Sulfate by *Desulfovibrio desulfuricans*

H. D. Peck, Jr.

Introduction. — Cell-free extracts of *Desulfovibrio desulfuricans* utilize hydrogen for the reduction of sulfate only in the presence of ATP. Preliminary results have indicated that, in addition to an appropriate electron donor, at least two enzymes are required for the reduction of sulfate.[27] It was suggested that inorganic sulfate initially reacts with ATP to form adenosine 5'-phosphosulfate (APS). APS is subsequently reduced to sulfite and adenosine monophosphate (AMP). The enzyme catalyzing the latter reaction has not previously been described and will be referred to as APS reductase. Sulfate reduction by extracts of yeast also exhibits a

[25] O. Greengard and P. N. Campbell, Biochem. J. 72, 305 (1959).

[26] J. R. McLean *et al.*, J. Biol. Chem. 233, 3 (1958).

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

requirement for ATP;[28,29] however, the sulfate compound reduced appears to be 3'-phosphoadenosine 5'-phosphosulfate, rather than APS as in D. desulfuricans.

Results. – When ATP and $S^{35}O_4^{--}$ are incubated with a cell-free extract of D. desulfuricans, one radioactive compound is formed that has the electrophoretic mobility and acid lability of authentic APS. This labeled compound is converted to $PAPS^{35}$ when it is incubated with APS and a purified APS-kinase isolated from yeast. This enzyme, which catalyzes the formation of APS, has been separated from APS reductase by electrophoresis. It requires Mg^{++} and pyrophosphatase for activity and catalyzes a magnesium- and sulfate-dependent exchange of labeled pyrophosphate into ATP. This enzyme thus appears similar to sulfurylase, which has been described and purified from yeast.[30,31]

Reduction of sulfate in the presence of ATP and molecular hydrogen is completely inhibited by Versene and is destroyed by heating the extract at 60°C for 2 min. Under these conditions, however, the extracts retain the ability to reduce APS with hydrogen to sulfite and AMP. In contrast to yeast, $PAPS^{35}$ is neither reduced nor metabolized by these extracts. Using partially purified enzymes, we have reconstructed the sulfate-reducing system. When yeast sulfurylase is added to APS reductase, sulfate-reducing activity is restored and a further increment in activity is observed when pyrophosphatase is added. These data firmly establish that APS, not PAPS, is the immediate substrate for sulfate reduction by extracts of D. desulfuricans.

For these studies, an assay for APS reductase that makes use of the acid volatility of sulfite was developed. APS^{35} is incubated with enzyme and reduced methyl viologen as electron donor; acid is then added and the radioactivity trapped in alkali is determined. By this assay, the enzyme was purified tenfold; preliminary evidence suggests that it contains a flavin prosthetic group.

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

[29] H. Hilz and M. Kittler, Biochim. et Biophys. Acta 30, 650 (1958).

[30] L. G. Wilson and R. S. Bandurski, J. Biol. Chem. 233, 975–81 (1958).

[31] P. W. Robbins and F. Lipmann, J. Biol. Chem. 233, 686–90 (1958).

Discussion. – There seem to be two pathways by which sulfate may be reduced. In yeast,[28,29] PAPS is the form in which sulfate is reduced; this pathway appears to be primarily concerned with the assimilation of sulfur. D. desulfuricans uses inorganic sulfate as its primary electron acceptor, and APS is the form in which sulfate is reduced. In the latter pathway, respiratory sulfate reduction, one less high-energy phosphate is required than in assimilatory sulfate reduction.

The Effect of 2,4-Dinitrophenol on the Anaerobic Metabolism of Yeast

W. E. Jefferson, Jr.

Introduction. – When yeast and some bacteria ferment carbohydrates in the absence of a source of nitrogen, a phenomenon known as fermentative assimilation occurs. That is, a portion of the carbohydrate ranging up to 30% is taken into the cells rather than fermented.[32] The addition of 2,4-dinitrophenol (DNP) to such systems abolishes the assimilation, and the carbohydrate is quantitatively converted to fermentation products.[33] There is no obvious relation between this effect of DNP and its well-known ability to uncouple the phosphorylation of ADP from electron transport in aerobic respiration. In the aerobic systems, DNP seems either to prevent the formation of ATP from ADP and inorganic phosphate or to cause its immediate hydrolysis. Neither explanation appears adequate to explain the anaerobic effect since any interference with ATP formation should cause the fermentation to stop rather than speed up. Nevertheless, the intimate relation between assimilation and phosphorus metabolism suggested that this effect of DNP might also be involved in some way with phosphorus metabolism. To test this idea and possibly to obtain an indication of the point of action of DNP in anaerobic systems, experiments were planned in which the distribution of P^{32} in the various phosphate-containing compounds of yeast would be determined after radioactive orthophosphate was added to yeast fermenting in the presence and in the absence of DNP.

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[32] C. B. Van Niel and E. H. Anderson, J. Cellular Comp. Physiol. 17, 49–56 (1941).

[33] J. B. Reiner and S. Spiegelman, J. Cellular Comp. Physiol. 30, 347–58 (1947).

Results and Discussion. — A preliminary experiment has been completed and a more-extensive one is partially complete. Considerable difficulty was encountered in fractionating the phosphates of yeast. Since their high lipid and polyphosphate contents make the schemes used for animal tissues inapplicable, various modifications had to be devised.

In the incomplete data obtained so far the most obvious effect of DNP is on the specific radioactivity of the intracellular orthophosphate, which is much higher when DNP is added — suggesting an increased rate of phosphate uptake by the cells, when incubated with P^{32}. That this is not the case, however, was shown by incubating yeast in 0.005 M KH_2PO_4 in the presence and absence of DNP and measuring the disappearance of phosphate from the medium. In all cases, the yeast fermenting in the absence of DNP took up some phosphate from the medium but not in its presence. The increased radioactivity of the intracellular orthophosphate must therefore represent an increased rate of equilibrium between intracellular phosphate. An altered permeability may be the most likely explanation, but an enzymatic explanation has not been excluded.

The observation that DNP exerts a greater effect in the absence of glucose than in its presence is so far unexplained.

Changes observed in the nucleotide and acid-soluble labile phosphorus activities, both increased by DNP, probably reflect the higher activity of the intracellular orthophosphate. This does not appear to be true of the acid-insoluble and stable phosphate fractions, which were less active in the presence of DNP and glucose than with glucose alone.

Amino Acid Incorporation in Plants

R. Rabson

Introduction. — The purpose of this investigation was to develop from plant material a cell-free preparation that would incorporate amino acids into protein and possibly effect net protein synthesis.[34,35]

Results. — A cell-free system from developing maize endosperm was prepared that efficiently incorporates C^{14}-leucine into protein. Maize

[34] G. C. Webster and M. P. Johnson, J. Biol. Chem. 217, 641 (1955).

[35] I. D. Raacke, Biochim. et Biophys. Acta 34, 1 (1959).

endosperm is homogenized with sand in 0.5 M sucrose. The suspension is centrifuged for 1 hr at 100,000 × g and the sedimented particles are taken up in distilled water. When such particles are incubated with ATP, an ATP-generating system, and C^{14}-leucine, amino acid is rapidly incorporated into protein. If the particles are well washed, an additional requirement for GTP and supernatant can be seen. The supernatant can be effectively replaced by pH 5.0 enzyme from rat liver homogenate but not by incorporating enzyme of Beljanski and Ochoa. The incorporation by the maize system is destroyed by RNase and is inhibited by a full complement of amino acids.

Discussion. — This system seems to have a higher efficiency in promoting the incorporation of amino acids into protein than the comparable system from rat liver. Further exploration of this system may yield new information on factors involved in protein synthesis.

The Formate-Pyruvate Exchange Reaction in *Escherichia coli*

G. D. Duda

Introduction. — Previous reports[36,37] from this Laboratory suggested that an active C_1 compound might be formed during an exchange reaction between formate and the carboxyl group of pyruvate. This exchange reaction has now been studied in *E. coli* strain 26 and the effects of several metabolic inhibitors is reported.

Results and Discussion. — An earlier report[37] cast doubt on the reversibility of the hydrogenlyase reaction, $HCOOH \rightleftharpoons H_2 + CO_2$, in cell-free extracts of *E. coli*. The reversibility of this reaction has now been confirmed in both whole cells and cell-free extracts of *E. coli*, Crooks strain. In both cases, the equilibrium in the direction of synthesis is 1.3.

Cell-free extracts of *E. coli* 26 prepared by grinding with alumina or sonic oscillation were used for studying the formate-pyruvate exchange. The most active preparations were made from cells grown on enriched media in the absence of glucose. As reported earlier,[36] the rate of exchange increases

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[36] G. D. Novelli and H. K. Sherwood, Biol. Semiann. Prog. Rep. Aug. 15, 1956, ORNL-2155, p 54.

[37] G. D. Novelli and H. K. Sherwood, Biol. Semiann. Prog. Rep. Aug. 15, 1957, ORNL-2390, p 85.

rapidly (autocatalytically) for 20 min and then becomes constant for 10 to 15 min. Thereafter, the rate of exchange drops rapidly. This autocatalysis is not overcome by preincubating the extract with pyruvate or formate. Indeed preincubation with formate almost completely inhibits the exchange. Attempts to accumulate the product of autocatalysis have been unsuccessful. CoA stimulates the exchange but it does not abolish the autocatalysis.

Amethopterin at 1.5×10^{-4} M inhibits the exchange reaction 50%. In attempting to overcome this inhibition, we observed that tetrahydrofolic acid caused 50% inhibition at 3×10^{-4} M. Folic acid itself was only slightly inhibitory. Menadione is a potent inhibitor and has been extensively studied. A 50% inhibition is obtained at 0.5 to 1×10^{-4} M, but higher concentrations fail to inhibit more than 70%. It appears that menadione delays the onset of autocatalysis but fails to abolish it. A large variety of compounds including CoA, ATP, flavins, β-mercaptoethanol, biotic, and catalase fail to relieve inhibition by menadione.

