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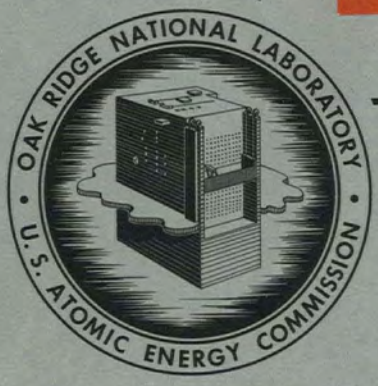
ORNL-2997
Progress

BIOLOGY DIVISION

SEMIANNUAL PROGRESS REPORT

FOR PERIOD ENDING AUGUST 15, 1960



OAK RIDGE NATIONAL LABORATORY
operated by
UNION CARBIDE CORPORATION
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U.S. ATOMIC ENERGY COMMISSION

ORNL-2997
Progress

Contract No. W-7405-eng-26

BIOLOGY DIVISION

SEMIANNUAL PROGRESS REPORT

For Period Ending August 15, 1960

Alexander Hollaender, Director
Stanley F. Carson, Assistant Director

DATE ISSUED

OCT 10 1960

OAK RIDGE NATIONAL LABORATORY
Oak Ridge, Tennessee
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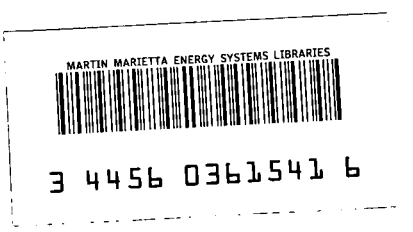

CONTENTS

PUBLICATIONS AND LECTURES

Publications. – The volume of papers and abstracts written and processed by the Biology Division in the past six months is up nearly 40% – a total of 281 in print or in press, as compared with 208 at the same time last year. Of this number 102 have been published and except for abstracts are listed in this report.

Two supplements to journals and a book are in press or published. Some Delayed Effects of Atom-Bomb Radiations in Mice* is appearing as a supplement to Cancer Research, September 1960. It was written by A. C. Upton, A. W. Kimball, J. Furth, K. W. Christenberry, and W. A. Benedict. Proceedings of the Symposium on Mammalian Genetics and Reproduction held at Gatlinburg last April will be published as a December supplement to the Journal of Cellular and Comparative Physiology. In press is Radiation Protection and Recovery, of which A. Hollaender is editor. D. Davidson, D. G. Doherty, T. Makinodan, T. T. Odell, Jr., L. H. Smith, G. E. Stapleton, S. Wolff, and A. Whiting all contributed chapters to this book, which is being published by the Pergamon Press.

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Adler, H. I.	Response of a Catalase-Negative Bacterium to Ionizing Radiation	In Immediate and Low Level Effects of Ionizing Radiations, ed. by A. A. Buzzati-Traverso (proc. of the Symposium held at Venice, June 22–26, 1959; suppl. to Intern. J. Radiation Biol.), Taylor and Francis Ltd., London, 1960, p 147–54
Anderson, B., and T. T. Odell, Jr.	Changes in Rat Cartilage Mucopolysaccharide with Age and Radiation	J. Gerontol. 15, 249–52 (1960)
Anderson, N. G., and R. E. Canning	A Method for Plotting Ultracentrifuge Diagrams for Polydisperse Systems	Biochim. et Biophys. Acta 38, 367–69 (1960)
Anderson, N. G., and C. B. Norris	Cell Division. III. The Effects of Amines on the Structure of Isolated Nuclei	Exptl. Cell Research 19, 605–18 (1960)

*Summarized in Biol. Semiann. Prog. Rep. Feb. 15, 1960, ORNL-2913, p 93.

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Arnold, W. A., and R. K. Clayton	The First Step in Photosynthesis: Evidence for Its Electronic Nature	Proc. Natl. Acad. Sci. U.S. 46, 769–76 (1960)
Auerbach, C.	Chemical Induction of Recessive Lethals in Neurospora crassa	Microbial Genetics Bull. 17, 5–6 (1960)
Auerbach, C.	Spontaneous Mutations in Dry Spores of Neurospora crassa	Z. Vererbungslehre 90, 335–46 (1959)
Auerbach, C., and G. Kølmark	Mutagen Specificity and Combination Treatments in Neurospora	Microbial Genetics Bull. 17, 23–24 (1960)
Bender, M. A	X-Ray-Induced Chromosome Aberrations in Mammalian Cells in Vivo and in Vitro	In Immediate and Low Level Effects of Ionizing Radiations, ed. by A. A. Buzzati-Traverso (proc. of the Symposium held at Venice June 22–26, 1959; suppl. to Intern. J. Radiation Biol.), Taylor and Francis Ltd., London, 1960, p 103–18
Bender, M. A, T. T. Phan, and L. H. Smith	Preservation of Viable Bone Marrow Cells by Freezing	J. Appl. Physiol. 15, 520–24 (1960)
Bernlohr, R. W., and G. D. Novelli	Some Characteristics of Bacitracin Production of Bacillus licheniformis	Arch. Biochem. Biophys. 87, 232–38 (1960)
Bollum, F. J.	Oligodeoxynucleotide Primers for Calf Thymus Polymerase	J. Biol. Chem. 235(5), PC18–PC20 (1960)
Brokaw, C. J.	Decreased Adenosine Triphosphatase Activity of Flagella from a Paralyzed Mutant of Chlamydomonas moewusii	Exptl. Cell Research 19, 430–32 (1960)
Brosseau, G. E., Jr.	Genetic Analysis of the Male Fertility Factors on the Y Chromosome of Drosophila melanogaster	Genetics 45, 257–74 (1960)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Clayton, R. K.	The Induced Synthesis of Catalase in Rhodopseudomonas spheroides	Biochim. et Biophys. Acta 37, 503–12 (1960)
Clayton, R. K.	An Intermediate Stage in the H_2O_2-Induced Synthesis of Catalase in Rhodopseudomonas spheroides	J. Cellular Comp. Physiol. 55, 9–14 (1960)
Clayton, R. K.	Kinetics of H_2O_2 Destruction in Rhodopseudomonas spheroides: Roles of Catalase and Other Enzymes	Biochim. et Biophys. Acta 40, 165–67 (1960)
Clayton, R. K.	Physiology of Induced Catalase Synthesis in Rhodopseudomonas spheroides	J. Cellular Comp. Physiol. 55, 1–7 (1960)
Clayton, R. K.	Protein Synthesis in the Induced Formation of Catalase in Rhodopseudomonas spheroides	J. Biol. Chem. 235, 405–7 (1960)
Cohn, W. E.	Pseudouridine, a Carbon-Carbon Linked Ribonucleoside in Ribonucleic Acids: Isolation, Structure, and Chemical Characteristics	J. Biol. Chem. 235, 1488–98 (1960)
Congdon, C. C., and A. Hollaender	Experimental Applications of Bone Marrow Transplantation	A.M.A. Arch. Pathol. 69, 605–12 (1960)
Cosgrove, G. E.	Therapy for Radiation Damage	In Oklahoma Conference – Radioisotopes in Agriculture, TID-7578, U.S. Government Printing Office, Washington, D.C., 1959, p 283–87
Darden, E. B., Jr.	Changes in Membrane Potentials, K Content, and Fiber Structure in Irradiated Frog Sartorius Muscle	Am. J. Physiol. 198, 709–14 (1960)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
de Serres, F. J.	Studies with Purple Adenine Mutants in Neurospora crassa. IV. Lack of Complementation Between Different ad-3A Mutants in Heterokaryons and Pseudowild Types	Genetics 45, 555–56 (1960)
Dolin, M. I.	The Streptococcus faecalis Oxidases for Reduced Diphosphopyridine Nucleotide. IV. Properties of the Enzyme-Substrate Complex Formed Between Reduced Diphosphopyridine Nucleotide Peroxidase and Pyridine Nucleotides	J. Biol. Chem. 235, 544–50 (1960)
Dolin, M. I., and N. P. Wood	The Streptococcus faecalis Oxidases for Reduced Diphosphopyridine Nucleotide. V. Isolation and Properties of a Flavin Mononucleotide-Containing Diaphorase	J. Biol. Chem. 235, 1809–14 (1960)
Friedberg, W.	Serum Albumin Metabolism in X-Irradiated Mice with Implanted Rat Bone Marrow	Intern. J. Radiation Biol. 2, 186–95 (1960)
Goff, R. A.	The Acute Lethal Response of the Chick Embryo to X Radiation	J. Exptl. Zool. 141, 477–97 (1959)
Goodman, J. W., and R. D. Owen	Red Cell Repopulation in Irradiated Mice Treated with Plethoric Homologous Bone Marrow	Am. J. Physiol. 198, 309–14 (1960)
Haber, A. H., and H. J. Luippold	Action of 6-(Substituted) Purines on Mitotic Activity in "Dormant" Lettuce Seeds	Physiol. Plantarum 13, 298–307 (1960)
Haber, A. H., and H. J. Luippold	Effects of Gibberellin on Gamma-Irradiated Wheat	Am. J. Botany 47, 140–44 (1960)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Haber, A. H., and H. J. Luippold	Effects of Gibberellin, Kinetin, Thiourea, and Photomorphogenic Radiation on Mitotic Activity in "Dormant" Lettuce Seeds	Plant Physiol. 35, 486–94 (1960)
Haber, A. H., and H. J. Luippold	Separation of the Mechanism Initiating Cell Division and Cell Expansion in Lettuce Seed Germination	Plant Physiol. 35, 168–73 (1960)
Haber, A. H., and J. D. White	Action of Maleic Hydrazide on Dormancy, Cell Division, and Cell Expansion	Plant Physiol. 35, 495–99 (1960)
Hollaender, A., and S. F. Carson	Biology Division Semiannual Progress Report for Period Ending February 15, 1960	ORNL-2913
Kameyama, T., and G. D. Novelli	The Cell-Free Synthesis of β-Galactosidase by Escherichia coli	Biochem. Biophys. Research Communs. 2, 393–96 (1960)
Kenney, F. T.	On the Nature of Adrenocorticoid-Induced Increase in Tyrosine-α-ketoglutarate Transaminase Activity of Rat Liver	Biochem. Biophys. Research Communs. 2, 333–35 (1960)
Kirby-Smith, J. S., and M. L. Randolph	Production and Lifetimes of Radiation-Induced Free Radicals in Some Molecules of Biological Importance	In Immediate and Low Level Effects of Ionizing Radiations, ed. by A. A. Buzzati-Traverso (proc. of the Symposium held at Venice, June 22–26, 1959; suppl. to Intern. J. Radiation Biol.), Taylor and Francis Ltd., London, 1960, p 11–24.
Kølmark, G., and C. Auerbach	Mutagenic Aftereffect in Neurospora Treated with Diepoxybutane	Microbial Genetics Bull. 17, 24–25 (1960)
Kondo, S., and M. L. Randolph	Effect of Finite Size of Ionization Chambers on Measurements of Photon Sources	Radiation Research 13, 37–60 (1960)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Kroeger, H.	The Development of Form in the Morphogenetic Field (in German)	Naturwissenschaften 47, 148–53 (1960)
Kroeger, H.	The Induction of New Puffing Patterns by Transplantation of Salivary Gland Nuclei into Egg Cytoplasm of Drosophila	Chromosoma 11, 129–45 (1960)
Lynn, W. G., and J. N. Dent	The Action of Various Goitrogens in Inhibiting Localization of Radioiodine in the Thyroid and Thymus Glands of Larval Tree Toads	Biol. Bull. 118, 430–38 (1960)
McGrath, R. A.	Cell Migration and Abnormal Crypt-Cell Enlargement in the Small Intestine of X-Irradiated Mice	Intern. J. Radiation Biol. 2, 177–85 (1960)
Makinodan, T., and N. Gengozian	X-Ray Depression of the Recognition Mechanism of Antibody-Forming Cells	In Mechanisms of Antibody Formation, Proceedings of a Symposium, Czechoslovak Academy of Sciences, Prague, 1960, p 339–52
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	Ibid., p 182–89
Mazur, P.	Physical Factors Implicated in the Death of Microorganisms at Subzero Temperatures	Ann. N.Y. Acad. Sci. 85, 610–29 (1960)
Novelli, G. D.	The Turnover of Pantothenic Acid	In Fourth International Congress of Biochemistry, vol XI, Vitamin Metabolism, ed. by W. Umbreit and H. Molitor, Pergamon Press, London, 1960, p 169–84

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Oakberg, E. F.	Effects of Radiation on the Gonads of Mice	In Oklahoma Conference – Radioisotopes in Agriculture, TID-7578, U.S. Government Printing Office, Washington, D.C., 1959, p 157–62
Oakberg, E. F.	Irradiation Damage to Animals and Its Effect on Their Reproductive Capacity	J. Dairy Sci. 43 (Suppl.), 54–67 (1960)
Oakberg, E. F., and R. L. DiMinno	X-Ray Sensitivity of Primary Spermatocytes of the Mouse	Intern. J. Radiation Biol. 2,. 196–209 (1960)
Phan, T. T., and M. A Bender	Protection of Mouse Bone Marrow by Inorganic Compounds During Freezing and Thawing	Proc. Soc. Exptl. Biol. Med. 104, 388–90 (1960)
Rabson, R., and G. D. Novelli	The Incorporation of Leucine-C^{14} into Protein by a Cell-Free Preparation from Maize Kernels	Proc. Natl. Acad. Sci. U.S. 46, 484–88 (1960)
Randolph, M. L.	The Location of Lost γ-Ray or Neutron Sources	Health Phys. 2, 408–9 (1960)
Russell, L. B., S. K. Badgett, and C. L. Saylors	Comparison of the Effects of Acute, Continuous, and Fractionated Irradiation During Embryonic Development	In Immediate and Low Level Effects of Ionizing Radiations, ed. by A. A. Buzzati-Traverso (proc. of the Symposium held at Venice, June 22–26, 1959; suppl. to Intern. J. Radiation Biol.), Taylor and Francis Ltd., London, 1960, p 343–59
Russell, W. L., L. B. Russell, and E. M. Kelly	Dependence of Mutation Rate on Radiation Intensity	Ibid., p 311–20
Smith, L. H., R. A. Popp, and W. St. Amand	Transplantation of Leukemoid Leukocytes in Irradiated Mice	Proc. Soc. Exptl. Biol. Med. 103, 232–34 (1960)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Stuy, J. H.	Studies on the Radiation Inactivation of Microorganisms. VI. X-Ray-Induced Breakdown of Deoxyribonucleic Acid in Haemophilus influenzae and in Other Bacteria	J. Bacteriol. 70, 707–15 (1960)
Tonomura, K., and G. D. Novelli	Cysteine Inhibition of the Release of Repression of Dihydroorotic Acid Dehydrogenase in Escherichia coli	Biochem. Biophys. Research Communs. 2, 31–34 (1960)
Vos, O., J. W. Goodman, and C. C. Congdon	Donor-Type Lymphatic Tissue Cells in Lethally Irradiated Mice Treated with Homologous Fetal Liver	Transplantation Bull. 7, 408–11 (1960)
Wolf, F. T., and A. H. Haber	Chlorophyll Content of Gibberellin-Treated Wheat Seedlings	Nature 186, 217–18 (1960)
(Members of the Biology Division)	Summary of Reports of Bone Marrow Conferences Organized by Members of the Biology Division, ORNL	Reprint No. 1508

Lectures. — Members of the Biology Division gave 116 lectures in the period covered by this report. Of this number 45 were part of the Traveling Lecture series and 71 were addresses before professional societies. Lectures were given at seven international meetings, six of which were held abroad. A. Hollaender gave the opening address "Finsen and Basic Research" at the Third International Congress of Photobiology in Copenhagen in July. J. G. Carlson (coauthors M. E. Gaulden and J. Jagger) and R. C. von Borstel were invited to speak at this meeting, called the Finsen Congress in memory of the great scientist. S. Wolff also presented a paper. Earlier in the year 20 Biology Division personnel spoke at the meeting of the Federation of American Societies for Experimental Biology held in Chicago.

SPEAKER (AND COAUTHORS)	TITLE	PLACE PRESENTED
Adler, H. I.	The Sequence of Events in Radiation Injury	1. Univ. Rochester, Rochester, N.Y. 2. Cornell Univ., Ithaca, N.Y.

SPEAKER (AND COAUTHORS)	TITLE	PLACE PRESENTED
Anderson, N. G. (W. D. Fisher and H. E. Bond)	Macromolecular Interactions Underlying Cell Division	2nd Conf. Mechanisms of Cell Div., N.Y. Acad. Sci.
Anderson, N. G.	Molecular Basis of Cell Function	East Tenn. Regional Collegiate Div., Tenn. Acad. Sci., Carson-Newman Coll., Jefferson City
Arnold, W. A.	Photosynthesis	Duke Univ., Durham, N.C.
Arnold, W. A.	Semiconducting Properties of Chloroplasts	Dartmouth Med. School, Hanover, N.H.
Astrachan, L.	Composition of Metabolically Active RNA in Phage-Infected Bacteria [Federation Proc. 19 (part 1), 304 (1960)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chemists), Chicago, Ill.
Bender, M. A	Chromosomal Evolution in Primates	Univ. Kentucky, Lexington
Bender, M. A	Chromosomes of New World Primates	Am. Assoc. Phys. Anthropologists, Washington, D.C.
Bernlohr, R. W. (and G. D. Novelli)	Bacitracin Biosynthesis and Spore Formation in Bacillus licheniformis	Soc. Am. Bacteriologists, Philadelphia, Pa.
Bollum, F. J.	Mechanism of DNA Synthesis	Duke Univ., Durham, N.C.
Bollum, F. J.	Primers for Calf Thymus Polymerase [Federation Proc. 19 (part 1), 305 (1960)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chemists), Chicago, Ill.
Bollum, F. J.	Structures of Oligodeoxynucleotide Primers for Calf Thymus Polymerase	Gordon Research Conf.: Proteins and Nucleic Acids; DNA Structure and Synthesis, New Hampton, N.H.
von Borstel, R. C.	Ultraviolet and Ionizing Radiation Induction of Nuclear Damage in Habrobracon Eggs	3rd Intern. Congr. Photobiology, Copenhagen, Denmark

SPEAKER (AND COAUTHORS)	TITLE	PLACE PRESENTED
Carlson, J. G. (M. E. Gaulden and J. Jagger)	Mitotic Effects of Monochromatic Ultraviolet Microbeam Irradiation of the Nucleolus	3rd Intern. Congr. Photobiology, Copenhagen, Denmark
Carson, S. F. (E. F. Phares and S. F. Carson)	Role of Methylmalonyl-CoA and Biotin in Bacterial Succinate Decarboxylation	Soc. Am. Bacteriologists, Philadelphia, Pa.
Celada, F. (and T. Makinodan)	A Quantitative Method for the Study of Hematopoietic Transplantation Antigens [Radiation Research 12, 427 (1960)]	Radiation Research Soc., San Francisco, Calif.
Chu, E. H. Y.	Chromosome Numbers in the Prosimiae and Catarrhina	Am. Assoc. Phys. Anthropologists, Washington, D.C.
Clayton, R. K.	H_2O_2-Induced Catalase Synthesis in Rhodopseudomonas spheroides [Federation Proc. 19 (part 1), 347 (1960)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chemists), Chicago, Ill.
Cohn, W. E.	Minor Nucleotides in Ribonucleic Acids; Pseudouridine	1. Tulane Univ. School of Med., New Orleans, La. 2. New Orleans Biochem. Club, New Orleans, La. 3. Univ. Alabama Med. Center, Birmingham
Congdon, C. C.	Lymphatic Tissue Reaction in Normal Mice Given Foreign Bone Marrow [Federation Proc. 19 (part 1), 216 (1960)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Exptl. Pathol.), Chicago, Ill.
Congdon, C. C.	Radiation and Cancer	Am. Soc. Med. Technologists, Atlantic City, N.J.
Cosgrove, G. E. (and A. C. Upton)	Delayed Effects in Mice Irradiated with Partial Body Shielding [Federation Proc. 19 (part 1), 353 (1960)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Exptl. Pathol.), Chicago, Ill.

SPEAKER (AND COAUTHORS)	TITLE	PLACE PRESENTED
Cosgrove, G. E.	Effects of Radiation on Mammals	Research Center, Tennessee Polytech. Inst., Cookeville
Countryman, J. L.	Heterogeneous Ribonucleic Acid Fractions in Young Cultures of *Escherichia coli* B	Soc. Am. Bacteriologists, Philadelphia, Pa.
Cudkowicz, G. (and G. E. Cosgrove)	Mortality and Changes in Irradiated F_1 Hybrid Mice Injected with Parental Adult Liver Cells [Radiation Research 12, 430 (1960)]	Radiation Research Soc., San Francisco, Calif.
de Serres, F. J.	Consequences of Spontaneous and Induced Mutation in the *ad-3* Region of *Neurospora* [Radiation Research 12, 430 (1960)]	Radiation Research Soc., San Francisco, Calif.
de Serres, F. J.	Recent Advances in *Neurospora* Genetics (two lectures)	Western Reserve Univ., Cleveland, Ohio
de Serres, F. J.	Some Aspects of Interallelic Complementation Among *ad-3* Mutants of *Neurospora*	1. Western Reserve Univ., Cleveland, Ohio 2. Univ. Washington, Seattle 3. Univ. California, Berkeley 4. California Inst. Technol., Pasadena
Doherty, D. G.	Chemical Protection Against Radiation Damage in Mammals	1. Kanawha Valley Sect., Am. Chem. Soc., Charleston, W. Va. 2. Univ. California, Berkeley
Doherty, D. G.	Protective Effect of Aminoalkylisothioureas, Their Rearrangement Products, Mercaptoethyl- and Mercaptopropylamines, and Their Disulfides in the Bone Marrow System [Federation Proc. 19 (part 1), 355 (1960)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chemists), Chicago, Ill.

SPEAKER (AND COAUTHORS)	TITLE	PLACE PRESENTED
Friedberg, W. (and V. K. Jenkins)	Protein Metabolism in the Rat-Mouse Chimera [Federation Proc. 19 (part 1), 353 (1960)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Exptl. Pathol.), Chicago, Ill.
Goodman, J. W.	Homologous Bone Marrow Transplantation in Mice	California Inst. Technol., Pasadena
Goodman, J. W. (and C. C. Congdon)	The Killing Effect of Blood-Bone Marrow Given to Irradiated Mice [Radiation Research 12, 439 (1960)]	Radiation Research Soc., San Francisco, Calif.
Green, D. M.	Ethyl Methane Sulfonate-Induced Mutation of Extracellular Bacteriophage	Harvard Univ., Cambridge, Mass.
Grell, R. F.	Nonrandom Assortment of Nonhomologous Chromosomes in Drosophila melanogaster	Univ. Tennessee, Knoxville
Haber, A. H.	Physiology of Seed Germination	1. Marquette Univ., Milwaukee, Wis. 2. Univ. Wisconsin, Madison
Hollaender, A.	Introductory Remarks	Radiation Research Soc. Symposium on Energy Transfer in Photobiological Processes, San Francisco, Calif.
Hollaender, A.	Finsen and Basic Research	3rd Intern. Congr. Photobiology, Copenhagen, Denmark
Hollaender, A.	Studies on Microorganisms	Federation Am. Soc. Exptl. Biol. Symposium on Radiation Protection and Recovery, Chicago, Ill.
Horn, E. C. (M. L. Barber and A. A. Barber)	A Method for Isolating Fowl Serum Albumin [Anat. Record 136, 213 (1960)]	7th Intern. Congr. Anat., New York, N.Y.

SPEAKER (AND COAUTHORS)	TITLE	PLACE PRESENTED
Hyde, C. C. (and M. E. Gaulden)	Chromosome Breakage Frequency Induced by 3r of X Rays in Grasshopper Neuroblasts	Assoc. Southeastern Biologists, New Orleans, La.
Jacobson, K. B.	The ATP-PP Exchange Reaction and Growth of the Chick Embryo	Conf. Developmental Biol., Tallahassee, Fla.
Jacobson, K. B.	On the Mechanism of Acetyl Transfer [Federation Proc. 19 (part 1), 332 (1960)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chemists), Chicago, Ill.
Jagger, J.	Some Physical Characteristics of Photoprotection in Escherichia coli [Biophys. Soc. Program and Abstr., abstr. N-11 (February 1960)]	Biophysical Soc., Philadelphia, Pa.
Kenney, F. T.	Studies on the Nature of the "Adaptive" Increase in Tyrosine α-Ketoglutarate Transaminase Activity of Rat Liver [Federation Proc. 19 (part 1), 4 (1960)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chemists), Chicago, Ill.
Khym, J. X. (and W. E. Cohn)	Selective Reduction of a Single Aldehyde Group of Periodate-Oxidized Nucleosides [Abstr. of Papers, 137th Natl. Meeting Am. Chem. Soc., p 6D (1960)]	Am. Chem. Soc., Cleveland, Ohio
Kimball, R. F.	Studies on the Period Refractory to the Induction of Recessive Lethals in Paramecium [Radiation Research 12, 448 (1960)]	Radiation Research Soc., San Francisco, Calif.
Kirby-Smith, J. S.	Some Biophysical Approaches to Chromosome Breakage	Southeastern Sect., Am. Phys. Soc., Gatlinburg, Tenn.
Krieg, D. R.	EMS-Induced Mutations	Long Island Biol. Lab., Cold Spring Harbor, N.Y.

SPEAKER (AND COAUTHORS)	TITLE	PLACE PRESENTED
Krieg, D. R.	Induced Mutation of Bacteriophage	Johns Hopkins Univ., Baltimore, Md.
Krieg, D. R.	Mutations Induced by Exposure of Free Phage to Ethyl Methane Sulfonate	Gordon Research Conf.: Proteins and Nucleic Acids; Chemical Mutation of DNA and RNA, New Hampton, N.H.
Krieg, D. R.	Ultraviolet-Induced Mutation of Extracellular Bacteriophage T4 [Radiation Research 12, 450 (1960)]	Radiation Research Soc., San Francisco, Calif.
McGrath, R. A.	Cytidine Labeling of Abnormally Enlarging cells [Federation Proc. 19 (part 1), 358 (1960)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Exptl. Pathol.), Chicago, Ill.
Maisin, J. R. (and D. G. Doherty)	Chemical Protection Against Radiation Damage [Federation Proc. 19 (part 1), 356 (1960)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Exptl. Pathol.), Chicago, Ill.
Maisin, J. R. (and D. G. Doherty)	Chemical Protection of Mammalian Tissues	Federation Am. Soc. Exptl. Biol. Symposium on Radiation Protection and Recovery, Chicago, Ill.
Makinodan, T.	Advances in Radiation Immunology	1. Federation Am. Soc. Exptl. Biol. Symposium on Radiation Protection and Recovery, Chicago, Ill. 2. Med. Coll. Virginia, Richmond
Makinodan, T.	Problems and Aspects of Sensitivity of Marrow Transplants to Immune Responses	Comm. on Tissue Transplantation of the Natl. Research Council, Washington, D.C.
Makinodan, T. (and W. J. Peterson)	Quantitative Assay of Radiosensitivity of Antibody-Forming Cells [Radiation Research 12, 455 (1960)]	Radiation Research Soc., San Francisco, Calif.

SPEAKER (AND COAUTHORS)	TITLE	PLACE PRESENTED
Mans, R. J. (and R. Rabson)	The Incorporation of Amino Acids in Protein by Cell-Free Preparations from Maize Endosperm and Germinating Seedlings [Federation Proc. 19 (part 1), 344 (1960]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chemists), Chicago, Ill.
Mortimer, R. K. (and R. C. von Borstel)	Dose-Effect Relations in Haploid and Diploid Sperm of the Wasp Mormoniella [Radiation Research 12, 458 (1960)]	Radiation Research Soc., San Francisco, Calif.
Novelli, G. D. (T. Kameyama and G. D. Novelli)	Appearance of Particle-Bound β-Galactosidase of Escherichia coli During Its Induction After Photoreactivation	Soc. Am. Bacteriologists, Philadelphia, Pa.
Oakberg, E. F.	Radiation Genetics of Mammals	Univ. Texas, Southwestern Med. School, Dallas
Odell, T. T., Jr. (and T. P. McDonald)	Peripheral Counts and Survival of Blood Platelets of Mice [Federation Proc. 19, 63 (1960)]	Federation Am. Soc. Exptl. Biol. (Am. Physiol. Soc.), Chicago, Ill.
Odell, T. T., Jr.	Platelet Labeling with Radioisotopes and in Vivo Platelet Survival	Intern. Symposium on Blood Platelets, Henry Ford Hosp., Detroit, Mich.
Peck, H. D.	Evidence for Oxidative Phosphorylation During the Reduction of Sulfate with Hydrogen by Desulfovibrio desulfuricans	Soc. Am. Bacteriologists, Philadelphia, Pa.
Peck, H. D.	The Reduction of Sulfate to Sulfite by Soluble Enzymes of Desulfovibrio desulfuricans [Federation Proc. 19 (part 1), 242 (1960)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chemists), Chicago, Ill.

SPEAKER (AND COAUTHORS)	TITLE	PLACE PRESENTED
Peck, H. D.	The Utilization of Inorganic Sulfate by Microorganisms	1. Univ. Georgia, Athens 2. Emory Univ., Emory University, Ga.
Peterson, W. J.	Job Opportunities	Austin High School, Knoxville, Tenn.
Phan, T. T. (and M. A Bender)	Preservation of Viable Bone Marrow by Freezing	3rd Australasian Conf. Radiobiology, Sydney, Australia
Popp, R. A.	The Genetics of Mouse Hemoglobins	California Inst. Technol., Los Angeles, Calif.
Popp, R. A.	Proteins in Hematopoietic Tissues of Irradiated Rat-Mouse Chimeras	Tri Beta, Berea Coll., Berea, Ky.
Popp, R. A. (and S. A. Sapp)	Repopulation of Hematopoietic Tissues in Irradiated Mice [Radiation Research 12, 463 (1960)]	Radiation Research Soc., San Francisco, Calif.
Popp, R. A.	Work with Radiation Chimeras	Tri Beta, Winthrop Coll., Rock Hill, S.C.
Prescott, D. M.	Cell Life Cycle	Washington Univ., St. Louis, Mo.
Prescott, D. M.	Nucleic-Cytoplasmic Interactions	Radiobiology Div., U.S. Army Med. Research Lab., Fort Knox, Ky.
Randolph, M. L. (and A. H. Haber)	Production and Decay of Free Radicals Induced by X Irradiation of Dry Lettuce Seeds	Symposium on the Effect of Ionizing Radiation on Seeds and Its Significance for Crop Improvement, Karlsruhe, Germany
Randolph, M. L.	Quantitative and Qualitative Changes During Decay of Radiation-Induced Magnetic Centers in Crystalline Amino Acids	Symposium on Free Radicals in Biological Systems, Stanford Univ., Palo Alto, Calif.

SPEAKER (AND COAUTHORS)	TITLE	PLACE PRESENTED
Russell, L. B. (and C. L. Saylors)	Factors Causing a High Frequency of Mice Having the XO Sex-Chromosome Constitution [Science 131, 1321 (1960)]	Natl. Acad. Sci., Washington, D.C.
Russell, L. B. (and W. L. Russell)	Genetic Analysis of Induced Deletions and of Spontaneous Nondisjunction Involving Chromosome 2 of the Mouse	Symposium on Mammalian Genetics and Reproduction, Gatlinburg, Tenn. (ORNL Biology Div. Symposium)
Russell, L. B.	Genetic Aspects of Radiation-Induced Chromosomal Changes in Mammals	Biomedical Research Group, Los Alamos Sci. Lab., Los Alamos, N.M.
Russell, W. L.	Genetic Effects of Radiation	1. Combined chapters, Sigma Xi, St. Louis and Washington Univ., St. Louis, Mo. 2. Univ. Rhode Island, Kingston 3. Harvard Univ., Cambridge, Mass. 4. Los Alamos Sci. Lab., Los Alamos, N.M.
Russell, W. L. (and E. M. Kelly)	Mutation Frequency at Low Radiation Intensity [Science 131, 1320 (1960)]	Natl. Acad. Sci., Washington, D.C.
Schwartz, D.	Coding Problems in Proteins	Duke Univ., Durham, N.C.
Schwartz, D.	Genetic Studies on Maize Enzymes	Univ. Oregon, Eugene
Schwartz, D.	Genetic Studies on Proteins	1. Univ. Pittsburgh, Pittsburgh, Pa. 2. Cornell Univ., Ithaca, N.Y. 3. Univ. Rochester, Rochester, N.Y.

SPEAKER (AND COAUTHORS)	TITLE	PLACE PRESENTED
Smith, L. H. (M. Lion and L. H. Smith)	Low-Temperature Protection of X-Irradiated Mouse Bone Marrow Cells [Federation Proc. 19 (part 1), 354 (1960)]	Federation Am. Soc. Exptl. Biol. (Am. Physiol. Soc.), Chicago, Ill.
Stapleton, G. E. (M. S. Engel and H. I. Adler)	The Influence of Cultural Conditions on Radiosensitivity of Escherichia coli	Soc. Am. Bacteriologists, Philadelphia, Pa.
Swanson, H. D.	Some Effects of Intracellular Protein on Nuclei	Univ. Tennessee, Knoxville
Taylor, M. B. (G. D. Novelli and R. J. Mans)	Studies on Polysaccharide Synthesis by a Soil Diphtheroid	Soc. Am. Bacteriologists, Philadelphia, Pa.
Taylor, W. H. (K. Tonomura and G. D. Novelli)	The Synthesis of Aspartyltranscarbamylase and Dihydroorotic Dehydrogenase by Cells and Protoplasts of Escherichia coli	Soc. Am. Bacteriologists, Philadelphia, Pa.
Upton, A. C. (E. P. Sniffen and F. F. Wolff)	Coleukemogenic Effects of Myleran on Lymphoma Development in Irradiated RF Mice [Proc. Am. Assoc. Cancer Research 3, 158 (1960)]	Am. Assoc. Cancer Research, Chicago, Ill.
Upton, A. C. (and A. W. Kimball)	Effects of Whole-Body Irradiation on the Age Distribution of "Senile" Changes in Mice [Radiation Research 12, 482 (1960)]	Radiation Research Soc., San Francisco, Calif.
Upton, A. C. (and E. P. Sniffen)	Further Dose-Response Studies on Radiation-Induced Myeloid Leukemia in RF Mice [Federation Proc. 19 (part 1), 353 (1960)]	Federation Am. Soc. Exptl. Biol. (Am. Assoc. Exptl. Pathol.), Chicago, Ill.

SPEAKER (AND COAUTHORS)	TITLE	PLACE PRESENTED
Upton, A. C.	Significance of the Greenhouse Project to the Design of Studies on Man	Annual Meeting of the Advisory Committee on the Atomic Bomb Casualty Commission: Symposium on Physical Dosimetry and Pathological Effects, San Francisco, Calif.
Volkin, E.	Biologic Activity of RNA in T2 Infection [Federation Proc. 19 (part 1), 305 (1960)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chemists), Chicago, Ill.
Wells, O. C.	Radiobiology of Tetrahymena pyriformis	Assoc. Southeastern Biologists, New Orleans, La.
Wolff, S.	Mechanisms of Chromosome Breakage and Repair	Conf. Exptl. Advances in Radiotherapy, Carmel, Calif.
Wolff, S.	On the Apparent Synergistic Effect of Far Red and X Rays in the Production of Chromatid Aberrations	3rd Intern. Congr. Photobiology, Copenhagen, Denmark
Wolff, S.	Postirradiation Storage and the Growth of Barley Seedlings	Symposium on the Effect of Ionizing Radiation on Seeds and Its Significance for Crop Improvement, Karlsruhe, Germany
Wust, C. J.	Coenzyme Protection of Tryptophan Synthetase from Antibody Neutralization	Soc. Am. Bacteriologists, Philadelphia, Pa.

Visiting Lecturers. – An average of two guest lecturers a week took part in the Visiting Lecturer Seminar Program. Scientists from laboratories, universities, and research organizations in the United States, Canada, Europe, South America, and Japan gave lectures as follows:

SPEAKER	AFFILIATION	SUBJECT
P. Koller	Chester Beatty Research Inst., Royal Cancer Hosp., London, England	Immunological Behaviour in Radiation Chimaeras
H. H. Kramer	Dept. Agron., Purdue Univ., Lafayette, Ind.	Genetic Control of Endosperm Carbohydrates
J. L. Bateman	Medical Research Center, Brookhaven Natl. Lab., Upton, N.Y.	Relation of RBE to Neutron Energy
O. H. Scherbaum	Dept. Zool., Univ. California, Los Angeles	Synchronous Division in Microorganisms
I. Raw	Univ. São Paulo, São Paulo, Brazil	Mitochondrial Cytochrome b5
F. Wilt	Lab. Exptl. Embryol., Paris, France	Molecular Aspects of Vision During Metamorphosis
L. H. Gray	Mount Vernon Hosp., Northwood, Middlesex, England	Oxygen Effects in Radiobiology
L. Augenstine	Div. Biol. and Med., U.S. Atomic Energy Comm., Washington, D.C.	Thermal Luminescence of Irradiated Proteins
P. Howard-Flanders	Dept. Radiol., Yale Univ. School of Med., New Haven, Conn.	Chemical Factors in the X-Ray Inactivation of Bacteria and Bacteriophage
K. A. Stacey	Dept. Radiol., Yale Univ. School of Med., New Haven, Conn.	Ionizing Radiations and DNA: The Death of a Hypothesis?
N. R. Di Luzio	Dept. Physiol., Univ. Tennessee, Memphis	Possible Mechanisms of Radiation-Induced Hyperlipemia
J. Papaconstantinou	Dept. Embryology, Carnegie Inst. of Washington, Baltimore, Md.	Protein Changes in the Development of the Lens
E. Goldwasser	Argonne Cancer Research Hosp., Chicago, Ill.	Some Studies on the Purification of a Mouse Leukemia Agent

SPEAKER	AFFILIATION	SUBJECT
D. G. Harnden and P. A. Jacobs	Dept. Radiotherapy, Western Gen. Hosp., Edinburgh, Scotland	Chromosome Aberrations in Men
G. Hoecker	Catedra de Biologia, Inst. Biologia "Juan Noe," Santiago, Chile	Antigens in the Mouse
R. Auerbach	Zool. Dept., Univ. Wisconsin, Madison	Studies on the Development of the Mouse Thymus
J. W. Drake	Dept. Microbiol., Univ. Illinois, Urbana	Host-Directed Variation in Newcastle Disease Virus
A. H. Mehler	Dept. Health, Education, and Welfare, National Insts. of Health, Bethesda, Md.	Structure and Function of Aldolase
J. I. Valencia and R. Valencia	Dept. Biol. and Med., Atomic Energy Comm., Buenos Aires, Argentina	Induction of Mutations at Different Stages of Oogenesis in Drosophila
L. Jaffe	Dept. Biol., Brandeis Univ., Waltham, Mass.	Orientation of Cell Growth by Polarized Radiation
K. Hirschhorn	Dept. Med., New York Univ., Bellevue Med. Center, New York	Virus Resistance and Susceptibility: A Possible Genetic Marker in Mammalian Cell Culture
A. B. Stavitsky	Dept. Microbiol., Western Reserve Univ., Cleveland, Ohio	Recent in Vivo and in Vitro Studies of the Primary and Secondary Antibody Response
O. Scott	Research Unit in Radiobiol., Mount Vernon Hosp., Northwood, Middlesex, England	Some Problems Involved in the Use of Homografts for Radiobiological Research
A. Portela	Dept. Anat., Emory Univ., Atlanta, Ga.	The Effects of X-Irradiation on Muscle Membrane
G. H. Bourne	Dept. Anat., Emory Univ., Atlanta, Ga.	Histochemical Studies on Peripheral Nerves and Spinal Ganglia

SPEAKER	AFFILIATION	SUBJECT
M. J. Moses	Dept. Anat., Duke Univ. Med. Center, Durham, N.C.	Chromosome Structure and Function
J. M. Mitchison	Brown Univ., Providence, R.I.	Growth During the Cell Cycle
J. F. Duplan	Wistar Inst., Philadelphia, Pa.	Incidence of Spontaneous and Radiation-Induced Leukemias in Irradiated Mice Treated with Fetal Liver Cells
A. H. Doerman	Dept. Biol., Vanderbilt Univ., Nashville, Tenn.	Structure and Regeneration of Heterozygotes of T4
L. Schwinck	Queen's Univ., Kingston, Ontario, Canada	Effect of Red, Blue, and White Light on the Photosynthetic Assimilation of $^{14}CO_2$ of Detached Tobacco Leaves
A. Bussard	Inst. Pasteur, Paris, France	1. Antibody Synthesis by Spleen and Lymph Node Cells by Short Time Tissue Culture 2. Glucose PO_4 Dehydrogenase Antibodies in Immune and Tolerant Rabbits
E. Zeuthen	Biol. Inst., Carlsberg Foundation, Copenhagen, Denmark	The Synchronous Tetrahymena System
A. V. Beatty	Dept. Biol., Emory Univ., Atlanta, Ga.	Chromosome Recovery from Radiation Damage
W. G. Downs, Jr.	Tennessee Polytech. Inst., Cookeville	Studies on Effects of Stress on Blood of Rats
P. Doty	Dept. Chem., Harvard Univ., Cambridge, Mass.	Strand Separation and Specific Recombination in DNA
R. M. S. Smellie	Dept. Biochem., The University, Glasgow, Scotland	RNA Synthesis in Extracts of Mammalian Cells

SPEAKER	AFFILIATION	SUBJECT
M. Whittinghill	Dept. Zool., Univ. North Carolina, Chapel Hill	Crossing Over in Drosophila and Other Organisms
J. L. Jinks	Div. Biol., California Inst. Technol., Pasadena	Mapping and Complementation Studies on the Host Range and Related Mutants of Phage T4
J. M. Wiame	Dept. Microbiol., New York Univ. Coll. Med., New York	Growth and Repression of Enzyme Synthesis in Bacteria
C. Pavan	Dept. Gen. Biol., Univ. São Paulo, São Paulo, Brazil	DNA-RNA and Protein Synthesis in Polytene Chromosomes
T. Yamada	Fac. Sci., Nagoya Univ., Chikusa, Nagoya, Japan	A Protein Causing Mesodermal Differentiation in the Newt Ectoderm Cultured in Vitro

Foreign Travel. – In addition to making the opening address at the Finsen Memorial Congress in Copenhagen last July, A. Hollaender in his capacity as president of the Comité International de Photobiologie presided on two special occasions honoring the Danish scientist. He spoke at the unveiling ceremony of a memorial tablet at Dansk Esso, and he awarded the Finsen medal to P. B. Rottier of Holland at a luncheon given by the Finsen Memorial Hospital. As a representative of this committee he attended a meeting in Leiden, Holland, sponsored by the Dutch Society of Biophysics to discuss an international organization in biophysics and the program of the first International Biophysical Congress in Stockholm in 1961.