Although attempts to purify the systems have been unsuccessful, a boiled juice that increases the activity of the extract tenfold has been prepared. The factors involved in this stimulation are now being examined.

Purification of Ornithine Transcarbamylase from *Escherichia coli*

P. Rogers, Jr. R. M. Flora

Introduction. – This study is an extension of the isolation and purification work previously reported.[38] As of this report, we have been able to purify ornithine transcarbamylase (OTC) to about 100-fold the activity contained in the original extract.

Results. – One and one-half pounds of *Escherichia coli* W cells were grown in a biogen, ground with alumina, and extracted with 0.05 M Tris buffer at pH 8.5, as was previously reported.[38] Heating the extract at 65°C for 5 min gave a slight increase in specific activity. This was followed by an ammonium sulfate fractionation resulting in a tenfold increase in specific activity over the original extract in the best fractions. The

[38] P. Rogers, G. D. Novelli, and H. Maclay, Biol. Semiann. Prog. Rep. Aug. 15, 1958, ORNL-2593, p 84.

next step consisted of an ethanol fractionation with pH adjustments. At the higher pH values (pH 8.5), a higher concentration of ethanol was required to bring about precipitation of the desired material than was necessary at more acid pH values (pH 7.0 and 5.5). Because of the denaturating effects of high ethanol concentrations, a method was used whereby much of the inactive protein was removed by precipitation with 48% ethanol at pH 7. The remaining supernatant was then diluted to 21% ethanol and adjusted to pH 5.5. The resulting precipitate contained the OTC at a higher specific activity. Table 27 shows the progress of the purification through the various steps. A calcium phosphate gel at a ratio of 3 gel to 1 protein

Table 27. Purification of OTC from E. coli W Cells

	Total Units ($\times 10^4$)	Specific Activity*	Percentage Yield
Original extract	52.5	8.3	100
Heat 65°C 5 min	38	43	72
Ammonium sulfate precipitation	24	82	46
Ethanol fractionation and pH adjustment	12.7	350	24
$CaPO_4$ gel adsorption	6.2	700	12
Geon electrophoresis	2.7	800	5

*1 unit of activity = 1 μmole of citrulline produced per minute at 37°C, pH 8.5. Specific activity = units per mg of protein.

(w/w) adsorbed all of the protein contained at this stage. Specific elution of OTC with the substrate carbamyl phosphate was used as suggested from Heppel.[39] Carbamyl phosphate at a concentration of 0.005 M eluted the OTC activity and less than half of the protein from the gel. Eluted OTC was concentrated by lyophilization and subjected to zone electrophoresis, Geon being used as a supporting medium, for two days at 25 ma and 60 v. Sections cut from the tray were eluted with distilled water. The

[39]L. A. Heppel, in Methods in Enzymology, vol II,p 570, 1955.

enzyme activity migrated coincident with the majority of the protein, as shown in Fig. 20. The OTC eluted between the dotted lines contained the units and specific activity shown in Table 27.

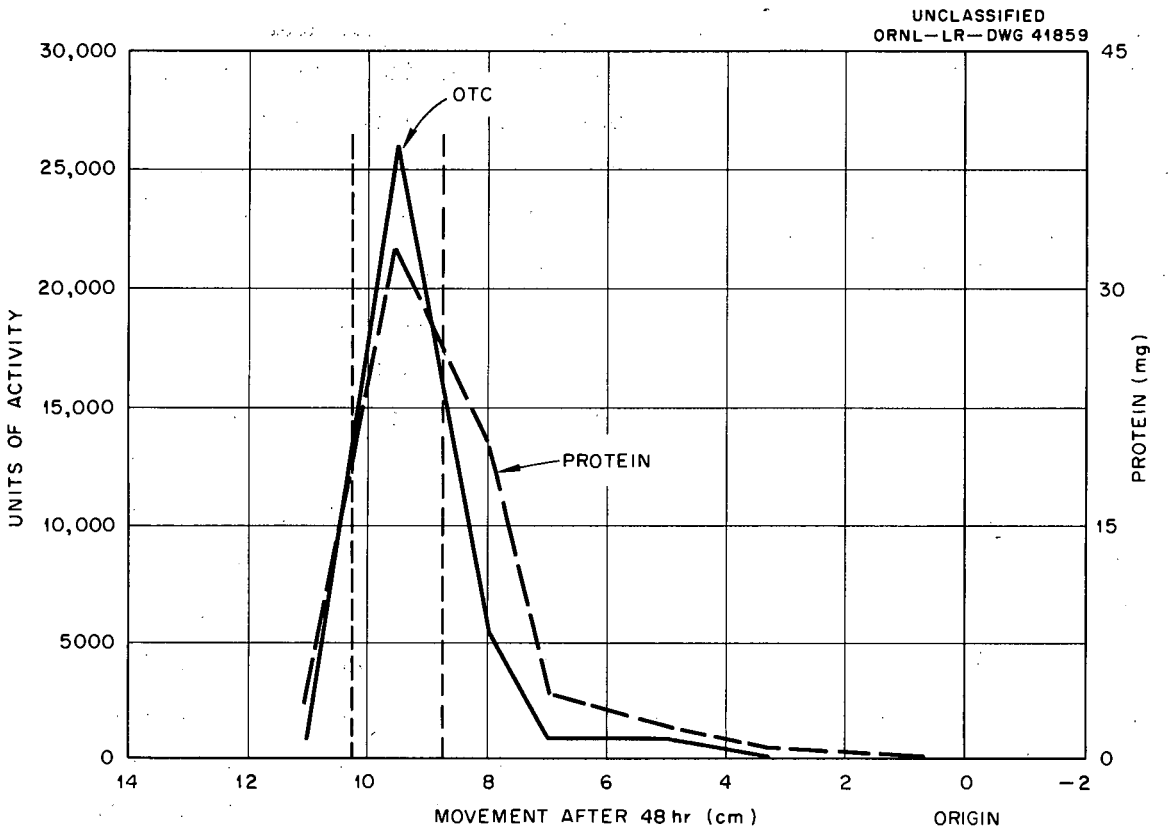

Fig. 20. Geon Electrophoresis of OTC.

Discussion. — A 100-fold purification of OTC has been obtained from cells that originally contained 10 times the steady state level of OTC. Crystallization of this enzyme will be attempted along with further purification. Since only a small fraction of the original total units remains, 2 lb of additional cells have been grown in order that more OTC of high purity can be isolated.

Studies on the properties of this enzyme and the production of a specific antiserum to be used in connection with our investigation on the mechanism of OTC synthesis will be undertaken.

Albumin-Bound Pigment of Chicken Serum

F. T. Kenney T. Makinodan[40]

Introduction. – Chicken serum albumin prepared by ammonium sulfate fractionation, and homogeneous by ultracentrifugal, electrophoretic, and immunological criteria, carries an intense yellow color that is not removed by dialysis or by salting out at various hydrogen-ion concentrations. The nature of the yellow pigment and the possible significance of its tight binding to albumin are being investigated.

Results and Discussion. – Treatment of the purified albumin with trichloroacetic or perchloric acid and then shaking with ether releases a small fraction of the yellow pigment into the ether phase. Since mineral acids are relatively ineffective, it would appear that extensive denaturation of the protein, rather than a hydrogen-ion effect, is involved in this release. The absorption spectrum of the pigment in ether or ethanol reveals the following: a high end absorption in the far ultraviolet (below 250 mμ), a minimum at 265 and maximum at 270 mμ, a shoulder at 310–330 mμ, and a gradually decreasing absorption extending to 450 mμ. The spectrum of the original albumin is similar except that absorption in the 270–280 mμ range is altered, owing to contributions of the protein. Albumin prepared by ethanol fractionation[41] is nearly colorless, but its absorption spectrum indicates that it also carries small amounts of the same pigment bound to the "yellow albumin." Antibody prepared to the latter reacts equally well to the albumin prepared by ethanol fractionation. The ether-soluble fraction of whole serum contains at least two yellow pigments, one of which has been provisionally identified as the carotenoid xanthophyll, by virtue of absorption maxima (in ethanol) at 475, 445, and 420 mμ. Destruction of this component by acid treatment reveals the second major component, with a spectrum identical to that of the pigment bound to albumin. Chromatographic purification of this pigment is now being undertaken.

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[40]Radiation Immunology Group.

[41]P. N. Campbell, Biochem. J. 61, 496 (1955).

Acetylation Reactions in Higher Animals

K. B. Jacobson

Introduction. – Previous workers have characterized the acetylation of arylamines, such as sulfanilamide, as consisting of two reactions. First, the acetate ion is raised to a high potential energy level by forming a thioester bond with coenzyme A; second, the acetyl-CoA reacts with the arylamine, transferring the acetyl group to the nitrogen of the amine. In pigeon liver these two reactions were separated some years ago by fractionation of the liver extract. Recently, Johnson *et al.*[42] observed that the acetylation of sulfanilamide was inhibited by amethopterin and the inhibition was noncompetitive with sulfanilamide. Amethopterin is generally considered an antagonist of reactions involving a folic acid derivative and appears incongruous as an inhibitor of a CoA-linked reaction.

Results. – For measuring the transfer of acetyl groups to an arylamine a spectrophotometric procedure was used. Instead of acetyl-CoA, an acetylated arylamine was used as the acetyl donor. Thus the reaction may be studied apart from CoA, which does not participate in the transfer reaction. By this system, the localization of the amethopterin inhibition was shown to lie in the acetyl-transferring step. This inhibition was not reversed by folic acid, leucovorin, tetrahydrofolic acid, the acceptor amine, or the acetyl donor. Thus the mechanism of inhibition by amethopterin remains obscure.

Further observations confirmed previous reports that CoA itself is an inhibitor of the acetyl transfer system. It was shown that cysteine reverses the inhibition by CoA provided that the enzyme is not preincubated with Versene.

Discussion. – The mechanism of acetylation reactions has an unexplained parameter in the inhibition by amethopterin. On the other hand, this inhibition may provide an insight into the mechanism of the reaction as well as indicate a type of amethopterin inhibition previously unrecognized.