In Brussels, Hollaender took part in outlining and launching the biology program of the Communauté Européenne de L'Energie Atomique. Three days were spent touring laboratories in Italy as adviser to the Division of Biology, Comitato Nazionale per le Recherche Nucleari of Italy, a program primarily concerned with the effects of radiation and uses of radioisotopes in biology, medicine, and agriculture.

C. C. Congdon was invited to Geneva, Switzerland, by R. L. Dobson of the World Health Organization to help plan a joint symposium with that organization and the International Atomic Energy Agency on "The Diagnosis and Treatment of Radiation Injury in Man."

T. T. Phan (coauthor, M. A Bender) attended the Third Australasian Conference on Radiobiology in Sydney where he presented a paper, "Preservation of Viable Bone Marrow by Freezing."

M. L. Randolph presented an invited paper in Karlsruhe at the International Atomic Energy Agency symposium on "The Effects of Ionizing Radiation on Seeds and Its Significance for Crop Improvement." On this trip abroad, Randolph also had discussions with scientists at several research organizations in England and attended the Third International Congress of Photobiology.

S. Wolff presented papers at the Finsen Memorial Congress in Copenhagen and at the International Atomic Energy Agency symposium in Karlsruhe. Wolff also visited scientific institutions in England and France for discussions with researchers.

Fourteenth Annual Biology Research Conference. – "Recovery of Cells from Injury" is the topic around which the 1961 Spring Conference has been planned. It will be held April 3–6 at the Mountain View Hotel in Gatlinburg, Tennessee. A committee, comprised of R. F. Kimball, chairman, H. I. Adler, N. G. Anderson, C. C. Congdon, G. D. Novelli, and S. Wolff has arranged the following tentative program:

MONDAY, APRIL 3

Morning: Postirradiation Processes in Dry Material

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

E. L. Powers, Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Illinois

Afternoon: Renaturation and Reactivation of DNA

Julius Marmur, Department of Chemistry, Harvard University, Cambridge, Massachusetts

C. S. Rupert, Department of Biochemistry, The Johns Hopkins University, Baltimore, Maryland

Evening: Smoker. Host – Union Carbide Corporation

TUESDAY, APRIL 4

Morning: Reactivation of Bacteriophage

Walter Harm, Institut für Genetik, Abteilung Strahlenbiologie, Universität Kőln, Kőln, Germany

A. H. Doermann, Department of Biology, Vanderbilt University, Nashville, Tennessee

Afternoon: Excursion

Evening: Reception and dinner

WEDNESDAY, APRIL 5

Morning: Reversible Processes in Cell Inactivation

H. I. Adler, Biology Division, Oak Ridge National Laboratory

M. M. Elkind (tentative acceptance), National Cancer Institute, National Institutes of Health, Bethesda, Maryland

Afternoon: Postirradiation Processes in Mutation Induction

Evelyn M. Witkin, College of Medicine, State University of New York, Brooklyn, New York

R. F. Kimball or Sheldon Wolff, Biology Division, Oak Ridge National Laboratory

Evening: Round table discussion on "Evidence for Recovery Processes in Germ Cells"

THURSDAY, APRIL 6

Morning: Biochemical and Cell Physiological Aspects

Maurice Errera, Université Libre de Bruxelles, Faculté des Sciences, Bruxelles, Belgium

G. D. Novelli, Biology Division, Oak Ridge National Laboratory

Bone Marrow Conferences. — Chicago was the location of the spring conference on chemical protection and marrow transplantation, attended by a number of Biology Division personnel. C. C. Congdon was invited to address this conference.

The next bone marrow conference will be held November 18 and 19, 1960, at the Cancer Research Institute, New England Deaconess Hospital, Boston, Massachusetts. S. Warren and W. Dameshek are planning the program for this meeting, which takes the place of a January 1961 conference in Oak Ridge.

Education. — Thirteen college students participated in the third annual Student Trainee Program held during the summer in cooperation with

the Oak Ridge Institute of Nuclear Studies. H. I. Adler is Education Coordinator. Students, their affiliations, and their supervisors at the laboratory follow:

Student Trainee	Institution	Assigned to –
Enochs, N. J.	David Lipscomb College, Nashville, Tenn.	A. H. Haber
George, D. B.	Furman University, Greenville, S.C.	S. F. Carson
Gibson, B., Jr.	Florida A & M University, Tallahassee	W. J. Welshons
Graziani, J. T.	Randolph-Macon Woman's College, Lynchburg, Va.	T. Makinodan
Hirsh, D. I.	Reed College, Portland, Ore.	E. H. Y. Chu
Jones, C. G.	East Texas Baptist College, Marshall	R. K. Clayton
Kahle, J. R.	Concord College, Athens, W. Va.	K. B. Jacobson
Lowe, A. O.	Virginia State College, Petersburg	C. C. Congdon
McMurry, J. F., Jr.	Central State College, Edmond, Okla.	M. S. Engel
Page, S. L.	Winthrop College, Rock Hill, S.C.	C. J. Wust
Renaud, F. L.	University of Puerto Rico, Santurce	D. M. Prescott and K. B. Jacobson
Sanfelippo, P. M.	Marquette University, Milwaukee, Wis.	N. G. Anderson
Sheils, S. J.	University of Minnesota, Duluth	N. G. Anderson

The purpose of this ten-week course is to give students from small schools an opportunity to work in a laboratory engaged in full-time basic research. Trainees, who must have completed three years or more of college work, are selected on the basis of scholastic achievement and interest in scientific research. They are assigned to a group and work on a project closely related to their interest. At the end of the session they write a paper and present an oral report of their findings. Trainees are free to visit and observe the work going on in other groups, attend Division seminars, and take part in informal discussions with research personnel.

Interest in small colleges is also expressed during the school year by visits of Biology Division members to present lectures or short courses in radiation biology. Eleven institutions were included in this activity in the past six months, as follows:

Lecturer	Topic	Institution
Adler, H. I.	Microorganisms and Radiation Biology	Spring Hill College, Spring Hill, Ala.
Anderson, N. G.	Molecular Biology	1. Converse College, Spartanburg, S.C. 2. Wofford College, Spartanburg, S.C. 3. Limestone College, Gaffney, S.C. 4. Winthrop College, Rock Hill, S.C.
Odell, T. T., Jr.	Acute Radiation Injury in Mammals	1. Madison College, Harrisonburg, Va. 2. Eastern Mennonite College, Harrisonburg, Va. 3. Bridgewater College, Bridgewater, Va.
Smith, L. H.	Radiation Injury in Mammals	1. Western Maryland College, Westminster 2. Gettysburg College, Gettysburg, Pa. 3. Hood College, Frederick, Md.

The National Science Foundation again supported a Summer Institute for High School and Small College Teachers of Science; six Biology Division members took part. They were:

Lecturer	Topic	Institution
Anderson, N. G.	The Origin of Life	Columbia College, Columbia, S.C.
Cosgrove, G. E.	Tissue Changes in Irradiated Mammals and Protection of Mammals Against Irradiation Changes (series of ten lectures)	Florida State University, Tallahassee
Doherty, D. G.	Chemical Protection Against Ionizing Radiation (series of three lectures)	Tulane University, New Orleans, La.

Lecturer	Topic	Institution
Smith, L. H.	1. Effects of Radiation on Cell Morphology and Viability 2. Protection Against Ionizing Radiation Other than Chemical	Tulane University, New Orleans, La.
Stapleton, G. E.	General and Cellular Radiobiology (series of three lectures)	Western Kentucky State College, Bowling Green
Upton, A. C.	1. Effects on Prenatal Development in Mammals 2. Pathophysiology in Mammals 3. Relative Biological Effectiveness	Tulane University, New Orleans, La.

E. F. Oakberg was invited to lecture on "Diagnostic Radiology" as part of a postgraduate course of the same title at the University of California Medical Center, San Francisco, California. D. M. Prescott also contributed to the educational activities of the Division, lecturing on "The Mitotic Cycle" at the summer course of the Tissue Culture Association given at the University of Wisconsin, Madison.

The Oak Ridge Institute of Nuclear Studies invited Biology Division members to speak at the Radioisotopes Techniques School, and to its Medical and Training Divisions. A. C. Upton gave five lecture series in the past six months on the general subject "Biological and Pathological Effects of Radiation." D. G. Doherty spoke on "Chemical Protection Against Radiation" and W. D. Gude lectured on "Strip Film and Liquid Emulsion Techniques."

M. A. Kastenbaum presented a course of nine 1-hr lectures in Elementary Biometrical Methods for personnel in the Biology Division.

CYTOLOGY AND GENETICS

Paramecium and Tetrahymena

R. F. Kimball
O. C. Wells[1]
D. B. Williams[2]
S. W. Perdue
H. M. Scandlyn
M. F. Gibbs[3]

Tradescantia, Vicia, Hordeum

S. Wolff
P. C. Bailey[2]
D. Davidson[4]
C. Garcia-Benitez[3]
H. E. Luippold

Maize and Phage

D. Schwartz
D. R. Krieg
D. M. Green[1]
B. A. Swomley[1]
R. M. Fulkerson
C. B. Kincaid
K. H. McGrath

Timothy

R. T. Brumfield[5]
A. P. Davis[3]
M. W. Scott

Neurospora

F. J. de Serres
H. G. Kølmark[4]
B. B. Webber[1]
I. R. Cox
A. P. Teasley

Cell Growth and Reproduction

D. M. Prescott
R. C. von Borstel[6]
J. G. Carlson[5]
M. E. Gaulden[5]
B. C. Kluss[2]
A. R. Whiting[5]
C. H. Shappert
F. L. Renaud[7]
B. J. Stevens[8]

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[1] Research associate.
[2] Research participant.
[3] Loanee.
[4] Visiting investigator from abroad.
[5] Consultant.
[6] Leave of absence, NSF Senior Postdoctoral Fellowship, Pavia, Italy.
[7] Student trainee.
[8] Temporary summer employee.

Drosophila

W. J. Welshons[9]
D. L. Lindsley, Jr.[10]
E. H. Grell
B. Nicoletti[4]
R. F. Grell[5]
G. T. Pratt
M. L. Rekemeyer
R. D. Wilkerson

CYTOGENETIC EFFECTS OF RADIATION

Further Studies on the Refractory Period for Mutation Induction in *Paramecium aurelia*

R. F. Kimball S. W. Perdue

Introduction. – Irradiation of *Paramecium aurelia* in the second half of the interdivision interval produces about 10 times fewer mutations in the micronucleus than irradiation in the first half. Previous studies[11] of this refractory period have shown that (1) it occurs with alpha particles as well as x rays, and (2) it is coextensive in log-phase growth with the period of synthesis, S, of macronuclear DNA and with the post-synthesis period G2 (i.e., the period after the chromosomes have divided and before mitosis) of the micronucleus. The first finding together with other considerations led us to favor the view that the refractory period is a time when recovery from initial radiation damage is very efficient rather than a time when protection against initial radiation damage is very great. Further experiments have now been done that support this view, and the relations between the macronuclear and micronuclear S and G2 periods have been further tested.

Results. – Two tests that ought to have different results on the protection and recovery hypotheses have been made. (1) The same refractory period has been found for 2537-A ultraviolet light as for x rays. Past experience with protective procedures in radiation biology, including some of our own earlier work with *Paramecium*, has shown that the effective procedures are usually different for ultraviolet and x rays. On the other

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[9] Temporary *Drosophila* Group Leader.

[10] Leave of absence, NSF Senior Postdoctoral Fellowship, São Paulo, Brazil.

[11] R. F. Kimball, N. T. Gaither, and S. W. Perdue, Biol. Semiann. Prog. Rep. Feb. 15, 1960, ORNL-2913, p 29–30.

hand, recovery processes for mutational damage in Paramecium seem to be quite similar for the two radiations.[12] Thus the existence of the same refractory period for the two favors a recovery and not a protection hypothesis. (2) The self-reproducing, DNA-containing entity, kappa, which occurs in the cytoplasm of certain strains of Paramecium, has been found to be equally or nearly equally inactivated by x irradiation in the first and second halves of the interdivision interval. Anoxia protects against x-ray inactivation of kappa and x-ray induction of mutations about equally in Paramecium.[13] Thus the absence of a refractory period for inactivation of kappa makes it improbable that the refractory period for mutation is the result of an anoxia-like protection.

It was found possible to shift the normal relations between the S period for the macronucleus and the G2 period for the micronucleus. Normally the two are alike or nearly so, but if 1.5 mM caffeine is added to the culture medium the relations are altered. Autoradiographic studies of the incorporation of tritiated thymidine into macronuclear DNA show that caffeine does not delay the onset of DNA synthesis but does decrease its rate; consequently, the S period begins at the usual time, but lasts considerably longer. Studies of the cross-sectional area of the micronucleus show that the abrupt increase in area, which we presume to mark the time of chromosome duplication, occurs between 4 and 5 hr after division instead of between 2 and 3. The refractory period likewise starts between 4 and 5 hr after division in the presence of caffeine instead of the normal 2 to 3 hr. Thus the beginning of the refractory period is the same as the time of chromosome duplication in the micronucleus and is not the same as the beginning of the macronuclear S period. The refractory period is coextensive with the micronuclear G2 period and with the latter part of the macronuclear S period.

Conclusions. — These studies make it nearly certain that the refractory period is caused by efficient recovery from rather than protection against initial radiation damage. They also show that the beginning of the refractory period coincides with micronuclear chromosome duplication

[12]R. F. Kimball, N. Gaither, and S. M. Wilson, Radiation Research 10, 490–97 (1959).

[13]R. F. Kimball, Ann. N.Y. Acad. Sci. 59, 638–48 (1955).

rather than the onset of macronuclear chromosome duplication. A distinction between the alternatives that (1) doubleness of the micronuclear chromosomes is the only condition necessary for recovery or (2) something additional is required, especially active macronuclear synthesis of DNA, cannot be made at present. There are some considerations that favor the second alternative,[11] but a direct test depends on finding conditions of growth in which a recently doubled micronucleus is present when macronuclear DNA synthesis is already completed.

A Long-Term Effect of Ionizing Radiation on the Micronucleus of *Tetrahymena pyriformis*

O. C. Wells

Introduction. — Recent reports that only amicronucleate cells of *Paramecium multimicronucleatum*[14] and *Tetrahymena pyriformis*[15] are recovered after massive x-ray dosages are contradictory to observations made in this laboratory that radiation-produced amicronucleate cells rarely survive. Only one amicronucleate clone of *T. pyriformis* has been found among several hundred survivors. The members of this clone are the vegetative progeny of a single organism of clone I which received 285 kr of x rays.[16] This cell gave rise to a mixed population of micronucleate and amicronucleate organisms; this mixture persisted at least three months. Only amicronucleate animals were found in the population seven months after irradiation.

Consequently a more detailed study of postirradiation micronuclei in clone I after 285 kr was made. A stationary-phase population was irradiated, and 215 single cells were isolated. Fourteen single cells produced clones; each of these, and 14 control survivors (survival = 145/150) were examined cytologically at monthly intervals after irradiation for three months. The number of micronucleate and amicronucleate cells per hundred was recorded for each clone.

One month after irradiation, 100 subisolations were made from one of the surviving clones in which micronucleate and amicronucleate cells were

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[14] R. Wichterman, Science 129, 207–8 (1959).

[15] C. C. Speidel and G. G. Holz, Jr., Biol. Bull. 117, 384–85 (1959).

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

present. Details of irradiation, isolation, and cytological methods have been reported elsewhere.[16,17]

Results. – All cells in four irradiated clones and in all 14 control clones contained micronuclei at every examination. Mixed populations of micronucleate and amicronucleate cells were observed in each of the remaining ten radiation survivors. One month after treatment a low percentage (range 1–25) of cells in each population were micronucleate; at the second examination the percentage had risen (range 58–80), and almost all of the cells in each population were micronucleate by the final study (range 90–100). A concurrent change in micronuclear morphology was noted. Most micronuclei in mixed populations were atypical one month after treatment. Instead of appearing as compact, densely staining bodies, as in controls, radiation-damaged micronuclei were loose, lightly staining aggregations of chromatin threads, often swollen to several times the diameter of control micronuclei. Many nuclei failed to undergo karyokinesis. Many micronuclei no different in morphology and karyokinesis from controls were found in the populations at the end of two months, and atypical nuclei were seen only rarely at the third examination.

An attempt to establish amicronucleate clones from amicronucleate cells in mixed populations was fruitless. Of 100 single cells isolated from a culture in which 25% micronucleate cells were present, only 32 subclones were obtained. Each of these subclones was a mixture of micronucleate and amicronucleate cells.

Discussion. – Amicronucleate cells are definitely formed from irradiated micronucleate cells of T. pyriformis for some time after treatment, probably by the failure of damaged micronuclei to divide properly at fission.[17] The subisolation study shows plainly, however, that most of these amicronucleate cells are incapable of producing viable clones. An occasional amicronucleate cell can produce a viable lineage, since one such cell line has been obtained in our laboratory. The difference between this rare type of cell and the amicronucleate cells which die is not known

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

at present. The results of Wichterman[14] and Speidel and Holz[15] can be explained on the basis of occasional viable amicronucleate organisms produced in their populations. It must be stressed, however, that most radiation-produced amicronucleate cells are inviable.

The long-term damage inflicted upon micronuclei by 285 kr and the subsequent repair (return to typical morphology and behavior at fission) of these damaged micronuclei are remarkable. It is doubtful that functionally normal micronuclei exist in these populations during the first month after irradiation, indicating that cells of this species are capable of producing thousands of progeny (probably at least three generations per day) even though long-term radiation damage is present. It is probable that the micronuclei in only a few cells of the population are repaired by some unknown mechanism, and that cells bearing these nuclei replace all other aberrant and dying cells with time.

Further Studies on X-Ray Effects in *Spathidium spathula*

D. B. Williams

Introduction. — Ciliates, in general, have been demonstrated to be quite resistant to x rays, requiring in the order of 100 kr before showing significant depression in daily growth.[18] *Spathidium spathula* is one ciliate, however, which suffers significant depression of daily division rate after exposure to doses as low as 10 kr.[19] This report presents results of three experiments: (1) x irradiation of *Spathidium* with 15 kr in atmospheres of air, oxygen, and nitrogen in an effort to demonstrate the presence or absence of an oxygen effect on daily division rate over a 14-day period; (2) effects of 1–15 kr on the length of the first interdivision interval following exposure; and finally, (3) an attempt to demonstrate induction of recessive deleterious and lethal mutations following conjugation (selfing) at 10 and 20 divisions following exposure.

Results. — When logarithmically reproducing animals are exposed to 15 kr in an atmosphere of air, oxygen, or nitrogen, they show a significantly depressed daily division rate when compared with unirradiated controls. Forty-five animals were included in each group to give the following numbers of divisions 24 hr after treatment: air without irradiation,

[18]R. F. Kimball, p 285–331 in Radiation Biology, ed. by A. Hollaender, vol I, McGraw-Hill Book Co., Inc., New York, 1955.

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

4.79; 15 kr in air, 2.57; oxygen without irradiation, 4.67; 15 kr in oxygen, 1.79; nitrogen without irradiation, 4.53; 15 kr in nitrogen, 2.21. By the second day, irradiated lines for all gas treatments showed a division rate 85–87% of that of the controls. From the third to the fourteenth day, growth of the three irradiated groups stabilized at about 80% of that of the controls when the experiment was ended. No significant differences existed among the irradiated lines receiving air, oxygen, or nitrogen when the data for the first 24 hr growth were compared using the chi-square method ($P = 0.95–0.90$), nor were there any significant differences at later times.

The increase in generation time by doses of a few kr was studied by irradiating 1 hr after division and ascertaining the time of the first postirradiation division. The generation time for controls was 6 hr, and doses of 1.00, 1.25, and 1.50 kr increased this time by about 30 min per kr. At higher doses precise generation times were not ascertained, but 8/15 and 10/15 Spathidia had not divided at 8 hr with doses of 2.5 and 4.0 kr, respectively; and 8/15, 14/15, and 13/15 had not divided at 10 hr with doses of 5, 10, and 15 kr, respectively.

Exconjugant survival and vitality were tested by irradiating recently divided animals (1 hr postfission) and allowing 10 or 20 fissions to occur before selecting a single animal from each line to grow, exhaust the food supply, and then conjugate. Ten exconjugants from each original line were then tested for survival and vitality (as measured by growth) three days after isolation. In the first experiment 4 kr was administered, and selection for conjugating lines was made ten generations after treatment. The controls gave 80% exconjugant lines showing fair to good growth, whereas exconjugants from irradiated lines gave 52% (250 exconjugants were observed in each case). At 6 kr the controls yielded 82% fair to good lines, whereas irradiated lines gave 42%. In this experiment selection was not made until 20 generations had elapsed following treatment.

Discussion. – This work has given additional data on the comparatively high radiosensitivity of S. spathula and has tested some possible explanations. A peroxide medium effect was previously shown not to be responsible for depressed daily growth.[20] The first experiment reported in this

[20]D. B. Willians, Biol. Semiann. Prog. Rep. Aug. 15, 1959, ORNL-2813, p 34–36.

paper shows that animals irradiated in an atmosphere of oxygen do not show a significant depression of division rate when compared with animals irradiated in nitrogen or air. Thus the sensitivity of this ciliate cannot be due to an oxygen effect. Paramecium, though much more resistant, also shows little or no oxygen effect on division delay.[21]

The second experiment focuses attention on the first division interval following x-ray treatment and has demonstrated that doses as low as 1–2.5 kr will cause appreciable increase in generation time. Indeed, 5, 10, and 15 kr cause increases of 2 hr and usually more. In this regard Spathidium appears to be much more sensitive than Paramecium.[22]

The results on exconjugant growth and viability indicate that Spathidium might be suitable material to test mutation induction. The selfing conjugation displayed by this form should, on certain simple assumptions, produce homozygosity for any micronuclear mutations that were induced by radiation, thus allowing them to come to expression. It is not possible, however, to exclude the hypothesis that damage noted in exconjugants of x-rayed Spathidia is vegetative rather than genetic. Tests of these two alternatives are feasible, however, so that a decision should be possible eventually.

The Limited Number of Nuclear Sites for Chromosome Exchange and the Distribution of Aberrations

S. Wolff H. E. Luippold

Introduction. – The chromosome aberrations that are induced by x radiation are the result of at least two processes: the breakage of the chromosomes and the subsequent rejoining (or not rejoining) of the breaks. Breakage has been found to occur randomly in the nucleus, but rejoining is nonrandom. Consequently, the numbers of most aberrations per cell are distributed according to the Poisson distribution, but chromosome exchanges that are dependent upon rejoining are not.[23] The deviation from the Poisson distribution occurs because there are always too few cells containing multiple exchanges. Accordingly, it has been postulated that within the

[21]R. F. Kimball, Proc. Soc. Exptl. Biol. Med. 82, 471–77 (1953).

[22]R. F. Kimball, Ann. Rev. Microbiol. 11, 199–200 (1957).

[23]S. Wolff, Radiation Research, Suppl. 1, 453–62 (1959).

nucleus there are a limited small number of sites where the chromosome threads are close enough to one another so that if broken they can rejoin to form exchanges.[23,24] The present experiments were designed to find the number of these sites and to find how the exchange aberrations were distributed per cell.

Results. – Mathematically the proportion of cells not having a two-hit chromosome exchange can be likened to the proportion of cells that survive a two-hit event. Thus the standard two-hit survival curve of

$$S = \left[1 - (1 - e^{-kD})^2\right]^n$$

can be fitted to the number of sites where exchanges can take place. In this case at dose D, e^{-kD} would be the probability of not breaking a strand in a site and S would be the proportion of cells without an exchange in all n sites.

A least-squares fit to this equation of extensive data on Tradescantia microspores gives a value of n = 4.1 (Fig. 1). Such a low value accounts for the lack of cells having multiple exchanges.

Knowing the number of sites, it was now possible to characterize the distribution of two-hit chromosome aberrations per cell. If in a given experiment an aberration yield per cell of Y was obtained, the yield per site for Tradescantia microspores would be Y/4. This would be the chance of getting an exchange in a site, and 1 – (Y/4) would be the chance of not getting an exchange in a site. The numbers of exchanges per cell would then be binomially distributed according to the formula

$$\left[\left(1 - \frac{Y}{4}\right) + \frac{Y}{4}\right]^4$$

where the first term of the expansion would give the expected proportion of cells not having an exchange in any of the four sites (and therefore none in the cell), the second term of having an exchange in only one of the sites (and therefore only one in the cell), etc. The data that do not fit the Poisson distribution have been found to fit the expected values determined from the binomial expression in this way.

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[24]S. Wolff et al., J. Biophys. Biochem. Cytol. 4, 365–72 (1958).

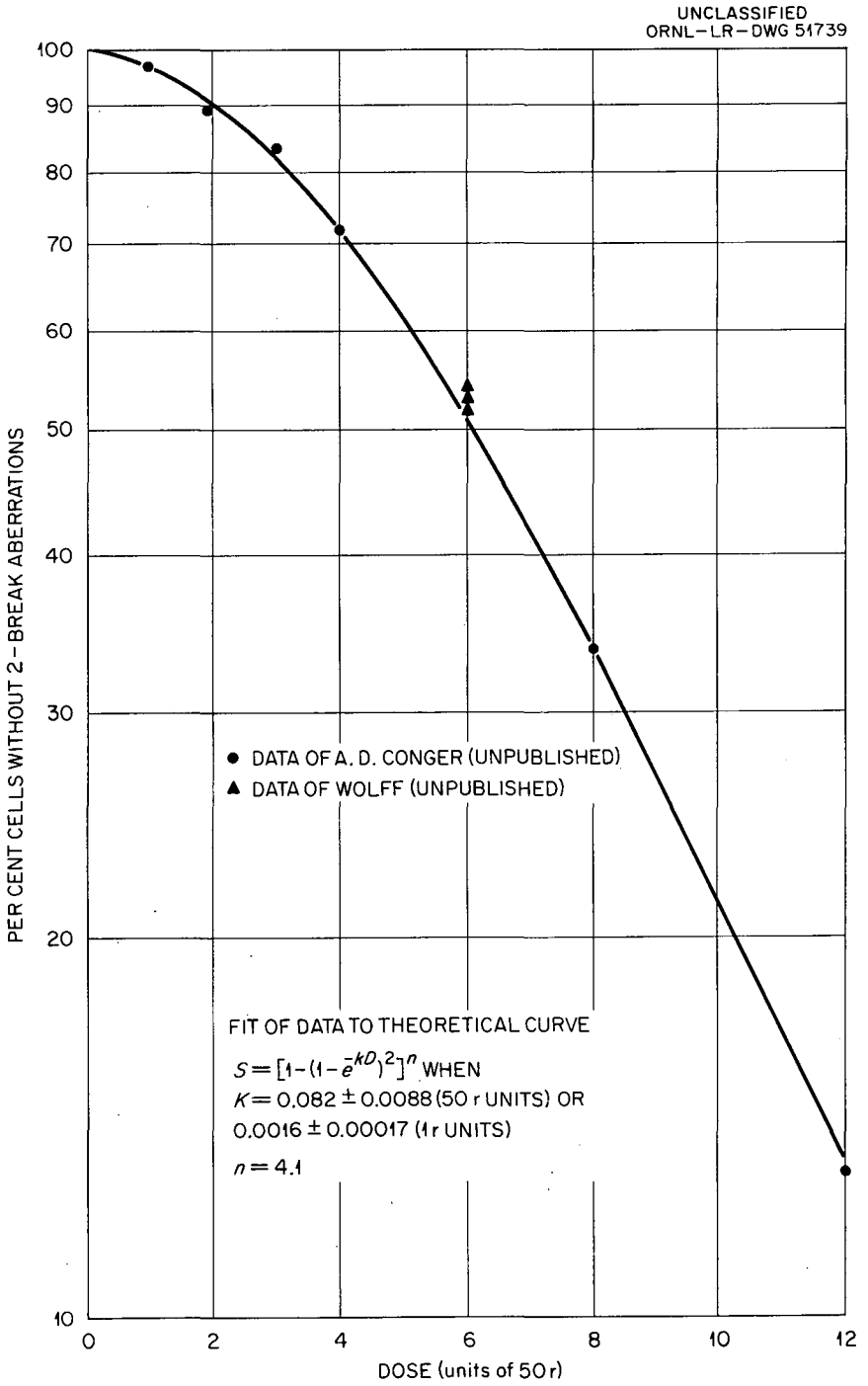

Fig. 1. Fit of Data on Numbers of X-Rayed Tradescantia Microspores Having No Aberrations to the Theoretical Curve. From unpublished calculations of Atwood and Wolff.

For the first division of Vicia faba root tips, it seems as though the site number is even lower and is about 2.

Conclusions. – The well-known lack of randomness throughout the whole nucleus in the rejoining of radiation-induced chromosome breaks is explained on the basis of there being only a limited small number of sites within the nucleus where the chromosomes are close enough to rejoin with one another if broken. For Tradescantia microspores, calculations have indicated that the site number is only 4, whereas in Vicia it is 2.

With these site numbers, it is now possible to characterize the observed distribution of chromosome exchanges. It is found to be a binomial distribution.

Cell Death and Root Growth in Vicia

D. Davidson

Introduction. – It had been reported[25,26] that cells with visible chromosome deficiencies induced by irradiation persist in roots of Vicia faba. Such cells are of particular interest because of the view that chromosome changes are directly responsible for cell death following irradiation.

Primary roots have also been examined two, five, and seven days following irradiation, to determine whether mitosis stops during the period of slow root growth caused by the irradiation.

Results. – 1. Chromosome Changes and Cell Death. – Chromosomes with visible deletions appear to be quite stable. They are present in 3.6% of cells, seen at metaphase, 21 days following irradiation (Table 1; see also Table 2 of ref 26). These results indicate that chromosome change does not necessarily lead to cell death, for even cells with a deficiency may survive over long periods. They emphasize the need to distinguish between actual cell "death" and the disappearance of a cell from an active meristem. It is misleading to assume that all cells seen with chromosome changes soon after irradiation are destined to die without passing through a second mitosis.

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

[26]D. Davidson, Ann. Botany (London), in press.

Table 1. Frequency of Cells with Aberrant Complements in Apical Meristems of Primary Roots of Vicia

Roots were fixed 21 days after having received 600 r

Root No.	Reciprocal Translocation					Deficiency			C_2''	Dupli-cation		Double Reciprocal Trans-location	Ring	Total Aberra-tions	Per Cent Aberra-tions
	A	B	C	D	E	A	B	C		A	B				
1	9	6	19	1		6	5							46	23.3
14	4	7	5	3	1	2			4					26	23.6
19	23	13	5			10			1	18	2			72	22.7
11	19											1		20	24.1
4	35	33	6			9	3	17	5				3	111	34.4
23[1]	34													34	29.9
20[1]	48													48	84.2
20[2]		14												14	22.9
10	3	7	3											13	15.5
25	6	17	7	6	3	7				11		5		62	22.1
31	6	7				4								17	13.6
Total		350					63		10	31		6	3	463	

2. X Rays and Root Growth. – Roots fixed two, five, and seven days following irradiation contain dividing cells; these roots pass through a period of very slow or no visible growth. It seems anomalous that a supposedly highly sensitive process – cell division – continues following irradiation, while cell elongation, which is responsible for visible growth, and which is less sensitive to irradiation, is stopped.

The presence of dividing cells in nongrowing roots and the eventual regeneration of irradiated roots is evidence that cessation of visible growth is not truly indicative of root "death" and that conclusions based upon this assumption require re-examination.

DNA Synthesis and Mitosis in Vicia Roots

D. Davidson

Introduction. – Although the primary roots of Vicia faba are in frequent use in radiation studies, nothing is known of the time of DNA synthesis in relation to the first mitotic divisions. Further, it has not been shown whether the first cells to undergo mitosis synthesize DNA during germination or have already completed it in the last interphase before the bean has been shed by the mother plant. Studies have therefore been made to determine the time of onset of DNA synthesis and of mitosis in the primary root of the germinating bean.

Results. – 1. Incorporation of H^3-Thymidine. – Incorporation of H^3-thymidine is almost absent during the first 32 hr of soaking and germination. Of 40,000 nuclei examined (ten roots per fixation; 2000 nuclei per root) after exposure to H^3-thymidine for 24–28 or 24–32 hr, only 0.66% of the nuclei had incorporated labeled compound. In the period 32–36 hr, 7.87% of the nuclei became labeled. The incorporation of labeled precursor in immediately subsequent periods is being determined.

2. The Onset of Mitosis. – A preliminary determination showed that mitotic figures were present in roots 48 hr after the beginning of germination. Beans were kept at 20°C in aerated water, and beginning at 44 hr, fixations of ten primary roots were made every 2 hr. The percentage frequency of mitosis is low at 44, 46, 48, and 50 hr, that is, about 1% or less, but increases to 3% at 52 hr.

The difference of about 12 hr in the time of DNA synthesis and onset of mitosis would seem to indicate synthesis had not occurred before the bean entered the period of dormancy. Mitosis in the germinating root can occur, it seems, only after DNA synthesis.

Hybrid Enzyme Formation in Maize Heterozygotes

D. Schwartz K. H. McGrath

Three alleles of a gene controlling the synthesis of a basic protein with esterase activity have been found in a survey of a number of different maize stocks. The alleles are designated E^F, E^N, and E^S. The allele most commonly found in the stocks tested is E^N, and the enzyme specified by this gene is referred to as normal. The esterase specified by E^F shows a faster migration rate, whereas that of E^S shows a slower migration rate. This enzyme occurs with highest concentration in the developing endosperm and in the germinating seedling. Electrophoretic studies are made with extracts from single kernels.

The homozygotes, E^F/E^F, E^N/E^N, and E^S/E^S, show only a single basic esterase type, either fast, normal, or slow, respectively. Heterozygotes between any two of the three alleles show in addition to both parental-type enzymes a third enzyme which occupies an intermediate position. Thus E^F/E^N heterozygotes show the fast band, the normal band, and an intermediate band labeled fast-normal. E^N/E^S heterozygotes show the normal band, the slow band, and an intermediate band labeled normal-slow. E^F/E^S heterozygotes show in addition to the fast and slow bands a third band in an intermediate position. The position of the normal-type enzyme band is not halfway between the fast and slow enzyme bands, but is closer to fast than it is to slow. It is interesting that the hybrid band formed in the E^F/E^S heterozygotes has precisely the same migration rate as the normal band specified by E^N. In heterozygous germinating seedlings, which are diploid, the intensity of the hybrid band as determined by esterase activity is greater than that of either parental enzyme band. In heterozygous developing endosperms, which are triploid, the hybrid band and the band which is formed by the enzyme type specified by the allele contributed by the maternal parent are heavy, showing about the same intensity, while the third band is light. For example, endosperms resulting from

the cross $E^N/E^N \times E^F/E^F$ ♂ show intense normal and fast-normal bands, while the fast band is light. These results are consistent with the hypothesis that this esterase enzyme exists as a dimer. The dimer can be composed of two fast, two normal, or two slow monomers or any combination of these. The migration rates of the hybrid dimers would be expected to be intermediate between the rates of the homozygous parental dimer types. The relative concentrations of the three enzyme types in endosperm heterozygotes would be in line with this hypothesis, since the endosperm receives two chromosome sets from the maternal parent and one from the male. Preliminary attempts to dissociate this enzyme have not been successful.

Currently we are making a survey of 15,000 strains of maize from all over the world in a search for other alleles. The enzyme is being purified in preparation for comparative studies on amino acid composition and fingerprinting.

Electrophoretic Studies on Maize Amylases

B. A. Swomley

Introduction. – Four protein bands with amylase activity separate out on starch gel during electrophoresis of developing maize endosperm.[27] β-Amylase activity has been found to be associated with the fastest moving band of the four. Only two protein bands with amylase activity result from starch gel electrophoresis of the scutellum of germinating maize seeds. One protein occurs at much higher concentration and exhibits greater activity than the other. When the developing endosperm and scutellar extracts are run electrophoretically side by side in the overlap test,[27] the two fast-migrating endosperm amylase bands coalesce with the faster of the two scutellar amylase bands. By selective precipitation at varying ammonium sulfate concentrations, it was possible to separate the faster-moving scutellar band into two components.

Comparisons also were made between electrophoretic banding patterns of amylases in maize plumule and coleoptile and in bean cotyledon, plumule, and root.

Results. – The two leading amylase bands of developing endosperm, a fast-moving band, A, and one which migrates directly behind it, B, can be

[27]D. Schwartz, Genetics 45(10) (1960), in press.

selectively precipitated at ammonium sulfate concentrations of 60–80% and 0–60%, respectively. These same concentrations applied to scutellar extract result in the separation of the foremost protein band into two distinct components. The component which precipitates at 0–60% AS migrates at the rate of the original protein. A second component, which precipitates at 60–80% AS, moves much less rapidly. When the 60–80% AS fractions from both developing endosperm and scutellum are run in the overlap test, they form a single continuous band on the gel, while the slower-moving 0–60% AS fraction of endosperm connects up with the rapidly migrating 0–60% AS fraction of scutellum.

Extract prepared from maize plumule and coleoptile exhibited a single protein band corresponding to protein band A of developing maize endosperm. Extracts from plumule, root, and cotyledon of the germinating bean seed produced an analogous protein band, considerably higher in concentration in the plumule than in the root and cotyledon. An additional band moving at a slightly faster rate was found in cotyledon extract, but no correlation could be found between this band and the amylase bands of maize.

Discussion. – A protein component found in developing endosperm of maize, and with which β-amylase activity has been found to be associated, occurs in high concentration in the scutellum of maize and in bean plumule, and to a lesser degree in maize developing endosperm, plumule, and coleoptile and in bean cotyledon and root. In the scutellum of maize, this component can only be identified after selective precipitation in 60–80% ammonium sulfate, as it normally migrates in association with a second scutellar component.

Genetic Effects of Ethyl Methane Sulfonate Exposure of Phage T4

D. R. Krieg B. C. Pal
D. M. Green R. M. Fulkerson

Induced mutations occur among the survivors of extracellular phage exposed to ethyl methane sulfonate.[28] This alkylating agent evidently reacts with the phage genes in vitro, and the induced mutant clones arise during the subsequent reproduction of the treated phage in untreated bacteria. This could be "delayed mutation" in which chemically altered genes

[28]D. M. Green, Biol. Semiann. Prog. Rep. Feb. 15, 1960, ORNL-2913, p 44–45.

are capable of producing either normal or mutant copies at any of their duplications.[28] The evaluation and clarification of this hypothesis require further knowledge of the effects of the chemical treatment on the phage.

The effects of changes in various parameters during the exposure of the phage have been explored in recent experiments. It appears that the rates of killing and mutation are proportional to the concentration of ethyl methane sulfonate, and that both are strongly accelerated by increasing the temperature from 30 to 45°. The rate of killing increases progressively during an exposure. Although inactivation has been studied to less than 0.01% survival, the survival curve does not become exponential. The accelerated killing is not attributable to a gradual production of some toxic substance, and 0.5 M phosphate buffer is used to limit the pH drop from hydrolysis of the chemical. It is tentatively inferred that some killing is due to accumulation of nonlethal phage damages.

It appears that phage exposed to doses giving 1% or less survival adsorb to bacteria more slowly than untreated phage, and most of the treated phage no longer are capable of killing the host bacteria. This probably indicates a block in the injection of the genetic material into the host cell. Multiplicity reactivation (the production of phage in bacteria infected by several inactivated phage) and cross reactivation (the "rescue" of genes from inactivated phage in bacteria also infected by viable, untreated phage) occur to a much lesser extent than with ultraviolet-irradiated phage.

Stocks have been prepared, and optimal conditions are being determined, for a systematic screening of many T4r_{II} mutants for ethyl methane sulfonate–induced reversion. This will test a hypothesis developed during our earlier work that the mutagen is quite specific as to the genetic sites affected and the types of mutation produced.

Chemical evidence is being sought on the nature of the products formed by the reaction of the phage with the alkylating agent. Preliminary experiments with pure bases and nucleotides have demonstrated that a variety of reaction products are formed and that purines are more subject to reaction than pyrimidines.

Indole-3-acetic Acid and Root Growth

R. T. Brumfield M. W. Scott A. P. Davis

Introduction. – Unilateral exposure to ultraviolet radiation induces negative curvatures (stimulation) in timothy roots. Nonirradiated roots are stimulated after contact with irradiated ones, the stimulus being mediated through the transfer of an extracellular gelatinous material covering the root tip. Very low concentrations of certain substances (i.e., IAA) influence root growth, and it is pertinent to determine the effective concentrations of such compounds before attempting to isolate and identify the responsible component of the gelatinous substance.

IAA and Geotropism (M. W. Scott). – Seedlings are grown and treated as described elsewhere,[29] and growth is recorded by time-lapse cinephotography using an "identical twin" technique. One-half of the film is masked, the root tip aligned with a horizontal cross hair in the camera viewer, and its growth to the bottom of the frame recorded on the unmasked half frame. This control period varies from 80 to 120 min with individual roots and is 700 μ of root growth. The film is rewound to the starting frame, the exposed half frame is masked, the root treated, and subsequent growth recorded on the other half frame for a number of frames equal to the control period. When projected, growth before and growth after auxin treatment are seen simultaneously, and inhibition or stimulation is detected without measurement. Time intervals are 7.5 sec, and projection time is 40–60 sec at 16 frames/sec. The root is again treated and then turned horizontally, and geotropism is recorded. The path of the root tip is traced during projection. Control roots curve upward for the first 5–10 min (2.5–5 sec on screen), then turn downward. The growth movement is not uniform, but undulates downward and forward with a period of 4–5 min. The delicate balance between stimulation and inhibition is striking. IAA, 10^{-16}–10^{-13} M, stimulates growth or is ineffective, while 10^{-12}–10^{-7} M inhibits. Of 15 roots treated with 10^{-16}–10^{-13} M, eight showed the ageotropic response induced by 2,4,6-trichlorophenoxyacetic acid, in which the curvature per unit of growth is less than that of controls. In higher concentrations (10^{-12}–10^{-7} M), the curvature per unit of growth is greater than that of controls.