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[42] W. Johnson, G. Corte, and R. Jasmin, Proc. Soc. Exptl. Biol. Med. 99, 677–80 (1959).

The inhibition of acetyl transfer to aromatic amines by CoA may play a regulatory role. When acetyl groups are required for respiratory or synthetic purposes, the level of free CoA may rise and thus be available to accept newly produced acetyl groups and also inhibit the acetylation of aromatic amine, thus preserving the acetyl groups for other purposes. This possibility is speculative since the physiological role of the acetyl transfer enzyme is not known.

Growth and Metabolism of Mammalian Cells *in vitro*

K. B. Jacobson M. A. Bender[43]

Introduction. — The ability to grow animal cells and tissues *in vitro*, isolated from the whole organism, represents a significant advance in the study of differentiated cells. Ideally, it should make possible large-scale production of defined cell types and allow detailed biochemical study of their metabolic patterns, hormonal requirements, and interrelations with other cells. Reproduction of a specific cell with a unique function would have important ramifications in studies of hemoglobin synthesis, hormone synthesis, nerve impulse generation and transmission, and radiation protection by bone marrow, to name just a few.

A major obstacle to the realization of these objectives lies in the cell culture procedure itself. The techniques currently used inhibit the growth of highly differentiated cells. It has been observed[44] that cells remain diploid for only a few generations after isolation from an organ, and after the active multiplication of diploid cells ceases, the cultures enter a static phase during which marked morphological and cytochemical changes take place. After some time, cells may emerge that are characterized by hyperdiploidy, by the ability to grow as single isolated cells (clone formation), and by vigorous growth for an apparently unlimited number of generations. These characteristics probably apply to all the standard cell lines currently in use in various laboratories, regardless of the species or organ from which it was derived and whether the original

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[43]Mammalian Recovery Group.
[44]M. A. Bender, Science 126, 974–75 (1957).

tissue was normal or neoplastic. They do not apply to newly cultured diploid cells. An understanding of the metabolic properties of a cell strain before, during, and after this "neoplastic transformation" might well enable us to prevent the change.

Results. — A commonly cited property of cultured mammalian cells is an energy-yielding metabolism that is mainly glycolytic. Metabolic measurements have been made on a clonal derivative of a human heart cell line (Girardi Heart, clone 7A6) that we have isolated. These measurements are consistent with a glycolytic metabolism. Respiratory measurements indicate that there is only extremely low oxidative activity. All of the glucose utilized by these cells can be accounted for as lactic acid.

Additional measurements have been made on the Ehrlich ascites cell. We have developed a suspended culture system that allows multiplication *in vitro*. The metabolism of these cells is peculiar, in that they produce large amounts of an unidentified acid in addition to lactic acid. The unfortunate propensity of these cells to stick to the glass walls of the culture vessel has prevented accurate measurements of the increase in cell number *in vitro*. Nevertheless, measurements of the total cells in the fluid phase have revealed that the cells multiply by at least one division every 24 hr.

Discussion. — We hope that the pursuit of these joint studies will eventually enable us to control both the growth of undifferentiated cells and also the differentiation of cells *in vitro*. We might then produce cells such as the protective bone marrow cell in large quantities.

Purification of β-Amylase from Maize

A. N. Best G. D. Novelli

Introduction. — During an investigation of the effect of mutant genes on the enzymes involved in starch synthesis,[45] β-amylase activity was observed in developing endosperm of waxy maize. Because various investigators have stated that β-amylase is not present in maize endosperm,[46]

[45] D. Schwartz *et al.*, Biol. Semiann. Prog. Rep. Feb. 15, 1959, ORNL-2702, p 46–48.

[46] R. L. Whistler and W. M. Corbett, chap. XII in The Carbohydrates, ed. by W. Pigman, Academic Press, New York, 1957.

it became desirable to purify the activity and determine whether it was in fact β-amylase. The present report indicates the progress of this project.

Results and Discussion. – Extracts were prepared by grinding frozen developing endosperms of waxy maize with one volume of 0.005 M Tris buffer pH 7.0 in a Lourdes Hi-speed blender. After centrifugation, first at low speed to remove coarse material and then at 100,000 × g, the extract was dialyzed for 3 hr against 0.005 M Tris pH 7.0. The extract was fractionated with solid ammonium sulfate and the β-amylase activity was precipitated between 0.55 and 0.9 saturation. Dialysis of this fraction against acetate buffer at pH 4.5 resulted in denaturation of considerable protein without loss of activity. The extract was again fractionated with ammonium sulfate at pH 8.0 and the activity precipitated between 0.6 and 0.7 saturation. After dialysis, the preparation was fractionated with acetone and the activity appeared in the 0.57–0.79 fraction. These steps resulted in an over-all purification of 75-fold. The amount of this enzyme in the developing endosperm is very low and the material available to us until recently has been too scant to permit the isolation of the enzyme in sufficient amounts for proper characterization. We have just harvested this summer's crop of corn and are now in a position to isolate the enzyme in quantity. In germinating seeds, on the other hand, the β-amylase is present in 10 to 20 times the amount in developing endosperm. Preliminary studies with ammonium sulfate fractionation of this enzyme suggest that it may be different from the similar enzyme in endosperm. We expect to purify the β-amylase from both sources and compare their respective characteristics.

Biochemical Properties of Antigens Responsible for Bone Marrow Transplantation Immunity

P. F. Lincoln T. Makinodan[40] G. D. Novelli

Introduction. – This is a continuation of a joint effort of the Enzymology and Radiation Immunology Groups to isolate and characterize the antigens involved in bone marrow transplantation immunity.[47]

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[47]A. N. Best et al., Biol. Semiann. Prog. Rep. Feb. 15, 1959, ORNL-2702, p 155.

Results and Discussion. — As mentioned in a previous report, studies with rat liver mitochondria were initiated, since a larger quantity of material would be available. Attempts to prepare the active material from whole rat liver homogenates by papain digestion or autolysis led to complete inactivation. The material from mitochondria seemed to become increasingly labile on alternate freezing and thawing. These observations suggested that with mitochondria we might be dealing with a different substance from that which we started to isolate from microsomes. We therefore returned to rat liver microsomes for further work, repeating the experiments on dialysis, heating, and freezing and thawing, and found little or no loss in activity with these treatments. When the microsomes were treated with 0.5 mg/ml of sodium lauryl sulfate for 1 hr at 37°C about half of the active material was no longer sedimentable in the Spinco. The activity could be precipitated with acetone and was soluble in sodium chloride. Since, at about this time we began to see early deaths in our control mice, further experiments were suspended.

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

G. D. Novelli P. F. Lincoln

Introduction. — This is a continuation of a joint effort of the Enzymology and Mammalian Recovery Groups to isolate and characterize substances producing a characteristic change in the white pulp of the spleen.[48]

Results and Discussion. — Because of the observation that different lots of horse serum contained different amounts of the substance, R. D. Owen[49] suggested that the substance might be related to the secretor substance. Hence C. C. Congdon[50] examined human saliva and found it to be a rich source of the substance. Accordingly, we started isolating the active

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[48]G. D. Novelli, A. N. Best, and P. F. Lincoln, Biol. Semiann. Prog. Rep. Feb. 15, 1959, ORNL-2702, p 154.

[49]Consultant to Radiation Immunology Group.

[50]Mammalian Recovery Group.

material from our own saliva. The active material is stable to boiling for 15–20 min, is precipitable by five volumes of ethanol, and stable to freezing and thawing and dialysis. Suspecting that we might be dealing with one of the blood group polysaccharides, we started a standard isolation procedure for such substances. The first step requires a two-week digestion with crystalline pepsin. Such treatment completely inactivated the saliva. Stability to heating and lability to peptic digestion together with precipitability by low alcohol and by acetic acid suggested that the substance might be a muco- or glycoprotein. Curtain and Pye described the isolation, from bovine submaxillary glands, of a mucoprotein that inhibited influenza virus hemagglutination of red cells. We prepared the substance from slaughterhouse material through the following steps. Bovine submaxillary glands were freed from fat and connective tissue and homogenized for 3 min with an equal weight of water. After centrifugation at 5000 × g for 30 min, the supernatant was adjusted to pH 4.0 with 5 N acetic acid. The precipitate was removed and discarded, and the supernatant was neutralized and dialyzed for 48 hr against two changes (20 liters) of distilled water. The dialyzed extract was made to 0.1 M barium acetate, and the active material was precipitated by bringing the solution to 64% methanol. The precipitate was collected, dissolved in 0.1 M disodium versenate, and dialyzed for 60 hr against distilled water. This procedure has yielded the most active material seen to date. We are now in the process of collecting submaxillary glands from the rat, in the hope that a similar substance can be isolated from this source and thus permit us to return to the rat-mouse system.

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

T. Kameyama

Introduction. – Previous investigation of ultraviolet effect on RNA and protein biosynthesis in *Escherichia coli* indicated that the most striking effect of ultraviolet (UV) was the suppression of the formation of ribonucleoprotein (RNP) as observed by starch zone electrophoresis and DEAE cellulose chromatography.[51] This observation suggested that normal

[51] T. Kameyama, Biol. Semiann. Prog. Rep. Feb. 15, 1959, ORNL-2702, p 156.

biosynthesis of cellular macromolecular components might be disturbed by UV radiation. The UV-induced suppression of RNP synthesis is gradually restored during further incubation, although preceding macromolecular alterations might give rise to serious inhibition of cellular metabolism and finally to ultimate death.

Some abnormal properties of RNA synthesized after UV irradiation could be determined with P^{32} radioactivity,[52] already incorporated into RNA, to a definite level during subsequent incubation with nonradioactive phosphate medium. There are also alterations in the amounts of cytidylic acid and uridylic acid, and an increase in the amount of soluble RNA extractable with molar solution of NaCl from the bulk RNA prepared by phenol method.

Results and Discussion. — *E. coli* B and K12 were grown in minimum medium containing succinate. The cells in log phase were irradiated in physiological saline with various UV doses. For the purpose of photoreactivation, the cells were kept constantly under white light for 60 min after UV irradiation. Cells were washed and resuspended in reaction medium containing thiomethyl β-galactoside (TMG) or thioethylgalactoside (TEG) for induction of β-galactosidase and radioactive precursors for labeling of protein and nucleic acids. After incubation the cells were disrupted by French pressure cell. The crude extract was fractionated by DEAE cellulose column and Geon zone electrophoresis.

UV sensitivity of induction of β-galactosidase was relatively higher than that of bulk synthesis of RNA and protein. This suppression of enzyme induction could be easily reversed by a dose of white light smaller than that required for recovery of survival, RNA and protein synthesis.

UV treatment prolonged the lag period of initiation of increase of total RNA and enzyme induction, even though a considerable amount of P^{32} and S^{35} could be incorporated into cellular material during initial lag period of RNA and protein synthesis. This lag period was quite dependent on UV dose. When irradiated cells were incubated for 30 min without addition of inducer (TMG or TEG), the lag period for induction of enzyme synthesis was considerably shortened, suggesting that a certain type of RNA might be necessary for enzyme induction in the initial period. This

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[52] K. Suzuki and J. Ono, Nature 183, 395 (1959).

is completely consistent with other findings in respect of requirement of simultaneous synthesis of RNA for enzyme biosynthesis.[53,54]

To determine more in detail the changes of cellular components provoked by UV, we fractionated nucleic acid and protein by DEAE cellulose and electrophoresis. An accumulation of relatively large amounts of soluble RNA could be observed by both fractionations as compared with normal. This result could be explained on the basis that the newly synthesized soluble RNA might not be transferred to ribosomal RNA by either some abnormality of molecular configuration or by blocking of other metabolic systems.

Photoreactivation was also successful not only in the recovery of the RNP fraction, but also in obtaining a normal ratio of nucleic acid to protein in this fraction.

Activity of the β-galactosidase induced in normal cells could be found in three fractions on Geon, but one of these three peaks was lost for enzyme induced after UV and photoreactivation. Whether these fractions of the β-galactosidase might reflect any biologically important significance is under investigation.

The Biosynthesis of Dihydroorotic Dehydrogenase in *Escherichia coli*

K. Tonomura G. D. Novelli

Introduction. — The synthesis of three enzymes required for the synthesis of orotic acid in *E. coli* is controlled by the intracellular concentration of metabolites that are not substrates of the enzymes but are end products of the metabolic pathway. The synthesis of all three of these enzymes could be almost completely suppressed by growing the cells in the presence of uracil or cytosine. As discussed in several previous reports,[55,56] an enzyme-forming system that can be so readily

[53] P. Rogers and G. D. Novelli, Biochim. et Biophys. Acta 33, 423 (1959).

[54] A. B. Pardee, Proc. Natl. Acad. Sci. U.S. 40, 233 (1954).

[55] J. A. DeMoss and G. D. Novelli, Biol. Semiann. Prog. Rep. Aug. 15, 1957, ORNL-2390, p 83–84.

[56] P. Rogers, Jr., and G. D. Novelli, Biol. Semiann. Prog. Rep. Feb. 15, 1958, ORNL-2481, p 62–63.

controlled has considerable potential for studies on the mechanism of cell-free protein synthesis. Accordingly, we started to investigate the requirements for synthesis of dihydroorotic dehydrogenase in whole cells before attempting to obtain the synthesis of the enzyme in cell-free preparations. The reason for our selection is that the activity of the enzyme can be measured quickly and conveniently in the spectrophotometer by taking advantage of the absorption of the product orotic acid at 290 mμ.

Results and Discussion. – Using a uracilless mutant of *E. coli* K12, we could observe tenfold increases in dihydroorotic dehydrogenase by releasing suppression through removal of uracil from the growth medium. The standardized procedure for these studies involves the growth of the cells in minimal medium containing glucose and 100 μg/ml uracil as suppressor. Enzyme synthesis is observed with 3-hr cells washed and resuspended in minimal medium by removing aliquots at various time intervals and determining enzyme activity with toluenized cells. We have demonstrated that appearance of enzyme activity after the release of suppression requires a source of energy, a nitrogen source, and is inhibited by sodium azide and chloramphenicol. These observations suggest that the appearance of enzyme activity is a result of synthesis of new enzyme rather than the activation of preformed enzyme. Since we are dealing with a uracil-requiring mutant and suppression is released by removal of uracil, the synthesis of dihydroorotic dehydrogenase can occur without simultaneous synthesis of RNA. This is similar to the synthesis of ornithine transcarbamylase in the absence of simultaneous RNA synthesis.[57] Unlike OTC synthesis, we have been unable to observe synthesis of dihydroorotic dehydrogenase in protoplasts. We are continuing to attempt to observe synthesis with protoplasts under varying conditions.

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[57] P. Rogers, Jr., and G. D. Novelli, Biochim. et Biophys. Acta 33, 423 (1959).

MICROBIOLOGY

ENZYME AND TRACER STUDIES ON BACTERIAL METABOLISM

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

Studies on Flavoproteins

M. I. Dolin

Introduction. – Previous work[1,2] from this laboratory demonstrated that the flavoprotein, DPNH peroxidase, forms a visible complex with its substrate, DPNH. Since similar findings were made for acyl-CoA dehydrogenase[3] and continue to be made for other flavoproteins, it appears that the phenomenon has general significance. The present work was performed in order to investigate further the nature of the complex formed between DPNH peroxidase and DPNH. The work deals with the spectral and kinetic properties of the complexes formed between this flavoprotein and a series of pyridine nucleotide analogues.

Results and Conclusions. – With DPNH peroxidase, the changes accompanying complex formation with DPNH consist of partial reduction in intensity of the 450-mμ band of the flavoprotein and formation of a broad absorption band centered around 540 mμ. It has now been found that these two absorption changes can be dissociated from each other by choosing the proper substrate. For instance, TPNH can cause the change at 450 mμ but not the formation of the 540-mμ band. Conversely, pyridine aldehyde deamino DPNH causes the formation of the 540-mμ band, but not the absorption change at 450 mμ. However, when a pyridine nucleotide analogue that causes both changes is used, the absorbance changes at 450 and 540 mμ are linked in a constant ratio, as would be expected if a single spectral intermediate were involved. These results are difficult to reconcile with the hypothesis that the complexes represent semiquinone radicals similar to those that have been postulated for some flavoprotein

[1] M. I. Dolin, Arch. Biochem. Biophys. 60, 499 (1956).
[2] M. I. Dolin, J. Biol. Chem. 225, 557 (1957).
[3] H. Beinert and E. Page, J. Biol. Chem. 225, 479 (1957).

systems.[3] The substrate specificity for complex formation has been investigated with a series of DPNH analogues. Pyridine nucleotide analogues that are peroxidase substrates are also able to elicit the characteristic absorption changes; those nucleotides that do not cause the typical absorption changes are not substrates. Both gross spectral changes and results obtained in titrating reduced enzyme with pyridine nucleotide analogues lead to the conclusion that the amino group of adenine, the amino group of the carboxamide moiety, and the 5' phosphate of the NMN moiety function as binding sites. The adenylic acid moiety of DPN is necessary for formation of the 540-mμ band but not of the 450-mμ band.

Two nucleotides, TPNH and NMNH, cause the formation of only the 450-mμ band. These compounds are poor peroxidase substrates, but in common with active nucleotides, they serve as hydrogen donors for the weak oxidase activity of DPNH peroxidase. Correspondence between the kinetic and spectral properties of the complexes formed with TPNH and NMNH suggests that the 5' phosphate of TPNH prevents the binding of the adenylic acid moiety, thus making TPNH and NMNH identical as substrates.

With the six nucleotides that showed detectable enzymatic activity, there is good correspondence between K, the dissociation constant of the visible complex, and K_s, the Michaelis constant determined from kinetic studies. All the criteria that have been used lend strong support to the hypothesis that the visible intermediate formed in the DPNH peroxidase system is a kinetically active enzyme-substrate complex.

Induced Synthesis of Catalase in *Rhodopseudomonas spheroides*

R. K. Clayton

The induced synthesis of catalase in *Rhodopseudomonas spheroides* continues to be investigated.

Through C^{14}-incorporation experiments it has been shown[4] that the enzyme-protein is synthesized *de novo*.

Centrifugation of a sonic extract of *Rps. spheroides* reveals that the catalase, together with respiratory activity, is not precipitated in

[4] R. K. Clayton, J. Biol. Chem. (1959) (in press).

2 hr at 105,000 × g and thus does not reside in the pigment-bearing chromatophores.

Chance[5] has stated that substances such as nitrite and alcohols, which are electron donors for a peroxidatic reaction with catalase and H_2O_2, will accelerate the destruction of H_2O_2 in the presence of catalase. This conclusion was invoked to explain the protection afforded by nitrite[6] and alcohols[7] against damage by ionizing radiation. The reaction kinetics pertinent to Chance's system predict, however, that these electron donors will retard the destruction of H_2O_2.[8] The foregoing explanation for protection against irradiation must therefore be rejected.

Current work is centered in identifying the inducers of catalase synthesis in Rps. spheroides and elucidating the kinetics of induction. Some parameters of this problem are:

1. The effectiveness of various inducers (e.g., O_2 and H_2O_2) added externally.
2. The uniqueness or multiplicity of internal inducers (organic peroxides?) formed in response to external inducers.
3. Factors affecting the dissipation or the effective utilization of external and internal inducers.

Inducers of catalase synthesis in Rps. spheroides are (among others) O_2, H_2O_2, and CH_3OOH. Saturation with air, which gives an O_2 concentration of 240 μM, produces the same effect as a sustained H_2O_2 concentration of about 0.5 μM. Induction by O_2 is probably mediated through the intracellular generation of H_2O_2. This assumption is supported by the fact that nitrite and alcohols, which retard the destruction of H_2O_2, potentiate the inducing effects of both H_2O_2 and air (a single result to the contrary, reported earlier, has been overruled by about 20 subsequent experiments).