[29] R. T. Brumfield, Am. J. Botany 42, 958 (1955).

Unilateral Application of IAA in Lanolin (A. P. Davis). — Aqueous IAA solutions are added to anhydrous lanolin V/V, heated to 32°C to melt the lanolin, then thoroughly mixed. A bit of the mixture is taken up on the end of a glass rod 0.1 mm in diameter and applied to the root, grown in air, by means of a micromanipulator. Curvatures and growth are recorded photographically. Lanolin and water produce only minor positive or negative curvatures. Lanolin containing 10^{-15} or 10^{-16} M IAA applied ca. 250 μ behind the tip induces negative curvatures up to 40° ca. 1000 μ behind the tip. Although the response is variable, it is clear that the stimulations induced by irradiated roots can be duplicated with 10^{-16} M IAA.

IAA and the Cell Growth Pattern (R. T. Brumfield). — The growing points of living roots are photographed (183X) at 5-min intervals and increments in cell and root length measured. Controls show wavelike fluctuations in growth rates as seen in the time-lapse motion picture studies. In an experiment with 10^{-12} M IAA, the rate of root growth fluctuated from 28 μ in 5 min to 46 μ in 5 min prior to treatment. Twenty minutes after treatment the rate dropped to 12 μ in 5 min, and 45 min after treatment it increased to 42 μ in 5 min, nearly the maximum rate existing before treatment. All cells were affected, but only those near the tip regained the original rate of elongation. Higher concentrations inhibit for a longer period. In a root treated with 10^{-15} M IAA, the rate varied from 18 μ in 5 min to 29 μ in 5 min in six successive 5-min intervals before treatment; in ten successive 5-min intervals after treatment the rate varied from 24 μ in 5 min to 46 μ in 5 min. Cells in all regions along the length of the root were stimulated.

These results show that 10^{-16}–10^{-15} M IAA stimulates growth of the timothy root, and therefore unusually sensitive methods for bioassay must be employed in the isolation and identification of the stimulatory substances released by ultraviolet.

Evidence for Complexity in the Complementation Map of the ad-3 Region of Neurospora crassa

F. J. de Serres

Introduction. — Previous experiments on allelic complementation in the ad-3 region demonstrated extensive interaction with tester strains in

a sample of leaky ad-3B mutants with 100% (42/42) of the mutants giving positive tests, but only very limited interaction in a sample of nonleaky ad-3B mutants with only 5% (12/256) giving positive tests.[30] Since these mutants were not tested in all possible pairwise combinations, no definite conclusions were reached as to the nature of the complementation map of mutants in this region. Since the complementation map is a result of interaction at the cytoplasmic level, it is interesting to determine whether the complementation map is linear or nonlinear and just what type of correspondence exists between the position of individual mutants on this map and that on the genetic map of the same region. The present analysis is concerned solely with the nature of the complementation map. To approach this problem a mixed population of 99 leaky and nonleaky ad-3 mutants showing allelic complementation were tested in all possible pairwise combinations to determine whether there was an internal consistency in the interaction patterns such as one might expect if the map were two-dimensional and linear.

Results. – Heterokaryon tests were made as described previously[31] with a total of 2.5×10^5 conidia per tube in a total volume of 2 ml of minimal medium, and detailed observations were made over a period of ten days. The tubes were kept for a total of six weeks, and no evidence was obtained for further change. Fifteen of the 99 mutants grew too rapidly (very leaky mutants) under the present experimental conditions to be mapped with certainty and will have to be mapped more precisely in subsequent analyses. However, among the remaining 84, the interaction patterns of various ad-3A-ad-3B combinations showed that the ad-3A region can be subdivided into at least four subgroups, and the reaction patterns of various ad-3B-ad-3B' combinations show that the ad-3B region can be subdivided into at least 23 different subgroups. Thirty-one strains showing distinctly different complementation patterns have been selected for use as tester strains for this region (Fig. 2). Most of the mutants can be represented on the map as continuous straight lines, and the majority of the

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[30] F. J. de Serres, Biol. Semiann. Prog. Rep. Feb. 15, 1960, ORNL-2913, p 32–34.

[31] F. J. de Serres, Biol. Semiann. Prog. Rep. Feb. 15, 1960, ORNL-2913, p 34–35.

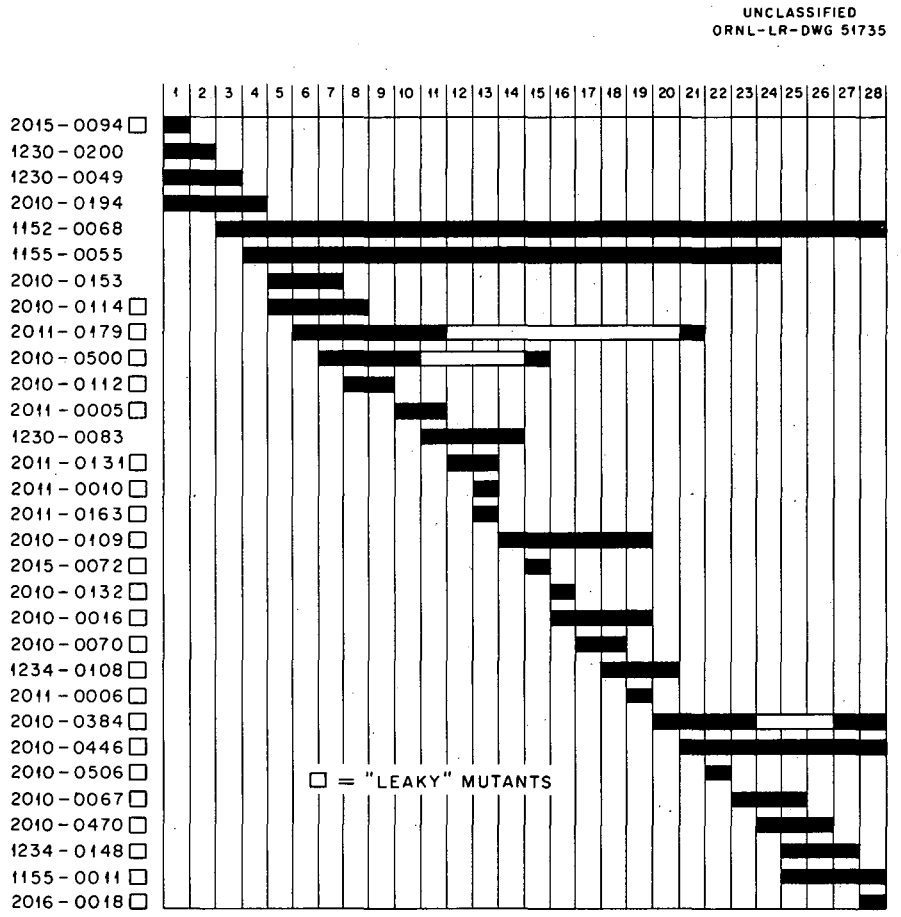

Fig. 2. Complementation Map of the ad-3 Region of Neurospora Showing the Extent of Functional Alteration in 31 Tester Strains.

mutants can be arranged in a consistent linear pattern, starting on the left with ad-3A mutants and ending up on the right with ad-3B mutants in accord with the genetic map of this region. However, 14 of the 84 mutants do not map well with the others and can be represented only with noncontinuous lines. Such mutants give positive tests with tester strains in the middle of the ad-3B region of the map but negative tests with tester strains on either side. Thus, these mutants map as apparent double mutants, and it is especially noteworthy that all 14 map in essentially the same place on the map! Considering these 14 apparent double mutants, the unexpected positive results obtained with individual mutants or groups of mutants in the middle part of the map of the ad-3B region were as follows: one mutant, subunits 10 through 13; eight mutants, subunits 11 and 12; and five mutants, subunit 12. Of equal importance in this consideration is

the fact that 26 of the remaining 70 mutants gave negative tests with tester strains for regions 4 through 16, showing that the tester strains have been placed in proper linear order. Attempts to rearrange the tester strains create an even larger group of apparent double strains than those under consideration.

Discussion. – The results of this experiment show quite clearly that it is not possible to represent the interaction patterns of this group of 84 mutants in terms of a linear complementation map. This is due to the presence of a large number of mutants that cannot be represented with a continuous straight line. It seems unlikely that these mutants are actually double mutants: (1) from what is known about artifically constructed double mutants at other loci,[32] (2) because they constitute such a large proportion of the total population of mutants, and (3) because they are not randomly distributed on the complementation map of the ad-3 region. Since all available direct evidence indicates that allelic complementation results from interaction between enzyme molecules, most probably in the formation of a polymer,[33] the most attractive hypothesis to explain the complexity is that it is a direct reflection of interaction between three-dimensional enzyme molecules of complex structure. However, it is still quite possible that the complexity may be due either to some unknown property of leaky mutants per se or to some uncontrolled variable in the experimental procedure.

The Significance of the Changes in the Complementation Patterns of Individual ad-3B Mutants with Time

F. J. de Serres

Introduction. – According to a hypothesis by Woodward,[34] allelic complementation results from the dissociation and reassociation of differentially defective polypeptide chains to form enzyme molecules of four different types: two types with singly defective polypeptide chains (the

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[32]M. E. Case and N. H. Giles, Proc. Natl. Acad. Sci. U.S. 46, 659–76 (1960).

[33]J. R. S. Fincham, J. Gen. Microbiol. 21, 600–611 (1960).

[34]D. O. Woodward, Proc. Natl. Acad. Sci. U.S. 44, 1237–44 (1958).

parental classes) and (the recombinant classes) one type with doubly defective polypeptide chains, and another type with nondefective polypeptide chains (corresponding to the unaltered wild-type enzyme). Under optimum conditions in heterokaryons growing at wild-type rate, the four types of enzyme molecules would be present in equal proportions and an enzyme assay would show 25% of the wild-type enzyme activity. In addition to the fact that no heterokaryon between mutants showing allelic complementation had an enzyme activity in excess of the 25% predicted by the hypothesis, further support came from the observation that there was a positive correlation between the degree of complementation and the position of mutants on the complementation map (i.e., mutants in adjacent subunits grow slowly and show low enzyme activity). If the hypothesis were true one would expect an orderly progressive change in the interaction patterns of mutants showing allelic complementation with time. Mutants reacting with the most distal mutants would show on the map early in the experiment, and those in adjacent subunits much later. Such is not the case in tests of mutants in the ad-3 region. To illustrate the complexity, the interaction patterns of a sample of 25 ad-3B mutants were recorded with change in time.

Results. – The test tube method[31] was used with an inoculum of 2.5×10^4 conidia per tube in 2 ml of minimal medium. Tubes were kept at 25°C and observations made daily for a total of 288 hr after inoculation. On the complementation map in Fig. 3, the interaction patterns of individual mutants are presented as recorded 144 and 288 hr after inoculation.

Discussion. – Figure 3 shows that the defective regions in 23 of 25 mutants can be presented as a continuous straight line on the map, whereas two mutants appear to be double mutants as discussed in the previous report. The changes in the interaction patterns of these mutants with each other do not progress in an orderly fashion. When the mutants are arranged in linear order on the basis of the final reactions, it is especially noteworthy that many mutants give positive tests very late with mutants separated from them by many subunits and very early with mutants in adjacent subunits. The complexity involved in mapping mutants showing such complex reactions is well illustrated by imagining the effect of stopping the observations 144 hr after inoculation. This is a common practice where such tests are made on petri plates with several combinations being tested per

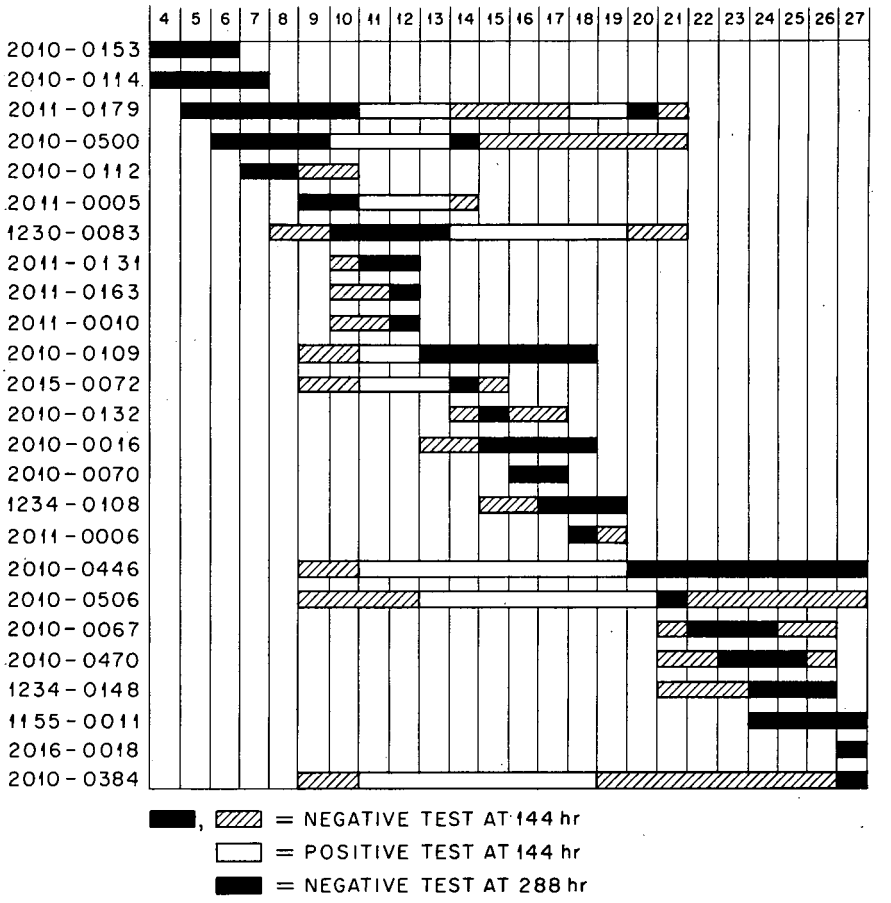

Fig. 3. Complementation Map of the ad-3B Region of Neurospora Showing the Change in Complementation Patterns of Selected Mutants at 144 and 288 hr After Inoculation (10^4 Conidia per Tube, ~ 25°C).

plate (since the plates are soon overgrown by any combination giving a positive test).

The complex complementation patterns that occur during the course of time with mutants in this region do not lend support to the Woodward hypothesis; instead they seem to be more the type of change expected if the interaction were between differentially altered enzyme molecules during formation of a polymer.

Protection with AET·Br·HBr Against the Mutagenic and Lethal Effects of X Rays in Neurospora crassa

H. G. Kølmark

Introduction. — It has been reported earlier[35,36] that AET·Br·HBr in experiments with Neurospora crassa failed to give any substantial protection against the mutagenic and lethal effects of x rays under a variety of conditions tested. On the other hand, it was reported by Hollaender and McCarthy[37] that treatment of Aspergillus terreus with AET·Br·HBr and AET·Cl·HCl (rearranged to MEG at pH 7) increased survival and decreased mutation rates after 100 kr of x rays. It has been shown[38] that the molecule of AET can be rearranged in a number of ways according to the pH of the solution. The rearrangement compound MEG formed at pH 7 is considered the most important for radiation protection.[39] It appeared that the influence of pH should be further investigated, since this factor is of main importance for the chemistry of AET. A few such conditions have now been tested and found to give a positive protective effect of AET in Neurospora crassa.

Results and Discussion. — The same strain, K 3/17 ad-3A (38701), was used as in earlier reports, and adenine reverse mutations were scored. All procedures, including the preparation of the AET solutions, were carried out at ice-water temperature. A phosphate buffer[40] and a phosphate-citrate-borate buffer[41] used in these experiments were both diluted four times from the standard and used here in ca. 0.02 M concentrations. This was

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

[36]H. G. Kølmark, Biol. Semiann. Prog. Rep. Aug. 15, 1958, ORNL-2593, p 27–28.

[37]A. Hollaender and A. M. McCarthy, Biol. Semiann. Prog. Rep. Feb. 15, 1960, ORNL-2913, p 64–65.

[38]J. X. Khym, R. Shapira, and D. G. Doherty, J. Am. Chem. Soc. 79, 5663–66 (1957).

[39]R. Shapira, D. G. Doherty, and W. T. Burnett, Jr., Radiation Research 7, 22–33 (1957).

[40]C. R. N. Strouts, J. H. Gilfillan, and H. N. Wilson (eds.), Analytical Chemistry: The Working Tools, p 239, Clarendon Press, Oxford, 1955.

[41]T. Teorell and E. Stenhagen, Biochem. Z. 299, 416–19 (1938).

done to keep the total salt concentration low, as the toxic effect of AET at pH's above 7 found earlier[36] might be partly due to a high total salt concentration. A 0.03 M solution of AET·Br·HBr in the diluted buffer[41] has no toxic effect in the pH range 4–8, at 0°C, over a period of 4 hr, and this concentration of AET was used here.

1. Influence of Preadjustment of Conidia to Different pH's Before AET Treatment. – Conidia were kept in buffer solutions from pH 4 to 10 for 2–3 hr, and afterward treated for 90 min with AET (rearranged to MEG before it was added to the buffered conidia suspensions) at pH ca. 7. In general, hardly any effect was obtained at preadjustments below pH 7, but protection was obtained at all pH's above 7. The protection found by this procedure, however, is less than that obtained by the procedure outlined in the following section.

2. Influence of pH During AET Pretreatment. – AET was neutralized in four times diluted buffer.[41] In a separate experiment pH was determined as a function of the volume of 1 N NaOH added to this AET solution, starting at pH 6.75. During the experiment 1 M NaCl, 0.075 M AET, and 1 N NaOH were added to the conidia in an irradiation tube, in this order, and calculated so that the final concentration of AET was 0.03 M and the total salt concentration was constant at all pH's used. Survival and mutation curves for pretreatments pH 6.75 and 7.0 are plotted in Fig. 4. At pH 8 the survival was essentially as at pH 7, and at higher pH's the survival was lower, which could be traced to a toxic effect of AET, whereas the fraction killed by x rays remained essentially constant at all pH's above 7. A protection factor of ca. 1.5 is found for survival, and ca. 2.4 for mutation at the lower doses. The curves approach a maximum and intersect at ca. 40 kr, which is similar to the curves obtained after cysteamine and anoxia treatment. No decline after the maximum is seen at pH 7 at the doses of x rays given, but such a decline might possibly take place at higher doses, as is found with cysteamine.

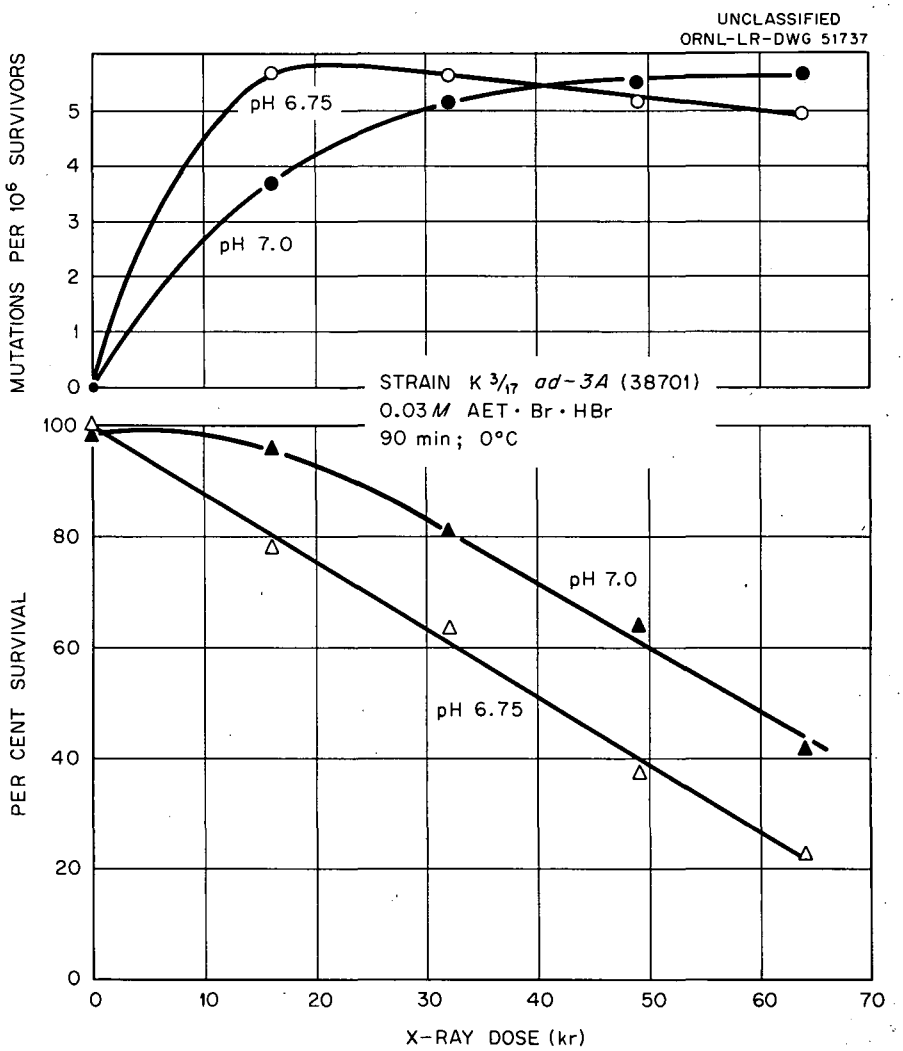

Fig. 4. Effect of pH on Protection with AET·Br·HBr Against X Rays. 2.5 ml suspensions, containing 40.3×10^6 viable conidia per milliliter during pretreatment and irradiation. Pretreatment with 0.03 M AET for 90 min. 0°C during all procedures. Each point is a mean of two tests.

Distinction Between Endogenous Anoxia and Other Physiological Factors as the Cause of Increased Resistance Against X Irradiation After Temperature Treatment of Conidia of *Neurospora crassa*

H. G. Kølmark

Introduction. – In previous experiments[42] it was found that conidia of the strain K 3/17 ad-3A (38701) of *Neurospora crassa* became resistant to the mutagenic and lethal effects of x rays, if suspensions in saline

[42]H. G. Kølmark, Biol. Semiann. Prog. Rep. Feb. 15, 1960, ORNL-2913, p 38–39.

in airtight tubes were incubated at 20–30°C for 75–90 min before x irradiation at 0°C. It was considered most likely that the increased radiation resistance was obtained because the conidia consumed the limited amount of oxygen in the irradiation tube by endogenous metabolism. Some experiments have now been carried out to test the possibility that the increased radiation resistance after temperature treatment might be due to some type of physiological change in the conidia rather than to the development of an anoxic condition.

Results. – In these experiments the temperature treatment was performed in such a way that an anoxic condition could either be canceled or prevented from developing. Both these types of experiments are plotted in Fig. 5.

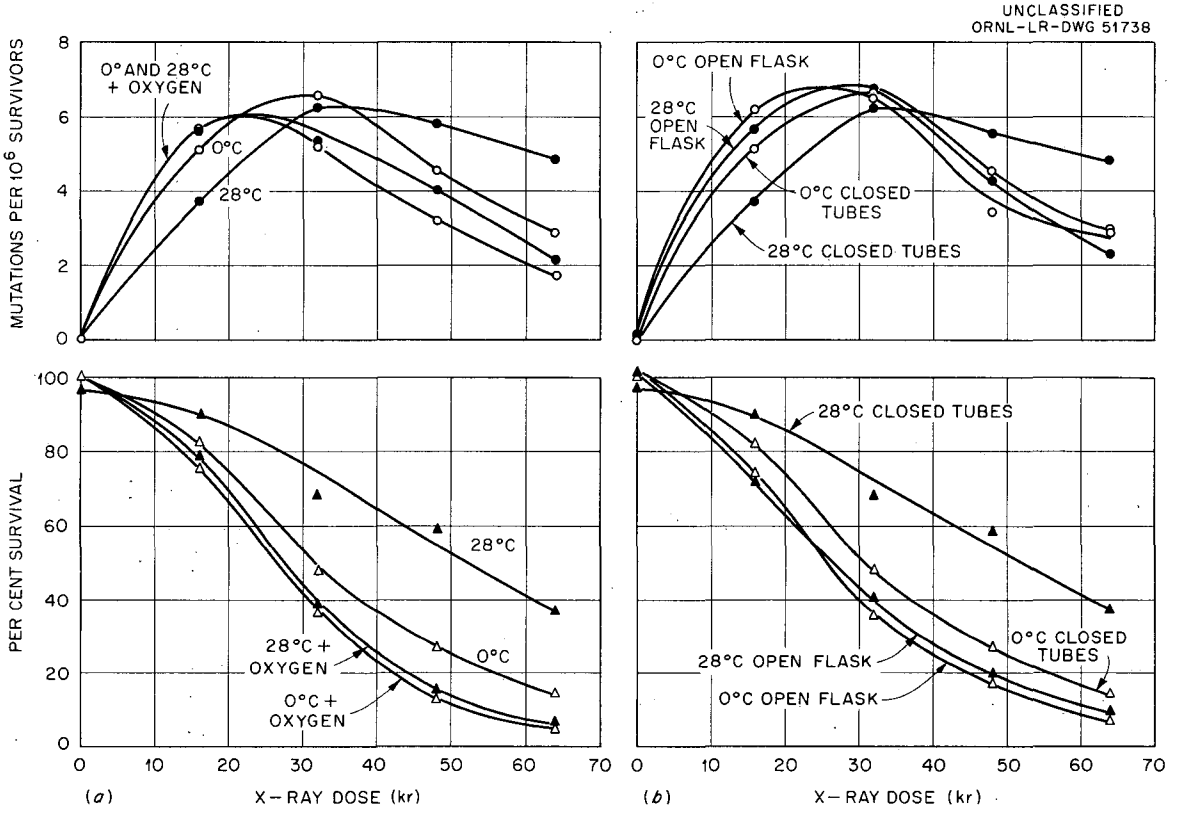

Fig. 5. (a) Cancellation of Temperature-Induced Protection by Subsequent Oxygen Bubbling Before X Irradiation. (b) Prevention of Temperature-Induced Protection by Access of Atmospheric Oxygen. 2.2 ml of conidia in suspensions in airtight tubes. 56.0×10^6 viable conidia per milliliter during pretreatment and irradiation. X irradiation at 0°C. All treatments done simultaneously and with the same batch of conidia. Each point is a mean of two tests.

1. Cancellation of Temperature-Induced Protection by Oxygen Bubbling. — Figure 5a is a graph of an experiment where 2.2-ml conidia suspensions, in saline, in 3-ml closed tubes, initially kept at 0°C, were incubated at 28°C for 90 min, then moved back to ice water. Half the 28°C pretreated tubes were then bubbled through with precooled oxygen for 2 min (25 ml of oxygen per minute). Similar tubes were kept cool throughout, and half of these were also bubbled through with oxygen. The tubes were then irradiated at ice-water temperature. The protective effect seen after incubation at 28°C (increased survival and decreased mutation frequencies as compared with tubes kept at 0°C) is completely canceled when the conidial suspensions are subsequently bubbled through with oxygen before irradiation. The lethal and mutagenic effects are even increased as compared with the effect on conidia kept at 0°C without oxygen treatment, presumably because the suspensions become more completely saturated with oxygen. At increased x-ray doses the mutation frequency curve passes through a maximum value and then decreases. At high doses the highest mutation frequency is found in the anoxic suspensions. This effect is believed to be caused by a differential killing, by x rays, of induced mutations in the presence of oxygen. A similar mutation response to x rays was found in experiments with Streptomyces.[43,44]

2. Prevention of Temperature-Induced Protection by Access of Atmospheric Oxygen. — Figure 5b is the result of a comparison between conidia kept in closed tubes and in open flasks, respectively, during incubation at 28°C before irradiation. Aliquots were subsequently irradiated in closed tubes at 0°C. The results are essentially the same as those obtained with oxygen bubbling, as judged by a comparison of survival and mutation rates.

Discussion. — The results of these experiments support the view that temperature treatment of Neurospora conidia leads to increased radiation resistance because of the development of anoxia as a result of endogenous metabolism, and give no evidence that other physiological changes contribute to the protective effect obtained under the conditions tested.

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[43]A. Kelner, J. Bacteriol. 56, 457–65 (1948).

[44]H. B. Newcombe and J. F. McGregor, Genetics 39, 617–27 (1954).

The present results are in agreement with results from studies with E. coli[45] and a variety of other microorganisms,[46] in which it was observed that a protective effect of high concentrations of cells could be eliminated when the cell suspensions were treated with air or oxygen.

Genetic Relationships of hist-3 Mutants of Neurospora crassa

B. B. Webber

Introduction. – Because of the biochemical heterogeneity exhibited by the hist-3 mutants,[47] interallelic crosses between hist-3 mutants containing contrasting markers at linked loci were analyzed. The study was to determine whether mutants of each biochemical class might exhibit distinctive behavior in such genetic experiments.

Results. – The markers arg-3 and nic-2 (six to seven map units to the left of hist-3 and two to three units to the right of hist-3, respectively) were used. In each cross an arg-3 hist-3 double mutant strain was crossed to a hist-3 nic-2 double mutant strain. From each cross the number of histidine-independent progeny and the total number of viable ascospores were determined by appropriate overplating techniques. These figures were used to determine the frequency of histidine-independent progeny from each cross. Only pairs of hist-3 mutants that do not complement strongly in heterokaryon tests were used in crosses; consequently, pseudowild-type progeny were not expected and not screened for. In these crosses histidine-independent progeny constituted less than 0.013% of viable spores plated. The length of the hist-3 locus is thus probably less than 0.026 conventional map unit. The genotypes of the histidine-independent progeny with respect to the arg-3 and nic-2 loci were also determined. The relative order of hist-3 mutant sites was determined by the quantitative plating data, whereas the question of which side of the locus is distal was answered by observations concerning the frequency with which the mutant

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[45]A. Hollaender and G. E. Stapleton, Federation Proc. 12, 70 (1953), and personal information.

[46]S. E. Gunter and H. I. Kohn, J. Bacteriol. 72, 422–28 (1956).

[47]B. B. Webber, Biol. Semiann. Prog. Rep. Feb. 15, 1960, ORNL-2913, p 39–41.

and wild-type alleles at the arg-3 and nic-2 appeared among the histidine-independent progeny from various crosses.

The map of the hist-3 locus may be divided into a proximal and a distil half. Seven mutants scattered through the distal half of the map are all subgroup 1 mutants (deficient for histidinol dehydrogenase activity). Seven subgroup 2 mutants (deficient for an early reaction in the histidine biosynthetic scheme) and four subgroup 3 mutants (deficient for an early reaction in the histidine biosynthetic scheme and for histidinol dehydrogenase) are mixed together in the proximal part of the map.

Discussion. — It appears that the proximal portion of the hist-3 locus carries information concerning protein structure essential to two separate enzymatic reactions in histidine biosynthesis (involving either two separate enzyme molecules or a single bifunctional enzyme). The distal portion, on the other hand, bears information related only to the structure of the histidinol dehydrogenase molecule (if two enzymes are involved) or the histidinol dehydrogenase active site (if a single bifunctional enzyme is involved). It is especially interesting that certain mutants, which are located in the proximal portion of the hist-3 locus and which yield histidine-independent progeny in crosses with all mutants in the distal half of the map, exhibit a loss of both enzymatic activities.

Histidinol Dehydrogenase Assays of Some hist-3 Mutants of Neurospora crassa

B. B. Webber C. J. Wust[48]

Introduction. — The hist-3 mutants of Neurospora crassa share the following three characteristics: (1) all require histidine for growth; (2) all fail to complement in heterokaryon tests with a single common tester used in selecting hist-3 mutants; (3) all are located in a small genetic region in linkage group I. However, the hist-3 mutants are biochemically heterogeneous and may be divided into the following three subgroups: (1) mutants deficient for histidinol dehydrogenase activity; (2) mutants deficient for one of the first two enzymatic reactions in histidine biosynthesis; (3) mutants deficient for both the early enzymatic activity

[48]Enzymology Group.

and histidinol dehydrogenase. Subgroup 1 mutants accumulate histidinol in their growth medium and so can be identified by positive diazotized sulfanilic acid (Pauly) tests of their medium. Subgroup 2 and subgroup 3 mutants fail to accumulate imidazole derivatives in their growth medium. However, mutants from these two subgroups may be distinguished by in vitro assays for histidinol dehydrogenase activity. For this purpose the histidinol dehydrogenase assay method described by Ames, Garry, and Herzenberg[49] has been modified for use with a Beckman spectrophotometer. The reaction assayed is:

$$\text{L-histidinol} + 2\text{DPN}^+ + \text{H}_2\text{O} \rightarrow \text{L-histidine} + 2\text{DPNH} + 2\text{H}^+$$

In the assay, diaphorase catalyzes the reduction of dichlorophenol dye by the DPNH formed in the oxidation of histidinol. One modification involved the use of potassium phosphate buffer instead of triethanolamine-HCl buffer for preparing the Neurospora homogenate, thus avoiding the interference of triethanolamine with protein determination (method of Lowry et al.[50]). The concentration of dichlorophenol indophenol dye was also decreased to permit more accurate determination of dye reduction rate with the Beckman spectrophotometer. One unit of enzyme is defined as the amount which reduces 0.1 micromole of dye per hour under the new assay conditions.

Results. – Table 2 lists the specific activity of some hist-3 mutants that fail to accumulate imidazole derivatives in their medium (subgroup 2 and 3 mutants). A distinction between subgroup 2 mutants (which exhibit histidinol dehydrogenase activity) and subgroup 3 mutants (which are deficient for histidinol dehydrogenase activity) is evident. Eleven additional hist-3 mutants which accumulate histidinol in their growth medium (subgroup 1 mutants) were assayed and shown to be deficient for histidinol dehydrogenase activity.

Discussion. – It is of considerable interest that a single genetic region should bear specificity for the enzyme (or enzymes) involved in two separate reactions in histidine biosynthesis and that mutations in

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[49]B. N. Ames, B. Garry, and L. A. Herzenberg, J. Gen. Microbiol. 22, 369–78 (1960).

[50]O. H. Lowry et al., J. Biol. Chem. 193, 265–75 (1951).

Table 2. Histidinol Dehydrogenase Specific Activity for Some hist-3 Mutants of Neurospora crassa

Mutant	Specific Activity*	Mutant	Specific Activity*
Wild type		Subgroup 2 (continued)	
74A	4.4	Y234-M681	5.0
Subgroup 2		Y234-M707	6.2
Y226-M408	14.5	Y226-M589	13.9
Y234-M566	10.2	Y234-M1352	3.4
Y226-M584	12.8	Y226-M551	4.2
Y224-M24	9.0	Y226-M68	4.0
Y226-M606	7.5	Y234-M698	3.3
Y226-M92	9.6	Y234-M1435	3.9
Y226-M77	8.7	Subgroup 3	
Y193-M16	8.1	Y226-M511	0
Y226-M433	10.3	Y226-M494	0
Y226-M416	7.1	Y152-M111	0
Y226-M94	7.4	Y189-M83	0
Y226-M216	8.8	Y226-M498	0
Y226-M419	8.9	Y226-M565	0
Y234-M1288	8.7	Y226-M585	0
Y193-M8	8.8	Y226-M496	0
Y193-M14	7.5	Y234-M574	0
Y193-M17	10.4	Y234-M711	0
Y234-M1439	5.8		

*Enzyme units per milligram of protein.

this region may result in the loss of one or the other or both of these two enzyme activities.

CELL GROWTH AND REPRODUCTION

Ribonucleic Acid Synthesis in the Cell Nucleus and Transfer of Ribonucleic Acid from Nucleus to Cytoplasm

D. M. Prescott C. H. Shappert

Introduction. — A current hypothesis states that transfer of genetic information from the chromosomes within the nucleus to points of protein synthesis in the cytoplasm might be mediated by ribonucleic acid. The hypothesis requires, among other things, that the nucleus be capable of ribonucleic acid synthesis and that this macromolecule be transferred to the cytoplasm. Synthesis of at least some ribonucleic acid in the cell nucleus is an established fact, but transfer of this molecule from the nucleus to the cytoplasm is not. The experiments described below support the thesis that the nucleus not only synthesizes ribonucleic acid, but is the major site of such synthesis, and this ribonucleic acid is transferred to the cytoplasm.

Results. — The incorporation of tritium-labeled cytidine into the ribonucleic acid of the nucleus and cytoplasm of cells (Tetrahymena pyriformis) was followed with time. Within the first 15 to 20 min, all incorporated radioactivity is localized in the nucleus. At 15 to 20 min radioactivity begins to accumulate in the cytoplasm, and by 40 min, the total amount of label is equally distributed between nucleus and cytoplasm. After 35 min the nucleus continues to increase in radioactivity at a slower rate, while the cytoplasm continues to be labeled rapidly. By 60 min the cytoplasm contains several times more activity than the nucleus.

In a second experiment, cells were incubated in the presence of tritium-labeled cytidine for 12 min and transferred to a nonlabeled medium. At 12 min the incorporated radioactivity is localized in nuclear ribonucleic acid. Because the cells build up a large pool of tritiated cytidine or its derivatives during the initial 12 min, the nucleus continues to be labeled further after removal of the exogenous tritiated cytidine. At about 40 min the pool is exhausted, and the nucleus no longer increases in radioactivity. At 50 min the activity in the average nucleus begins

to decline, and it falls to nearly zero by 100 min. About one-third of the nuclei are now completely devoid of activity. As in the previous experiment, the cytoplasm begins to show radioactivity at about 20 min and continues to gain activity even after the nucleus has begun to lose activity. The cytoplasmic increase stops when the nucleus has lost most of its radioactivity.

Discussion. – The experiments give a more quantitative basis to phenomena which have been observed in several cell types.[51-53] The initial accumulation of isotope into ribonucleic acid of the nucleus and the delay in the appearance of cytoplasmic label are interpreted as evidence that the nucleus is the major site of ribonucleic acid synthesis and that nuclear ribonucleic acid is the precursor of ribonucleic acid found in the cytoplasm. The gradual disappearance of labeled ribonucleic acid from the nuclei of cells that have been transferred to a nonradioactive medium and the concomitant increase in radioactive cytoplasmic ribonucleic acid is further evidence of a nucleus-to-cytoplasm transfer of this macromolecule. The presence of a nonradioactive nucleus surrounded by a cytoplasm heavily labeled with tritium suggests in addition that the transfer of ribonucleic acid does not occur in the direction of cytoplasm to nucleus. These experiments support a similar conclusion suggested in nuclear transplantation experiments with Amoeba.[54]

Acceleration of Mitotic Rate by Microbeam Irradiation of the Nucleolus with Small Doses of Ultraviolet Radiation

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

Previous studies have shown that irradiation of one of the two nucleoli of the grasshopper neuroblast with a microbeam of ultraviolet radiation retards or, in the case of high doses, permanently inhibits mitosis.[55,56] Some evidence in the experiments utilizing a microbeam of

[51]M. Zalokar, Exptl. Cell Research 19, 559 (1960).

[52]P. S. Woods and J. H. Taylor, Lab. Invest. 8, 309 (1959).

[53]D. M. Prescott, Exptl. Cell Research 12, 196 (1957).

[54]L. Goldstein and W. Plaut, Proc. Natl. Acad. Sci. U.S. 41, 874 (1955).

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

[56]M. E. Gaulden, J. G. Carlson, and J. Jagger, Biol. Semiann. Prog. Rep. Aug. 15, 1959, ORNL-2813, p 59–63.

specific wavelengths suggested that a small dose of ultraviolet to a nucleolus might stimulate rather than retard mitosis. In order to test for stimulation, two mitotic stages, early and middle prophase, were selected for careful study.

Embryos of the grasshopper Chortophaga viridifasciata were oriented in hanging drops so that the neuroblasts situated on the ventral surface of the embryo were uppermost against a quartz cover. The culture medium was the glycine–glutamic acid solution described by Shaw.[57] The nuclear diameter is large in relation to the size of the neuroblasts; therefore in a small proportion of the cells at least one of the two nucleoli will, purely by chance, lie close to the cover. This is important in order to ensure that there is little protoplasmic material to absorb the ultraviolet radiation before it reaches the nucleolus. Nucleoli selected for irradiation were within 5 μ of the quartz cover.

The microbeam apparatus used was of the type designed by Uretz and Perry.[58] Monochromatic radiation was obtained by focusing the beam of a high-pressure mercury arc lamp (G-E AH-6 or Philips SP-500) on the entrance slit of a quartz monochromator. The image of the exit slit was focused on the primary aperture of the microbeam apparatus. The aperture used in these experiments gave a microspot 3 μ in diameter. This is slightly smaller than the diameter of the nucleolus. Photoreactivation was avoided by passing all microscope illumination through appropriate filters. Ultraviolet intensity was measured at the beginning and end of each day's experiments by inserting under the reflection objective of the microbeam apparatus an end-window photomultiplier with uranium glass cover that converts the ultraviolet to radiation of longer wavelengths detectable by the photomultiplier.

Data were collected by locating in an embryo two cells at exactly the same point of development within a given mitotic stage (early or middle prophase); one was treated and both were timed to metaphase. Of the 12 cells in early prophase given small doses (0.0002–0.007 erg in incident microbeam) of 2804 A, ten reached metaphase ahead of their controls and two after their controls. Of eight cells in middle prophase given small

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

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

doses of 2804 A, the seven with the smallest doses reached metaphase ahead of their controls, and the remaining one reached it at the same time. On the other hand, 2650 A showed no such accelerating effect on mitosis — in both early and middle prophase the ratios of control to treated cell division times were about evenly scattered above and below 1.00. It appears from these results that the protein rather than the RNA is the effective component of the nucleolus in producing ultraviolet-induced acceleration of mitosis. The reason for this is not yet known.

Chromosome Breaks Induced in Grasshopper Neuroblasts by 1 and 3 r of X Rays

M. E. Gaulden C. H. Shappert

Since the first reports of the mutagenic action of x rays, a number of investigations covering a wide range of doses have revealed that the frequency of gene mutations and chromosome breaks is directly proportional to dose. Little experimental work has been done with only a few r of x rays, but from the linear dose relation it can be inferred that there is no dose of radiation below which gene mutations and chromosome breaks cannot occur. The paucity of experiments with very low doses of x rays is probably attributable in the main to the large number of cells or organisms required for statistically significant results. Since many rapidly dividing neuroblasts occur in the easily obtained grasshopper embryo, low-dose studies with this cell are feasible. Another advantage of the neuroblast is the fact that spontaneous chromosome breakage has never been observed in it.

Fourteen-day-old eggs of the grasshopper Chortophaga viridifasciata were subjected to 1 and 3 r of x rays at room temperature (25 ± 2°C). About 18 embryos were irradiated at a time. In order to obtain equilibrium of secondary radiation, the eggs were irradiated in a plastic dish 38 mm in diameter with an 8-mm solid base; a plastic insert 9 mm thick covered and was in contact with the eggs. For monitoring, the end of a 25-r Victoreen thimble chamber was inserted in a plastic replica of the irradiation dish. A G-E Maxitron 250-kvp unit was used at 125 kvp and 1.0 ma with 3.5 mm of aluminum filtration and 49.3 cm target distance; the dose rate was 3 r/min.

Twenty-one minutes after irradiation the eggs were changed from 25 to 38°C. At four intervals during the period of recovery from mitotic inhibition — 110, 176, 242, and 353 min after irradiation — embryos were separated from egg membranes and yolk and fixed 5 min in 3:1 absolute alcohol–glacial acetic acid. They were stained in aceto-carmine 20 min and made into temporary squash preparations with a 3:3:2 mixture of water, aceto-carmine, and white Karo syrup, one embryo to a slide. Untreated embryos were similarly handled. All eggs were kept on moist filter paper throughout operations up to fixation.

All neuroblasts in late anaphase and very early telophase were analyzed for the presence or absence of chromosome fragments. These are the only stages in this cell in which acentric fragments can be positively identified as such, because they lie at or near the center of the spindle, the unbroken chromosomes and the centric fragments being at the poles of the spindle. Acentric fragments will hereafter be referred to as fragments. The doses of radiation used produce only simple terminal deletions and no rearrangements.

Neuroblasts contain a large number of morphologically indistinguishable chromosomes (23 in male and 24 in female); fragments cannot, therefore, be traced to their specific chromosome origin. Since sister chromatids repel each other at anaphase in the neuroblast,[59] it is impossible to distinguish between a chromatid fragment and a fragment resulting from permanent fusion of broken ends of sister fragments that have repelled each other to form a single straight fragment. In view of these difficulties no attempt was made to classify fragments as chromatid, isochromatid, or chromosome; they were simply scored as either single or double (two fragments of the same size lying relatively near each other), each type assumed to have been produced by a single radiation event and scored as one break.

Results and Discussion. — A total of 2768 irradiated cells (650,580 chromosomes) were analyzed. Single and double fragments of widely varying lengths were observed after both 1 and 3 r of x rays. After 1 r, 1.48%

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[59] J. G. Carlson, Proc. Natl. Acad. Sci. U.S. 24, 500–507 (1938).

of the cells have chromosome fragments with 0.063% of the chromosomes broken; after 3 r comparable figures are 2.05 and 0.093%, respectively (Table 3). Carlson,[60] analyzing neuroblasts exposed to doses of x rays from 7.8 to 125 r, found that the relation of the frequency of single chromosomal breaks to dose was a simple linear one. A regression line fitted to his data and passing through the origin gives expected percentages of chromosomes broken by 1 and 3 r as 0.042 and 0.127, respectively; these expected values are within the 95% confidence limits of the observed percentages (Table 3). Thus in the neuroblast we have experimental verification of the linearity principle for chromosome breaks down to 1 r of x rays.

Table 3. Induction of Chromosome Breaks in Grasshopper Neuroblasts by Low Doses of X Rays (110–352 min After Irradiation)

Figures in parentheses are 95% confidence limits

	1 r Dose	3 r Dose
Cells with no fragments*	1920	805
Cells with fragments*	28	15
Percentage cells with fragments**	1.48 (0.99–2.12)	2.05 (1.20–3.27)
Total chromosomes irradiated	45,777	19,270
Number of breaks	30	18
Percentage broken chromosomes	0.063 (0.042–0.091)	0.088 (0.053–0.141)
Number of embryos treated	227	88

*Neuroblasts in late anaphase and very early telophase.
**Estimated from the nonzero classes of the Poisson distribution.

DROSOPHILA CYTOLOGY AND GENETICS

Male Sterility of Translocations Between the X Chromosome and the Major Autosomes

M. Warters D. L. Lindsley M. L. Rekemeyer

In a previous investigation of sex-linked recessive lethals whose expression is suppressed by the Y chromosome (Lindsley, Edington, and Von

[60]J. G. Carlson, Proc. Natl. Acad. Sci. U.S. 27, 42–47 (1941).

Halle, in press) it was observed that there was a nearly perfect correlation between those lethals that were male sterile and those that carried T(X;2)'s or T(X;3)'s. The correlation between X;autosome translocation and male sterility was previously noticed by Schultz,[61] but the cytological difference between male fertile and male sterile translocations has not been investigated.

Induced translocations are usually recovered from the sons of irradiated parents, because the absence of crossing over in males facilitates the recognition of a male as a translocation heterozygote from his test cross progeny. The translocated X chromosomes thus recovered have therefore been carried in the haploid state for one generation; consequently any translocation associated with sex-linked recessive lethal or sterile changes will be eliminated from the sample before it is recorded. That this is an important factor in biasing a sample of translocations recovered from sons of irradiated parents is shown by the results of Patterson et al.,[62] who found that in a sample of 1792 translocations, only 429 involved the X chromosome, whereas the expectation on the basis of arm length is roughly T(X;2R) = T(X;2L) = T(X;3R) = T(X;3L) = T(2L;3L) = T(2L;3R) = T(2R;3L) = T(2R;3R); that is, the X chromosome should be involved in approximately half of all translocations.

Evidently, in order to obtain a more representative sample of induced translocations, all the irradiated chromosomes should be kept in heterozygous condition. This requires recovering the translocations from daughters of irradiated parents, but in order for the translocation to be recognizable in the test cross, recombination must be virtually eliminated in these daughters. A method has been devised for accomplishing this by recovering an irradiated paternal genome in combination with a maternal genome that is FM6, SM1, Ubx^{130} e; each of these chromosomes is multiply inverted and undergoes virtually no recombination with its homolog. From a sample of 650 irradiated genomes tested, we recovered 45 translocations, of which 15 were T(X;2)'s, 9 were T(X;3)'s, 18 were T(2;3)'s, and 3 were T(X;2;3)'s. This sample is much more in agreement with the expectations

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[61] J. Schultz, Cold Spring Harbor Symposia Quant. Biol. 12, 179–91 (1947).

[62] J. T. Patterson et al., Am. Naturalist 68, 359–69 (1934).

on the basis of arm length than is that of Patterson et al. Three of the T(X;A)'s proved to be male lethal and 14 were male sterile, so that had the same 650 genomes been recovered from sons rather than daughters, there would have been 28 translocations recovered instead of 45 and their breakdown would have been 6 T(X;2)'s, 1 T(X;3), 18 T(2;3)'s, and 1 T(X;2;3). These figures are comparable with those reported by Patterson et al.

The cytological analysis of the Y suppressed lethals already referred to revealed that the male sterile translocations had in common autosomal break points that were in the chromocentral heterochromatin; this was presumably because the method of recovery was designed to pick up variegated-type position effect lethals. The X chromosome breaks seemed to be distributed randomly along the X chromosome, both in euchromatic and proximal heterochromatic regions. It seemed as though any break in the X that was involved in a reciprocal translocation with a heterochromatic autosomal break point was male sterile, whereas indications were that insertional translocations and inversions did not lead to sterility. It was postulated that linear continuity between the tip and the base of the X chromosome is in some way important for male fertility and that reciprocal translocations but not insertional translocations or inversions interrupt this continuity. A preliminary cytological examination of four of the male fertile T(X;A)'s recovered in the present experiments has revealed that all four have both the X and the autosome broken in the proximal heterochromatin. Thus not every interruption in the continuity of the X leads to sterility; either linear continuity of the X chromosome is not important to fertility, or these fertile translocations are broken outside a region of the X in which continuity is important, for example, in X^R. This sample is being enlarged, and all translocations will be characterized by salivary chromosome analysis in order to learn what are the cytological correlates of male sterility in T(X;A)'s.

The Compound Generating Bar of Stone Duplications

D. L. Lindsley L. Sandler M. L. Rekemeyer

$T(1;4)B^S$ is a reciprocal translocation between the X and the fourth chromosomes in which the X is broken just to the left of polytene chromosome band 16A1 and the fourth chromosome is broken subterminally in region

102F. The proximal portion of the X of this translocation is slightly viable as a duplication in males and apparently completely viable in females. It may be designated $Dp(1;f)B^S$.

The attachment of $Dp(1;f)B^S$ to the end of the long arm (X^L) or to the minute heterochromatic short or right arm (X^R) of an X chromosome that is either completely inverted or in normal sequence can produce four different types of X chromosomes that carry the proximal half of $T(1;4)B^S$ as an attached duplication, that is, $Dp(1;1)B^S$. In a heterozygote carrying $Dp(1;1)B^S$ and a normal X chromosome, exchange between the duplicated segment and the base of the normal X will result in the replacement of the B^S duplication with the normal X and the consequent production of a compound X chromosome, that is, two X chromosomes attached to a single centromere. The four types of compound X's have been synthesized by Novitski[63] and classified by him as tandem if the relation between the two X's is A B - A B and reversed if it is A B - B A; a compound X is said to be acrocentric if the centromere is at one end of the pair and metacentric if it is between the two X's. Tandem compounds form a spiral during synapsis, whereas the reversed compounds pair by folding double in the middle. The four types of compounds, then, are the tandem acrocentric (TA), the reversed acrocentric (RA), the tandem metacentric (TM), and the reversed metacentric (RM); the $Dp(1;1)B^S$ chromosomes from which each can be produced may be referred to as the tandem acrocentrogenic (TAG), the reversed acrocentrogenic (RAG), the tandem metacentrogenic (TMG), and the reversed metacentrogenic (RMG) B^S duplications, respectively. The structure of the four different $Dp(1;1)B^S$ chromosomes and the event that generates each type of compound X are diagramed in Fig. 6.

Muller[64] was the first to recognize the utility of $Dp(1;1)B^S$ as a compound generating chromosome, and he produced the first one by detaching an attached X (RM) with $Dp(1;f)B^S$. He called this $Dp(1;1)B^S$ "doubler," and according to the present terminology it would be symbolized $Dp(1;1)B^S$, RMG. We have synthesized the other three: TAG by a complicated series of irradiation procedures from $Y^SX^\cdot$ and $Dp(1;f)B^S$; TMG by detachment of

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[63]E. Novitski, Genetics 39, 127–40 (1954).

[64]H. J. Muller, Drosophila Inform. Serv. 6, 8 (1936).

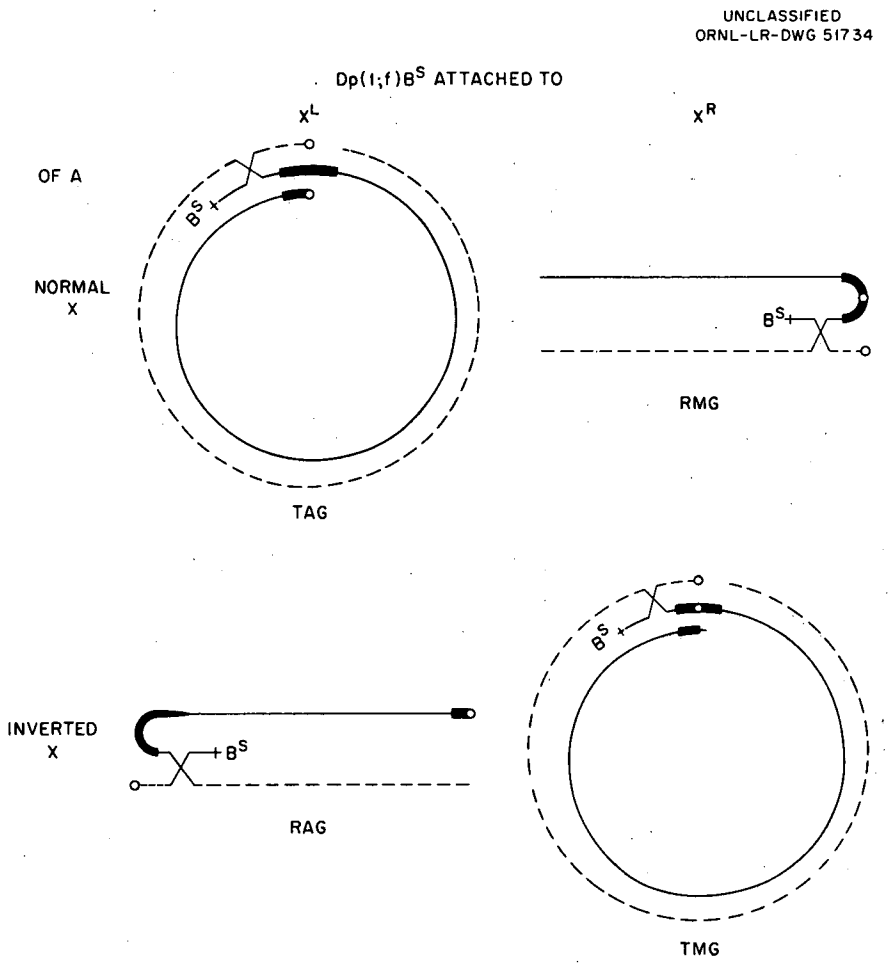

Fig. 6. The Four Different Compound Generating Dp(1;1)B^S Chromosomes and the Exchange with a Normal X Chromosome That Leads to the Production of the Corresponding Four Different Compound X Chromosomes. The broken line represents the normal X chromosome, and the solid line represents the Dp(1;1)B^S with the heavy line indicating the distribution of chromocentral heterochromatin in the Dp(1;1)B^S.

TM with Dp(1;f)B^S; and RAG by detachment of RA with Dp(1;f)B^S. These chromosomes generate compound X's at a rate of about 0.001 and are very useful in the production of compound X chromosomes carrying appropriate markers for various types of investigations. It is also anticipated that they will prove useful in the production of tandem ring compound X chromosomes and ring XY chromosomes, both of which we have desired for projected experiments for several years.

Allelic Interactions and Dosage Effects at the Pin Locus in *Drosophila*

E. H. Grell

Introduction. – Two new mutant alleles at the Pin locus on the right end of the second chromosome of *Drosophila* have been discovered. Both occurred spontaneously (at different times) in the same stock. They are phenotypically different from the first Pin allele and are different from each other. Heterozygous Pin^1 is described by Ives as causing shortened bristles that are thick at the base and sharp at the point. The homozygote is viable and has the general appearance of the heterozygote, but mutant effect is more exaggerated. One of the new alleles, Pin^{Yt} (Pin–yellow tip), in the heterozygous condition produces nearly normal length bristles with twisted yellow tips. The homozygote is lethal. The other new allele, Pin^2, when heterozygous with wild type, produces small bristles about one-eighth of the normal size if the flies are cultured at 25°. If the animals are reared at 17°, the heterozygote appears wild-type. At both temperatures homozygous Pin^2 is lethal. Neither Pin^1 nor Pin^{Yt} are affected by temperature.

Chromosomes are available that carry duplications including the wild-type allele of Pin, and there are second chromosomes deficient for the Pin locus. The number of doses of the wild-type allele may be varied by combining the appropriate chromosomes. In so doing some unusual properties of the locus have been demonstrated.

Results. – In Table 4 are presented the genotypes that were produced and descriptions of their phenotypes. Flies were reared at 25°. Some of the more important comparisons are the following: $Pin^2/+/+$ has bristles of a length intermediate between $Pin^2/+$ and wild type. $Pin^1/+/+$ and $Pin^{Yt}/+/+$ are unchanged by one more dose of + and resemble $Pin^1/+$ and $Pin^{Yt}/+$ respectively. Pin^2/Df (deficiency) is lethal. That situation is the usual one for homozygous lethal mutants. On the other hand, Pin^{Yt}/Df is not only viable but closely resembles $Pin^{Yt}/+$. The fact that $Pin^{Yt}/+/+$ also resembles $Pin^{Yt}/+$ further differentiates this allele from Pin^2.

The doses of the mutant alleles may be varied as well as doses of the wild type. $Pin^2/Pin^2/+$ flies are rarely viable and have very tiny

Table 4. The Phenotypes of Combinations of Alleles of Pin

	Genotype	Phenotype
1.	Pin^1/Pin^1	Bristles shorter than normal, wide at base, tapering rapidly
2.	$Pin^1/+$	Similar to above but slightly less extreme
3.	$Pin^1/+/+$	Like 2
4.	Pin^2/Pin^2	Lethal
5.	$Pin^2/+$	Short bristles
6.	$Pin^2/+/+$	Medium length bristles
7.	$Pin^2/Pin^2/+$	Very short bristles, rarely viable
8.	Pin^2/Df	Lethal
9.	Pin^{Yt}/Pin^{Yt}	Lethal
10.	$Pin^{Yt}/+$	Slightly shortened bristles, twisted and yellow at tip
11.	$Pin^{Yt}/+/+$	Like 10
12.	$Pin^{Yt}/Pin^{Yt}/+$	Bristles as 10, but animals rarely viable
13.	Pin^{Yt}/Df	Like 10
14.	Pin^1/Pin^2	Very short bristles
15.	Pin^1/Pin^{Yt}	Lethal
16.	Pin^2/Pin^{Yt}	Lethal
17.	$Pin^1/Pin^2/+$	Very short bristles
18.	$Pin^1/Pin^{Yt}/+$	Very short bristles; often only bristle sockets present
19.	$Pin^2/Pin^{Yt}/+$	Short bristles like 5

short bristles. $Pin^{Yt}/Pin^{Yt}/+$ is also only rarely viable, but the bristles appear like those of $Pin^{Yt}/+$. $Pin^{Yt}/Pin^1/+$ flies have very short bristles, and many of the bristles are represented only by the sockets. $Pin^2/Pin^1/+$ also has very small bristles. The bristles of $Pin^2/Pin^{Yt}/+$ are fairly short and resemble $Pin^2/+$.

Discussion. – If one trys to apply Muller's classification of genes (based on dosage response), Pin^2 and its wild-type allele are antimorphs. The mutant allele and the wild-type allele appear to be in competition. Addition of doses of Pin^+ causes the phenotype to be more normal, and addition of doses of Pin^2 makes the phenotype more mutant.

Pin^{Yt} (and probably Pin^{1}, although it hasn't been tested as thoroughly) fits into Muller's classification of a neomorph. Doses of + seem to have little or no effect on the bristle phenotype. Even when Pin^{Yt} is heterozygous with a chromosome deficient for Pin, there is no enhancement of the phenotype over $Pin^{Yt}/+$. Pin^{+} by this criterion would have no gene product and be an amorph.

There is, of course, a contradiction. Pin^{+} has a product capable of competition in one case and appears to produce no product in the second case. The mutants may be pseudoallelic, and the reason for the paradox may lie in that condition. If two pseudoalleles control two separate gene products the problem is resolved, but if they control the same gene product, the basis of this behavior must lie elsewhere in the chain of command between gene and phenotype.

Enhancement of the Red Fat Cell Phenotype of *Drosophila* by Larval Starvation

E. H. Grell

The red fat cell phenotype is normally caused by the presence of two closely linked recessive mutants, *lys* (lysine) and *rc* (red cell). When one is homozygous without the other, no red fat cell phenotype is produced. The mutant *lys* has another phenotype with or without *rc* and that is an accumulation of the amino acid lysine within flies homozygous for the mutant.

The red fat cell condition of *lys rc* homozygotes was found to be enhanced by larval starvation. Third instar larvae, about 72 hr old, were placed on 2% agar with no nutrients. The animals pupated and produced small flies with many pigmented fat cells. The number of pigmented fat cells and the intensity of pigmentation was greater than in flies reared on normal *Drosophila* culture medium.

There has been some question as to the relationship between *lys* and *rc* in the production of the red fat cell phenotype. Is one of the mutants primarily responsible for the abnormal phenotype and the other an enhancer? Larval starvation has provided an answer to that question.

Experimental Procedure and Results. – Larvae of the two genotypes $+\ rc^{2}$ (an allele of *rc*) and *lys* + were placed on 2% agar. The late pupae

and young adults were examined for the presence of red fat cells. In both cases pigmented cells were visible in all flies. If the flies had developed from normally cultured larvae, there would have been no pigmented fat cells.

Discussion. – Since each mutant without the other appears to be able to produce the red fat cell phenotype, they do not act as a primary-effect gene and an enhancer gene. Rather, there is an additive sort of interaction between two mutants each of which has a tendency to produce red fat cells. Starvation somehow lowers the threshold and the single mutants can express their tendencies.

The biochemical basis for the accumulation of lysine in flies homozygous for lys appears to be their inability to degrade lysine. The pigment which is deposited in the fat cells is a tryptophan-derived ommochrome. A connection between the metabolism of the two amino acids is unknown at this time. Studies are being made concerning the way that one mutant can produce the two effects.

MAMMALIAN GENETICS AND DEVELOPMENT

Section Chief – W. L. Russell

Genetic Effects of Radiation in Mice

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L. Wickham
J. R. Inman
J. S. Gower[1]
J. S. Bangham
M. B. Cupp
G. M. Guinn
P. R. Hunsicker
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Effects of Radiation on Mammalian Gametogenesis

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Developmental Effects of Radiation in Mice

L. B. Russell
U. H. Ehling[2]
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Mammalian Cytogenetics

W. J. Welshons
E. H. Y. Chu
B. Gibson, Jr.[3]
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Mutation Frequency and Radiation Intensity

W. L. Russell E. M. Kelly

In the latest Semiannual Progress Report[4] we presented results from one of several experiments started to investigate some of the implications of our finding that a given dose of chronic irradiation produces

[1]Consultant.
[2]Visiting investigator from abroad.
[3]Student trainee.
[4]W. L. Russell and E. M. Kelly, Biol. Semiann. Prog. Rep. Feb. 15, 1960, ORNL-2913, p 53.

fewer mutations than does the same dose of acute irradiation. The data accumulated in another experiment in this series are now adequate for the reaching of some important conclusions.

In this experiment, the specific locus mutation frequency is being measured in spermatogonia of mice irradiated at an intensity of 48 r/hr for a total dose of 600 r (Cs^{137} source). This intensity is approximately the geometric mean of the two intensities (90 r/week and 90 r/min) used in our original discovery of the intensity effect. It is approximately 100 times greater than the lower of these two intensities and 1/100 of the higher intensity. Six presumed mutations have so far been observed in a total of 21,337 offspring of the irradiated males. This is in close agreement with the mutation frequency obtained in the earlier experiments at the low radiation intensity of 90 r/week. It is significantly ($P < 0.001$) below the mutation frequency obtained from the same total dose of x rays delivered at the intensity of approximately 90 r/min.[5] It is also significantly ($P = 0.01$) below the mutation frequency obtained to date in a gamma-ray (Co^{60} source) experiment described elsewhere[6] in which the intensity was 24 r/min. This latter intensity is only 30 times the 48-r/hr dose rate used in the experiment described here.

The original finding of a dose-rate effect on mutation frequency involved a radiation intensity difference of approximately 10,000 fold. The new experiments have shown that most, or all, of the marked effect occurs in a particular range which has a mere 30-fold difference in intensity. This information should help in the important basic problem of elucidating the mechanism for the intensity effect.

The practical implications are also important. For example, the radiation intensity at the gonads in some medical exposures, particularly in fluoroscopy, does not exceed 48 r/hr. It appears that the genetic hazards from such exposures may be somewhat less than had been estimated on the basis of mutation rates obtained at higher radiation dose rates.

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[5]W. L. Russell, L. B. Russell, and E. M. Kelly, Science 128, 1546–50 (1958).

[6]W. L. Russell, L. B. Russell, and E. M. Kelly, Intern. J. Radiation Biol., Suppl., 311–20 (1960).

Further Analysis of Variegated-Type Position Effects from X-Autosome Translocations in the Mouse

L. B. Russell J. W. Bangham

In an earlier report of a case of variegated-type position effect at the b locus,[7] it had been inferred from preliminary indications that the chromosomal change involved was a translocation between chromosome 8 and the X chromosome. Linkage tests now completed with the sex-linked marker Ta (on b/b background) support this earlier conclusion. Frequency of crossing over between the break point and Ta is about 8%.

In maintenance of the brown-variegated stock, semisterile black (i.e., nonvariegated) females, an unexpected type, occasionally appear. These exceptional animals, in tests with Ta males, have yielded hemizygous-Ta daughters, indicating that they are deficient for a portion of the X chromosome containing the Ta locus, or may even lack the entire X chromosome. It can thus be inferred that absence of one X chromosome, or X-chromosome portion, suppresses variegation of $+^{b}$. This would also explain why male translocation heterozygotes are black, rather than brown-variegated (as are translocation females bearing two complete X chromosomes).

In a second case of position effect involving variegation at the c locus, results from matings of variegated females with c^{ch}/c^{ch} males are completely analogous to those described earlier for b-locus variegations. It is, therefore, concluded that the chromosomal basis is a translocation between the X and chromosome 1. The two cases differ in proximity of the break to the locus involved: crossover frequencies for the c and b variegations are < 1.5 and 16%, respectively.

Changes in Oocyte Numbers with Age

E. F. Oakberg

The necessity for more information on the dynamics of oogenesis has become imperative in our attempts to elucidate the bases for fertility effects in irradiated females. Knowledge of the number of primary oocytes required for normal fertility, the rate of elimination of oocytes (both by ovulation and by degeneration), the distribution of oocytes among stages

[7] L. B. Russell and J. W. Bangham, Genetics 44, 532 (1959).

of different radiation sensitivity, and the time spent in each stage of oocyte development will be required for understanding the response to different radiation doses and dose rates, and for making valid comparisons both between species and between diverse genotypes within species.

Before such information can be gained, it will be necessary to resolve the seemingly simple yet difficult problem of distinguishing between normal and degenerating oocytes. It is common knowledge that most oocytes degenerate before ovulation, but the extent of this loss has not been determined, and insufficient attention has been given to early degenerative changes. In the counts given here, nucleolar and nuclear morphology and staining reaction with hematoxylin azure II-eosin have been used as criteria of classification. Most of the 300-plus abnormal oocytes observed in ovaries of young adult females are in follicles ranging from those with two cell layers to follicles showing the presence of a small antrum, that is, in intermediate stages of follicular development. Accordingly, it is necessary to distinguish normal developmental changes in the nucleus and nucleolus during growth of the oocyte from degenerative changes in order to establish the relationship between number of oocytes and fertility. Numbers of oocytes in mice of different ages should indicate whether the loss can be accounted for by ovulation alone, or whether the decrease is in closer agreement with the frequency of presumed degenerating cells. Total counts of serial sections of both ovaries of mice of different ages are given in Table 5. Comparison of columns 6 and 7 reveals that the decrease is much greater than can be accounted for by ovulation alone, even though the estimated ovulations have been maximized by assuming a four-day estrus cycle and the actual decrease with age has been minimized by using total oocyte counts instead of apparently normal cells.

A precise relationship between frequency of presumed degenerating cells at any one time and decrease in number of oocytes with age could be established only if time from appearance of recognizable pathological changes and disappearance of the oocyte were known. A general relationship, however, can be established on the basis of the observation that after irradiation, degenerating oocytes in follicles with two or more layers of cells persist for as long as 20 days. This easily would allow accumulation of 200 to 300 degenerating oocytes in the normal ovary. Thus,

Table 5. Total Oocyte Counts of Female Mice of Different Ages

Age (days)	Number Counted				Decrease with Age	Estimated Number Ovulated*	Lost by Degeneration
	Normal	Degenerating	Total	Mean			
13	4706	279	4985				
15	5564	295	5859	5503		0	?
17	5337	329	5666				
69	4207	333	4540			93	
72	3756	309	4065	4173	1330	93	1234
77	3577	337	3914			103	
303	666	169	835			690	
304	1132	288	1420	1060	4443	690	3729
331	692	233	925			762	

*Calculated on basis of four-day estrus cycle, 10.3 ovulations per estrus (L. B. Russell and W. L. Russell, p 187–92 in Progress in Radiobiology, ed. by J. S. Mitchell, B. E. Holmes, and C. L. Smith, Oliver and Boyd, Ltd., Edinburgh, 1956).

the data indicate that our present techniques of classifying cells are giving results in agreement with the decrease in the number of oocytes with age; however, further experiments will be required in order to fully elucidate the changes occurring during both normal development and degeneration of the primary oocyte. It is clear that most of the loss can be attributed to cell degeneration.

The Cytological Detection of Translocations in the Mouse

W. J. Welshons

Introduction. – If an animal is heterozygous for a translocation, four different types of gametes can be produced, of which two are aneuploid and generally result in the formation of lethal zygotes when such gametes function in fertilization. Consequently, translocations are generally detected in the mouse by noting a reduction in the size of litters produced by a prospective parental animal. This method cannot be expected to work if both aneuploid gametes give rise to viable zygotes, and if only one type of aneuploid gamete were to form a viable zygote, the litter size data might be ambiguous.

Methods. – To identify an animal heterozygous for a translocation in the event that at least one of its aneuploid gametes is capable of forming a viable zygote, we devised a simple technique for the cytological identification of the translocation during late prophase and metaphase of the first meiotic division in the primary spermatocytes of a male. The details of the technique will not be presented, but the requirements which must be fulfilled by such a technique before it can be considered reliable will be briefly discussed.

If an animal is heterozygous for a translocation one might expect to detect at late diakinesis or metaphase a ring or chain composed of four associated chromosomes. However, since the chromosomes of the mouse are quite small, one might have considerable trouble recognizing a ring or chain of four. On the other hand, if a translocation configuration does occur, but is not distinguishably different from the remaining 18 pairs of chromosomes, one could still detect its presence by counting 19 stained "elements" corresponding to 18 pairs plus one group of four chromosomes. If on the other hand a translocation configuration that is not visibly different from the remaining 18 pairs were to occur in only a fraction of the cells examined, one might still be able to detect its presence by counting many cells and noting the frequency of counts totaling 19 "elements" as opposed to 20 "elements."

Results and Conclusions. – With these considerations in mind it is apparent that a cytological technique for the routine recognition of translocations in the mouse should be rapid and simple. It must be possible to obtain many late prophase or metaphase figures; the chromosomes should be well stained, sharply defined, and well spread; and cell fragmentation should be minimal. Such a technique has been devised and tested.

For test purposes 12 different mutants and two inbred lines have been examined. Five of the mutant stocks were known to be semisterile, and, upon cytological examination of these five, a ring or a chain was clearly visible in most cells and corresponded to a count of 19 stained "elements." When a ring or chain was not visible the count was 20 "elements." In the eight remaining mutants and two inbred lines, no evidence of a translocation could be found.

Experiments are now under way in which litter-size data will be collected from prospective male translocation heterozygotes, and conclusions concerning the actual presence of a translocation will be directly checked by cytological examination of the same male parent. In this way a direct comparison can be made of the two methods of detection.

Cytogenetic Studies of the Mouse Using Tissue Culture Techniques

E. H. Y. Chu

Introduction. – The existing cytological procedures utilizing meiotic bone marrow or regenerating liver cells of mammals, while extremely useful, have limited applications. The present report summarizes recent development of simple and reliable methods of in vitro cultivation and cytological preparation of somatic cells, thus opening new avenues for experimental cytogenetic studies of the mouse, which is probably the genetically best known mammal.

Results and Discussion. – Three types of cultures have proved feasible for investigating a variety of problems. First, the mass culture of actively proliferating embryonic skin fibroblast-like cells derived by trypsin dissociation has been employed for an analysis of x-ray-induced chromosome aberrations.[8,9] Second, plasma-clot cultures of skin biopsies from young or adult animals without sacrificing them permit karyotypic analysis and verification of a certain known genotype such as XO.[10] Usually, tail tips or small pieces of skin were aseptically removed for this purpose. To date, better than 90% of over 50 such trials have yielded successful cultures. The fact that tissues from different body sites or organs can be so cultivated makes it possible to study somatic variations. Third, from individual fetuses of a known cross, tissue explants or short-term cultures of cells dissociated by means of a combination of trypsin digestion and mechanical separation through a syringe have been made. These techniques serve to reveal the chromosomal basis for lethals or developmental abnormalities, both spontaneous and induced, in early embryonic stages.

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[8]E. H. Y. Chu and V. C. Monesi, Biol. Semiann. Prog. Rep. Feb. 15, 1960, ORNL-2913, p 59.

[9]E. H. Y. Chu and V. Monesi, Records Genet. Soc. Am. 29, 63 (1960).

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

The times of peak mitotic activity for different methods and materials have been determined. Partial mitotic synchronization was obtained by pH adjustment and nutritional stimulation. Permanent preparations of well-spread, colchicine-blocked, and Feulgen-stained metaphases were used for chromosome studies.

Karyotypic analyses have led to the construction of an idiogram of the mouse mitotic chromosomes. All 20 pairs of chromosomes are acrocentric; they range from about 1 to 3.5 μ in length at metaphase. Four autosomal pairs in addition to the Y chromosome are morphologically distinct and can be individually identified. The Y is the smallest element of the complement; the X is about 2.5 μ long and ranks sixth or seventh in the decreasing order of length. The presence or absence of the Y enables the cytological diagnosis of sex in somatic cells. Experiments on stocks having the XO constitution and other peculiar sex-linked mutant characteristics which cause death of embryos are in progress.

Reproductive Capacity of Female Mice

U. H. Ehling

Introduction. – F_1 hybrid female mice from the cross of 101 × C3H inbred strains have an average of only 1.4 litters after 400 r, and only 4.0 litters after 50 r of acute x rays, then are sterile for the remainder of their life. Comparable controls have 13 or 14 litters.[11] On the other hand, female beagles receiving x-ray exposure up to a median lethal dose are not seriously affected in their reproductive capability.[12] Thus, there are obviously great species differences. The present experiments were performed to determine whether there was also intraspecific variation, and, if so, to find its cause.

Methods and Results. – From 14 to 18 female mice of each of the strains SEC, A, SEA, NB, and DBA were irradiated with 50 r (80 r/min). Heads were shielded with lead. One day after exposure each female was caged separately with a nonirradiated C3H male for the duration of the

[11]L. B. Russell, K. F. Stelzner, and W. L. Russell, Proc. Soc. Exptl. Biol. Med. 102, 471–79 (1959).

[12]A. C. Anderson and F. T. Shultz, Radiation Research 12, 417–18 (1960).

experiment. Equal numbers of nonirradiated females were mated in the same way. Litters were recorded and killed at birth.

Total production of offspring per female, for a period of 210 days after exposure with 50 r, expressed as percentage of the comparable control group, was as follows for the various strains: SEC, 17.5%; A, 20.3%; SEA, 24.4%; NB, 30.0%; and DBA, 68.8%. For the most radiosensitive strain (SEC), the total numbers of offspring per female (mean and standard error) in the control and 50-r groups were 63.4 ± 2.6 and 11.1 ± 0.9, respectively ($P < 0.001$); for the most radioresistant strain (DBA), the correspondent values were 32.4 ± 3.1 and 23.3 ± 1.6, respectively ($P = 0.01$). It should be noted that the difference in the breeding performance between the control groups of the two strains are highly significant too ($P < 0.001$; 50-r SEC vs 50-r DBA, $P < 0.001$). The reproductive performance of the DBA control group is only 51.1% of the correspondent value of the SEC strain (Fig. 7).

Discussion. – The results clearly demonstrated strain differences in reproductive performance following irradiation. The explanation for the intraspecific variation may be: (a) differential radiation sensitivity of the oocytes, (b) differences in the number of oocytes present before irradiation, (c) a combination of both mechanisms.[13,14] Experiments are now in progress to distinguish between these alternatives. So far, counts of corpora lutea (representing number of ovulated eggs) and examination of the uterine contents indicate that a combination of both mechanisms may be responsible for the interstrain differences in radiation response.

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[13]E. F. Oakberg, J. Dairy Sci. 43, Suppl., 54–67 (1960).
[14]U. H. Ehling, Records Genet. Soc. Am. 29, 67 (1960).

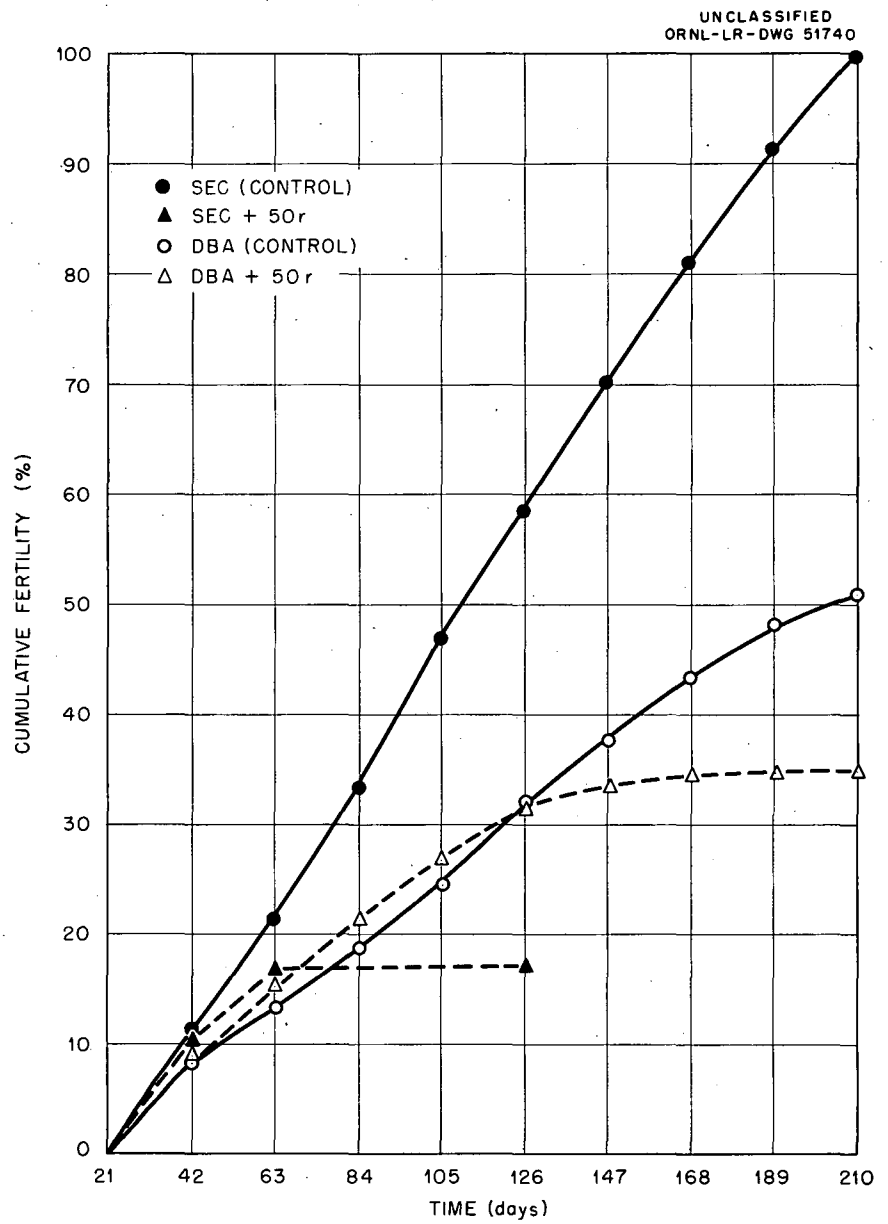

Fig. 7. Reproductive Performance of the Radiosensitive and the Radioresistant Strain over a Period of 210 Days.

A. Hollaender
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Culture Conditions as Determinants of X-Ray Sensitivity of *Escherichia coli*

G. E. Stapleton M. S. Engel

Introduction. — Several strains of *Escherichia coli* show a dramatic change in sensitivity to inactivation by x rays, ultraviolet radiation, and heat that results from the metabolic activity of the cells during their growth prior to irradiation.[2,3] In previous reports[3,4] we have noted that the extreme differences can be demonstrated between cells grown in unbuffered peptone or nutrient broth and those grown in the same medium with glucose added (0.2 to 1%). The former cultures (sensitive) reach a final pH of 8.5, whereas the latter cultures (resistant) attain a final pH of 4.6 to 4.8. Sensitive cells yield exponential survival curves as a function of dose; resistant cells, however, yield highly sigmoidal curves with a lower limiting slope, indicating a high multiplicity for inactivation. The resistance is not a function of intra- or extracellular pH during irradiation. Moreover, attempts to demonstrate the presence of a metabolically produced protective agent in resistant cells have been unsuccessful. A number of alternative explanations for the difference in resistance have now been investigated.

Results and Discussion. — Oxygen Effect. — Alper[5] suggested recently that part of the resistance of budding yeast cells may be attributable to

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*Visiting investigator from abroad.

[1]Student trainee.

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

[3]M. S. Engel and G. E. Stapleton, Biol. Semiann. Prog. Rep. Feb. 15, 1960, ORNL-2913, p 60-62.

[4]G. E. Stapleton and M. S. Engel, J. Bacteriol., in press.

[5]T. Alper, Intern. J. Radiation Biol. 1, 414-19 (1959).

a reduced oxygen dependence for inactivation of these cells as compared with the more sensitive nonbudding portion of the population. We have been able to show no significant difference in the magnitude of the oxygen-dependent inactivation of sensitive or resistant cells.