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[5] B. Chance, Science 116, 202 (1952).

[6] L. J. Cole, V. P. Bond, and M. C. Fishler, Science 115, 644 (1952).

[7] A. Hollaender, G. E. Stapleton, and W. T. Burnett, Jr., Abstr. 120th Natl. Meeting Am. Chem. Soc., p 14C, 1951.

[8] R. K. Clayton, Biochem. and Biophys. Research Commun. (1959) (in press).

Distinct from the foregoing action of air is an inducing effect produced by low concentrations of O_2 (about 1 μM) in the presence of strong light. This "photo-oxidative" induction may be of interest with regard to the protection of photosynthetic tissues against photosensitized oxidations.

If resting cells of Rps. spheroides are exposed for a short time to H_2O_2 or to O_2 and then placed in darkness at 0°C, the inducing effect can be preserved for many hours. Apparently, H_2O_2 causes the formation of an "internal inducer" that is stable at 0°C but not at room temperature. Some properties of this internal inducer are being studied.

At concentrations of 1 mM and higher, the destruction of H_2O_2 in Rps. spheroides is mediated mainly by catalase. At concentrations of 0–100 μM, H_2O_2 is destroyed largely by one or more peroxidases. The kinetics of this destruction are of the usual Michaelis-Menten form, with K_m = 30 μM. Sodium azide, 10^{-3} M at pH 7, inhibits this peroxidase activity about 50% while inactivating the catalase completely.

The kinetics of the induced synthesis of catalase in Rps. spheroides can now be seen to depend on several factors: the manner in which H_2O_2 or other inducers are introduced, the rate at which H_2O_2 is destroyed by catalase and one or more peroxidases, and the efficiency with which "internal inducer" is generated and utilized. Further study of the interplay of these variables should clarify the steps through which aeration of a photosynthetic bacterium causes it to synthesize catalase.

Role of Methylmalonyl-CoA and Biotin in Bacterial Succinate Decarboxylation

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

Introduction. – Cell-free extracts of Propionibacterium pentosaceum and Micrococcus lactilyticus (Veillonella gazogenes) decarboxylate succinate to propionate and CO_2. The total reaction is CoA and ATP dependent; deacylases, transphorases, and activation of succinate and lower fatty acids were demonstrated.[9] The two principal steps consist of:[9,10]

[9] E. A. Delwiche, E. F. Phares, and S. F. Carson, J. Bacteriol. 71, 598 (1956).

[10] E. F. Phares, E. A. Delwiche, and S. F. Carson, J. Bacteriol. 71, 604 (1956).

(1) the decarboxylation of succinyl-CoA by the Propionibacterium extract, yielding propionyl-CoA but little free CO_2 and (2) the production of virtually stoichiometric amounts of free CO_2 when a Micrococcus washed particle preparation is also present.

Results and Discussion. — We now have evidence that these two reactions involve methylmalonyl-CoA, methylmalonyl isomerase[11] (transcarboxylase[12]), and a biotin-requiring decarboxylase:

$$\begin{array}{ccc} \text{succinyl-CoA} & \xleftrightarrow[\text{(isomerase)}]{\text{Propionibacterium extract}} & \text{propionyl-CoA} \\ + & & + \\ \text{propionyl-CoA} & & \text{methylmalonyl-CoA} \end{array}$$

(Net: succinyl-CoA → methylmalonyl-CoA) (1)

$$\text{methylmalonyl-CoA} \xleftrightarrow[\text{(biotin-requiring decarboxylase)}]{\text{Micrococcus particles}} \text{propionyl-CoA} + CO_2 \quad (2)$$

Succinyl-1,4-C^{14}-CoA is converted to C^{14}-labeled methylmalonyl-CoA by the Propionibacterium extract, and a washed Micrococcus particle preparation decarboxylates methylmalonyl-2-C^{14}-CoA to C^{14}-labeled propionyl-CoA. In the presence of both of the bacterial preparations, avidin (directly added) inhibited propionyl-CoA and CO_2 production from succinyl-CoA, as well as CO_2 exchange into succinyl-CoA; biotin fully overcame these inhibitions. However, avidin did not inhibit propionyl-CoA exchange into succinyl-CoA. Avidin pretreatment of the Micrococcus particles inhibited the decarboxylation and exchange reactions [forward and reverse reactions of Eqs. (1) and (2), respectively] much more than pretreatment of the Propionibacterium extract did. These results indicate that the site of biotin function is in the Micrococcus particle preparation, which was

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[11] W. S. Beck, M. Flavin, and S. Ochoa, J. Biol. Chem. 229, 997 (1957).

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

previously termed the "CO_2 enzyme." Hence this latter "enzyme" appears to be more closely related to Lynen's microbial biotin-enzyme,[13] which carboxylates β-methylcrotonyl-CoA, than to Ochoa's mammalian nonbiotin-requiring propionyl carboxylase.[14]

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[13] F. Lynen and J. Knappe, Sitzber. math.-naturw. Kl. bayer. Akad. Wiss. München, Feb. 6, 1959, p 1.

[14] A. Tietz and S. Ochoa, J. Biol. Chem. 234, 1394 (1959).

PLANT PHYSIOLOGY AND PHOTOSYNTHESIS

A. H. Haber F. T. Wolf[1]
H. J. Luippold W. A. Arnold[2]

Dormancy Resulting from Gamma Irradiation of Lettuce Seed

A. H. Haber H. J. Luippold

Introduction. – We have found that ionizing radiation administered to dry lettuce seed can prevent germination; 250 kr of Co^{60} gamma radiation prevents about half the population of originally viable seeds from germinating when they are subsequently moistened with water. The work reported here was performed to explore possible explanations for this effect of gamma radiation. The results show that gamma radiation prevents germination of lettuce seeds by inducing (or maintaining) a physiological state of dormancy similar to that effected by certain other treatments, such as exposure to far-red light.

Results. – Respiratory rates were studied to investigate the extent to which germination percentages might reflect possible killing of the seeds by gamma rays. Irradiation of dry seeds resulted in a lower respiratory rate as soon after the beginning of water imbibition as oxygen uptake can be measured with confidence.[3] The decrease in respiratory rates was, however, proportionately less than the decrease in germination percentages effected by the gamma rays.[3] These results suggest that the failure of irradiated seeds to germinate may not be simply explained in terms of killing, but perhaps in terms of some altered physiological state, another consequence of which is the lowered respiratory rates. This suggestion is corroborated by the data reported in Table 28. Populations of gamma-irradiated seeds that did not germinate in water did germinate in solutions of gibberellic acid, kinetin, or thiourea under continuous white light. These chemicals are known to be capable of

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[1] Research Participant.

[2] On official leave, summer 1959.

[3] A. H. Haber and H. J. Luippold, Intern. J. Radiation Biol. (1959) (in press).

Table 28. Effects of Gibberellic Acid, Kinetin, or Thiourea on Gamma-Irradiated Lettuce Seed

Dose (kr)	Seeds Germinated in Water Supplemented with	Percentage Germination After Eight Days (25°C, 35 ft-c white light)
0		98
	G*	100
	K	98
	T	94
214		58
	G	86
	K	100
	T	92
427		24
	G	87
	K	95
	T	95
641		17
	G	79
	K	95
	T	87
854		4
	G	90
	K	51
	T	50
1068		7
	G	74
	K	18
	T	17
1282		2
	G	15
	K	4
	T	0

*G = 2.5×10^{-3} M gibberellic acid; K = 10^{-5} M kinetin; and T = 3×10^{-2} M thiourea.

breaking dormancy in lettuce seed.[4,5] The germination-stimulating effects of gibberellic acid, kinetin, or thiourea include both cellular expansions and cell divisions, since mitotic figures (the late prophase, metaphase, anaphase) and micronuclei were detected in root and shoot meristems from those irradiated seeds that did germinate, irrespective of radiation dose and chemical treatment.[3] Two other treatments also known to break dormancy of lettuce seed, light[6] and pricking,[7] similarly increased the germination of gamma-irradiated seeds.

Since these five agents are known to break dormancy of unirradiated lettuce seed under certain conditions, it seems likely that the inhibition of germination was the result of dormancy imposed (or maintained) by the gamma radiation. To conclude rigorously, however, that the failure of gamma-irradiated seeds to germinate was caused by seed dormancy, it is necessary to show that agents that reversed the effect of gamma rays on germination afforded no reversal of other effects of gamma radiation. It was found, however, that the chemical treatments that greatly affect germination percentages of irradiated seeds had no effect on their respiratory rates before radicle protrusion.[3]

Gamma radiation prevented mitosis only when it prevented germination, since no combination of radiation dose and chemical treatment could enable seeds to germinate without also permitting mitotic activity in the shoot and root meristems. This suggests that the block in germination imposed by gamma radiation cannot be identified with the prevention of cell divisions. It might, nevertheless, still be argued that the radiation did prevent germination by somehow preventing mitotic activity. If so, then such a block in mitotic activity would have to be the locus of action of the germination-promoting chemicals. The absence

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[4] R. C. Thompson and W. F. Kosar, Plant Physiol. 14, 567–73 (1939).

[5] A. H. Haber and N. E. Tolbert, in Photoperiodism and Related Phenomena in Plants and Animals (in press).

[6] E. H. Toole *et al.*, Ann. Rev. Plant Physiol. 7, 299–324 (1956).