Growth Rates of Resistant and Sensitive Cells. — A number of cellular radiobiologists[6] have proposed that postirradiation conditions that reduce the growth rate of bacteria may increase the viability of irradiated cells. Our data indicate that unirradiated resistant and sensitive cells grow at the same rate on a variety of media. Moreover, irradiated cells show an induced lag in growth rates on all media that is not significantly different for sensitive and resistant populations.

Size and Chemical Analysis of Sensitive and Resistant Cells. — We have previously shown[4] that resistant cells are uniformly larger cells than are sensitive cells. Total counts and size distribution studies made with an electronic cell counter (Coulter) indicate that resistant cells have about twice the cell volume of sensitive cells. The DNA, RNA, and protein, dry weight, and packed cell volume per cell (based on total cells and viable cells) accord very well with the differences in cell size. Sigmoid survival curves of the type obtained for resistant cells have often been analyzed to obtain a number which might be useful in estimating the multiplicity of "targets" required for inactivation. For cells with known differences in ploidy or number of nuclei this type of analysis has been useful. It is not clear however that such analyses are helpful in cases in which very high multiplicities are indicated. In the case of resistant cells, crude chemical analyses show no simple relationship between amount of DNA, RNA, or protein per cell and the estimated target number obtained by analysis of the sigmoid survival curves. It is possible, of course, that the concentration or activity of important minor constituents of any of the components mentioned could be specifically increased by a large factor without being revealed by the measurements of total nucleic acids or protein per cell.

Influence of Postirradiation Medium. — Although we have mentioned that no significant differences were found for growth rates of resistant

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

and sensitive cells on several media, a large differential effect was found in the relative response of resistant cells to a simple minimal medium (M-9) as compared with a natural, complete medium [nutrient agar, peptone or peptone-glucose agar (PGA)]. Both resistant and sensitive irradiated populations show a lower survival at any x-ray dose on M-9 than on PGA (Fig. 8). No such medium effect is detectable for unirradiated cells. The postirradiation medium seems to alter the shoulder of the survival curve for resistant cells more than the limiting slope,

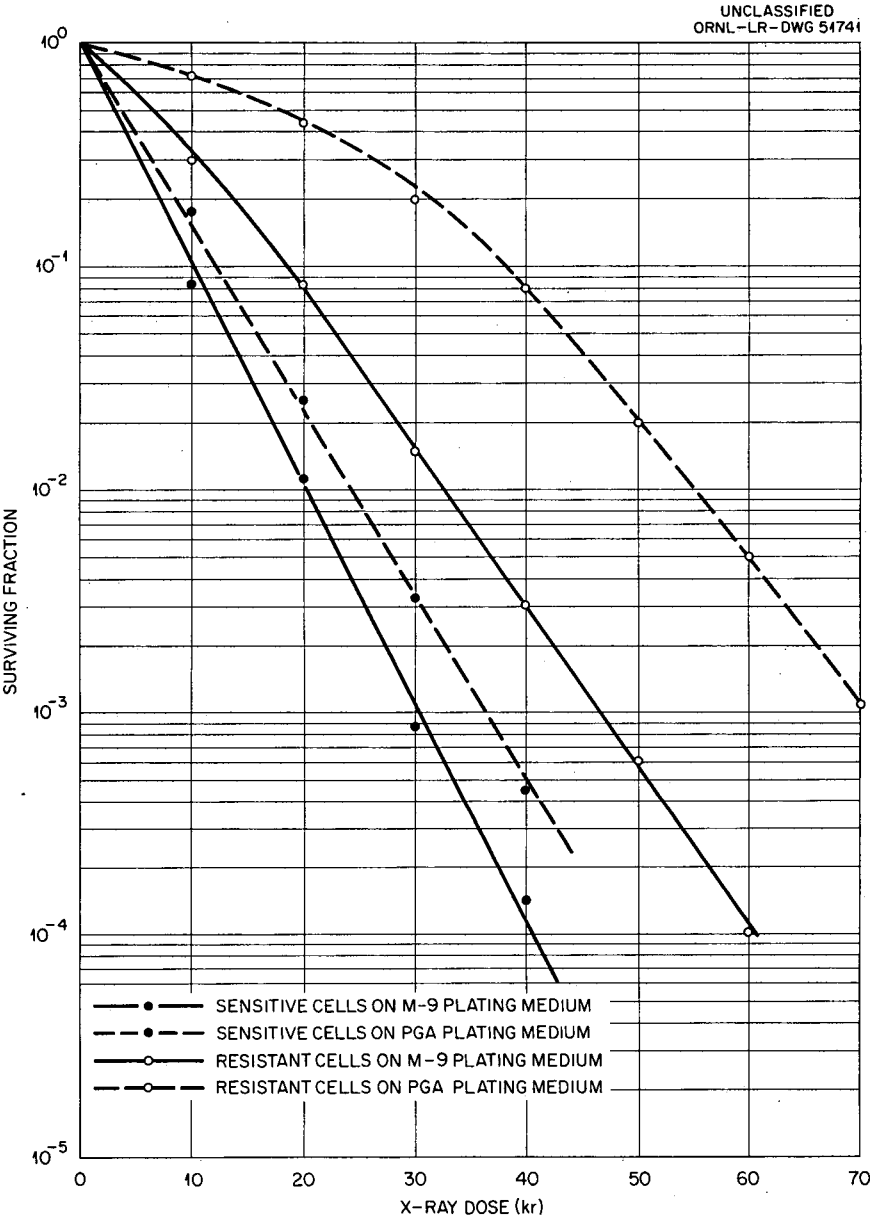

Fig. 8. X-Ray Sensitivity of Escherichia coli B/r (CSH) as a Function of Postirradiation Plating Medium.

suggesting that the resistance of these cells can be expressed to the utmost only in the presence of certain nutritional factors such as amino acids. This finding suggests that the resistant cells have a capability of repairing or recovering from radiation damage if they are supplied with appropriate substrate. It will be important to determine the specific requirements for expression of radioresistance. Also, since the resistance seems not to be specific for ionizing radiation, but for ultraviolet radiation and heat as well, it will be of interest to determine if a medium effect also exists for expression of altered resistance to these damaging agents.

Catalase Activity, Ionizing Radiation Sensitivity, and Hydrogen Peroxide Sensitivity in *Escherichia coli*

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

Introduction. — Recent reports in the literature dealing with the relationships between catalase activity, radiation sensitivity, and hydrogen peroxide sensitivity prompted us to investigate these factors making use of bacteria in our collection.

Results. — The data in Table 6 indicate that, for three closely related organisms, there is no correlation between radiation sensitivity

Table 6. Characteristics of Peptone-Grown *E. coli* Strains

Strain	Catalase Activity[a]	Radiation Sensitivity[b]	Hydrogen Peroxide Sensitivity[c] (%)
E. coli B_S	204	4.55×10^{-6}	2.0
E. coli B	192	8.35×10^{-3}	17
E. coli B/r ORNL	194	5.10×10^{-1}	45
E. coli H_{7+}	75	1.07×10^{-4}	

[a]Catalase activity = microliters of oxygen per minute per 10^{10} cells.

[b]Radiation sensitivity = fraction surviving 20 kr.

[c]Hydrogen peroxide sensitivity = per cent surviving 0.6% H_2O_2 at 24°C for 20 min.

and catalase activity. There is, however, a good correlation between the order of radiation sensitivity and hydrogen peroxide sensitivity. Escherichia coli H_7+, an organism not closely related to the first three, has less than half the catalase activity of E. coli B but a radiation sensitivity intermediate between B_S and B.

In order to study these relationships further, we have grown the organisms in a glucose-containing medium, a procedure known to increase radiation resistance (Engel and Stapleton[3]). Table 7 shows that this growth procedure has increased the radiation resistance of all these organisms (compare to Table 6) but the catalase activity only of E. coli B_S. The other organisms have all decreased in catalase activity. The

Table 7. Characteristics of Peptone-Glucose-Grown E. coli Strains

Strain	Catalase Activity[a]	Radiation Sensitivity[b]	Hydrogen Peroxide Sensitivity[c] (%)
E. coli B_S	240	7.70×10^{-5}	8.0
E. coli B	170	2.40×10^{-2}	45
E. coli B/r ORNL	70	5.5×10^{-1}	70
E. coli H_7+	7.8	2.55×10^{-3}	

[a]Catalase activity = microliters of oxygen per minute per 10^{10} cells.

[b]Radiation sensitivity = fraction surviving 20 kr.

[c]Hydrogen peroxide sensitivity = per cent surviving 0.6% H_2O_2 at 24°C for 20 min.

hydrogen peroxide sensitivity is apparently independent of catalase activity and closely parallels the radiation sensitivity. Data on the hydrogen peroxide sensitivity of E. coli H_7+ will be accumulated in the near future.

Discussion. — These data demonstrate that total catalase activity of these cells is not related to radiation sensitivity or, for that matter, to hydrogen peroxide sensitivity. It should be pointed out that catalase determinations were made manometrically and that toluenization

of the cells did not increase the activity. The data also indicate a striking correlation between the sensitivity of the cells to ionizing radiation and added hydrogen peroxide. It should be emphasized that the level of hydrogen peroxide added is very much higher than that produced by the doses of radiation under consideration.

The somewhat surprising lack of correlation between catalase activity and hydrogen peroxide sensitivity is being explored further in cooperation with R. Clayton of the Microbiology Group.

Effect of Products Formed During Ultraviolet Irradiation of Methyl Linolenate on Growth and Cell Division in Yeast

J. H. Fränz B. T. Cole[7]

Introduction. — Previous studies have demonstrated the presence of substances not clearly defined but judged to be peroxides extractable in aqueous solution from methyl linolenate following irradiation. These substances are known to have an inhibitory effect upon cell division.[8,9] The growth of E. coli is said to be inhibited by peroxides formed by oxidation of linolenic acid.[8] Methyl linolenate previously exposed to ultraviolet irradiation inactivates several enzymes including succinoxidase, cytochrome oxidase, and choline oxidase.[10] This study was initiated in order to investigate the possibility that with proper experimental design, one might find some correlation between the formation of organic peroxides and the effects of ultraviolet irradiation upon yeast. This could suggest that formation of organic peroxides in vivo might be a contributing factor in the inhibition of growth and cell division due to ultraviolet irradiation.

Results. — One milliliter of pure fatty acid was irradiated with an AH_4 lamp without filter, operating at 110 v and 5 amp for 1 hr 30 min. Five milliliters of distilled water was added to each of three tubes containing 1 ml of irradiated fatty acid, 1 ml of linolenate which had

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[7]Research participant, Cell Physiology Section.

[8]K. M. Wilbur, Exptl. Cell Research 13, 503–9 (1957).

[9]B. T. Cole et al., Biol. Semiann. Prog. Rep. Feb. 15, 1960, ORNL-2913, p 95.

[10]F. Bernheim et al., Arch. Biochem. Biophys. 38, 177–84 (1952).

stood for an equivalent time at room temperature, and 1 ml of material which had been refrigerated for an equal period of time. Material was shaken thoroughly and allowed to stand at room temperature for 2 hr and the aqueous phase decanted.

One milliliter of a yeast suspension growing in a synthetic medium with 2% glucose was inoculated with 1 ml of aqueous extract from each of the three samples, and cell division studies reflecting growth were made using turbidity measurements and counting of the cells as end points. Figure 9 shows the observed inhibitory effect. After 48 hr the cell number per milliliter was only 60% of the controls. Control tubes containing no added extracted material, addition of extract of fatty acid

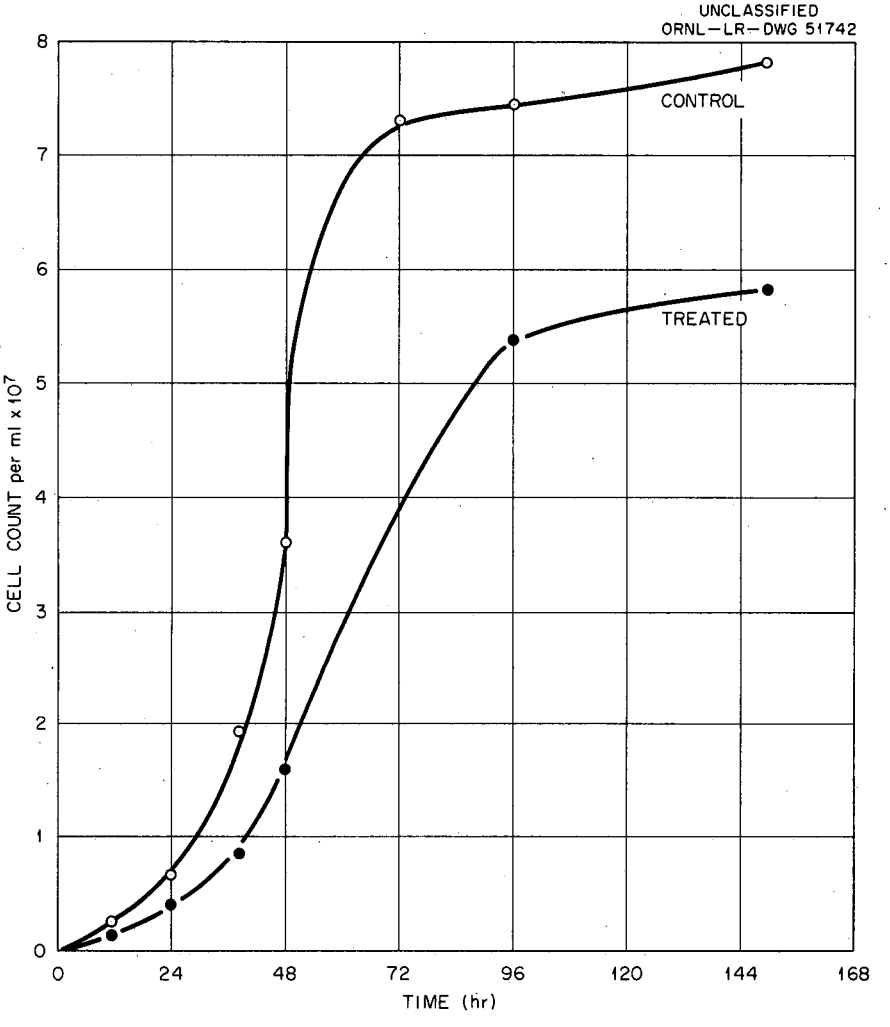

Fig. 9. Effect of Substance Extractable from Irradiated Methyl Linolenate on Cell Division in Yeast S.

held at room temperature, and addition of extract of fatty acid held at low temperature were employed. Cytologically, the treated cells had a much higher granule content (light-reflecting bodies) than the control types.

Warburg studies demonstrated that the endogenous respiration of a yeast culture starved for 48 hr in phosphate buffer (pH 6) was 5 μl per 3×10^8 cells after 2 hr. If one adds an aqueous extract of irradiated methyl linolenate, the endogenous respiration increases from 0 to 40 μl per 3×10^8 cells after 2 hr. Exogenous respiration is not affected by aqueous extracts of either irradiated or unirradiated methyl linolenate.

Discussion. — These results demonstrate that a water-soluble substance, probably an organic peroxide, is extractable from irradiated methyl linolenate and is capable of inhibiting growth or cell division of yeast in very low concentration. The results do not allow for specific conclusions to be drawn concerning the means by which this inhibition is manifest. The characteristic pattern of growth inhibition, however, is likewise seen in yeast following ultraviolet irradiation.[11,12] Whether what we are seeing is simply another "toxicity syndrome" manifesting itself in an analogous way, or whether the formation of organic peroxides in vivo during ultraviolet irradiation is a contributing factor in the effects of irradiation, remains to be clarified.

Protection of *E. coli* by β-Mercaptoethylamine

C. A. Elias G. E. Stapleton

Introduction. — Several reports[13,14] claim that the protection afforded by sulfhydryl protective agents against x-ray inactivation depends on the conditions under which they are used (pH, temperature, suspending medium, presence of certain ions). Some of these factors

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[11]R. H. Oster, J. Gen. Physiol. 18, 71–88 (1934).

[12]A. C. Giese and W. H. Swanson, J. Cellular Comp. Physiol. 30, 285–301 (1947).

[13]A. Hollaender and C. O. Doudney, Radiobiol. Symposium, Proc., Liege, 1954, p 112, Butterworths Scientific Publ., London, 1955.

[14]H. I. Kohn and S. E. Gunter, Radiation Research 11, 732 (1959).

may explain, of course, the variable results published. We have investigated one of the reported variables, ionic constitution of the suspending medium, and have compared its influence on cells grown in a complete and a minimal synthetic medium and their subsequent protection. The protective agent used was β-mercaptoethylamine (MEA).

Results. — An important consideration in estimating protective activity is the choice of a suitable base line. The large dose-reduction factors (DRF) reported in the literature are based on a comparison of oxygen-saturated suspensions with those reacted with maximally effective concentration of MEA. Since the compound is autoxidizable it was considered more practical to study its protective activity in air-free suspensions; therefore the activity referred to represents that obtained over and above the protection from oxygen depletion. An unexpected finding shown in Table 8 is that the suspending medium was found to be a significant variable in estimating the sensitivity of the cells even in the absence of protective chemical agents.

Table 8. X-Ray Sensitivity of *E. coli* B/r (ORNL) Grown in Synthetic (M-9) Medium or Nutrient Broth (0.5% NaCl) and Irradiated in Several Suspending Media

Growth Medium	Surviving Fraction at 10 kr (Oxygen-Free)		
	H_2O	NaCl	PO_4 Buffer
Minimal (M-9)	0.246	0.53	0.65
Nutrient broth	0.475	0.64	0.67

The relative magnitude of the oxygen effect was determined for cells suspended in the three media and was found to be approximately the same for cells irradiated in NaCl and in PO_4 buffer (i.e. a DRF = ~ 3). However, the difference between oxygen and nitrogen sensitivity is reduced in a water suspension (DRF = 1.0–1.5) (Table 9). Since experiments have been reported by other investigators in which water was the suspending medium, it is surprising that this effect has not been reported. The difference in the sensitivity described in the present paper may be related to the culture medium. This also is being

Table 9. Comparison of Survival of Oxygen- and Nitrogen-Saturated Water Suspensions of E. coli B/r (ORNL)

X-Ray Dose (kr)	Surviving Fraction	
	Oxygen	Nitrogen
2	0.77	0.79*
	(0.75–0.78)	(0.75–0.87)**
4	0.645	0.69
	(0.61–0.68)	(0.66–0.74)
6	0.55	0.64
	(0.49–0.60)	(0.63–0.65)
8	0.46	0.57
	(0.39–0.55)	(0.53–0.62)
10	0.37	0.475
	(0.30–0.42)	(0.43–0.50)

*Mean of four experiments.
**Range of survival values.

investigated in an attempt to explain the variable results reported in the literature.

Preliminary experiments in which MEA is added to suspensions of cells in H_2O or PO_4 buffer show a protection over and above that obtained in O_2-free suspensions (DRF = 1.5–3). It is clear that the maximally protective concentration may be dependent on the suspending medium. The present data suggest that the base line for comparison of protection may be different and may explain the almost contradictory results obtained in different laboratories.

MAMMALIAN RADIATION RECOVERY

Hematopoietic Recovery

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G. Doria[2]
S. Rogers[1]
P. G. Delker
R. A. McGrath
D. C. Swartzendruber
A. O. Lowe[3]

Bone Marrow Physiology

L. H. Smith
J. W. Goodman
M. B. Lion[2]
P. G. Lankford
T. W. McKinley, Jr.

Tissue Culture

M. A Bender
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Histologic Pattern of Normal Organized Lymphatic Tissue in the Mouse

C. C. Congdon P. G. Delker

Introduction. — Studies at different time intervals of mice receiving lethal irradiation and foreign bone marrow treatment have pointed to the organized lymphatic tissues as the major site of specific pathologic changes in the foreign bone marrow reaction or secondary disease.[5] It is the radiation damage to these tissues that allows foreign bone marrow to transplant after injection. When these tissues are intact, as in the normal animal, the foreign bone marrow is quickly rejected. It is known that the organized lymphatic tissues are the major component of the immune mechanism, and they show cellular alterations after an injection of antigenic material. The variation in the normal histology of these tissues, however, has made precise localization of the origin of the cellular changes difficult to determine. Our knowledge is therefore limited as to

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[1]Consultant.
[2]Visiting investigator from abroad.
[3]Student trainee.
[4]Research participant.
[5]C. C. Congdon and I. S. Urso, Am. J. Pathol. 33, 749–67 (1957).

exactly which cells are involved in radiation damage to the immune mechanism, which are involved in immunological disorders, such as secondary disease, or which are involved in the phenomenon of tolerance to foreign marrow as seen most dramatically in fetal radiation chimeras.

In the mouse our studies[6] pointed to the germinal center structures as showing the primary change after injection of a wide variety of antigenic materials, including foreign bone marrow. During the course of this work many observations have been made on organized lymphatic tissues in normal mice, and the results are summarized schematically in Fig. 10.

Discussion. – In the longitudinal anterior-posterior section through the spleen, each section through the white pulp contains two hemispherical masses of germinal center cells. Each has a characteristic internal structure and orientation in relation to the central artery and the red pulp. The more intensely stained mass of cells nearest the central artery shows most of the mitotic figures in the germinal center, and contains the "tingible bodies." It is this dark-staining mass of cells that takes up tritiated thymidine in a greater amount than any other portion of the organized lymphatic tissue. A light-staining mass of cells is oriented toward the red pulp. A similar characteristic germinal center structure is present in the lymph node cortex and in the intestinal Peyer's patches. The light-staining part of the germinal center in the lymph node is oriented toward the marginal sinus and probably is in contact with this sinus. In the Peyer's patches the light-staining part of the germinal center is toward the lumen of the intestine, being in contact with the submucosa of the epithelium.

Reconstructions of the germinal center have not been made, but they appear to be roughly cup-shaped structures with the darker-staining mass of cells forming the bottom wall of the cup and the light-staining cells the upper portion of the cup. It is interesting that in the lymph node cortex the germinal center abuts against the marginal sinus, through which antigenic materials get to the parenchyma of the lymph node. In the spleen and Peyer's patches the special orientation may also reflect the way in which the antigenic materials get to the organized lymphatic tissue.

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[6]C. C. Congdon, Federation Proc. 19, 216 (1960).

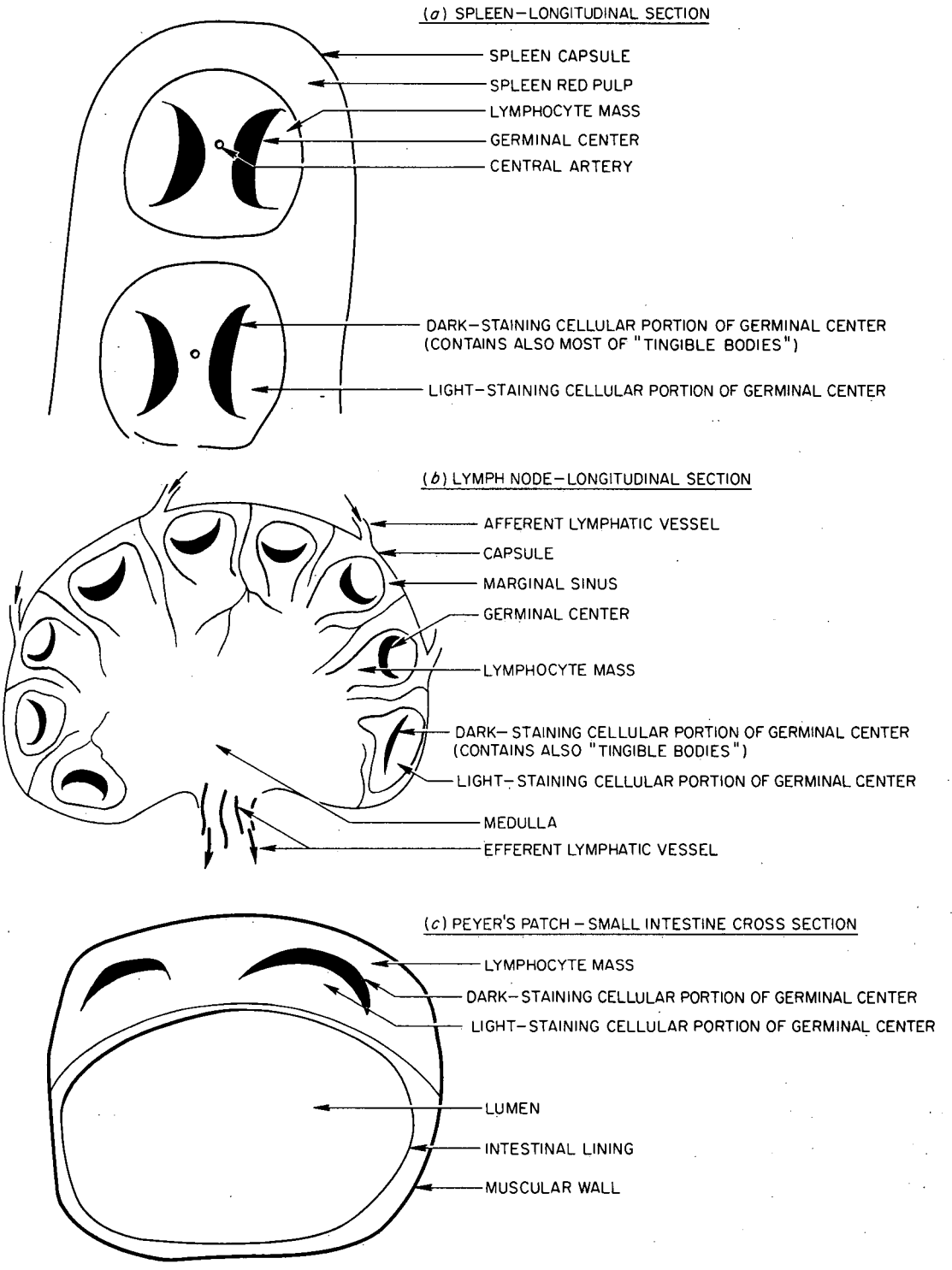

Fig. 10. Histologic Pattern of Normal Organized Lymphatic Tissue in the Mouse. (a) Spleen - longitudinal section; (b) lymph node - longitudinal section; (c) Peyer's patch - small intestine cross section.

Blood vessels penetrate the germinal center tissue too, and antigenic materials might reach it in this manner in addition to diffusion from adjacent tissues. From these studies, proliferation and migration of antibody-forming cells in the germinal center, or from the germinal center, is thought to be the histologic basis of the immune mechanism and to account for most of the reported findings on the histology of the immune mechanism.

Origin of Antibody-Forming Cells in the Spleen of Long-Term Homologous Bone Marrow and Fetal Liver Chimeras

G. Doria

Introduction and Methods. — Previous reports pointed out that long-term homologous bone marrow and fetal liver chimeras challenged with antigen foreign to both host and donor are capable of an immune response. The present work is a study of the relative immune capabilities of the host and donor cells in chimeras sensitized against sheep RBC.

The method is one in which lethally irradiated (900 r) test mice are used as a culture medium for a known number of spleen cells from donors injected with sheep RBC 18 days earlier. The irradiated recipients are injected with the test antigen at the time of the spleen cell transfer and bled six days later. The secondary response ($\log_2$ of titer) of the transferred spleen cells is a linear function of the number injected (from 0.75 to 24 $\times$ 10^6). If the irradiated recipients have been preimmunized to the donor, no antibody activity of the transferred spleen cells can be detected even for 24 $\times$ 10^6 injected cells.

Homologous bone marrow and fetal liver chimeras [BDF_1 donor and (101 $\times$ C3H)F_1 recipient] 120 days old were tested for erythrocyte type in the peripheral blood by the agglutination and hemolysis methods. Chimeras, all having only donor-type red cells, were injected intraperitoneally with 1 ml of 1% sheep RBC. Eighteen days after the antigen injection, spleen cells from five fetal liver or bone marrow chimeras were pooled in each experiment, and 12 $\times$ 10^6 cells injected intravenously into each irradiated recipient, which also received intraperitoneally 1 ml of 1% sheep RBC. The irradiated recipients were BDF_1; (101 $\times$ C3H)F_1; BDF_1 preimmunized against (101 $\times$ C3H)F_1; and (101 $\times$ C3H)F_1 preimmunized against BDF_1. All

were irradiated at 900 r 2 hr before receiving any injection. The sera were collected at the sixth day after the spleen transfer and individually tested.

Results and Discussion. — In both cases (fetal liver and bone marrow chimeras) the chimeric spleen cells gave positive titer in the first three groups of recipients and a negative one in the 900 r (101 × C3H)F_1 mice preimmunized against BDF_1. There was no significant difference between the averages of the $\log_2$ of titers of the positive groups.

These results provide evidence for the donor origin of antibody-forming cells in the spleen of homologous chimeras challenged with sheep RBC. The failure to detect activity of host-type cells can be due to their absence or functional impairment.

Biochemical Changes and Increased Liver Weight in Bone Marrow Chimeras

A. L. Kretchmar C. C. Congdon

Introduction. — Profound metabolic disturbances develop in lethally irradiated mice treated with foreign bone marrow. Loss of body weight in the presence of a normal food intake and the failure of hair growth are indications of the metabolic disorder. At autopsy mice that died of secondary disease show grossly the picture of starvation.

In the present series of experiments liver, plasma, and skeletal muscle have been taken from irradiated homologous-bone-marrow-treated mice before and during the secondary disease syndrome in order to make determinations of the amino acid distribution in these tissues. At the same time similar specimens have been collected from appropriate control groups.

Results. — In collecting liver specimens a rather striking increase in the liver weight of homologous-marrow-treated mice was present at 12 days after treatment. Somewhat less liver enlargement developed in the isologous bone marrow control group.

In both groups treated with bone marrow, total liver nitrogen was increased; glutamine and glutathione were reduced in the liver at 12 days, whereas the concentrations of β-aminoisobutyric acid and of β-alanine were greatly increased. Analyses on other tissues and at other time

intervals are still being carried out. Histologic study of the enlarged livers indicated that the enlargement was caused by individual liver cord cell hypertrophy with increased basophilic material in the cytoplasm.

Discussion. — A biochemical characterization to supplement the clinical, pathologic, and immunologic descriptions of secondary disease is needed. The present studies show that marked gross, histologic, and biochemical changes are present in the liver of bone-marrow-treated mice, and that these are greater with homologous than isologous bone marrow treatment.

Effect of Removal of Lymphatic Tissue on Anti-Sheep RBC Production in Mice

D. C. Swartzendruber R. R. Bigelow

Introduction. — In earlier studies it was observed that extensive lymphatic tissue removal in mice prolonged skin homograft survival for about a week longer than in sham-operated controls.[7] Skin grafting was studied, rather than homologous bone marrow transplantation, because it was difficult to get survival of irradiated bone marrow chimeras after such an extensive surgical procedure.

As another immunological parameter the hemagglutinin response to a heterologous antigen, sheep RBC, in lymphadenectomized mice was measured. The standard antigen dose of 0.5 ml of 0.2% sheep RBC was given intraperitoneally.

Results and Discussion. — Figure 11 shows the poor hemagglutinin production in mice subjected to the extensive lymphatic tissue removal procedure compared to normal controls or those that had been splenectomized. When a much larger antigen dose was administered, the differences were not as great as those in Fig. 11.

The results of these studies on skin homografting and hemagglutinin production in lymphadenectomized mice, although showing a definite impairment of the immune mechanism, also indicate that functional recovery can occur, probably from residual tissue.

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[7]D. C. Swartzendruber and R. R. Bigelow, Biol. Semiann. Prog. Rep. Feb. 15, 1960, ORNL-2913, p 75–76.

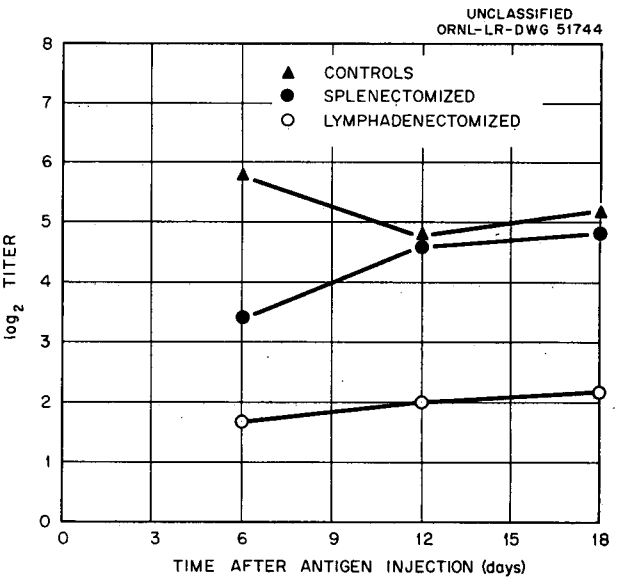

Fig. 11. Anti-Sheep RBC Production in Mice Injected with 0.5 ml of 0.2% RBC.

Popliteal Lymph Node Weights in Normal and Irradiated Mice After Foot Pad Injection with Rat Spleen Cells

A. O. Lowe

Introduction. — In earlier histologic studies[8] the spleen white pulp has been used as a lymphatic tissue site for evaluating the effect of intravenous foreign tissue antigen injections on normal and irradiated mice. A gross evaluation of the effect of these antigens on spleen weight was made difficult by the variable amount of blood in the spleen red pulp.

To get a different lymphatic tissue site and avoid the use of intravenous injections, a preliminary series of experiments were carried out using the foot pad as injection site and the popliteal lymph node weight as a criterion of the effect of antigen injection.

Results. — Figure 12 shows pooled popliteal lymph node weights of normal mice given different concentrations of rat spleen cells into the foot pad. A marked increase in popliteal lymph node weight occurred with

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[8]C. C. Congdon, Federation Proc. 19, 216 (1960).

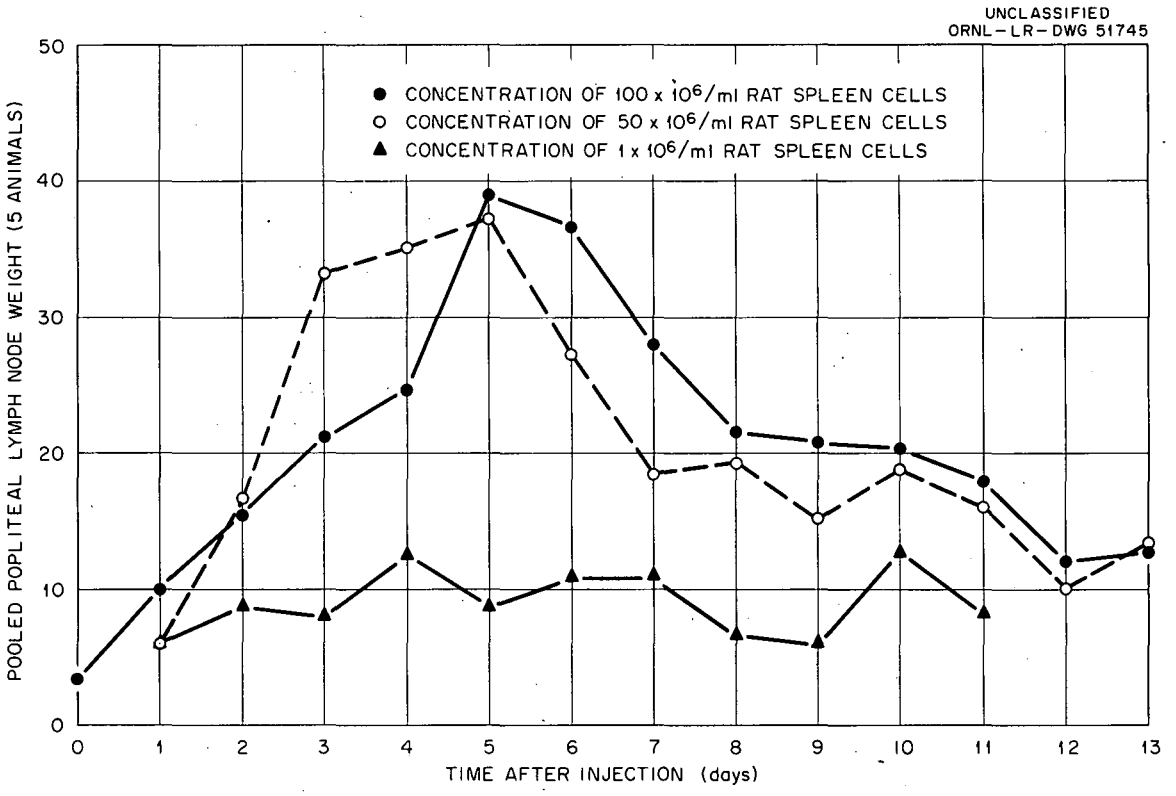

Fig. 12. Weight of Right Popliteal Lymph Node.

the two largest doses. Figure 13 shows the same data for irradiated mice. At 950 r only the highest concentration of rat spleen cells injected into the foot pad gave a difference for the popliteal lymph node weight from that of the x-ray controls.

Discussion. — These findings show that the normal popliteal lymph node gives a striking response to rat tissue antigens. Furthermore, the irradiated mouse also shows a weight change in the popliteal lymph node that is related to the rat cell injection. There is a possibility that the popliteal lymph node weight might be used as an assay for investigating the chemical nature of the tissue transplantation antigens.

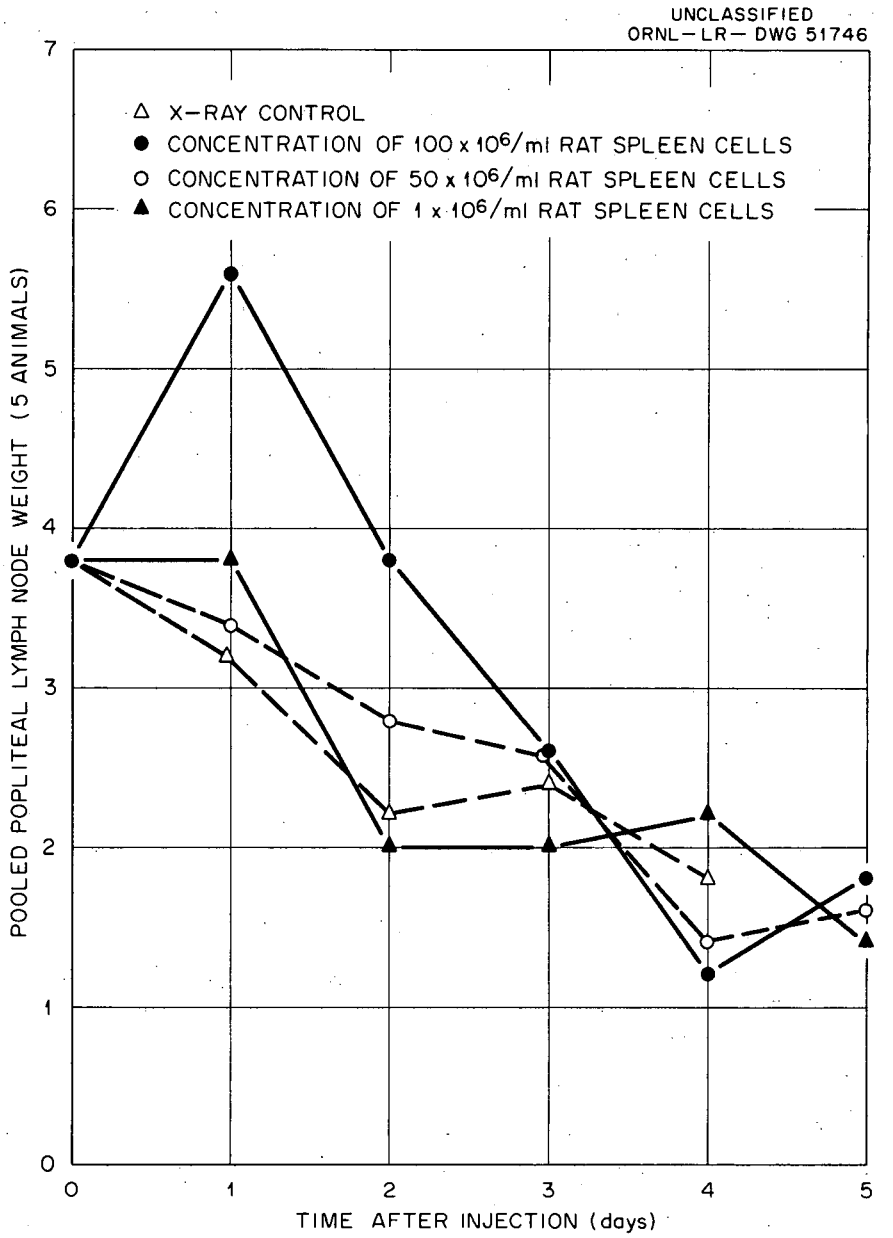

Fig. 13. Weight of Right Popliteal Lymph Node in Irradiated Mice.

Leucine and Cytidine Incorporation into Abnormally Enlarging Mouse Intestinal Crypt Cells After X Irradiation

R. A. McGrath

Introduction. – McGrath and Congdon[9] reported hypertrophy of some mouse intestinal crypt cells after 2500 r of x rays. They proposed that

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

the hypertrophy was an abnormal form of differentiation resulting primarily from radiation-induced mitotic inhibition. Further study of abnormal enlargement[10] showed that between x-ray doses of 500 and 10,000 r, enlargement was greater with increasing dose; that greatest enlargement was seen 48–60 hr after all exposures; and that enlargement was not associated with increased H^3-thymidine incorporation.

An analysis of autoradiograms of mouse small intestine, made after repeated H^3-cytidine and H^3-leucine injection, was undertaken to determine the relationship of protein and RNA synthesis to abnormal enlargement. Half of the mice used were nonirradiated, the others received 5000 r.

Results and Discussion. – Abnormal crypt cell enlargement during the first 48 hr after 5000 r is associated with increase of total RNA and protein and a dilution of the existing DNA. This assumes that tritiated thymidine is a specific label for DNA, that tritiated cytidine is specific for DNA and RNA, and that tritiated leucine is specific for protein.

Erythrocyte Life Span in Strain-2 Guinea Pigs

L. H. Smith T. W. McKinley, Jr.

Introduction. – The purpose of these experiments was to determine the survival time of autologous, Cr^{51}-labeled guinea pig erythrocytes. Seven strain-2 guinea pigs[11] of both sexes were used. About 1.0 ml of blood was collected from an ear vein and the erythrocytes chromated for 1 hr (15 μc of Cr^{51} per milliliter of whole blood). After chromation, the autologous labeled cells were injected into a neck vein. A 50-λ sample was taken 24 hr later and its activity taken as 100%. Weekly 50-λ samples were obtained and their activities expressed in percentage of the 24-hr sample.

Results. – The Cr^{51} activity disappeared from the peripheral circulation curvilinearly, the 50% level occurring on about day 16. By day 70, the level was about 8%. A log plot of the activity against time suggested

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[10] R. A. McGrath, Intern. J. Radiation Biol. 2, 177 (1960).

[11] Breeder stock of strain-2 guinea pigs was obtained from the National Institutes of Health. The animals used in these experiments were inbred isologous offspring of these breeders.

that the disappearance was exponential, although some residual curvilinearity was present. Extrapolation of the line beyond day 70 intersects the abscissa (activity less than 2%) at about 80 days.

Conclusion. – The T 1/2 for autologous guinea pig erythrocytes is about 16 days, and the extinction point occurs about day 80. The results suggest a large component of Cr^{51} elution from the cells and/or random destruction and an average life span of approximately 80 days.

Donor-Type Lymphoid Cells in Homologous and Heterologous Bone Marrow Chimeras

J. W. Goodman P. G. Lankford

Introduction and Methods. – The secondary disease usually seen in mice that have been irradiated and treated with homologous or heterologous bone marrow is believed to have an immunological basis. Much research and discussion concerning the syndrome and its etiology center around the origin and reactivity of immunologically competent cells in chimeras. This study makes use of a technique described by Gorer and Mikulska[12] in which cytotoxic antibodies are employed to distinguish cells of a particular mouse strain from those of another strain.

Late chimeras, at least 90 days after lethal x irradiation and bone marrow treatment, were studied, and in all cases erythrocytes were found to be 100% donor-type before mice were sacrificed for cytotoxic study.

Specific antisera were prepared by multiple immunizations of (C57BL/6 × DBA/2)F_1 mice against the (C3H × 101)F_1 strain and of (C3H × 101)F_1 mice against the (C57BL/6 × DBA/2)F_1. Rat anti-mouse and mouse anti-rat sera were similarly prepared. Cell suspensions from spleens, and separately from lymph nodes, of chimeras and appropriate control animals were made and, after an initial count, diluted in Tyrode's solution. Aliquots of cell suspensions were reacted for 15 min at 37°C with antiserum (usually 1/8 to 1/16 in Tyrode's solution) and rabbit complement that had been absorbed with mouse and rat red cells. Each cell suspension was reacted with anti-donor, anti-host, and normal mouse sera. After incubation, suspensions were cooled immediately and were kept at approximately 4°C until they

[12]P. A. Gorer and Z. B. Mikulska, Cancer Research 14, 651 (1954).

were diluted with Tyrode's solution containing eosin and counted in a hemocytometer. Those cells not stained with eosin were scored as living, and those stained as dead. At least 100 cells were counted for each determination.

Results and Discussion. — Data are presented in Table 10, and it can be seen that lymphoid cells of 18 homologous and 4 heterologous chimeras reacted in every case like control cells of the bone marrow donor type. If these cells, predominantly lymphocytes, are part of or give rise to cells of the immune system, this study affords a basis for graft-vs-host reactions in the chimera.

Several points should be mentioned with regard to the test itself and to control data: (1) some cells, around 10% minimally, are dead before the cytotoxic test is begun; (2) lymph node cells are more readily killed in normal serum, either alone or with complement, or in nonspecific antiserum than are spleen cells; (3) rabbit complement, at the level necessary to yield usable cytotoxicity in antiserum, is cytotoxic in itself (see last column of Table 10); normal serum alone is not toxic; (4) in general the normal serum percentage-dead values for chimeric lymph node cells are higher than those for comparable controls.

Chromosome Aberrations Induced in Vivo by X Irradiation of the Chinese Hamster

M. A Bender P. C. Gooch

Introduction. — A number of studies[13,14] have shown that the x-ray-induced chromatid-type chromosome breakage rate in mammalian cells in vitro is about 0.004 break $cell^{-1}$ r^{-1}, and that the spontaneous rate varies from a maximum of about 0.22 break/cell for "fibroblasts" to less than 0.01 break/cell for epithelioid cells. Preliminary studies on the spontaneous and induced aberration rates in vivo in the bone marrow cells of the monkey Ateles showed that, while the induced rate was similar to that found for

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

[14]M. A Bender, p 103–18 in Immediate and Low Level Effects of Ionizing Radiations, ed. by A. A. Buzzati-Traverso (Proceedings of Symposium held in Venice, June 22–26, 1959, published as a supplement to Intern. J. Radiation Biol.), Taylor and Francis, Ltd., London, 1960.

Table 10. Identification of Donor-Type Lymphoid Cells in Bone Marrow Chimeras

Irradiated Host	Bone Marrow Donor	Number of Mice[a]	Tissue	Average Percentage of Cells Dead in Serum		
				Anti-host	Anti-donor	Normal Mouse
$(C3H \times 101)F_1$	$(C57BL/6 \times DBA/2)F_1$	5	Spleen	23.7	98.3	24.7
			Lymph node	59.6	96.1	52.6
$(101 \times C3H)F_1$	$(C57BL/6 \times DBA/2)F_1$	8[b]	Spleen	21.0	87.2	21.8
			Lymph node	36.0	77.7	33.9
$(C57BL/6 \times DBA/2)F_1$	$(C3H \times 101)F_1$	5	Spleen	23.4	94.8	22.7
			Lymph node	45.8	96.5	41.8
$(C3H \times 101)F_1$	Sprague-Dawley rat	4	Spleen	14.9	49.9	19.8
Nonirradiated Controls						
$(C3H \times 101)F_1$		2	Spleen	80.2	21.3[c]	12.1
		1	Lymph node	94.2	26.6	26.3
$(101 \times C3H)F_1$		3	Spleen	84.2	22.1	28.6
		4	Lymph node	94.3	46.3	42.5
$(C57BL/6 \times DBA/2)F_1$		4	Spleen	93.8	25.5	26.1
		5	Lymph node	99.7	44.1	49.7
Sprague-Dawley rat		1	Spleen	55.3	9.7	10.5

[a] Number of mice represented in average percentages.

[b] Original suspensions in Tyrode's solution left overnight in refrigerator before cytotoxicity test. Averages include three suspensions containing very few cells and much debris before, and very low cell death in all sera after, incubation.

[c] Antiserum specific for a strain different from that of control mouse.

cells in culture, the spontaneous rate was much lower. For a more detailed analysis, we selected the Chinese hamster, Cricetulus griseus, because of its smaller size, favorable karyotype, and genetic uniformity.

Results.— Diploid bone marrow cells from inbred Chinese hamsters were examined for chromosome aberrations by a modification of the technique of Ford and Hamerton.[15] Three unirradiated control animals were examined. No aberrations were seen in a total of 299 cells. A series of 12 animals were used to investigate the influence of the time between irradiation and fixation on the yield of visible aberrations. All were given 100 r of total body hard x irradiation (hvl = 2 mm Cu). The results appear in Table 11. The yield of chromatid aberrations fell as a logarithmic function of time. Two-hit chromatid aberrations were seen only in the 6- and 10-hr samples. Chromosome-type aberrations did not appear until 36 hr after irradiation. Dose curves were made at 2 and 6 hr after irradiation. These results also appear in Table 11. The slopes of the linear regression lines calculated to pass through the origin are 0.0051 break $cell^{-1}$ r^{-1} at 2 hr and 0.0021 break $cell^{-1}$ r^{-1} at 6 hr.

Discussion. — The logarithmic relation between breakage rate and time and the late appearance of chromosome-type aberrations suggest that the bone marrow cells have a long interdivision time, and that the interdivision time is quite variable in the cells sampled. The induced breakage frequencies a few hours after irradiation are not very different from those previously found for mammalian cells in vitro. The spontaneous aberration rate appears to be much lower in vivo than in vitro. It appears, then, that epithelioid cells in vitro are more nearly similar to somatic cells in vivo than are "fibroblasts" in vitro, but that even the epithelioid cells have an abnormally high spontaneous rate of chromosome breakage.

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[15]C. E. Ford and J. L. Hamerton, Stain Technol. 31, 247 (1956).

Table 11. X-Ray-Induced Chromosome Aberrations in Chinese Hamster Bone Marrow Cells in Vivo

Time (hr)	Dose (r)	Number of Cells Scored	Chromatid Deletions	Isochromatid Deletions	Chromatid Exchanges	Chromosome Breaks	Chromatid Breaks* (%)
Control		299	0	0	0	0	0.0
2	100	86	35	8	0	0	50.0
4	100	91	20	7	0	0	29.7
6	100	101	8	7	3	0	18.8
8	100	100	13	4	0	0	17.0
10	100	100	8	7	1	0	17.0
12	100	80	3	6	0	0	11.3
15	100	100	4	5	0	0	9.0
18	100	98	0	4	0	0	4.1
24	100	98	2	2	0	0	4.1
30	100	100	0	1	0	0	1.0
36	100	38	0	0	0	6	0.0
48	100	100	1	0	0	6	1.0
2	25	100	10	4	0	0	14.0
2	50	100	19	5	0	0	24.0
6	25	100	5	0	0	0	5.0
6	50	89	4	3	0	0	7.9
6	150	74	12	15	5	0	50.0
6	200	100	21	13	2	1	48.0

*Chromatid deletions plus isochromatid deletions plus twice chromatid exchanges.

Visible Chromosome Aberrations Induced by Irradiation of the Male Germ Cells of the Mouse

C. B. Blair, Jr. M. A Bender

Introduction. — A number of investigations have been made of spontaneous and x-ray-induced visible chromosome aberrations in mammalian cells, both in vitro and in vivo.[13,14,16] No corresponding investigation has been made of visible aberrations induced in mammalian germ cells. The results of such a study will be of value both for comparison with the existing data on somatic aberrations, and for comparison with the extensive data available for genetically detected aberrations. Because most of the mammalian genetic data have been obtained from the mouse, this species was selected for a cytological investigation.

Method. — Adult male C3H inbred mice were irradiated with 100 r of hard x rays. Each male was mated with three separate C3H females, one during the first postirradiation week, one during the third week, and one during the fifth week. Control matings of unirradiated C3H mice were also made. Cytological preparations were made from the liver and spleen of each of the resulting embryos by a modification of the bone marrow technique of Ford and Hamerton.[15] The modification consisted in injecting the pregnant female with Colcemid and, after 2 hr, dissecting the liver and spleen from each embryo, and dissociating their cells by passage through a fine hypodermic needle. After the usual hypotonic treatment and fixation, the cells were spread by dropping a small amount of the suspension onto the wet surface of a slide, and staining with aceto-orcein. At least six good mitotic figures from each embryo are to be studied and any abnormalities in chromosome number or karyotype recorded.

Results and Discussion. — Preparations from 39 embryos of the first-postirradiation-week sample, 14 embryos of the third-week sample, and 63 embryos of the fifth-week sample have been made. In addition, preparations were made from 106 control embryos. All of the first- and third-week embryos have been examined. No aberrations were seen. None of the fifth-week or of the control preparations have as yet been examined.

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

Karyotype analysis of the embryo spleen and liver cells prepared by the present method is relatively easy. The chromosomes are more contracted and the figures less well spread, however, than those of tissue cultures of the mouse. This is probably due to the small cytoplasmic volume of the hematopoietic cells. For these reasons, it has been impossible to distinguish the Y chromosome in our preparations. The present incomplete results cannot, of course, give any indication of chromosome aberration rates. They have shown, however, that an investigation by this method, while tedious, is perfectly feasible.

T. Makinodan
N. Gengozian
E. H. Perkins
E. E. Capalbo[1]
F. Celada[1]
A. Ramos[1]
R. R. Carter
W. J. Peterson
M. A. Robinson
J. T. Graziani[2]
P. Urso[3]

Radiosensitive Nature of Primary and Secondary Antibody-Forming Cells

T. Makinodan W. J. Peterson

Introduction. — Our previous work[4] revealed that, in the nonlethal x-ray dose range, the primary antibody response of intact mice was noticeably depressed but the secondary antibody response was not; in the mid-lethal dose range, both responses were depressed. Using heavily x-rayed isologous mice as in vivo cultures for known numbers of spleen cells from donors exposed to various doses of total-body x irradiation, we have been able to determine the relative radiosensitivity of primary and secondary antibody-forming cells.

Results. — As shown in Fig. 14, no significant difference in the magnitude of depression of activity was observed after 250 r between the primary and secondary antibody-forming cells. Comparable results were obtained after 150 r. Furthermore, the slopes of the regression lines (titer vs cell number) between non-x-rayed and x-rayed primary and secondary responses were comparable.

Discussion. — These results clearly show that the radiosensitivitie of primary and secondary antibody-forming cells are comparable and that the difference observed among intact animals exposed to nonlethal x-ray doses, therefore, must be due mainly to the difference in the absolute number of such cells.

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[1]Visiting investigator from abroad.

[2]Student trainee

[3]ORINS Fellow.

[4]T. Makinodan, Symposium on Radiation Protection and Recovery, to be published in Federation Proc.

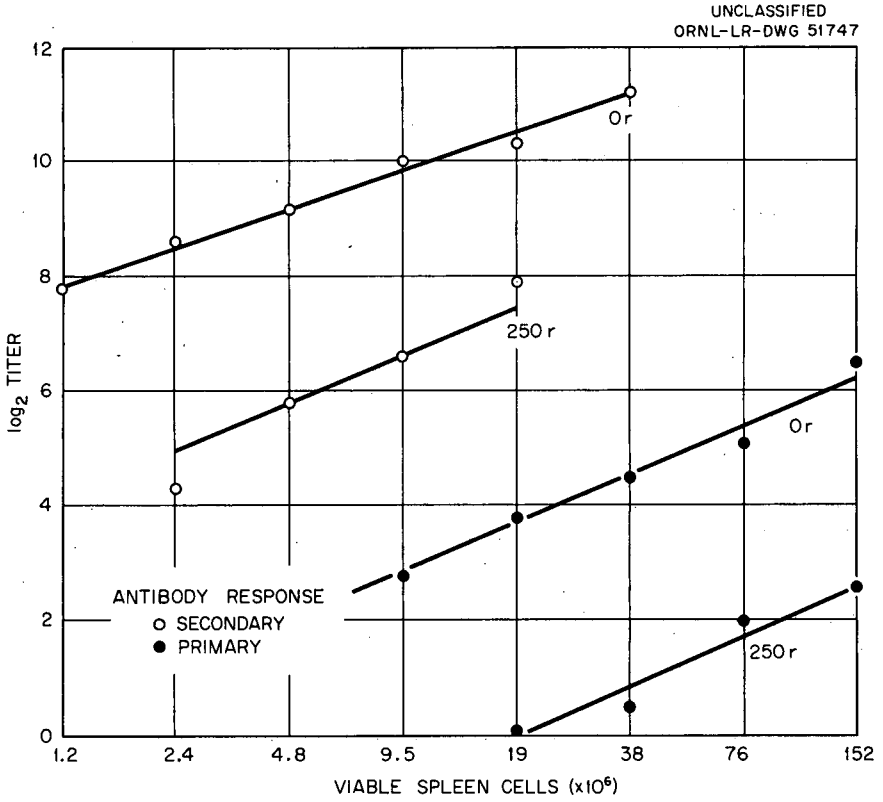

Fig. 14. Relative Radiosensitivity of Primary and Secondary Antibody-Forming Cells.

Fate of H^3-Thymidine-Labeled Antibody-Forming Cells in in Vivo Tissue Culture

E. E. Capalbo W. D. Gude

Introduction. — It is known that heavily x-rayed animals are incapable of responding to an antigenic stimulation for a given period of time. We have therefore used these x-rayed animals as in vivo cultures for isologous antibody-forming cells and found that a linear $\log_2$ relation exists between antibody response and injected spleen cells. Information on the distribution of these cells and on the significance of cell division and maturation vectors with regard to antibody formation is still lacking. Recently preliminary attempts have been made to determine the fate of spleen cells in in vivo cultures using labeled (tritiated thymidine) spleen cells.

Results. — Most of the labeled cells were observed in the spleen, thymus, mesenteric lymph node, and bone marrow, few in the lung and small

intestine, and none in the brain, skin, kidney, testis, and muscles (pectoral and cardiac). Determination of the per cent labeled cells in spleen imprints 1 to 73 hr after cell infusion revealed that a twofold decrease in the generation time and the time of maximum per cent labeled cells occurred when the infused cells were stimulated with a secondary antigen injection (Fig. 15). Antibodies were detected in the circulation three days after antigen injection with the peak titer occurring on the fifth or sixth day.

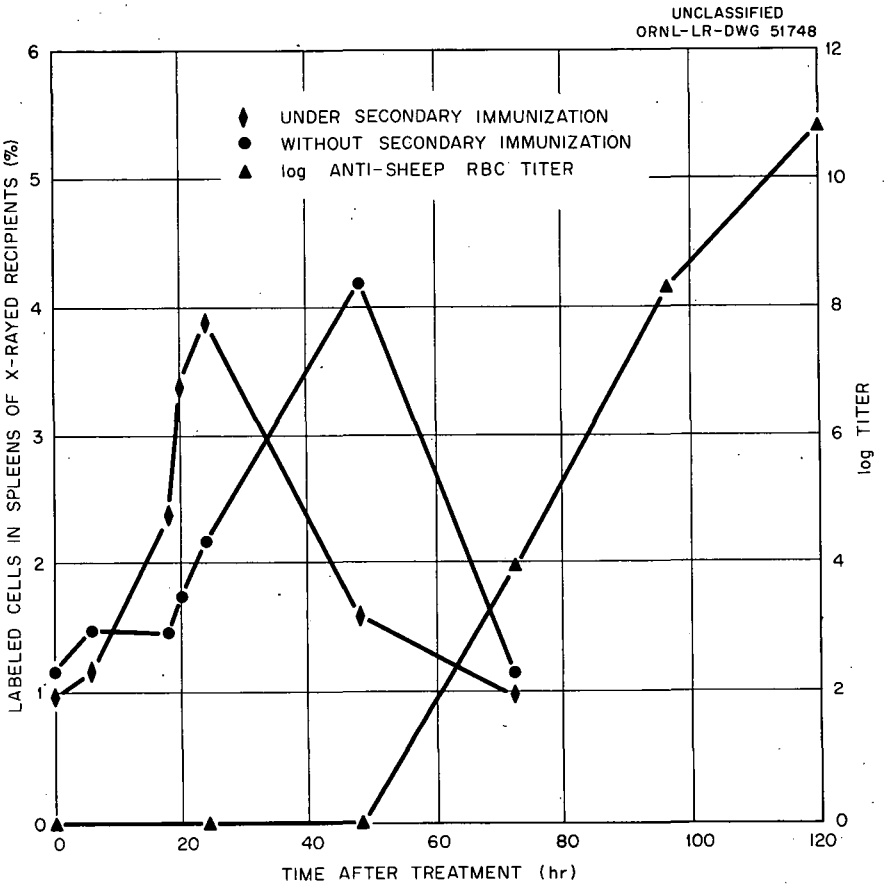

Fig. 15. Effect of Antigen Injection on H^3-Thymidine-Labeled Spleen Cells in in Vivo Cultures.

Discussion. — These results show that spleen cells when injected into heavily x-rayed isologous mice localize preferentially in hematopoietic organs, and that the generation time of these cells can be shortened by an antigenic stimulation. The precipitous drop in the per cent labeled cells observed both in the experimental and control recipients suggests

that the labeled cells are either being diluted among the rapidly proliferating nonlabeled cells or killed by internal radiation of H^3.

Phagocytosis of Heterologous Antigens (RBC) by Mouse Peritoneal Exudate Cells

E. H. Perkins M. A. Robinson

Introduction. — Agreement on the sequential cellular events of antibody formation is not unanimous. Thus, it is not known whether phagocytosis is coincidental to, or truly the initial phase (antigen uptake) of this process. To enable us to evaluate the role of phagocytic cells in antibody formation preliminary studies have been initiated.

Results. — In vitro studies on the phagocytic capacity (per cent phagocytosis) of mouse (101 × C3H)F_1 peritoneal exudate cells to RBC antigens revealed that the more distantly related the RBC is to the phagocytic cells, the greater the per cent of phagocytosis. Thus, the phagocytic capacity to RBC of six mammalian species was as follows: Sheep > rabbit > guinea pig > rat > hamster > mouse (C57BL/6 × DBA/2)F_1. These observations could not be correlated with natural antibody. The efficiency of phagocytic cells could be enhanced with specific antibodies, and the degree of enhancement appeared to be dependent on the concentration of antibodies.

Discussion. — These results suggest that phagocytosis is coincidental to the phenomenon of antibody formation. However, further work is necessary to fully understand the role of these cells during the initial phase of antibody formation.

Transplantation Antigens

F. Celada

Introduction. — Recently we described a new model for studying hematopoietic transplantation antigens which is based on the rejection of spleen cells transferred into preimmunized homologous mice exposed to 800 r.[5] Several variables have since been studied and the results are presented here.

[5] F. Celada and T. Makinodan, Radiation Research 12, 427 (1960).

Results. – The following observations were made: (1) The rate of appearance of agglutinins in the circulation when spleen cells are transferred into 800-r isologous recipients was slightly higher than that in the 800-r homologous recipients; the most significant difference in titer was observed on the sixth day. (2) The survival of spleen cells in homologous recipients increased with increase in the radiation dose to the recipients. Thus, the per cent rejection of homologous spleen graft with respect to isologous spleen graft was 0, 33, 46, 66, and 98% after 900, 850, 800, 700, and 500 r, respectively. (3) A two-day preimmunization period was sufficient to provoke a response in recipient mice to reject about 90% of the subsequent spleen graft, and a maximum preimmunization period was reached in four days. (4) The RD_{50}, defined as the dose of antigen capable of stimulating the recipient to reject 50% of the grafted cells, was 6×10^2 for viable bone marrow cells and about 10^5 for liver and testicular cells. When cellular antigens were killed by 2000 r exposure or by freezing and thawing, their antigenic potency decreased by a factor of about 10^2 (Fig. 16).

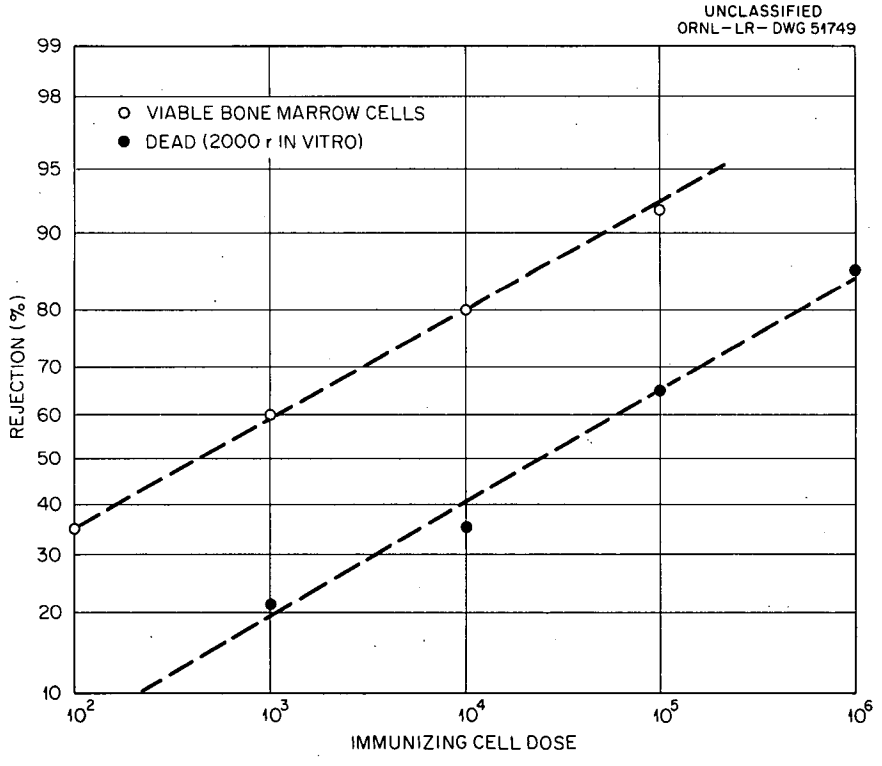

Fig. 16. Determination of the RD_{50} for Viable and Nonviable Bone Marrow Cell Antigens.

Discussion. — Our data demonstrate that the present model fulfills the requirements for a quantitative bioassay and that its sensitivity is considerably higher than any other proposed method. This model is now being used to evaluate the antigenicity of cell fractions of Snell's coisogenic lines of mice.

Passive and Reverse Passive Cutaneous Anaphylaxis

F. Celada A. Ramos

Introduction. — Previous work has shown that the passive cutaneous anaphylaxis (PCA) test in mice is almost as sensitive as in guinea pigs when mouse antiserum is used,[6] and that in guinea pigs it is negative when distantly related antisera are employed.[7] On the other hand, the reversed passive cutaneous anaphylaxis (RPCA) is positive with any antiserum when rabbit gamma globulin is used as the test antigen.[7] As a possible model for the homograft reaction phenomenon we have studied the PCA and RPCA reactions with antibodies from different species.

Results. — The PCA test was performed in mice and in chickens and the RPCA test in mice with anti-BSA sera of known antibody content obtained from chicken, rabbit, rat, and mouse. In the PCA test 0.05 ml of antiserum is injected intradermally, followed 3 hr later by an intravenous injection of 1.25 mg of antigen (BSA) dissolved in 0.5% Evans blue in saline. The reactions were evaluated 30 min later. The RCPA test was modified slightly from that of Ovary: antigen (2.5 mg BSA) was injected intravenously; 3 hr later the antiserum was injected intradermally; 10 min later the dye was injected intravenously; reactions were evaluated 30 min later. The results on our PCA and RPCA tests in mice are summarized in Table 12. Analogous results were obtained in chickens.

Discussion. — Our results show that the sensitivities of both the PCA and the RPCA reactions are dependent on the phylogenetic relation between test animal and the source of antiserum. Studies are in progress to determine whether this relationship is limited to gamma globulins or shared with all other serum proteins.

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[6] J. Munoz and R. L. Anacker, J. Immunol. 83, 640 (1959).

[7] Z. Ovary, Immunology 3, 19 (1960).

Table 12. PCA and RPCA in Mice (BSA–Anti-BSA Reaction)*

Micrograms of Antibody N	Source of Antiserum							
	Chicken		Rabbit		Rat		Mouse	
	PCA	RPCA	PCA	RPCA	PCA	RPCA	PCA	RPCA
2.0	–	–	+++	++	++++	++++	++++	++++
1.0	–	–	++	++	+++	++	++++	++++
0.5	–	–	+	+	+++	++	++++	+++
0.25	–	–	–	+	++	+	+++	++
0.125	–	–	–	–	+	+	+++	++
0.062	–	–	–	–	+	–	++	+
0.031	–	–	–	–	–	–	++	–
0.015	–	–	–	–	–	–	+	–
0.007	–	–	–	–	–	–	+	–
0.003	–	–	–	–	–	–	–	–

*The antigen amount per animal was 1.25 mg for the PCA and 2.5 mg for the RPCA.

Fatal Anaphylaxis in Mice as Influenced by Dose and Route of Sensitization and Time of Challenge

P. Urso

Introduction. – In a previous study[8] it was found that the frequency of fatal anaphylaxis in mice sensitized to 20 mg of bovine serum albumin (BSA) emulsified in Freund's adjuvant and challenged 35–47 days later was dependent on the route of injection and the challenging dose. In experiments described here the following variables were studied: (1) route and dose of sensitization, (2) time of challenge, and (3) challenging dose of antigen.

[8]P. Urso and T. Makinodan, Biol. Semiann. Prog. Rep. Feb. 15, 1960, ORNL-2913, p 80.

Results. – (C57BL × 101)F_1 mice (12–15 weeks old) were sensitized with 0.01, 0.1, 1.0, 5–7, or 20 mg of BSA and challenged 9, 21, 35, or 47 days (induction period) later. The routes of sensitization were subcutaneous (SC), intraperitoneal and subcutaneous (IP-SC), intraperitoneal (IP), and intramuscular (IM). It was found that the frequency of fatal shock can be influenced by the route of sensitization, induction period, and challenging dose (Fig. 17). Thus, for example, a decrease in the

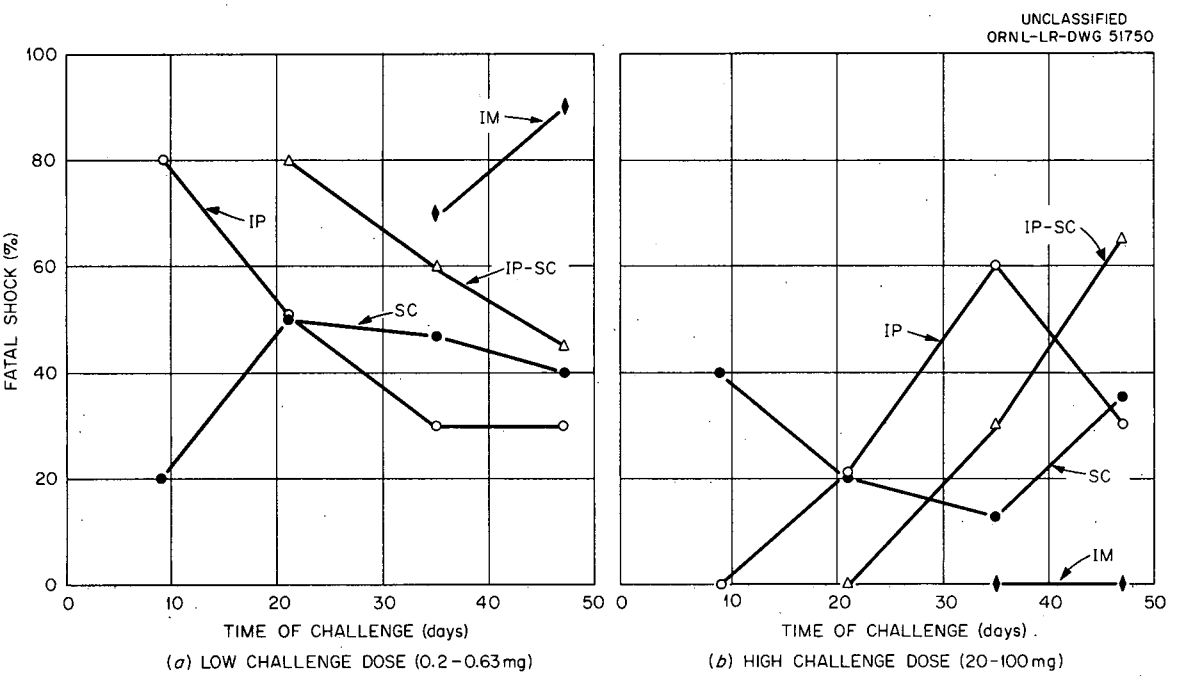

Fig. 17. Frequency of Fatal Anaphylaxis in (C57BL × 101)F_1 Mice Sensitized with 20 mg of BSA by Various Routes and Challenged with High and Low Doses of Antigen at Various Intervals After Sensitization.

frequency of fatal anaphylaxis was seen among mice sensitized with 20 mg of BSA IP-SC and IP with increase in the induction period when they were challenged with a low dose of antigen (0.2–0.63 mg). On the other hand an increase in the frequency of fatal shock occurred when these mice were challenged with a high dose of antigen (20–100 mg).

In general among IP-SC-sensitized mice a decrease in the frequency of fatal shock occurred with a decrease in the sensitizing dose. On the other hand among SC-sensitized mice the frequency of fatal shock was independent of the sensitizing dose when the induction period was 35 days.

When the induction period was 21 days the frequency of fatal shock was higher with lower sensitizing dose (52% vs 18%).

Discussion. — These results show that the frequency of fatal anaphylactic shock in mice is a function of dose and route of sensitization, induction period, and dose of challenge. Valid interpretation of these results must await analyses of the amount and nature of the circulating antibodies at the time of challenge.

Studies on Passive Anaphylaxis in the Chicken

N. Gengozian J. T. Graziani

Introduction. — There is considerable variation among mammals in susceptibility to anaphylaxis, both active and passive; however, under suitable experimental conditions, marked symptons leading to death of the animal can be obtained. In chickens, Makinodan et al.[9] have shown a direct correlation between the antibody concentration in the serum and the quantity of challenging antigen injected on the ability to induce fatal active anaphylaxis in these animals. To clarify further the mechanism and conditions necessary for anaphylaxis in chickens as compared with other species, studies on passive anaphylaxis have been initiated.

Results. — Groups of 11-day-old chickens weighing approximately 100–130 g were injected intravenously with 1 ml of chicken anti-bovine serum albumin (BSA) containing either 1000 or 100 μg of Ab N. At intervals of 2, 24, and 96 hr after antiserum the challenging BSA antigen of either 0.1, 1.0, or 10.0 mg of protein was injected intravenously. Five chickens were used for each of the three variables studied: sensitizing antibody dose, challenging antigen dose, and the time interval between injections. Scoring of anaphylaxis was as follows: —, no reaction; 1+, inability to stand, deep breathing; 2+, inability to stand, falling to one side, deep breathing; 3+ immediate loss of balance, marked convulsive movements of legs and wings, very deep breathing; 4+, death. Changes in body temperature were recorded at intervals of 0, 5, 10, 15, and 30 min after the challenging antigen. The data are shown in Table 13. Definite symptoms of anaphylaxis were obtained with the higher sensitizing dose of antiserum,

[9]T. Makinodan et al., J. Immunol. 68, 219–26 (1952).

Table 13. Passive Anaphylaxis in 11-Day-Old Chicks After Intravenous Injection of Antiserum and Antigen

Sensitizing antibody dose: 1000 μg of Ab N

Antigen Challenging Dose (mg protein)	Degree of Reaction		
	Time Interval Between Ab and Ag (hr)		
	2	24	96
10	1+	2+	–
	2+	3+	1+
	1+	1+	1+
	2+	1+	1+
	2+	1+	1+
1	1+	3+	2+
	2+	1+	2+
	–	–	2+
	3+	2+	1+
	–	1+	1+
0.1	1+	1+	1+
	–	1+	1+
	–	1+	1+
	1+	1+	2+
	–	1+	1+

and there appeared to be some correlation with the dose of antigen and time interval of injection. Thus, on the basis of this scoring system, the 24-hr interval would appear optimum in that all antigen concentrations used showed some degree of reaction. The reactions at 96 hr, though graded slightly lower, were often characterized by long periods of deep breathing and inactivity. The chickens generally recovered from the reactions within a period of 3–6 min, even those which had experienced a marked 3+ reaction. In no case was there fatal anaphylaxis. Body temperature changes were absent or very slight (–1 to –3°C) during the 30-min observation time for all chickens and have not been tabulated. In contrast to the

sensitization with 1000 μg of Ab N, anaphylactic symptoms were not apparent with injection of 100 μg of Ab N and antigen injection at the various intervals (not tabulated).

Discussion. — The data obtained thus far suggest a definite correlation between the amount of circulating antibody and the challenging dose of antigen in inducing passive anaphylaxis. Additional studies to verify this quantitative relation and the conditions necessary to induce fatal passive anaphylaxis are anticipated.

PATHOLOGY AND PHYSIOLOGY

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Modification of Homologous Disease Following Transplantation of Parental Bone Marrow and Recipient Liver in Irradiated F_1 Hybrid Mice

G. Cudkowicz G. E. Cosgrove

Introduction. — Although the foreign spleen reaction may be inhibited by exposing the donor-type spleen cells in vitro to recipient-type liver cells,[5] the liver contains immunologically competent cells which themselves can induce wasting disease[6] or confer adoptive immunity.[7] Therefore, the influence of the liver incubation procedure has been reevaluated.

Methods and Results. — Young adult (C57BL × 101)F_1 mice were injected intravenously within 2–3 hr after 900 r of whole-body x radiation with mixtures of C57BL bone marrow cells (10×10^6 nucleated cells) and recipient liver cells (equivalent to 0.1 liver) incubated for about 20 hr at 10°C (group 1). A second group of irradiated (C57BL × 101)F_1 mice received fresh unincubated parental marrow (10×10^6 nucleated cells) intravenously and concomitantly recipient liver (equivalent to 0.5 liver) intraperitoneally (group 2). A third group of irradiated (C57BL × 101)F_1 mice received parental marrow (10×10^6 nucleated cells) which had been previously incubated with recipient-type liver cells but separated from them by centrifugation before injection (group 3). A fourth group (control) received parental marrow prepared and incubated with saline solution under the same conditions as the bone-marrow–liver mixture (group 4). Marrow and liver were derived from young adult donors killed by bleeding. The animals receiving a mixture of parental marrow and isologous F_1 liver showed increased mortality 15 to 60 days after treatment, although their later mortality was about the same as in the group receiving parental bone marrow alone (Fig. 18). When, however, preincubated C57BL bone marrow cells were separated from the liver cells before transplantation, mortality among the recipients was markedly reduced; that is, death occurred in only 18% of the 21-day survivors as

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

[6] G. Cudkowicz and G. E. Cosgrove, Biol. Semiann. Prog. Rep. Feb. 15, 1960, ORNL-2913, p 91–93.

[7] C. C. Congdon and D. B. Duda, A.M.A. Arch. Pathol., in press.

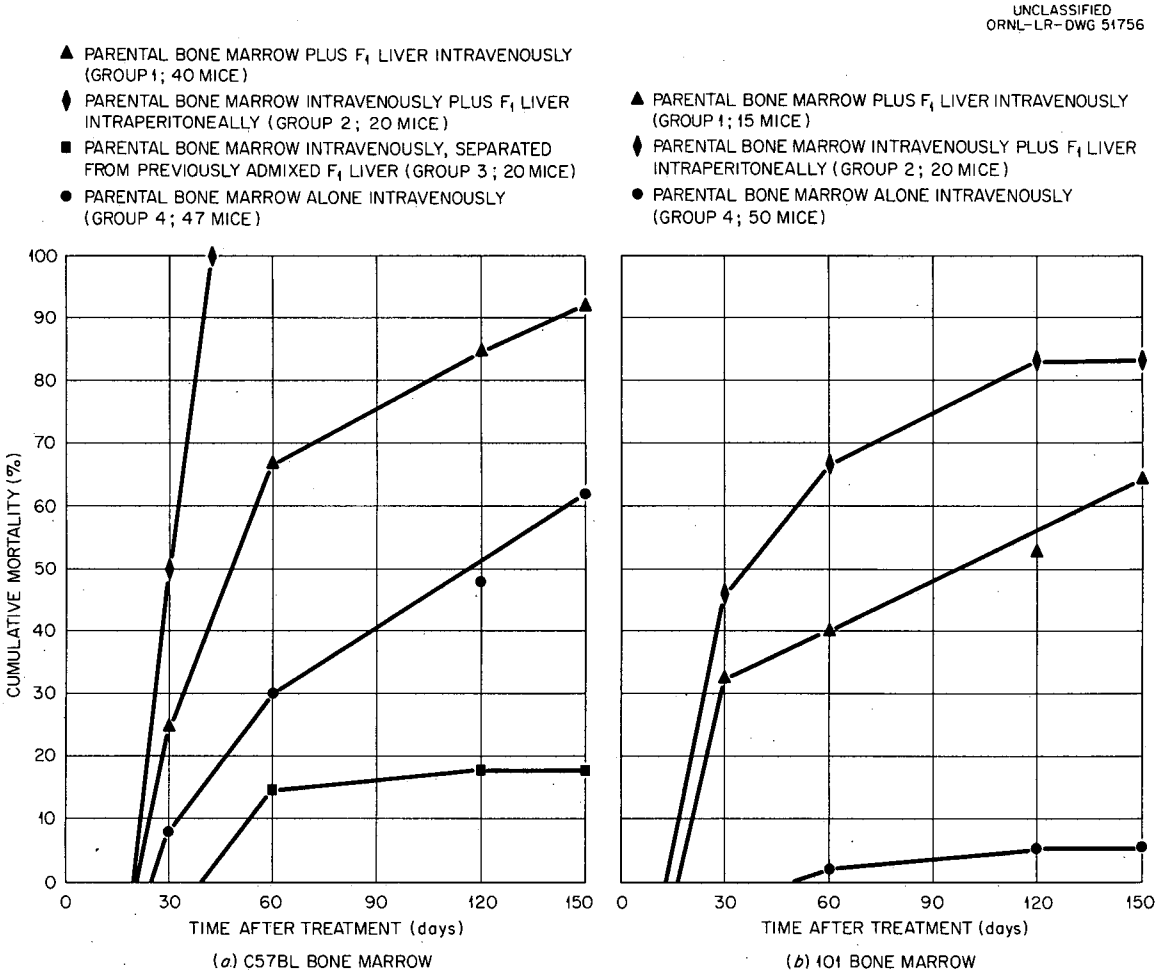

Fig. 18. Effect of Isologous F_1 Liver Cells on the Mortality of 900 r X-Irradiated (C57BL × 101)F_1 Mice Injected with Parental Bone Marrow.

opposed to 62% of the controls. The relatively low mortality (8%) in F_1 recipients of 101 bone marrow is consistent with previous results, confirming unexplained differences between the two parental strains in the effects of their marrow on the survival of irradiated F_1 recipients.

Discussion. – The low mortality of irradiated F_1 hybrids given pre-incubated C57BL bone marrow (group 3) is tentatively ascribed to reduction of the immunologic reactivity of the implanted marrow, with inhibition of the graft-anti-host reaction, as was suggested by Cosgrove et al.[5] for incubated spleen cells. The comparable experiment with 101 marrow was not performed, since 101 marrow does not normally induce a high mortality in irradiated (C57BL × 101)F_1 mice (Fig. 18b). Conversely, the heightened early mortality of mice given isologous F_1 liver as well as

parental marrow is tentatively attributed to an immunological reaction by the injected liver cells against the implanted marrow. This postulated F_1-anti-parent reaction, although unconventional, can be justified on theoretical grounds[8] and is supported by collateral experimental data.[9]

Acute Effects in Mice of Various Alkylating Agents.