[7] M. Evenari and G. Neumann, Bull. Research Council Israel 2, 75–78 (1952).

of cell division until one or two days after germination for irradiated (214–641 kr) seeds rules out the last-mentioned possibility. When seeds were given 27.2 kr and later were examined for chromosomal abnormalities in the root tip, the numbers of chromosomal aberrations (either as deletions or as breaks resulting in detectable exchanges) were unaffected by gibberellic acid or kinetin.[3] The results with thiourea were especially interesting, since treatment with this chemical actually increased the number of chromosomal aberrations even though it (like the other chemicals) decreased the gamma-radiation-induced inhibition of germination.[3] In the absence of ionizing radiation, thiourea had no obvious effect on aberrations.[3] These results show that reversal of the gamma-ray-induced inhibition of germination by germination-stimulating chemicals could not be related to any detectable reversal of radiation effects on chromosomal abnormalities. A final piece of evidence showing that the reversal of radiation effects afforded by the germination-stimulating chemicals was peculiar to the germination process was that the delayed effects of radiation on seedling growth were not influenced by the chemicals.[3]

Discussion. – Our results show that the failure of lettuce seed to germinate after receiving 300–1000 kr of gamma radiation was caused by a state of physiological dormancy resulting from the radiation. This explanation was suggested by the fact that such gamma-irradiated seed did germinate when they were subsequently treated by each of five agents known to break dormancy of lettuce seed: gibberellic acid, kinetin, thiourea, pricking, and light.[4–7] For all three of the chemical agents investigated, the germination was always accompanied by cell expansion and cell division. Alternatives to the seed-dormancy explanation have been ruled out. The respiratory studies preclude the possibility that the inhibition of germination by gamma radiation, as well as the reversal of such inhibition by chemical treatment, could be explained in terms of killing. The prevention of germination by gamma radiation cannot be explained by interference with mitosis, since the lack of mitotic activity could be correlated only with failure to germinate and not with radiation dose. Moreover, germination preceded the first mitoses in irradiated seeds. Also, the chemical treatments that reversed the effects of gamma radiation on germination percentages did not reverse radiation effects on chromosomal aberrations. Finally,

we may consider the reversal of the radiation effects by the aforementioned agents specific for the germination process, since there was no reversal by gibberellic acid or kinetin on the radiation-induced lowering of respiratory rate; no reversal by gibberellic acid, kinetin, or thiourea on the frequency of radiation-induced chromosomal aberrations; and no reversal by gibberellic acid or kinetin on the radiation-induced reduction in seedling height.

We have previously shown that injurious treatments, including X irradiation during water imbibition, can induce a light requirement in these seeds.[8] The results reported here show that germination-stimulating agents, other than light, can also promote the germination of these seeds when they are suitably damaged. The gibberellic acid, kinetin, thiourea, and pricking treatments each promote germination of irradiated seeds, even under continuous white light. We have shown that Grand Rapids lettuce seed would respond to germination-promoting light under conditions in which they did not respond to either gibberellic acid or kinetin.[5] The results reported here thus demonstrate converse results: the efficacy of gibberellic acid or kinetin (as well as thiourea or pricking) under conditions in which the response to light was poor.

The dormancy in lettuce seed resulting from gamma irradiation, as well as that imposed by far-red light, can be broken by any of the five agents described in this paper. The "thermodormancy" induced in lettuce seed by continuous exposure to temperatures of ~35°C can also be broken by kinetin or thiourea but cannot be broken by gibberellic acid, pricking, or light.[5,9] The significance of this similarity between dormancy induced by far-red light and dormancy induced by gamma radiation remains to be evaluated fully. Such dormancy may be used as a tool for studying the effects of ionizing radiation and could supplement other known gross biological responses that have been used, for example, killing or growth inhibition.

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[8] A. H. Haber, Physiol. Plantarum (1959) (in press).

[9] A. H. Haber, unpublished data.

Effects of Gibberellin on Gamma-Irradiated Wheat

A. H. Haber H. J. Luippold

Introduction. — It has been suggested that gibberellin stimulates growth by cell expansion, cell division, or both.[10] In general, the problem of whether gibberellin-stimulated growth can be attributed to increased cell expansion, to cell division, or to both is complicated by the fact that both processes occur during normal growth. Grains given ~500,000 r of gamma radiation can develop into small seedlings by cell expansion only, mitosis being completely prevented by the irradiation.[11] In this work, we show that such irradiated wheat can give typical growth responses to gibberellic acid (GA) in the complete absence of cell division. The data also give an indication of the contribution of cell division to the gibberellin growth responses of young wheat seedlings.

Results. — Wheat grain were irradiated, then germinated and grown in either water or 3×10^{-4} M GA. At daily intervals, the shoot and a root meristem from one plant in water and one in GA were fixed for observation of mitotic figures. The total numbers of mitoses observed among each set of 48 meristems during the 12-day period were 5080, 89, 2, and 0 for doses of 0, 204, 408, and 612 kr, respectively. Plants from grain given 612 kr also lacked micronuclei, which further supports the conclusion that no mitosis occurred.

To determine whether young seedlings incapable of mitosis can respond to gibberellin, we exposed wheat grain to various doses of gamma rays, then allowed them to germinate and grow in water or 3×10^{-4} M GA, and measured the shoot height after 12 days. It can be seen from Fig. 21 that gibberellic acid-treated plants grow taller than controls for all radiation doses studied. The gibberellin effect is evident in plants grown from grain given 1225 kr, which is twice the dose that abolished all detectable mitotic activity. Consequently, the growth-stimulating effect of GA on plants from such heavily irradiated seed must be restricted

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[10] B. B. Stowe and T. Yamaki, Science 129, 807–16 (1959).

[11] D. Schwartz and C. E. Bay, Am. Naturalist 90, 323–27 (1956).

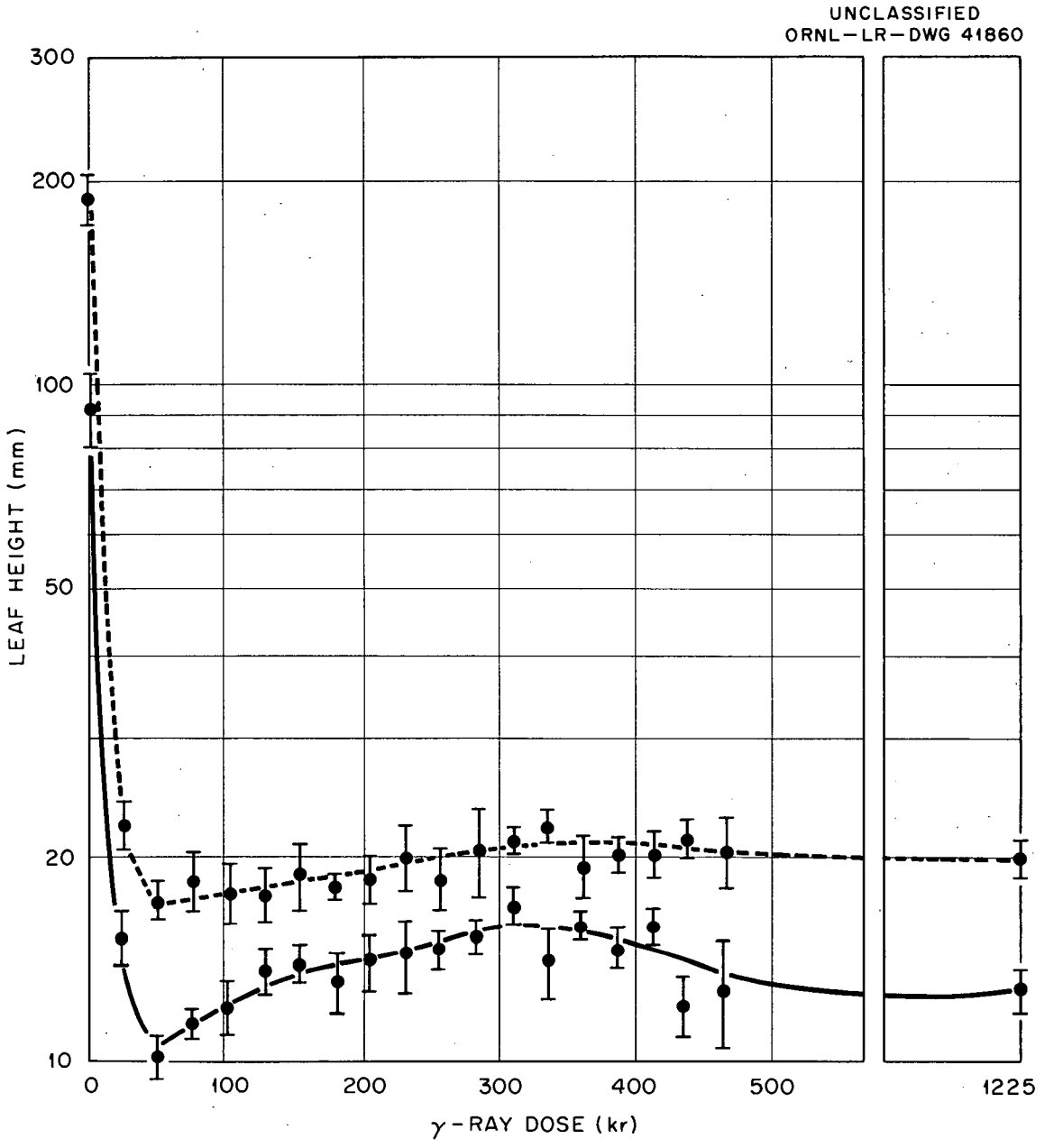

Fig. 21. Effect of Gibberellic Acid on Height of Wheat Seedlings 12 Days After Sowing the Irradiated Grains. Solid line, plants grown in water; broken line, grown in 3×10^{-4} M GA. Bars represent 95% confidence limits.

to cell expansion. The seedling height-dose curve for plants grown in water shows a minimum point for shoot height at ~50 kr. This reversal may be attributed to an interaction of chromosome breakage and mitotic inhibition.[11] For our present considerations, the conclusions of Schwartz and

co-workers suggest that, for doses of 50–80 kr, mitotic activity actually tends to decrease shoot height. Hence, if GA had any significant direct effect on increasing mitotic activity, we should predict that the seedling height-dose curve for plants in GA would differ from the curve for water controls in two ways: (1) The dip in the gibberellin curve should be proportionately greater than the dip in the curve for water, since increased mitotic activity for this dose range would tend to decrease shoot height. (2) The minimum in the gibberellin curve should correspond to lower doses of radiation. However, neither of these two predictions is fulfilled by the data in Fig. 21. The two predictions presuppose that the proportion of radiation-induced chromosomal aberrations per cell division is unaffected by the gibberellin treatment. This supposition seems warranted, since we have found that the frequency of chromosomal aberrations in root tips after irradiation of lettuce seed was unaffected by gibberellin treatment. After 27.2 kr of Co^{60} gamma rays, the percentage of normal metaphase figures, as well as the number of deletions or the number of breaks resulting in exchanges per 100 metaphase figures, were each unaffected by GA at a concentration active in stimulating seedling height and germination[3] and inhibiting root growth.[9] It thus appears that GA stimulates shoot growth after radiation doses for which (1) mitosis contributes to increasing shoot height (less than 25 kr), (2) mitosis presumably reduces shoot height (50 kr), and (3) mitosis is completely absent (1225 kr). The fact that the gibberellin and water-control curves in Fig. 21 tend to be roughly parallel (on the semilog plot) suggests that the GA effect on the growth of young wheat seedlings is not directly mediated to any great extent by a stimulation of cell division.