I. Comparison of HN2 and TEM with X Rays

M. Asano T. T. Odell, Jr. T. P. McDonald

Introduction. — Reports have indicated that nitrogen mustard derivatives are similar in their biological action to x rays, but are especially damaging to lymphatic tissue. They have, therefore, been used in the therapy of lymphoid neoplasms. Pathological changes in tissues and organs after administration of nitrogen mustard derivatives have been described, but there is relatively little information about the details of these changes in relation to dose. We have, therefore, studied hematological and pathological effects of these two drugs, methyl-bis(β-chloroethyl)amine (HN2) and triethylene melamine (TEM), and of x rays, all administered in the LD 10/30 to LD 80/30 dose range. An attempt has also been made to determine the relative sensitivities of different tissues and organs to these agents and to relate tissue and organ damage to cause of death.

Methods and Results. — Methyl-bis(β-chloroethyl)amine was injected intravenously and TEM intraperitoneally into ten-week-old (101 × C3H)F_1 and RF mice. Irradiation was carried out with a 300-kvp, 20-ma G-E Maxitron unit. At intervals of 1 to 28 days after treatment peripheral WBC and RBC counts were made, mice were killed, and sections were taken for pathological study. Thirty-day survival curves were obtained from additional groups of mice.

The median time of death in the LD 50/30 days groups was 10–12 days after x rays and 4–7 days after HN2 and TEM. Peripheral WBC counts declined rapidly in all groups to their lowest levels on the third to fifth days, with recovery beginning about the fifth day in the drug-treated

[8] R. D. Owen, J. Med. Educ. 34, 366 (1959).

[9] R. A. Popp and S. A. Sapp, Radiation Research 12, 463 (1960).

groups and later in the x-ray group. Lesions that appeared after exposure to these agents included immediate depression and prolonged atrophy of hemopoietic organs (bone marrow, spleen, lymph nodes, and thymus), and changes in the mucosa of the small and large intestines. Damage to the small intestine was less severe after x rays than after HN2 and TEM. Glandular ectasia of the stomach was found only in the irradiated animals. Bone marrow recovery was more rapid in the TEM and HN2 groups than in the x-ray group. Damage to the brain was observed only in the HN2 group and was most severe from the sixth to the fifteenth day in the LD 50/30 days group. The most severe atrophy of lymph nodes was noted in the x-ray group and the least severe in the TEM group. Damage to the thymus was similar in the two drug-treated groups and less than in the x-ray group.

Discussion. — In this study, atrophy of hemopoietic organs was more severe and damage to the small intestine less severe in x-irradiated mice than in those given HN2 or TEM in comparable doses. The three syndromes, related to injury of the hemopoietic, gastrointestinal, and central nervous system, respectively, associated with acute death by ionizing irradiation have been described and related to the total dose of x rays, but correlation of symptoms with dose in animals treated with alkylating agents is less well defined. Our results suggest that the cause of death after administration of doses of x rays and TEM lower than the LD 50/30 days is destruction of hemopoietic tissue. In animals given doses of HN2 greater than the LD 30/30 days, damage to the central nervous system is probably a major contributing factor to death.

Influence of Dose Rate on the Effectiveness of Gamma Rays for Delayed Somatic Effects in Mice

J. W. Conklin W. D. Gude M. L. Randolph[10]

Introduction. — Earlier investigations have indicated that a given dose of gamma rays administered at a low intensity over an appreciable portion of the life span is substantially less effective in causing life shortening and other delayed effects in mice than when given in a single, brief exposure. There has been little quantitative study, however, of

[10]Biophysics Group.

the influence of dose rate under conditions such that accumulated radiation is not "wasted."[11] The following are a few of the preliminary results of a large-scale investigation that further elucidate this question.

Results. — Female RF mice in groups of 100–150 were exposed 23 hr daily to various doses of Co^{60} gamma rays, beginning at ten weeks of age. At each dose level, the effectiveness of the radiation for shortening the life span (Fig. 19) and for induction of myeloid luekemias (Fig. 20), thymic lymphomas (Fig. 21), ovarian tumors (Fig. 22), and lens opacities (Fig. 23) varied in relation to the dose rate.

Discussion. — The observed intensity dependence for the induction of these delayed effects conforms, in general, to expectations based on earlier results with protracted gamma irradiation. The disproportionate life shortening at dose rates of about 15–30 rads/day was correlated with a relatively high incidence of lymphomas, confirming previous observations on the existence of optimal dose rates for lymphoma induction in mice. It is noteworthy that a similar optimum was not present at intermediate dose rates for the induction of myeloid luekemia. The absence of ovarian tumor induction by 600 rads delivered at a rate of 1 rad/day is also noteworthy, since tumorigenesis did occur in response to 300 rads delivered at 5 rads/

[11] R. H. Mole, J. Natl. Cancer Inst. 15, 907 (1955).

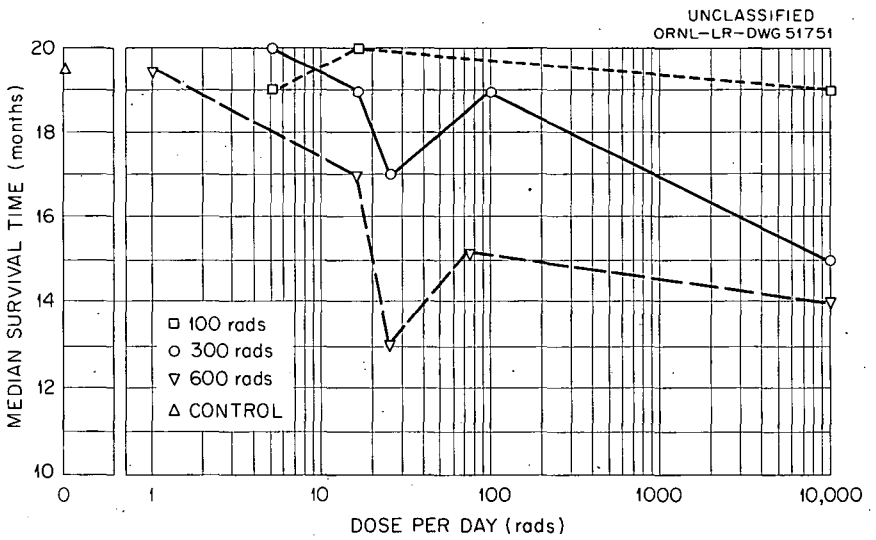

Fig. 19. Median Survival Time in Relation to Dose Rate of Daily Gamma Irradiation.

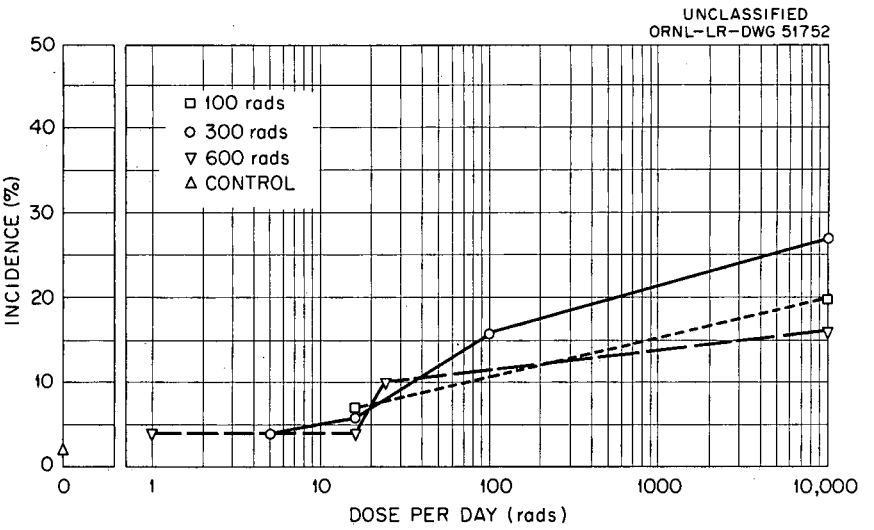

Fig. 20. Incidence of Myeloid Leukemia in Relation to Dose Rate of Daily Gamma Irradiation.

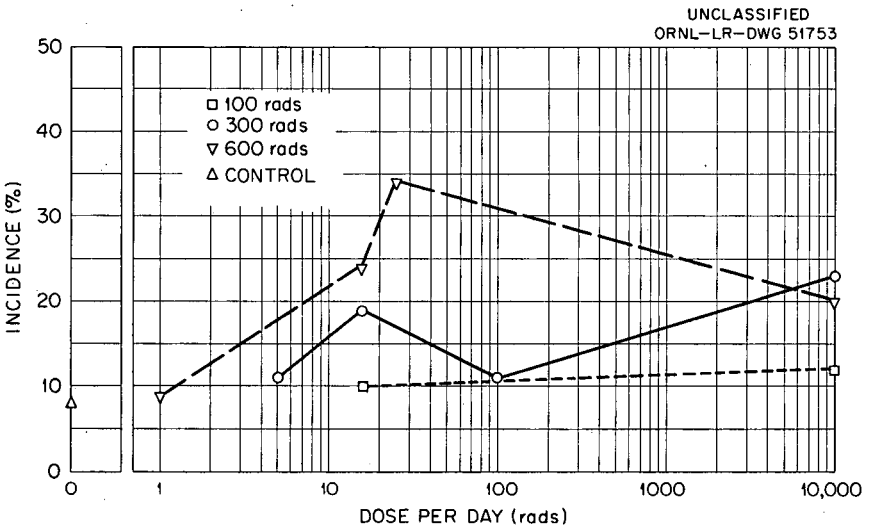

Fig. 21. Incidence of Thymic Lymphoma in Relation to Dose Rate of Daily Gamma Irradiation.

day. These results suggest a complex interaction between irradiation injury and homeostatic mechanisms but their practical and theoretical implications cannot yet be fully assessed. It is evident, however, that the effectiveness of gamma rays for delayed somatic effects is highly

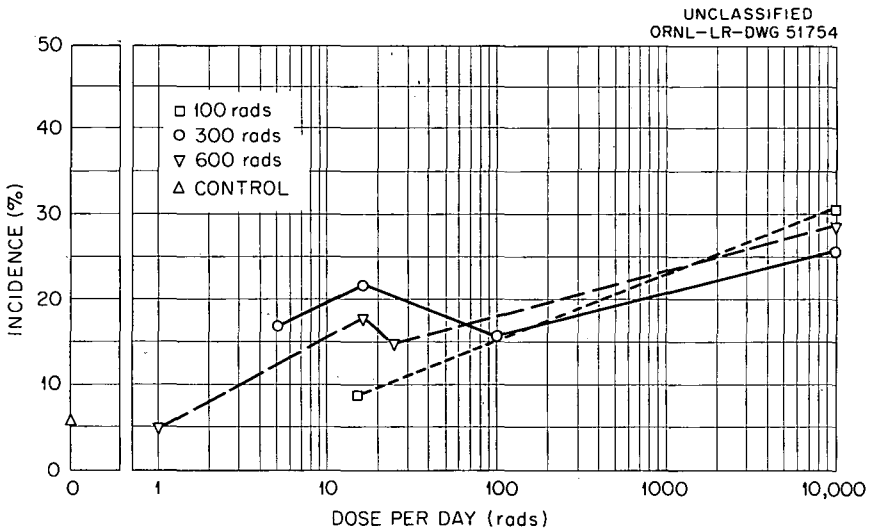

Fig. 22. Incidence of Ovarian Tumors in Relation to Dose Rate of Daily Gamma Irradiation.

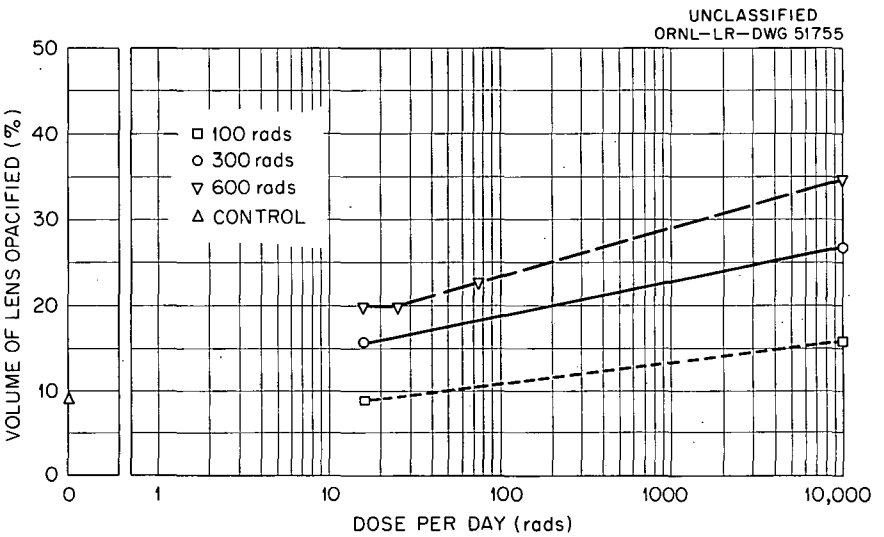

Fig. 23. Average Severity of Lens Opacities in 500-Day-Old Mice in Relation to Dose Rate of Daily Gamma Irradiation.

intensity dependent and that the influence of the intensity varies somewhat from one effect to another. This is in sharp contrast with the relatively low intensity dependence of the effectiveness of neutrons for similar delayed effects in mice of the same strain.[12]

[12]A. C. Upton, unpublished data (1960).

Effect of Induced Thrombocytopenia on Peripheral Platelet Count in Rats

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

Introduction. — For the purpose of investigating mechanisms that regulate platelet production, a series of experiments were undertaken to determine the effect of depletion of the circulating platelet mass on subsequent peripheral platelet counts in Sprague-Dawley rats.

Methods and Results. — Forty to sixty-five per cent of the blood volume (taken as 5% of body weight) was removed by cardiac puncture or from the jugular vein either in two or three partial removals during a 24-hr period or in a single removal. Tyrode's solution or saline was injected (usually intraperitoneally, but sometimes intravenously) into some of these rats to restore liquid volume. In others, blood volume was restored by intravenous injection of defibrinated blood (platelet-free) prepared from Sprague-Dawley donors. In some groups, daily serial platelet counts were taken for 7 to 18 days (more frequently during the first 24 hr), while in another experiment platelet counts were taken only at the beginning of the experiment and again when the rats were killed at intervals after bleeding. Peripheral platelet counts of control rats ranged between 1.3 and 0.865×10^6 platelets/mm^3 with an average of 1.033 for 34 rats. After the initial drop due to platelet removal, peripheral platelet counts of the bled rats rose to levels of 144 to 206% of the original count with the peak occurring during the fourth to sixth day period (Table 14). By the eighth to tenth day peripheral platelet counts were approaching the prebleeding level. No consistent differences in response to the different methods used to bleed rats are so far apparent.

Discussion. — These results indicate that removal of platelets from the peripheral circulation stimulates release of platelets into the blood stream in rats. Since there are no known reservoirs of blood platelets, the data also imply that the production of platelets is increased. In addition, the period of several days required to reach the peak number of platelets in the circulation also indicates an increase in production rather than release from a reservoir. Since an increase in platelet number is apparent by the third day, the megakaryocytes are presumably in some way able to increase platelet production in less than three days

Table 14. Thrombocytosis in Bled Rats

Group	Number of Rats	Total Volume Bled (% of blood volume)	Number of Bleedings	Replaced with –	Peak Platelet Count (% of original)
A	4	40	2*	Tyrode's	173
C	6	60	3*	Tyrode's	183
D	3	60	1	Defibrinated blood	174
E	4	42	1	Saline	157
F	5	65	2**	Tyrode's	164
G	14	65	1	Defibrinated blood	184

*Two or three bleedings were carried out within a 24-hr period.

**Two bleedings were carried out within a 6-hr period.

after the initial stimulus. Whether this results from the production of more platelets by mature megakaryocytes already present in hemopoietic tissue or from maturation of additional megakaryocytes is not yet known.

An increase in peripheral platelet count soon removes the stimulus to, or actually inhibits, platelet production, since the high platelet count is not maintained. Indeed, increased production is apparently arrested by the fourth to sixth day, and the platelet count is reduced to normal levels by about the eighth to tenth day, a period of time (about four days) equal to the average platelet life span in rats.

Some regulatory factor or factors must be present that stimulate or inhibit the production of platelets, or both. The nature and source of these factors are yet to be determined.

Effects of Intravenous Administration of Nitrogen Mustard on the Mouse Brain

T. P. McDonald M. Asano

Introduction. – Nitrogen mustard has been widely used during the past 14 years in the treatment of intracranial neoplastic diseases.

Several reports have suggested that normal nerve tissue is more resistant to this drug than neoplastic tissue cells. Since, however, functional aberrations in HN2·HCl-treated animals that might result from damage to the central nervous system have been described, female RF mice were injected intravenously with single graded doses of this radiomimetic chemical to assay by histologic methods the effects on the brain.

Results. – The LD 50/30 days of nitrogen mustard was found to be 0.127 mg/mouse (95% confidence with intervals of 0.103–0.162). The following symptoms were observed in intoxicated animals: extreme emaciation, ruffling of fur, inactivity, decreased muscular coordination, hyperirritability, convulsions, diarrhea, and lacrimation. It was found that a direct relationship existed between the dose of HN2·HCl and the pathological changes produced in the brain. Brain damage and death rate for higher dose groups reached maximum on days 6–8. Brains of mice receiving doses larger than the LD 50/30 days (0.15 mg/mouse) showed neuronal shrinkage, localized neuronal homogenisierende changes, severe hemorrhage, and multiple foci of necrosis; doses near the LD 50/30 days (0.10 and 0.12 mg/mouse) produced selective neuronal shrinkage in early stages and hemorrhage and focal necrosis later. At lower doses (0.05 mg/mouse), the most significant damage was shrinkage of the nerve cells. Sagittally, the sites of predilection for brain damage in all groups were the neocortex, pyriform cortex, hippocampus, cerebellum, and medulla oblongata.

Discussion. – These results demonstrate the damaging effect of HN2·HCl on the brain tissue of normal mice. This damage may account for the physiological and behavioral abnormalities that have been reported in animals treated with the drug. The localized pathological changes in the mouse brain were similar to those seen in anoxic brains. The reasons for this localized injury and why some cells are particularly sensitive to the action of the β-chlorethyl derivative remain obscure.

Protein Metabolism in the Rat-Mouse Chimera

W. Friedberg V. K. Jenkins

Introduction. – The purpose of the present study was to determine whether the elevated fractional rate of loss of I^{131}-labeled human serum

albumin (HSA-I^{131}) in rat-mouse bone marrow chimeras[13] results from leakage of protein into the gastrointestinal tract. To ascertain whether such leakage might occur, the loss via the gastrointestinal tract of injected radioiodine-labeled polyvinylpyrrolidone (PVP-I^{131}) was determined.

Results and Discussion. – Although the feces of rat-mouse chimeras injected with PVP-I^{131} generally are more radioactive than those of normal age controls or isologous bone marrow chimeras (Table 15), individual mice with an obviously elevated fractional rate of HSA-I^{131} loss were observed to have a fecal PVP-I^{131} loss in the normal range. In rat-mouse chimeras, therefore, the elevated fractional rate of loss of albumin is not always accompanied by a high loss of PVP in the feces. Both may, nevertheless, result from gastrointestinal leakage, which is being explored further. The excessive gastrointestinal loss of PVP-I^{131} in many rat-mouse chimeras also suggests that the gastrointestinal tract is abnormal in such animals and warrants further investigation in relation to their characteristic wasting disease.

Erythropoiesis by Normal Nucleated Peripheral Blood Cells Injected into X-Irradiated Recipient Mice

R. A. Popp D. M. Popp

Introduction. – Earlier experiments showed that transplanted leukocytes of leukemoid blood produce immunologically competent cells[14] and erythroblasts,[15] as well as promoting survival, in lethally irradiated recipient mice. This study was undertaken to determine whether circulating leukocytes in normal blood can similarly repopulate damaged hematopoietic tissues of irradiated recipient mice.

Methods and Results. – Coisogenic strains of mice differing at the hemoglobin locus are being produced, following the scheme of Snell.[16] The "single" type hemoglobin of strain C57BL mice was genetically introduced into strain 101 mice, which normally possess "diffuse" type

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[13] W. Friedberg, Intern. J. Radiation Biol. 2, 186–95 (1960).

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

[15] L. H. Smith, R. A. Popp, and W. St. Amand, Proc. Soc. Exptl. Biol. Med. 103, 232 (1960).

[16] G. D. Snell, J. Genet. 49, 87 (1948).

Table 15. Output of Injected PVP-I^{131} in the Feces of Rat-Mouse Chimeras*

Fecal Output of PVP-I^{131} (% of injected radioactivity)	Number of Mice		
	Treatment		
	Controls	950 r plus Isologous Bone Marrow	950 r plus Rat Bone Marrow
0–1	3		
1–2	19	18	6
2–3	7	5	15
3–4	3	1	9
4–5		1	2
5–6	1	1	
6–7	2	3	1
7–8			3
8–9			1
9–10			1
10–11			4
11–12		1	2
19–20			1
29–30			1
49–50			1
53–54			1
68–69			1
82–83	1		

*Compiled from several experiments carried out during the period, 11–49 days after x irradiation, when rat-mouse chimeras have a high fractional rate of HSA-I^{131} loss. Feces collected during the first 24 hr after PVP injection.

hemoglobin (hemoglobin types identified by starch gel electrophoresis[17] and hemoglobin solubility[18]). Five 16-week-old coisogenic 101 mice heterozygous for diffuse and single hemoglobins (progeny of fifth backcross) were given 600 r of x rays, and approximately 100 million buffy coat cells from ten normal 101 mice were injected intravenously into each recipient. The proportion of donor-type red cells derived from the injected white cell mixture was then determined periodically in each recipient (Table 16).

Table 16. Erythropoiesis by Grafted Peripheral Leukocytes

Experiment Number	Recipient Number	Percentage of Donor-Type Erythrocytes				
		Days After Treatment				
		15	29	46	49	63
I	1	0		0		
	2	30		60*		
II	3		**		40	25
	4		0		0	0
	5		60		100	100

*Mouse died on day 69.

**Analysis indicated that the graft was partially successful, but the blood was so anemic that the quantity of donor-type erythrocytes could not be determined accurately.

Discussion. — The data show that circulating leukocytes normally include cells capable of differentiating into erythroblasts. Perhaps one of the morphological types we classify as relatively mature (i.e., the monocyte or large lymphocyte) is really a free stem cell, as has been suggested by earlier observers. The identity of the stem cell and its range of potentialities are the subjects of continuing studies, as are the implications of these results for the unitary theory of hemopoiesis.

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

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

Electron Microscopy of Plasma-Cell Tumors of the Mouse

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M. A. Bender[20] G. T. Pratt[19]

Introduction. – Transplanted plasma cell tumors of C3H and BALB/c mice[21] were investigated by electron microscopy and tissue culture in an attempt to establish the significance of viruses in their etiology.

Results. – Large numbers of particles of tumor virus morphology were found in both lines of tumors grown *in vivo*. The particles present in the BALB/c tumor and in early C3H tumors were of Bernhard's type A,[22] but late C3H tumors showed particles of more complex morphology. The particles were formed inside endoplasmic reticulum in the BALB/c and early C3H tumors but inside large inclusions within the cytoplasmic matrix in the late C3H tumors. Few particles were observed outside the tumor cells or in blood or ascites fluid. In contrast with the results *in vivo*, cultures of the C3H tumor cells showed large numbers of particles forming by a budding process at the plasma membrane. These particles appeared more complex in type and are thought to represent a more mature form of the virus.

Discussion. – The results strongly suggest a virus etiology for these tumors and suggest a reason for the failure of previous workers[23] to transmit them by cell-free filtrates; that is, the virus present in the BALB/c and early C3H tumors appears to be incomplete and hence may be noninfective. Even the particles observed in late C3H tumors are not so complex in morphology as those found in tissue culture. The development of the virus may, therefore, be inhibited *in vivo* but not *in vitro*. Consequently, cell-free transfers of tissue culture fluids to newborn C3H mice have been carried out, and results are awaited.

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[19]Cytology and Genetics Group.

[20]Mammalian Recovery Group.

[21]Obtained from M. Potter, National Institutes of Health.

[22]W. Bernhard, Cancer Research 18, 491–509 (1958).

[23]A. F. Howatson and E. A. McCulloch, Nature 181, 1213–14 (1958).

CELL PHYSIOLOGY

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The Isolation and Characterization of Soluble Tissue Proteins

H. E. Bond N. G. Anderson

Introduction. — Investigations using cellulose cation and anion exchange materials to isolate the soluble proteins from normal rat liver tissues have continued. DEAE- and carboxymethyl-cellulose have proved so versatile for the separation and isolation of the tissue proteins that supplementary methods of protein isolation have not been instituted at present. Apparently the limits of resolution on the cellulose materials have not yet been attained, and the methods will continue to be refined before studies on additional methods and on other tissues will be attempted.

Results. — The gradient-elution system for DEAE-cellulose has been refined so that 26 recognizable components may be discerned in the chromatogram of rat liver soluble proteins. The chromatogram has been subdivided, and antibodies have been produced against some of these regions. A poorly defined basic fraction from the DEAE chromatogram has been partially separated on carboxymethyl-cellulose (CM-cellulose). The gradient-elution system for the CM-cellulose is undergoing further modification.

A protein component present in the liver of the male rat and absent from the female has been discovered in the basic fraction of the DEAE

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[1]Consultant.
[2]Research associate.
[3]Research participant.
[4]Loanee.
[5]Student trainee.
[6]ORINS Fellow.

chromatogram. The entire female basic fraction represents 16% of the total 280 mμ extinction of the material placed onto the columns. The entire male basic fraction correspondingly represents 28% by 280 mμ extinction and 24% by nitrogen determinations. The peak missing in the female but present in the male, the "male component," represents 8% of the total as judged by 280 mμ extinction and has a 280:260 ratio of 1.78 at the peak. The $s_{20,w}$ is 3.13, and the material appears homogeneous during ultracentrifugation. The mobility of the material at pH 7.5 is -1.6×10^{-5} cm^2 v^{-1} sec^{-1}. Electrophoretically, there is evidence of a minor slower-moving contaminant in the material being studied. Further attempts to characterize the "male component" will be deferred until it has been properly purified.

Discussion. — Antigenic differences between the tissues of male and female inbred rats and mice prevent skin homotransplants when the donor is male and the recipient female. However, the reverse procedure is typically successful. In both the rat and the mouse, the reaction has been attributed to histocompatibility genes on the Y chromosome. The presence of the "male component" is highly suggestive of Y chromosome activity, and the possibility that it may be involved in the histoincompatibility is presently under investigation.

Fatty Acids from Lipids of Rat Liver Microsomes

B. T. Cole N. G. Anderson

Introduction. — The work of Bucher and McGarrahan[7] has shown that the lipid-rich membranous or vesicular structures of rat liver microsomes are the most probable sites of cholesterol biosynthesis. Redman[8] proposed an effective role of phospholipid in the transport of enzymes across membranes of the endoplasmic reticulum. Hokin[9] demonstrated the ability of diglyceridekinase to catalyze the production of phosphatidic acid from diglyceride and ATP in rat brain microsomes. This work is a part of a continuing effort in the Cell Physiology Section to describe the lipid

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[7]N. L. R. Bucher and K. McGarrahan, J. Biol. Chem. 222, 1–15 (1956).
[8]C. M. Redman, J. Biophys. Biochem. Cytol. 6, 207 (1956).
[9]L. E. Hokin, J. Biol. Chem. 234, 1387 (1959).

characteristics of protoplasm and to correlate this knowledge with function.

Results. — Gravimetric determination of total lipids extractable from lyophilized microsomes from brain, kidney, liver, and testis of rats, shows them to be 20.8, 10.4, 14.6, and 17.6% lipids respectively.

A 322.5-mg sample of lipid, extracted from rat liver microsomes with chloroform-methanol (2:1) at room temperature, was taken up in hexane, placed on a silicic acid column, and eluted in accordance with a scheme devised by Horning,[10] modified by Turner et al.[11] Eleven peaks were obtained, and by various quantitative tests on each peak fraction, total cholesterol, phosphorus, total nitrogen, glycerol, ester linkages, and total lipid were determined. Analysis showed the lipid to be 17.5% cholesterol, 7.75% triglyceride, 6.98% diglyceride, 5.43% monoglyceride, and 62.7% phospholipid. The phospholipid fraction was eluted as six peaks. Specific identification of the phospholipid classes has not been made.

Two-milliliter samples of each peak were taken to dryness under nitrogen and hydrolyzed, and constituent fatty acids were methylated by a procedure described by Turner et al.[11] Qualitative fatty acid analysis was made by gas liquid chromatography under conditions described in Fig. 24. Suitable standards are being formulated for the purpose of fatty acid identification and quantitative analysis. Gas chromatography revealed the presence of nine different fatty acids esterified with cholesterol, ten associated with triglycerides, 15 with diglycerides, and 10, 14, 22, and 22 with phospholipid peaks 6 through 9 inclusive. Phospholipid peak 10 was inadvertently destroyed, and peak 11 gave evidence of containing only six fatty acid moieties. Gas liquid chromatography of fatty acids from peak 5 (monoglyceride) gave a single, large peak with a retention time of 1.11 min, height of 55 mm, and width at half height of 12 mm. The peak was badly "tailed," and repetitive analysis altered neither the peak shape characteristics nor its retention time. Other analyses revealed this peak to contain 5.43% of the total lipid, 0.30 mg of cholesterol, 219 mg of phosphorus, 0 nitrogen, 3.088 mg of glycerol, and 3.32 microequivalents of ester.

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[10]E. C. Horning (1960), in press.

[11]D. A. Turner (1960), personal communication.

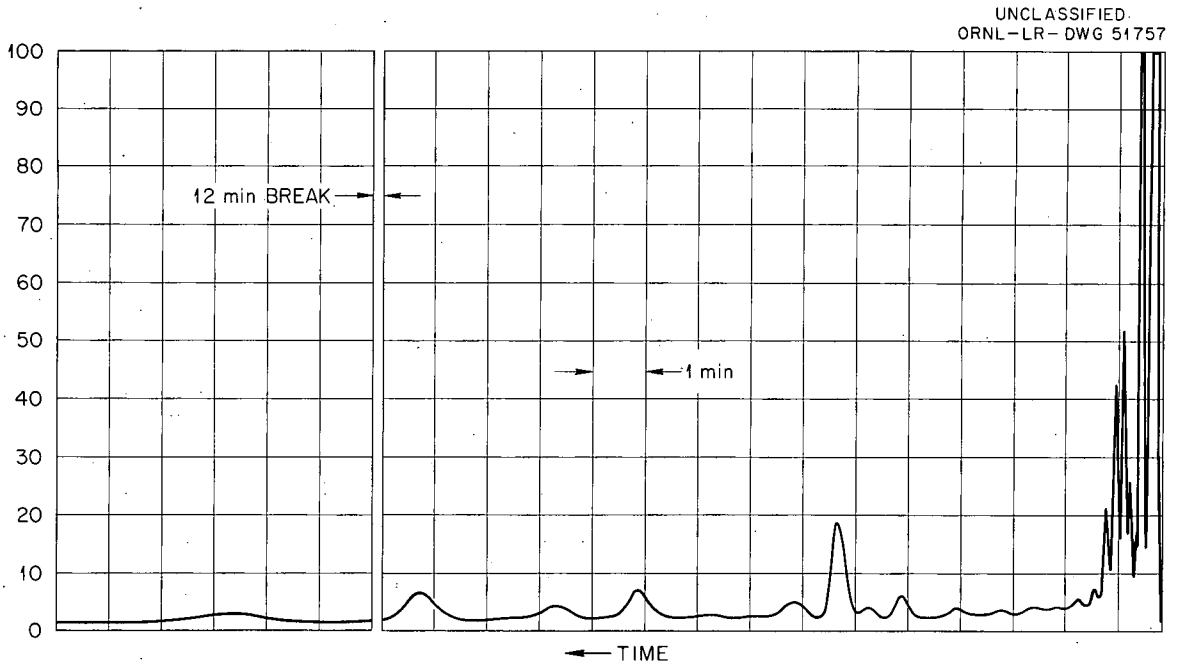

Fig. 24. Gas Chromatogram Showing Qualitative Separation of Fatty Acid Moieties of Phospholipids of Peak 9, Eluted by Silicic Acid Column Separation of Lipids of Rat Liver Microsomes. Packing, ethylene glycol succinate on Chromosorb W 80–100 mesh; 5-1/2-ft column; temperature, 200°C; flow rate, 60 ml/min; filament current, 265 ma; chart speed, 60 in./hr.

Discussion. – MacFarlane, Gray, and Wheeldon[12] report on their gas liquid chromatographic analysis of fatty acids from lecithin and kephalin fractions of silicic acid chromatography that only 12 fatty acids could be separated on Reoplex 400. They do not, however, give the other parameters of their GLC analysis nor the manner in which they esterified their fatty acids following hydrolysis from glycerol. In light of recent work on the importance of lipid moieties in the biologic activities of protoplasm (Bucher and McGarrahan,[7] Palade and Seikovitz,[13] Redman,[8] Hokin and Hokin,[14] Johnson and Albert[15]), it becomes increasingly important to know more of the characteristics of the entities involved.

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[12]M. G. MacFarlane, G. M. Gray, and L. W. Wheeldon, Proc. Biochem. Soc. (1960).

[13]G. E. Palade and P. Siekovitz, J. Biophys. Biochem. Cytol. 4, 557 (1956).

[14]L. E. Hokin and M. L. Hokin, J. Biol. Chem. 234, 1389 (1960).

[15]R. M. Johnson and S. Albert, J. Biol. Chem. 235, 1299–1302 (1960).

Studies on Serum Inhibitors of Lipid Peroxide Formation

A. A. Barber

Introduction. – Many vertebrate tissues possess inhibitors of lipid peroxide formation when added to incubating rat liver homogenates. Large differences in the dialyzability, radiation sensitivity, substrate specificity, and electrophoretic mobility of inhibitors exist. An understanding of the basic mechanism of inhibitory activity is necessary to explain these differences. These studies report the nature of the vertebrate serum inhibitors and the mechanism of their activity.

Results. – All vertebrate sera studied inhibited lipid peroxide formation. Rat and chicken sera possessed both dialyzable and nondialyzable inhibitors, whereas turtle, frog, and fish had only nondialyzable inhibitors. Chicken, turtle, and frog sera had two electrophoretically distinct inhibitors, whereas rat and fish had only one. The rat serum inhibitor was electrophoretically in the β-globulin peak which contains the iron-binding protein transferrin. Iron reversal studies on whole serum and on the isolated inhibitors indicated that the mechanism of inhibition was one of chelation of the catalyst required for lipid peroxide formation by incubated tissue homogenates.

Specific iron-binding components were demonstrated in rat, chicken, turtle, frog, and fish sera by using starch gel electrophoresis and radioactive iron (Fe^{59}). One iron-binding component was identified in all sera except turtle, which had two. Serum iron values and unsaturated iron-binding capacities were determined for rat, chicken, turtle, and frog sera. The values obtained were compatible with the values obtained in the iron reversal studies on inhibitors of lipid peroxide formation. The electrophoretic mobility of the serum iron-binding components was also similar to the electrophoretic mobility of the inhibitors of lipid peroxide formation. The mechanism of inhibition in all sera therefore appears to be iron binding.

Although the mechanism of inhibition by all sera appears to be iron binding, there exist specific physicochemical differences in the inhibitors from various sera. The serum inhibitors from rat, chicken, turtle, and frog have different affinities for ion exchange resins (DEAE), different

pH dependence, and different electrophoretic mobilities. Further studies on the chemical nature of the active site of inhibition are being carried out.

Discussion. — Whole-body irradiation does not affect the inhibitory activity of serum but does destroy the activity of the intestinal mucosa. This can now be explained on the basis of the different mechanism of action of these inhibitors. The inhibitor in the mucosa has been identified as a phospholipase whose activity disappears following whole-body irradiation.[16] The activity of the blood serum, however, depends on the functional capacity of the iron-binding proteins, which have been shown to be radiation insensitive.[17] These studies have indicated the importance of examining the mechanisms of tissue inhibition of autoxidation reactions.

ATPase and Apyrase Determination

M. L. Barber N. G. Anderson

Introduction. — A rapid method is required for the determination of ATPase and apyrase activity in muscle and other tissue extracts based on the ion exchange separation of adenosine, AMP, ADP, and ATP. The method should be rapid, qualitative, and quantitative.

Results. — Dowex 1, 8% cross-linked, 200–400 mesh with fines removed, was used, following at first methods developed by Cohn and Carter. The variables studied were: (a) flow rate (controlled by an all-fluorocarbon-plastic pump), (b) temperature, 25–60°C, (c) column length, and (d) composition of the eluting solution.

As shown in Fig. 25, clean separations of ADP and ATP from adenosine and AMP have been achieved in as little as 12 min at 60°C using a 50-ml gradient, starting with 0.015 M HCl and 0.2 M NaCl, grading linearly into 0.15 M $CaCl_2$ and 0.01 N HCl, using a 0.9 × 15 cm column.

Further studies will probably enable separations to be made in an even shorter length of time.

Discussion. — A rapid method suitable for separation of as little as 0.05 micromolar amounts of adenosine nucleotides has been perfected.

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[16] A. Ottolenghi and F. Bernheim, Radiation Research 12, 371 (1960).
[17] A. Chanutin and S. Ludewig, Am. J. Physiol. 166, 380 (1951).

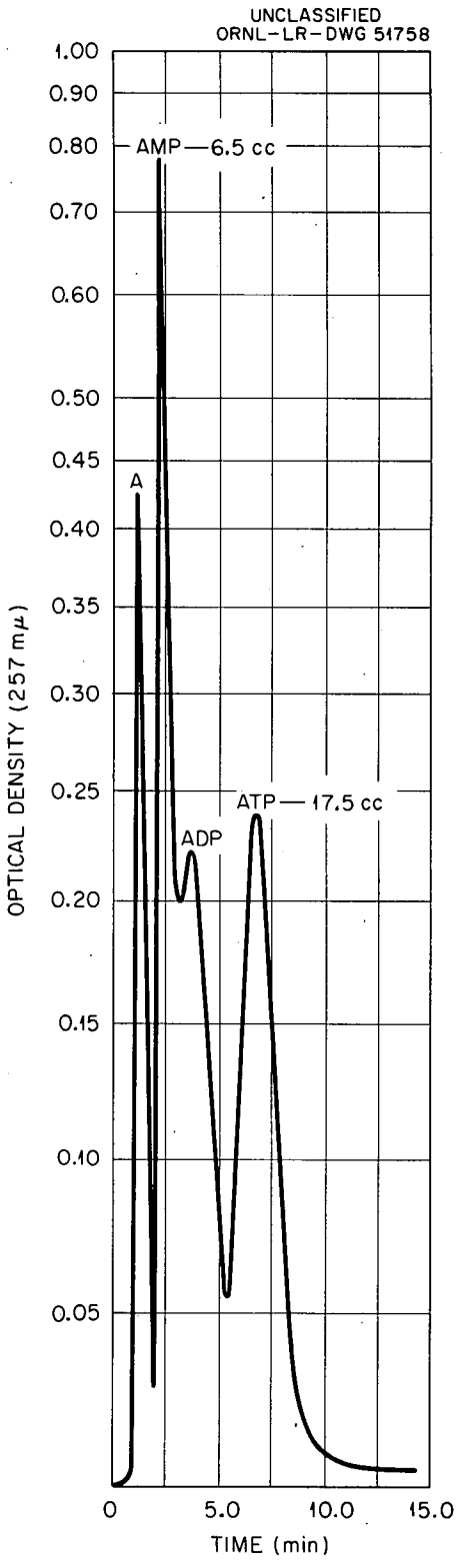

Fig. 25. Adenine Nucleotide Separation.

Preparation of Rat Serum Macroglobulins

R. E. Canning

Introduction. — In order to determine the partial specific volume, diffusion coefficient, and the amino acid analysis of rat serum macroglobulin (S20), it is necessary to obtain a larger quantity of the protein than previously obtained by any method.

Results. — We decided to use diethylaminoethyl cellulose (DEAE) column chromatography for this purpose. A pH and ionic strength gradient was worked out which separated rat serum into approximately 14 components when read at 280 mμ. When individual tubes from the fraction collector were examined using the Ouchterlony agar-diffusion method, the macroglobulin and albumin fractions were very close together. Therefore, we made a 40% ammonium sulfate (AmS) precipitate fraction of rat serum which would contain very little albumin. Using a Tris-phosphate, pH 8.0 gradient, the 40% AmS precipitate fraction was separated into two large fractions and one small. One of the large fractions is a γ-globulin, while the other is macroglobulin.

Discussion. — It is now planned to make a large 50% AmS precipitate fraction of rat serum and to use a large preparative column to separate out the macroglobulin in quantities sufficient so that the partial specific volume, diffusion coefficient, and amino acid analysis determinations can be made.

Automatic Sampling from Tiselius Electrophoresis Cells

A. A. Barber M. L. Barber N. G. Anderson

Introduction. — The localization of biological activities in complex free solution electrophoresis diagrams has been a tedious and often inaccurate procedure. A method has therefore been devised for moving sampling needles slowly down through both limbs of the electrophoresis cell while simultaneously pumping out a fixed ratio of protein solution and overlying buffer through a small peristaltic pump. The method has been adapted to the localization of inhibitors of lipid peroxide formation in several vertebrate sera.