For grain given 1225 kr, the effect of GA on cell expansion was paralleled by an increased dry weight. The dry weight of individual shoots after 12 days averaged 0.47 mg for plants in water and 0.92 mg for plants in GA. The gibberellin-treated shoots from grain given 1225 kr had other features that are also characteristic of the normal responses to gibberellin;[10] they were paler and proportionately thinner than plants grown in water from grain irradiated with the same dose.

The effects of GA on cellular expansion and dry-weight increase of irradiated plants growing without mitotic activity pertain to the total

extent of growth, not only to the rate.[12] Further studies on the time course of growth showed that by 12 days, growth is complete for such plants either in GA or in water.[12] Our data also suggested that sensitivity to gibberellin is not directly related to actual age but to whether the tissues are actively growing at the time of treatment.[12] Although this has been generally noted before, our results with seedlings growing without any cell divisions permit us to consider these relations on a per cell basis. Thus the general ineffectiveness of gibberellin on mature tissues cannot be explained by saying that the cells in the mature tissues had already reached the maximum size they had potentially been capable of attaining. Rather, it seems that the cells in mature tissues have somehow become physiologically incapable of responding to gibberellin.

Discussion. – The results just presented demonstrate that GA can stimulate growth of wheat grown from irradiated grain in the complete absence of cell division. Increasing the dose from 0 to 624 kr reduced the number of observed mitotic figures during the 12-day period from more than 5000 to 0. Approximately doubling the dose from 624 to 1225 kr thus gave a large safety factor for our conclusion that the gibberellin effects we observed were not accompanied by any cell division. Consequently, the effect of GA on plants from such heavily irradiated grain must have been entirely the result of cell expansion. Since the dry-matter content also increased in gibberellin-treated shoots from such heavily irradiated grain, the dry matter content per cell must have been increased by the gibberellin treatment. These results might suggest that the increased dry-matter content of gibberellin-treated plants[10] may generally be attributed largely to cell expansion.

An alternative explanation for our data might have been that the growth effects of GA we observed were not characteristic gibberellin effects, but that the GA somehow enabled the plants to overcome the effects of the gamma radiation by "restoration." This alternative explanation is most unlikely for two reasons. First, for all doses of radiation we

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

studied, the gibberellin-treated shoots had additional features characteristic of the gibberellin response; they were paler and proportionately thinner than untreated shoots from grain given the same dose of radiation. Second, GA does not have restorative activity on gamma-irradiated lettuce. It does not reverse gamma-radiation effects either on the decreased oxygen consumption per seed or on the expression of chromosomal aberrations per metaphase figure, even though lettuce does respond to gibberellin with respect to growth and germination.[3]

These results are especially pertinent to recent reports that GA stimulates growth by stimulating cell division,[10,13] and are discussed in a more detailed account of this work that is in press.[12]

Studies on Plant Pigments

F. T. Wolf A. H. Haber

Pteridines in Higher Plants. – It has been shown that unconjugated pteridines occur in spinach chloroplasts.[14] The distribution of pteridines in chloroplasts relative to the entire cell was determined by comparing the ratios of pteridines to chlorophyll in whole extracts and chloroplast fractions. Results show that the pteridines in spinach leaves are largely contained within the chloroplasts. Dark-grown Alaska peas produce a pteridine different from that of plants grown under usual greenhouse conditions.[15] Studies on the time course of the transformation of the "dark pteridine" to the "light pteridine" indicate that ~16 hr of illumination are required for nearly complete conversion.

Analysis of Gibberellin-Induced "Chlorosis." – The general belief has been that increased growth of plants treated with gibberellin is accompanied by chlorosis of the leaves, even under the best nutrient conditions.[16] It was not known whether such chlorosis was caused by

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[13] R. M. Sachs, C. F. Bretz, and A. Lang, Am. J. Botany 46, 376–84 (1959).

[14] R. C. Fuller et al., Proc. Natl. Acad. Sci. U.S. 44, 239 (1958).

[15] F. T. Wolf, unpublished.

[16] P. W. Brian, Biol. Rev. 34, 37 (1959).

chlorophyll destruction, reduced rate of synthesis, failure of synthesis to keep pace with increased growth, or a change in pigment composition.[16] We found that one-week-old wheat seedlings grown from gibberellin-treated grain, although much paler in appearance, had the same chlorophyll content per plant as controls, even though growth was increased by more than 60% by the gibberellin treatment. Thus, under the best nutrient conditions, there is no net decrease in chlorophyll, and therefore no real chlorosis, accompanying the increased growth of gibberellin-treated plants. After 12–30 days, the rate of net chlorophyll synthesis is less in gibberellin-treated plants than in controls; however, at these times the rate of growth (but not the extent) is no longer greater for gibberellin-treated plants. Examination of the ratio of chlorophyll a to chlorophyll b indicates that the "chlorosis" cannot be attributed to a change in pigment composition. Experiments in which green plants were transferred to continuous darkness showed no effect of gibberellin on the rate of chlorophyll destruction. Experiments in which leaves from etiolated plants were transferred to light showed no effect of gibberellin treatment during greening on the rate of chlorophyll formation. These results demonstrate that the apparent chlorosis in young seedlings results solely from failure of chlorophyll synthesis to keep pace with increased cell extension stimulated by gibberellin.[12] In addition to this simple dilution of chlorophyll, the apparent chlorosis in the older plants must also result from delayed effects that are not related to direct action of gibberellin on either the synthesis or the destruction of chlorophyll.

Photosynthesis

W. A. Arnold

Experiments made over the last few years leave little doubt that the production of light by green plants after they have been illuminated and the production of light by dried chloroplasts upon heating after illumination are both electron trapping phenomena. Parts, at least, of the chloroplast must act as electronic semiconductors. It is tempting to think that the first step in photosynthesis is the illumination of the

junction between a layer of chlorophyll and a layer of something else — perhaps lipid, containing carotene. The first act in photosynthesis would then be the transfer of charge across this boundary, much as in the solar battery.

The spring was devoted to experiments designed to show whether such charge transfer takes place in chloroplasts. These experiments are still inconclusive.

BIOPHYSICS

J. S. Kirby-Smith
M. L. Randolph
J. Jagger
M. Sidran[1]
O. H. Crawford[2]
M. L. Gwyn
H. G. Jones
D. L. Parrish

Comparative Studies of Electron Spin Resonances Induced by Ultraviolet and Ionizing Radiations

J. S. Kirby-Smith

Introduction. – The ability of low-energy electromagnetic radiation, visible as well as ultraviolet, to form free radicals in a number of systems is now well established from work in a number of laboratories. Our own current work has indicated a similar effect of ultraviolet radiation in a number of proteins and DNA. If a comparison is made of the relative effectiveness of ultraviolet and ionizing radiations in radical production on representative proteins and DNA, some quite interesting correlations with chromosome breakage induced by ultraviolet and ionizing radiations may be made.

Results and Discussion. – It was previously shown[3] that ultraviolet and ionizing radiations result in quite significant differences in the relative numbers of incomplete isochromatid aberrations in *Tradescantia* pollen and that, accordingly, the primary breakage and rejoining mechanisms must be considerably different for these radiations. These studies also indicated a close relation between the action spectrum for ultraviolet-induced aberration and the DNA absorption spectrum. Thus we may conclude that the site of primary damage to the chromosomes by ultraviolet radiation is the nucleic acid.

In addition, on the strict basis of energy absorption alone, ionizing radiation is several orders of magnitude more effective than ultraviolet for induction of chromosomal damage.

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[1] Research Participant.

[2] Temporary summer employee.

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

From our current studies of radical formation in DNA and protein, the following points are quite obvious: (1) radical production by ionizing radiation is much less in DNA than in proteins; (2) the effect of ionizing radiation on proteins is much greater per unit of absorbed energy than is ultraviolet for either protein or DNA; and (3) radical formation in DNA is somewhat more sensitive to ultraviolet than in proteins.

These findings all support the hypothesis that the primary site for chromosomal damage by ionizing radiation is in the protein portion of the strand and, for ultraviolet-induced damage, most if not all of the damage occurs in the DNA. This simple hypothesis also implicitly assumes that the chromosomal threads are made up of separate segments of DNA and proteins linked together in a continuous chain. Such a model has already been postulated by Schwartz[4] from genetic and cytological evidence, as well as from the ultraviolet-induced aberration studies of Kirby-Smith and Craig.[3]

Natural and Light-Induced Free Radicals in Riboflavin Systems

M. Sidran

Introduction. — The electron spin resonance spectra of three riboflavin systems before and after irradiation with visible light are under investigation. Isenberg and Szent-Györgyi[5] observed that concentrated solutions of riboflavin turn red in the presence of tryptophan or certain tryptophan derivatives. These investigators have presented spectral evidence that the red form is a riboflavin-tryptophan charge transfer complex in which a riboflavin molecule takes up one electron from the tryptophan. The riboflavin is then in the semiquinoid form. The formation of such a charge transfer complex might play a role in electron transport in the living cell.

The three systems under consideration are riboflavin 5-phosphate, riboflavin 5-phosphate plus tryptophan, and riboflavin 5-phosphate plus serotonin (5-hydroxytryptamine).

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[4] D. Schwartz, Nature 181, 1149 (1958).

[5] I. Isenberg and A. Szent-Györgyi, Proc. Natl. Acad. Sci. U.S. 44, 857–62 (1958).

Results and Discussion. — Concentrated solutions of riboflavin 5-phosphate and tryptophan were mixed in stoichiometric proportions, and the solutions were evaporated to dryness in vacuum, leaving behind a blood-red solid that appeared to be crystalline. Air was then admitted to the system. A paramagnetic resonance signal corresponding to one unpaired electron in every 10^5 molecules was obtained from this solid. The spectrum consisted of a single line typical of a free radical.

Upon exposure of this solid to ultraviolet radiation, the number of unpaired spins increased by a factor of 10. There was no change in the gross appearance of the solid. When the vacuum evaporation was performed in the dark, the gross appearance of the solid was considerably altered. It became very light and fluffy, and had a rust-brown color. About one-fourth as many free radicals were detected as for the solid prepared in the light. Thus the red color of the riboflavin-tryptophan system and the presence of unpaired spins appear to be light induced.

To eliminate the effect of impurities, tryptophan and riboflavin were tested separately for ESR signals. Solid tryptophan gave no measurable signal. Solid riboflavin from the stock bottle gave a signal corresponding to one spin in 10^6 molecules. Riboflavin dissolved in water in the dark and evaporated to dryness in vacuum gave a signal of about the same order of magnitude. Thus the signals obtained for the riboflavin-tryptophan system cannot be caused by impurities.

The effects of light on pure riboflavin were even more pronounced than they were for the riboflavin-tryptophan system. In this case also, a color change was produced by the light exposure. A signal corresponding to one spin per 5000–10,000 molecules was observed for riboflavin dried in vacuum in the light. The material turned a dark brown. The intensity of the brown color and the strength of the ESR signal could both be correlated with the light intensity. When the brown material was irradiated with ultraviolet light, the number of unpaired spins increased to one spin per 2000 molecules. The light-induced free radicals decay with time over a period of weeks. Naturally occurring free radicals observed for riboflavin in the dark are probably caused by previous exposure to light or small amounts of stray light.

Detection of free radicals in the riboflavin-tryptophan system is thus complicated by the presence of free radicals in riboflavin itself and by the strong sensitivity to light of free radical formation in both systems. It may be speculated that light produced in living systems actually plays a part in the electron transfer mechanism.

The riboflavin-serotonin system prepared in light gave a signal of about the same strength as that obtained from the riboflavin-tryptophan system, although the spectral evidence[5] indicates that complex formation should be seven times as strong in the former system.

Preliminary studies have also been made of the effects of oxygen and water on the formation of free radicals in these systems. It has been found that the presence of water accelerates the rate of formation of light-induced free radicals. The oxygen effects are not yet clarified.

Radiation-Produced Electron Spin Resonances in Crystalline Amino Acids

M. L. Randolph D. L. Parrish O. H. Crawford

Introduction. — Quantitative studies on the kinetics of electron spin resonance production by irradiation of dry crystalline amino acids have been continued. The principal efforts have been directed toward decay measurements, of resonant systems with short (less than two day) half lives and with long half lives, and toward the possibility of saturation limits on the number of resonances produced.

Methods. — Dry, crystalline amino acids, irradiated and stored in air were analyzed with the electron spin resonance spectrometer. The present instrument differs from that previously described[6,7] only in that a high Q ("Stalo") cavity tightly coupled to the klystron is inserted in place of the previous "Uniline," thus stabilizing the microwave frequency, and small separate field coils rather than the main magnet coils are oscillated for the 13-cps sweep frequency.

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

[7] A. D. Conger and M. L. Randolph, Radiation Research 11, 54–66 (1959).

Results and Discussion. – The decay of DL-leucine and methionine have been intensively studied. Each shows a complicated decay scheme requiring at least two exponential decay constants for mathematical description. Figure 22 illustrates the decay of DL-leucine measured by (1) differential peak height, (2) integral curve peak height, and (3) area of integral curve. Initially the differential curve repeatedly rises; but instead of an indication of increase in number of resonances, we believe this is an artifact caused by overlapping patterns canceling part of what would otherwise be the maximum differential peak height. We firmly believe the area of the integral curve is the best measure of the total number of resonances. Supporting the contention of canceling peaks in the differential curve is the observation that the initial integral curves show six peaks whereas later curves show but one, which appears to be the largest initial peak. Such change may represent transitions of resonances within the material within a few hours after irradiation or simply more rapid decay of the wings of the pattern than the largest peak.

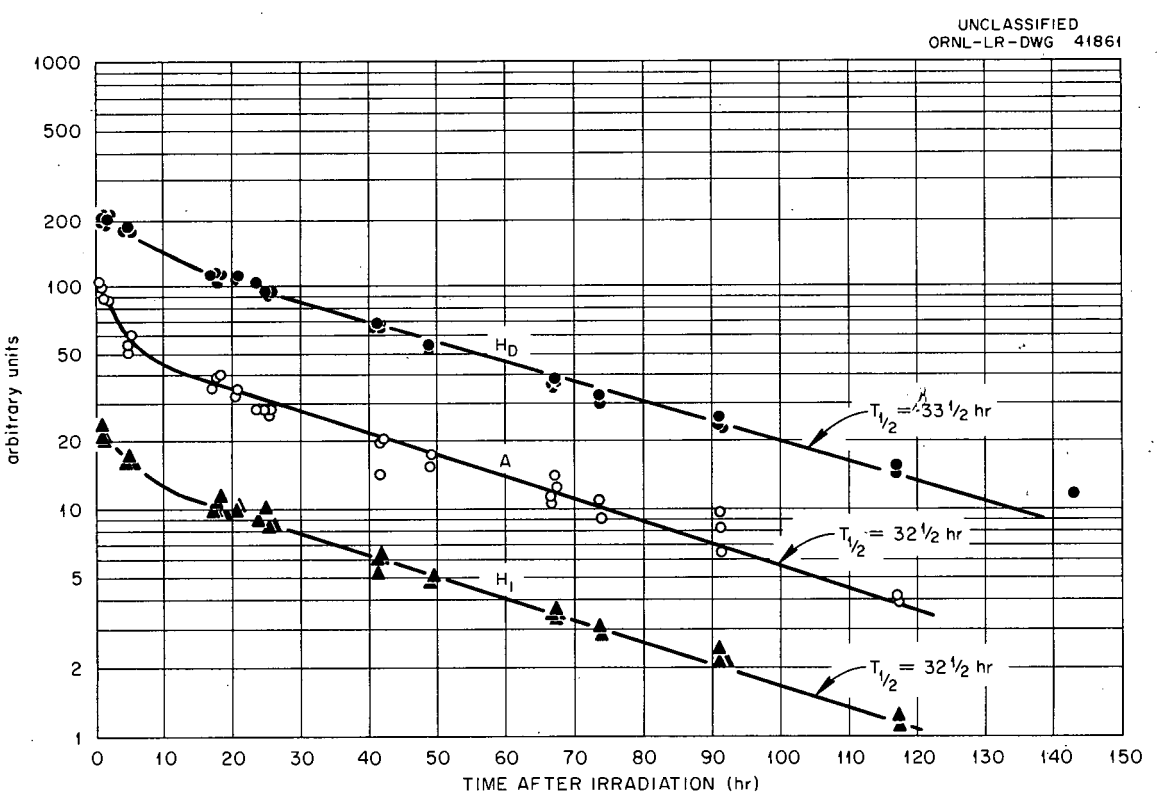

Fig. 22. DL-Leucine Decay; 250 krads of X Rays in 20 min.

A statistical check of the ratios for DL-methionine between integral curve area, integral curve height, and differential curve area showed no significant changes with time, indicating that the curve shape is constant. The decay is given approximately by:

$$N = N_0 \left(0.6e^{-0.315t} + 0.4e^{-0.0171t}\right) ,$$

where N_0 is the number of resonances immediately after brief irradiation and t the time in hours after the end of irradiation.

The study of the very long-term stability of resonances induced by heavy irradiation of glycine, glycylglycine, DL-α-alanine, and glutamic acid has been extended to more than eight months; very slow decay is still shown.[8] Protracted, 100-day irradiation of glutamic acid in the Co^{60}-gamma source to about 10^8 rads gave a signal little larger than 15-day irradiation. Since we find this irradiated material to have resonances stable for at least twice the duration of this irradiation, the result suggests a saturation limit when the number of resonances is very roughly 2:1000 the number of molecules. Such phenomena are also hinted at by our previous data.[8] Further very high dose experiments are planned to follow this lead. If saturation effects of this sort are the rule for crystalline amino acids, most of the observed resonances are probably some kind of lattice structural defects rather than biologically more significant molecular defects. Assuming the crystals are cubes 20 μ on a side, we find that the number of molecules on the surface is only 2×10^{-4} times the total number of molecules. Since this is but one-tenth the suggested saturation limit and the crystals are probably larger than assumed, it seems unlikely that the observed phenomena are primarily effects in the first surface layers of the crystals.

Extension of measurements of X-ray-induced resonances have yielded that one resonance is produced in DL-valine per 85 ev dissipated, only small signals from L(-)-cystine, and no signals from DL-isoleucine.

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

Photoprotection in *Escherichia coli* B

J. Jagger

Further experiments on photoprotection have been done by the techniques described in an earlier report.[9]

It has been found that there is (1) essentially no change in the reaction rate of photoprotection performed throughout the range 3–37°C, and (2) essentially no change in reaction rate when the intensity of the illumination is changed by a factor of 4.

These results strongly suggest that photoprotection is a purely photochemical reaction, thus differing basically from photoreactivation. Preliminary experiments indicate, however, that the range of effective wavelengths is the same for the two processes.

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

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