Results. – Frog serum inhibitors of lipid peroxide formation have been previously isolated using continuous flow electrophoresis on paper.[18] However, the electrophoretic separation of this serum in free cell electrophoresis results in more distinct separations of the serum components. Following free cell electrophoretic separation of the serum, a sampling needle advancing at the rate of 1.1 mm/min was introduced into the 11-ml quartz cell (both left and right limbs), and 0.6-ml samples were collected throughout the entire length of the cell. Thirty-four samples were obtained and analyzed for inhibitory activity. Photographs were taken of the needle progression, and the localization of inhibitory activity was compared with the position of the advancing needle. By this means the exact location of the inhibitors in the electrophoretic pattern was obtained as shown in Fig. 26. The results demonstrated two electrophoretically distinct inhibitors of lipid peroxide formation in frog serum, and their localization confirmed results obtained using the continuous flow paper curtain electrophoresis.

[18] A. Barber, Arch. Biochem. Biophys., in press.

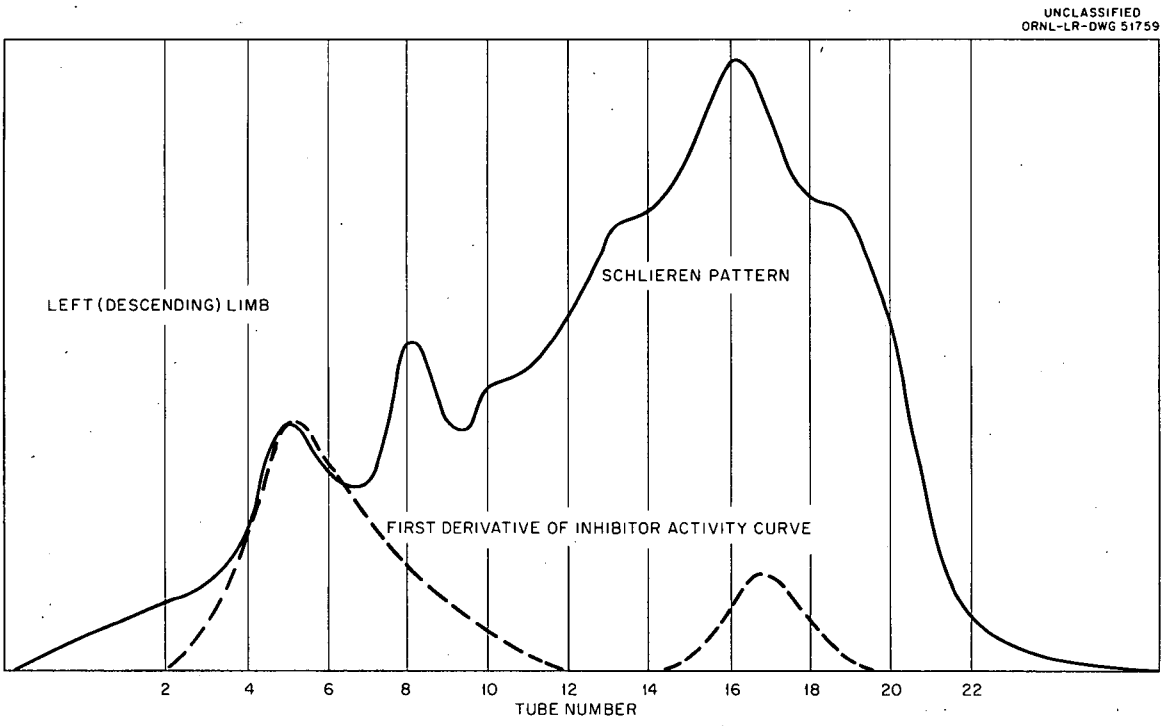

Fig. 26. Localization of Inhibitory Activity in Electrophoretic Pattern by Automatic Sampling.

Discussion. — A precise method of automatic sampling from free solution electrophoresis cells has been devised. The results obtained on the separation of frog serum inhibitors of lipid peroxide formation were comparable with those obtained using continuous flow paper curtain electrophoresis. The advantage of the present technique is that smaller samples can be analyzed with greater resolution and without the degradation often accompanying electrophoresis on paper.

Effects of Subzero Temperatures on Yeast Cells

P. Mazur

Introduction. — One aspect of the study on the effects of subzero temperatures on yeast has been to ascertain the relation between cell viability and physical and temporal factors such as temperature, rate of cooling and warming, and the presence of ice crystals in the system. Among the findings reported in the previous semiannual report,[19] one of the more striking was the sharp decrease in survival that occurs between −10 and −30°C when the water surrounding the cells is frozen. This decrease does not occur when the water remains unfrozen, that is, supercooled.[20] The relation between survival and temperature and the physical state of the suspending medium has been investigated further.

A second aspect of the study has been to determine some of the manifestations of injury in cells rendered nonviable by low-temperature exposure. Alterations in the morphology, size, volume, density, and solids concentration in "frozen and thawed" cells have been observed.

Results. — The drop in survival of yeast cells was found to occur between −10 and −30°C regardless of whether the cells were suspended in deionized distilled water or in 0.1 M solutions of KH_2PO_4, NaCl, or $CaCl_2$. Solidification of these four liquids (with and without cells) was found on the basis of electrical conductivity measurements to be completed at 0.3, −11, −30, and −70°C, respectively. Hence, there was no correlation between the temperatures at which freezing of the suspending medium was complete

[19] P. Mazur, Biol. Semiann. Prog. Rep. Feb. 15, 1960, ORNL-2913, p 100–103.

[20] P. Mazur, Ann. N.Y. Acad. Sci. 85, 610 (1960).

and the −10 to −30°C range in which survival dropped. The lack of correlation indicates that extracellular ice formation was not the cause of death even though it was a prerequisite for death.

When yeast cells are cooled rapidly to −30°C and warmed slowly, fewer than 0.01% survive. Correlated with this loss in survival were the following: (1) Cell volume decreased to about half that of uncooled cells. Volumes were measured directly by a "hematocrit" technique and estimated also by direct micrometry of cell diameters. (2) The density of the dead cells was greater than that of the control cells (approx 1.27 vs 1.16 g/ml). Densities were estimated by measuring the sedimentation rate of individual cells under $1 \times g$ and applying Stokes's law. (3) The large conspicuous vacuole in normal living yeast was absent in yeast killed by low-temperature exposure. (4) The concentration of solids in the "frozen-thawed" cells was greater than that of untreated living cells on the basis of interference microscope measurements. (5) The decreased volume of the treated cells was accompanied by or was a result of loss of water from the cells, water that contained about 7% solids as estimated from refractive index measurements of the exudate. In spite of these alterations and the very small percentage of survivors, the total numbers of cells (living and dead) in the suspensions remained unchanged, indicating that the low-temperature exposure did not result in cell disintegration.

Discussion. — The death of yeast cells is believed to be a result of ice crystal formation within the cell. The finding that death occurred between −10 and −30°C regardless of the temperature required for the complete solidification of the external suspending medium further supports such a hypothesis.

The observed alterations in cell morphology, composition, volume, and density suggest that death may be associated with increased cell permeability. But there is no way of knowing at present whether increased permeability is the cause of death or a post-mortem alteration. Also unknown are the mechanisms by which the postulated intracellular freezing might produce the manifestations of injury that are observed.

Some Effects of Cytoplasmic Proteins on Nuclei and Their Implications for Embryonic Differentiation

H. D. Swanson

Introduction. — It was previously shown that the average net charge on the proteins of the soluble phase of several organs[21] was correlated with nuclear size, in that liver and kidney, with relatively basic soluble proteins, contain diploid nuclei of about a quarter the volume of the most abundant diploid nuclei of brain, which has relatively acid proteins. As might possibly be predicted from this correlation, brain nuclei placed in liver protein decreased significantly in volume; liver nuclei placed in brain protein increased significantly in volume; while nuclei did not change volume when kept in homologous proteins.[22] These experiments with the whole soluble phase have now been extended to include the effects of the more basic soluble-phase proteins on nuclei.

Results. — Isolated nuclei of rat liver were incubated up to four days at 0°C in (1) rat liver soluble phase, (2) the basic fraction from rat liver,[23] and (3) several serum proteins, each previously dialyzed against a buffered sucrose solution. Only nuclei incubated in basic fraction showed a progressive shrinking, so that nuclear volume after four days was decreased by a factor of 2. Studies with the interference microscope indicated that in basic fraction and in γ-globulin (which did not shrink nuclei) the apparent nuclear dry mass was approximately doubled from about 3.5 to 7×10^{-6} g within a few minutes after the beginning of incubation, and that such nuclei were resistant to the action of DNase.

Discussion. — The data indicate that the basic proteins of the cytoplasm are present in different proportions in different tissues, probably help to establish the characteristic nuclear size of a tissue by direct action within the nucleus, and inhibit the interaction of nuclear DNA with its environment.

It is proposed that cytoplasmic basic proteins may contain tissue-specific arrays of protein species specific for portions of the genome,

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

[22] H. D. Swanson, Biol. Semiann. Prog. Rep. Feb. 15, 1960, ORNL-2913, p 103.

[23] H. E. Bond and N. G. Anderson, Biol. Semiann. Prog. Rep. Feb. 15, 1960, ORNL-2913, p 99.

and that the gene-inhibiting actions of these basic proteins are ultimately responsible for the stable differences among tissues. A hypothetical model describing the establishment and maintenance of differentiated cells has been developed, indicating how progressive genic inhibition by seven pairs of mutually exclusive basic protein could account for the epigenetic production of 128 stable cell types.

Fowl Serum Proteins: Albumin

E. C. Horn R. E. Canning
R. R. Becker E. W. Horn
S. L. Miller

Introduction. — Isolation and characterization of adult fowl serum proteins has been undertaken as a prerequisite to the study of their developmental history. To date fowl serum albumin has proved the least difficult to isolate by relatively gentle methods, namely, a stepwise progression through AmS precipitation, ultracentrifugation, and preparative paper curtain electrophoresis.

Results. — The product, which is bright yellow in solution, has been studied serologically, electrophoretically, and ultracentrifugally. In each of these tests only one component is detectable. The following are some physical data established for this preparation:

Electrophoretic mobility	−4.3 at pH 9.92, 0.1 ionic strength, and 0.9°C
Isoelectric point	pH 4.61
Apparent specific volume	0.717
Diffusion coefficient	6.39×10^{-7}

The amino acid analysis is given in Table 17.

The yellow color is difficult to remove from the albumin, but TCA precipitation followed by ether extraction separates most of the pigment in the ether phase. Spectrophotometric analysis of this solution shows it to have absorption maxima identical with those of β-carotene.

Discussion. — Rabbit antisera elicited by fowl serum albumin will be used to detect qualitatively and quantitatively serum albumin of the developing avian embryo. The completed physicochemical analysis will allow a reasonable basis for comparing adult with embryonic serum albumin.

Table 17. Amino Acid Analysis of Fowl Serum Albumin

Amino Acid	Micromoles per Milligram of Nitrogen	Amino Acid	Micromoles per Milligram of Nitrogen
Lysine	2.529	Histidine	0.772
Arginine	1.529	Aspartic acid	3.184
Threonine	1.306	Serine	2.048
Glutamic acid	4.728	Proline	1.748
Glycine	1.461	Alanine	2.466
1/2 Cystine	0.155	Valine	2.100
Methionine	0.965	Isoleucine	1.493
Tyrosine	1.104	Leucine	2.289
Phenylalanine	1.536	Ammonia	3.262

Basic Proteins of Frog Oocytes

E. C. Horn R. E. Canning
R. R. Becker E. W. Horn
S. L. Miller

Introduction. — Histochemical examination of frog oocytes and of the cells of the early frog embryo showed large quantities of substances in the yolk platelets which reacted to selective staining like basic proteins. The nuclei of these same cells failed to give the same reaction. At the beginning of gastrulation, however, these same nuclei demonstrate a positive staining reaction. During early organogenesis the yolk platelets, though still perfectly visible in most tissues of the embryo, react only weakly to the stain or not at all, suggesting a loss of some basic protein to the rest of the cell and/or to the nucleus. Extraction of material from the oocytes was attempted in order to establish by criteria more exacting than the staining reaction the presence of basic protein.

Results. — Large quantities of coelomic oocytes were acid-extracted with mild homogenization. The ultracentrifugal soluble portion was fractionated by progressive increases in pH, each followed by centrifugal separation. The soluble product at pH 7.0–7.5 was analyzed electrophoretically at pH 7.8 and found to consist of at least four components, two of which migrated toward the cathode.

Ultracentrifugal analysis showed three components, with estimated s_{20} values of 5.78, 3.87, and 1.62.

The fraction of lowest molecular weight was isolated by prolonged ultracentrifugation and subjected to amino acid analysis (Table 18).

Table 18. Amino Acid Analysis of Fraction of Lowest Molecular Weight

Amino Acid	Micromoles per Milligram of Nitrogen	Amino Acid	Micromoles per Milligram of Nitrogen
Lysine	4.559	Cysteic acid	?
Histidine	1.898	Aspartic acid	6.195
Arginine	2.288	Threonine	3.195
Serine	2.593	Glutamic acid	5.992
Proline	2.966	Glycine	3.534
Alanine	2.610	1/2 Cystine*	3.173 (?)
Valine	2.881	Methionine	0.771
Isoleucine	2.068	Leucine	2.246
Tyrosine	2.017	Phenylalanine	1.958
		Ammonia	7.229

*Two peaks overlapping (both cystine?).

The relative amounts of lysine and arginine suggest a basic protein.

Discussion. — The results indicate that basic proteins similar to histones are laid down in the yolk platelets of maturing frog oocytes and that during subsequent development, particularly during gastrulation and thereafter, these proteins move to the nuclei. If these observations and conclusions are correct, it may be hypothesized that these proteins operate in the nucleus at specific loci on the chromosomes in such a way as to determine the fate of future protein synthesis in particular cells and, hence, in their progeny. Such a mechanism might operate either by limiting specific portions of the genome ("blocking") or by providing the necessary prerequisites at specific chromosome sites to elicit specific activity.

NUCLEIC ACID CHEMISTRY

W. E. Cohn
F. J. Bollum
J. X. Khym
B. C. Pal[1]
A. J. Bandy

Nucleic Acid Chemistry

W. E. Cohn A. J. Bandy

Pseudouridine 5'-Phosphate. — The earlier finding of a 5'-phosphate form of pseudouridine in RNA hydrolyzed by snake venom diesterase, while settling the question of its occurrence in RNA, did not offer a practical source of this nucleotide for the attempt to produce di- and triphosphate forms of the new nucleotide as possible precursors of the RNA pseudouridine. Two other possible sources have now been examined, a crude commercially available digest from the Schwarz Laboratories, and an acid-soluble extract of a large amount of calf liver. The former, which contains the 5'-phosphates of the usual RNA nucleotides, does contain the 5'-phosphate of pseudouridine in the amounts expected from the crude RNA used as a substrate. In the latter (the liver acid-soluble fraction), nothing corresponding to pseudouridylic acid was found.

This negative result raises the question as to whether free pseudouridine 5'-phosphate is a precursor nucleotide for RNA as are all the other 5'-phosphates.

Tritiation of Pseudouridine. — A labeled pseudouridine would be useful for tracer studies, including the establishment of the nucleoside as a precursor of itself in RNA, and possibly as a specific RNA label. A large-scale tritiation of about 50 mg of our pseudouridine was performed by the Schwarz Laboratories, using a catalytic exchange process. We then submitted the material to detailed analysis. Although the tritiation seemed at first to have yielded material of about 100 μc/micromole, chromatography indicated that only about 1% of this activity was in what was left of the original pseudouridine C tritiated. Roughly equal amounts were found to appear at places on paper and on column chromatograms that were inseparable

[1]Research participant.

from added uracil, 5-hydroxymethyluracil, pseudouridines A and B, and hydrogenated (reduced) pseudouridine (5-ribityluracil). The isomeric A and B forms were not unexpected, but the appearance of high specific activities in the two bases and in reduced pseudouridine raises some interesting problems of specific tritium reactions. (Essentially no ultraviolet absorbance was detectable in these fractions, although the tritium activity was as much as that appearing in the many milligrams of pseudouridine C.) The active substances are being purified for further study and use.

Column Chromatography. – One of the inherent difficulties in ion exchange nucleotide preparation is that substantial quantities of ionic materials (salts, acids, bases) are left in the products. This has led to the general use of volatile materials, but salt volatility is not always sufficient to make the materials removable under conditions that are not deleterious to the products, and acids are usually a chemical hazard to nucleotide materials. Acetate and formate systems have achieved widespread popularity because their ammonium salts are somewhat volatile, but they still leave much to be desired in this respect.

Expanding the recovery procedure worked out for pseudouridylic acid, we have developed a separation scheme for the mono-, di-, and triphosphates of the common nucleosides and for ortho- and pyrophosphate, with ammonium bicarbonate as the eluting salt. This salt is completely volatile at low temperatures (under 60°) and at neutral pH (about 8). Alternatively, it can be removed with a sulfonic acid resin. The separated nucleotides are thus recovered in water solution.

This problem has been worked out in conjunction with F. J. Bollum and was motivated by his need for triphosphate separations.

Reactions of Periodate-Oxidized Nucleotides

J. X. Khym

Introduction. – As a corollary to the completed investigations of periodate-oxidized nucleosides,[2] experiments are now in progress to characterize more fully periodate-oxidized nucleoside 5'-phosphates. In the

[2] J. X. Khym and W. E. Cohn, J. Am. Chem. Soc., in press.

investigations mentioned, it was found that periodate-oxidized nucleosides show entirely different chromatographic properties after treatment with glycine buffer in contrast to treatment with solutions of sodium hydroxide or borax. This suggested the formation of a complex between the dialdehyde compounds and glycine. This concept is now being explored with particular attention being given to periodate-oxidized nucleoside 5'-phosphates.

Results. — Preliminary experiments indicate that periodate-oxidized adenosine 5'-phosphate forms a complex not only with glycine, but also with ammonia and primary amines, and that phosphate is not eliminated during the formation of these complexes. It appears that these complexes release inorganic phosphate slowly in the pH range of 8 to 11 and rapidly when the compounds are acidified.

Discussion. — These initial experiments seem to demonstrate that the release of inorganic phosphate from periodate-oxidized nucleoside 5'-phosphates occurs by a more complex mechanism than reports in the literature have indicated.

DNA Synthesis by Mammalian Enzymes

F. J. Bollum

Replication of DNA by the purified enzyme system from calf thymus gland has been examined by three criteria: (1) comparison of base incorporation ratios in primer DNA and product, (2) examination of physical and chemical properties of products synthesized in the presence of ϕX-174 (single-stranded) DNA, and (3) testing for biological activity (bacterial transformation) in enzymically synthesized products.

The comparison of base incorporation ratios with a variety of primers[3] indicates that DNA product reflects primer composition. Physical studies (performed by R. L. Sinsheimer, California Institute of Technology) show that the product formed from ϕX-174 DNA (single-stranded) has a melting curve similar to double-stranded DNA (Tm of products $\cong 80°$ in 0.1 M NaCl), while sedimentation measurements show that average molecular weight decreases from 1.5×10^6 to 0.5×10^6 during the enzymic synthesis as a result of contaminating DNase action. Synthetic products formed from ϕX DNA

[3]F. J. Bollum, J. Biol. Chem. 235 (August 1960).

do not act as primers for DNA synthesis unless subjected to denaturation, giving further support to the idea that the products are double-stranded. Attempts to synthesize biologically active DNA (transformants assayed by D. M. Green of the Cytology and Genetics Section) have failed, presumably also due to nuclease action. When synthetic activity and destruction of transforming activity are assayed in a series of samples from DEAE- or CM-cellulose columns, the resultant curves do not correspond precisely, and this indicates that it is possible to separate the synthetic activity from nuclease activity.

The evidence available suggests that DNA duplication is occurring in the enzyme system. Rigorous proof will be available only when precise duplication and synthesis of biologically active material is accomplished. Such an accomplishment depends upon obtaining a synthesizing enzyme essentially free of DNase activity.

NUCLEIC ACID ENZYMOLOGY

E. Volkin
L. Astrachan
T. N. Fisher[1]
M. H. Jones
K. H. Stephenson

RNA Function in Phage-Infected Bacteria

E. Volkin M. H. Jones

Introduction. — We have previously described experiments with the Escherichia coli mutant B94 which demonstrate a requirement for adenine and arginine for both growth and capacity to produce bacteriophage.[2] Although deoxyadenosine cannot ordinarily replace adenine in supporting T2 production, preincubation of infected bacteria in synthetic medium containing adenine alone will subsequently allow a definite, though limited, synthesis of T2 phage in media containing deoxyadenosine plus arginine, or arginine alone. The biochemical data lead us to believe that the synthesis of a specific RNA during incubation in adenine is responsible for the phage production. In the present report, a method is described for improving the yield of T2 phage upon incubation in deoxyadenosine:arginine medium. Experiments are also described which indicate that (1) "phage-specific" RNA is synthesized in the absence of protein synthesis, and (2) little, if any, RNA is synthesized during incubation in deoxyadenosine:arginine.

Results. — Centrifuging and washing infected cells, after incubation in adenine, in our medium (Tris, pH 8.0) results in about a 50% loss in newly labeled RNA, although there is no significant loss in infected bacteria. This loss can be largely prevented by adding phosphate buffer (final concentration 1/15 M, final pH = 6.5) before centrifuging and to the deoxyadenosine:arginine culture. As a result of this process, phage yields in deoxyadenosine:arginine are increased about 2–3 fold.

To test for the possibility that some protein synthesis may take place during incubation of infected cells in medium containing adenine alone, and thereby result in some RNA synthesis, we have added the protein synthesis inhibitor chloramphenicol to such cultures. The data from

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[1]Research participant.

[2]E. Volkin and M. H. Jones, Biol. Semiann. Prog. Rep. Feb. 15, 1960, ORNL-2913, p 110–11.

these experiments show that, though there is some time delay in T2 production, the ultimate phage yields in either deoxyadenosine:arginine or adenine:arginine are similar to the control cultures in which no chloramphenicol was added to the adenine preincubation.

The pyrimidine analog 6-azauracil completely inhibits T2 production by B94 incubated in complete medium (adenine plus arginine). However, infected bacteria preincubated in adenine alone produce equivalent phage yields (about 20 phages per bacterium) in subsequent culture in deoxyadenosine:arginine plus 6-azauracil as the latter medium without 6-azauracil. In addition, infected bacteria subsequently cultured in complete medium (adenine plus arginine) containing 6-azauracil produce yields of about 20 phages, instead of the normal yield of around 200. These experiments indicate that although 6-azauracil effectively inhibits any further RNA synthesis, the RNA that was preformed during the incubation in adenine medium will remain functional for phage production.

Conclusions. — A technique has been developed for preserving RNA synthesized in the absence of protein synthesis, thereby increasing subsequent phage yields. Experiments utilizing chloramphenicol reinforce the conclusion that protein synthesis is not necessary for the synthesis of RNA during incubations in adenine alone. Experiments with 6-azauracil indicate that no RNA synthesis occurs in deoxyadenosine:arginine cultures, after removal of adenine, but the preformed, specific RNA remains productive in its role in phage synthesis.

Resemblance of RNA and DNA in Phage-Infected Bacteria

L. Astrachan K. H. Stephenson

Introduction. — In experiments with Escherichia coli infected with bacteriophage T2, we have demonstrated the synthesis of an RNA which resembles the base composition of the infecting phage DNA. This evidence came from experiments in which P^{32}-labeled orthophosphate was incubated with phage-infected bacteria, following which the RNA was isolated and hydrolyzed with alkali and the specific activities of the resulting mononucleotides were determined. The distribution of radioactivity among the RNA mononucleotides paralleled the base composition of the phage DNA. It was recognized that the distribution of radioactivity can only reflect the

chemical composition of RNA if the precursor specific activities were equal. Since RNA was hydrolyzed with alkali before the mononucleotides were isolated, it was reasoned that even if the original acid-soluble 5'-nucleotide precursors had different specific activities, the isolated 2',3'-nucleotides would randomly select radioactive phosphorus from high- and low-specific-activity neighboring nucleotides. In this way the distribution of activity should reveal the chemical composition of the RNA being synthesized. This assumption of randomization has been tested and found valid by the experiments reported here.

Results. — Escherichia coli in the log phase of growth was infected with T2 bacteriophage. Three minutes after infection P^{32}-labeled orthophosphate was added, and aliquots of the culture were taken at various times thereafter. RNA was isolated from each aliquot and then divided so that half was hydrolyzed with alkali to yield 2',3'-mononucleotides and half was hydrolyzed with diesterase to yield 5'-mononucleotides. In addition, the acid-soluble material was fractionated to yield the 5'-mono-, di-, and triphosphates of ribo- and deoxyribonucleotides. It was found that the 2',3'-mononucleotides from RNA maintained a constant distribution of radioactivity and the distribution resembled the base composition of phage DNA. On the other hand, the distribution of activity among the 5'-mononucleotides from RNA varied greatly with time after infection. In the initial aliquots the radioactivity distribution did not resemble phage DNA, but with time the distribution changed gradually and eventually did resemble phage DNA. This suggested that the acid-soluble 5'-nucleotide precursors initially had widely different specific activities but later became uniform. Analysis of the acid-soluble precursors verified this point, and comparison of the precursor specific activities with the RNA 5'-nucleotide activities led to the calculation that the RNA synthesized after phage infection does reflect the composition of phage DNA.

Discussion. — All direct evidence about the synthesis of RNA indicates its synthesis from 5'-nucleotide precursors. Regardless of the number of phosphorus atoms on the precursor, only the proximal phosphorus enters RNA. When radioactive RNA is hydrolyzed with diesterase to yield 5'-mononucleotides, the distribution of activity depends on the composition of the RNA and the specific activities of the proximal phosphorus in the

precursors. Direct determination of the latter allows the calculation of RNA composition. It was found that at various sample times, when the precursor and RNA-derived 5'-mononucleotide activities varied greatly, comparison of the two always resulted in the same calculated composition of RNA, a composition which resembles phage DNA. This composition can be more easily, if indirectly, determined by alkaline hydrolysis. It appears safe to assume then that alkaline hydrolysis does result in sufficient randomization of precursor specific activities to allow a calculation of base composition from activity distribution. This finding is applied in the following report.

Resemblance of Bacterial RNA and DNA

L. Astrachan T. N. Fisher K. H. Stephenson

Introduction. — From the well-established concept that DNA is the hereditary substance, the question arises as to how the genetic information stored in DNA is transmitted to the sites of protein synthesis. A number of lines of evidence indicate that RNA has several important qualifications for this messenger service. Data from several microorganisms indicate that a small part of the cell RNA has the same base composition as the cell DNA, a similarity most suitable for the transmission of information from DNA. The evidence for this similarity is fragmentary, and it is to the amplification of this point that the present report is directed. Two organisms, Proteus vulgaris and Psuedomonas aeruginosa, were selected for the demonstration that some part of the cell RNA reflects the base composition of the cell's DNA. Although both organisms have essentially the same base composition in their total RNA's, the DNA's are greatly different. Proteus vulgaris is an A-T organism (this notation indicates that the DNA contains more adenine plus thymine than guanine plus cytosine) and Psuedomonas is a G-C organism (more guanine plus cytosine than adenine plus thymine). The working hypothesis for these experiments is that a small pool of DNA-like RNA exists, and this specific RNA is synthesized at approximately the same rate as the bulk of the cell's RNA, but that turnover limits the size of the pool. These assumptions were consistent with the data obtained.

Results. — Proteus vulgaris and Pseudomonas aeruginosa were separately grown in a low-phosphate broth. Aliquots of the culture were taken at various stages in the growth curve. Each aliquot was incubated with P^{32}-labeled orthophosphate for 30 sec and then immediately chilled and acidified. RNA was isolated from the bacteria and hydrolyzed with alkali. The mononucleotides were separated and purified before the total radioactivity associated with each mononucleotide was calculated. It was found that the distribution of label in the RNA mononucleotides matched the base composition of the DNA. Thus, in the A-T organism the counts associated with adenylic and uridylic acids were 1.5 times the counts in guanylic and cytidylic acids. In the G-C organism, guanylic and cytidylic acids contained the bulk of the radioactivity. Furthermore, the rules of DNA structure were obeyed by the radioactive RNA in that the counts in adenylic equaled the counts in uridylic and the counts in guanylic equaled the counts in cytidylic. In experiments with E. coli (A-T = G-C), the specific activities were, as expected, equal in the four nucleotides.

Discussion. — In our procedure in which RNA is hydrolyzed with alkali, we randomly select radioactive phosphorus from high- and low-specific-activity neighboring nucleotides. In this way the counts associated with each nucleotide are a measure of the amount of that nucleotide synthesized and the distribution of counts reflects the base composition. With the latter statement in mind, the data obtained indicate that a part of the cell RNA does reflect the base composition of the cell DNA.

CHEMICAL PROTECTION AND ENZYME CATALYSIS

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

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

Introduction. — Modifications of the basic structure of the aminoalkylisothioureas have been made in an effort to obtain radiation protective compounds with improved properties and to gain some insight as to how they function. In addition, several other compounds that could possibly function by one of the three current mechanisms for chemical radiation protection have been tested for protective activity as possible additives in mixtures of protective agents.

Results. — In view of the effectiveness of S,3-aminopropyl-N'-methylisothiourea dihydrobromide at radiation doses less than 1200 r, various substitutions in the amidine group of S,2-aminoethyl- and 3-aminopropylisothiourea (AET and APT) were made and tested both orally and intraperitoneally at 900 r. All the derivatives that were made rearranged in the normal manner to yield the corresponding mercaptoalkylguanidines. Substitution of an N'-ethyl group in place of the methyl increased the toxicity of both compounds without improving the protective activity. The addition of further methyl groups on the amidine nitrogen atoms both increased the toxicity and practically abolished the protective activity. Compounds such as S,2-aminoethyl- and 3-aminopropyl-N'-methyl-N''-hydroxyethylisothiourea were less toxic than the N'-methyl compounds as well as less active, although essentially complete 30-day survival could be obtained at the maximum compound dose level. The aminoalkyl N'-D-glucosylisothioureas as well as N-D-glucosylisothiourea itself were both nontoxic and completely inactive. Mono S,2-aminoethyl- and 3-aminopropylisodithiobiureas were also inactive, while the corresponding N'-guanylisothioureas exhibited marginal protective activity.

Bis(2-aminoethyl) sulfide, sulfone, and sulfoxide, though nontoxic compared to the parent compound cystamine, were completely ineffective

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as protective agents. 5-Hydroxytryptophane, N-acetyl-DL-homocysteine-thiolactone, 3-aminopropylthiosulfuric acid, D-penicillamine, and a series of dithiooxamides also provided no 30-day survival when given at their maximum compound dose level. 2-Aminoethylthiosulfuric acid and L-, D-, and DL-homocysteinethiolactone gave less than 50% survival at their maximum dose levels.

Conclusion. — Relatively small changes in the molecules of the parent compounds AET and APT markedly affect the protective activity. The introduction of hydrophilic groups into the molecule on the amidine nitrogen atoms reduces the toxicity but unfortunately also lowers the protective activity, while hydrophobic groups practically abolish protective activity.

Enzyme Substrate Complex Formation

F. C. Grimm D. G. Doherty

Introduction. — Further improvements in the protein fractionation procedure[2] have yielded a 90% pure beef heart malic dehydrogenase in reasonable quantity, and structural studies have been initiated.

Results. — Characterization of the molecule by physical chemical methods has shown it to have a sedimentation constant of 4.3×10^{-13} (at 20°C and infinite dilution), a partial specific volume of 0.71 ml/g, and an isoelectric point at pH 6.2. Contrary to previously reported results with a less pure enzyme,[3] it appears to have a rather low extinction at 280 mμ, $E^{1\%}_{1\ cm}$ 2.7. Kinetic studies on this malic dehydrogenase have shown that it will catalyze very slowly the DPN-dependent oxidation of mesotartrate and L-tartrate (unnatural form). At pH 9 the apparent K_S value for L-malate is 5×10^{-3} M, while the K_S value for DPN under the same conditions is 3.4×10^{-4} M. In addition to the above malic dehydrogenase the fractionation procedure has uncovered a new form of malic dehydrogenase in the supernatant cellular fraction of beef heart. This new enzyme is characterized by an entirely different electrophoretic

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[2]F. C. Grimm and D. G. Doherty, Biol. Semiann. Prog. Rep. Feb. 15, 1960, ORNL-2913, p 116.

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

behavior as well as different K_S values and relative rates with DPN and its analogs.

Conclusion. — Diffusion studies are in process to allow an estimation of the molecular weight of the purified enzyme. These values are essential for characterization of the protein and the study of the chemistry of the active site. The interesting new form from the supernatant fraction is being subjected to further purification and characterization. In view of the differences in charge and kinetic activity of the two enzymes, a comparison of their structure, particularly in the vicinity of the active site, should prove to be of considerable interest in understanding the mechanism of enzyme action.

ENZYMOLOGY

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Cell-Free Synthesis of β-Galactosidase

T. Kameyama W. H. Taylor, Jr. G. D. Novelli

Introduction. – During an investigation of the inhibition of the induced formation of β-galactosidase in cells of Escherichia coli by ultraviolet irradiation and the reversal of inhibition by photoreactivation, the presence of a particle-bound β-galactosidase was revealed by electrophoresis of cell extracts on Geon.[7] Further analysis of the behavior of the particle-bound enzyme during induction and after removal of the inducer gave results suggesting that the particle might be the site of synthesis of this enzyme.[8,9] The present experiments were therefore undertaken to determine whether the particle was capable of carrying out the synthesis of β-galactosidase in a cell-free preparation.

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[1]Research participant.

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

[8]T. Kameyama and G. D. Novelli, Biol. Semiann. Prog. Rep. Feb. 15, 1960, ORNL-2913, p 119.

[9]T. Kameyama and G. D. Novelli, Bacteriol. Proc. 60, 148 (1960).

Results. – Extracts were prepared from preinduced cells of E. coli by passage through a French pressure cell. Cells and cellular debris were removed by centrifugation at 8000 × g for 60 min followed by another centrifugation at 30,000 × g for 90 min. The supernatant was now centrifuged at 105,000 × g for 30 min to yield a particle fraction P_{30}. The supernatant was again centrifuged at 105,000 × g for 90 min to yield a second particle fraction P_{90} and a supernatant S_{90}. The particle fractions P_{30} and P_{90} were washed and recovered by centrifugation at 105,000 × g for 90 min. The supernatant S_{90} was dialyzed for 7 hr.

When P_{30} was incubated with ATP, an ATP generating system, and amino acids, an inducer-dependent increase in β-galactosidase was observed, as well as an incorporation of radioactive leucine into total protein. The synthesizing system is absolutely dependent upon inducer, a source of energy, and both the particle and supernatant fraction. To establish that the observed increase in enzyme activity actually represents synthesis of new protein molecules, the enzyme activity was precipitated with specific antisera, and the antigen-antibody precipitate was shown to contain radioactivity. Although there was some variable, nonspecific precipitation of radioactive protein by the antiserum the data show quite clearly that the newly formed β-galactosidase contains radioactive leucine and was therefore derived from the amino acid pool. This is most evident from the fact that the presence of inducer is absolutely necessary for enzyme synthesis and the inducer stimulates the incorporation of C^{14}-leucine into protein. All of the inducer-dependent increase in radioactive protein is precipitable by the specific antiserum.

The synthesizing system is stimulated by a full complement of amino acids and the nucleoside di- and triphosphates and is inhibited by chloramphenicol, RNase, DNase, and glucose.[10]

Discussion. – It is evident that a system has been uncovered that will permit the careful analysis of the factors involved in specific protein synthesis that obviates the difficulties of studies with whole cells or of incorporation experiments into nonspecific protein. The sensitivity

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[10]T. Kameyama and G. D. Novelli, Biochem. Biophys. Research Communs. 2, 397 (1960).

of the system to DNase together with the stimulation by nucleic acid precursors suggests that one may be able to study synthesis of specific RNA. The availability of various mutants of E. coli involving the β-galactosidase locus will permit a more careful study of the process of induction and of release of repression.

Studies on the Glucocorticoid-Requiring Activation of Tyrosine-α-ketoglutarate Transaminase

F. T. Kenney R. M. Flora

Introduction. – Recent experiments have demonstrated[11] that increased activity of rat liver tyrosine-α-ketoglutarate transaminase in vivo following administration of tyrosine or adrenal steroids[12] is due to an activation of an as yet unknown type. Lack of sufficient anti-transaminase has hindered further analysis of the activation mechanism, and accordingly the preparation of relatively large amounts of this enzyme has been undertaken. Second, a better understanding of the roles of substrate and adrenal hormone in the in vivo activation process has been sought by observing the effect on activation of various administered materials in intact and adrenalectomized rats. Finally, previous indications[13] that a small increase in activity can be observed in vitro have been extensively reexamined.

Results and Discussion. – 1. Purification. – In order to increase the yield of enzyme, and to investigate the possibility of a qualitatively different enzyme in the induced state, tyrosine was administered to rats 4–5 hr before liver extracts were prepared. Fractionation[14] has to date yielded a preparation containing several grams of enzyme about 50% pure; there is as yet no apparent difference in the enzyme due to induction. Further purification is expected to yield sufficient enzyme to support a rather large-scale production of anti-transaminase.

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[11]F. T. Kenney, Biochem. Biophys. Research Communs. 2, 333 (1960).

[12]E. C. C. Lin and W. E. Knox, Biochim. Biophys. Acta 26, 85 (1957).

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

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

2. Role of Substrate. – Tyrosine is fully effective as an inducer in intact rats, but is without effect in adrenalectomized animals unless hydrocortisone (or another glucocorticoid) is also given; in the latter case the amino acid causes a 3–4 fold stimulation over that obtained with the hormone alone. This has been interpreted[12] as reflecting two modes of induction – one hormonal, the other a substrate induction, in analogy with microbial enzyme induction. It has now been established that the amino acid methionine is as effective as tyrosine in either intact or adrenalectomized rats. In preliminary experiments ascorbic acid, which has been shown[15] to prolong the gluconeogenic and hematologic effects of cortisone, also appears to be effective in increasing the transaminase response to hydrocortisone. Methionine and ascorbic acid, like tyrosine, are ineffective in adrenalectomized rats unless hydrocortisone is also given. It is thus clear that activation requires the adrenal hormone, and that a given dose can be made more effective by simultaneous administration of several substances, which need not be related in any fashion to the catalytic capacity of the enzyme. It is possible that these materials exert a nonspecific pharmacological effect which increases the effective hormone concentration, perhaps by delaying its inactivation and subsequent excretion. This possibility is being investigated.

3. Activation in Vitro. – It is apparent that the mechanism of activation will not be completely understood until it can be observed in a cell-free system. Toward this end the increase in activity which was observed previously in liver homogenates was investigated further. Conditions were worked out under which as much as a doubling of activity could be observed when liver preparations were incubated at 37° for 2–3 hr. However, more extensive study of this phenomenon has made it clear that the apparent increase in activity was actually due to decay of a competing reaction, and this approach has therefore been temporarily suspended.

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[15]H. Bacchus, N. Altszuler, and M. H. Heiffer, Endocrinology 51, 302 (1952).

An Amino Acid Incorporating System from Mouse Liver

C. R. Lea G. D. Novelli

Introduction. — Maisin et al.[16] reported that an increased rate of protein synthesis is observed in the intestine of AET-protected mice given 1500 r. Following up these observations, we hoped to obtain a cell-free system from mouse intestinal mucosa that would incorporate amino acids into protein. Such a system would then permit analysis of which component of the system is responsible for the increased rate of synthesis and thereby cast light on the rate-limiting step in protein synthesis. However, all attempts to prepare such a cell-free system from mouse intestinal mucosa failed. Fortunately, at this time Kretchmar and Congdon[17] observed an increase in liver weight in mouse bone marrow chimeras, suggesting that the objectives listed above might be attainable with mouse liver. Accordingly, we shifted our attention from intestinal mucosa to mouse liver.

Results and Discussion. — An active amino acid incorporating system has been developed from mouse liver. The system is quite similar to that developed from rat liver by Zamecnik[18] and his co-workers. Mouse liver microsomes are prepared by homogenizing mouse liver and using differential centrifugation to obtain microsomes. The microsomes are then treated with a solution of sodium deoxycholate and again recovered by centrifugation, giving almost pure ribonucleoprotein particles. After washing the particles by resuspension in buffered salt solution to remove any remaining deoxycholate a preparation is obtained that will not incorporate amino acids into protein unless the particles are supplemented with a portion of the soluble fraction. The particles so prepared are quite stable to freezing and thawing and to storage at −20°. Thus a stable system is now available that should allow us to investigate the components of the soluble fraction that are required for incorporation. One or more of the components of the soluble fraction are unstable to dialysis or storage at −20 or 0° but are stable when stored under liquid nitrogen.

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[16] J. R. Maisin et al., Intern. J. Radiation Biol., in press.

[17] A. L. Kretchmar and C. C. Congdon, Am. J. Physiol., in press.

[18] E. B. Keller and P. A. Zamecnik, J. Biol. Chem. 221, 45 (1956).

We have observed that the pH 5 fraction of the soluble phase is less active than the supernatant after removal of the pH 5.0 fraction in stimulating incorporation into the particles. There is an additive effect when the two soluble fractions are recombined. As in other systems the incorporation process is rapid, being essentially complete in 15 min, and all of the incorporated radioactivity remains associated with the particle. The short time course of incorporation together with additivity when using a mixture of different C^{14}-amino acids led to the suggestion that the particles had only a limited number of sites available for attaching amino acids. However, when this idea was tested using various combinations we found that addition of more supernatant to the reaction mixture after the incorporation had ceased led to a further incorporation. Thus it would seem that the reaction stops because a labile component of the soluble fraction becomes exhausted. We are currently investigating the nature of the labile component.

Amino Acid Incorporation into Particles Isolated from Maize Seedlings

R. J. Mans G. D. Novelli

Introduction. — We are continuing our studies on the incorporation of radioactive leucine into trichloroacetic acid–precipitated protein in the presence of a particle fraction isolated from maize seedlings.[19] We have characterized the maize seedling system as to the requirements for the isolation of active particles and the properties and requirements for incorporation.

Results. — When particles isolated from a homogenate of maize seedlings by centrifugation at 105,000 × g are washed with distilled water, they lose approximately 80% of their ability to incorporate radioactive amino acids in the presence of ATP, GTP, an energy generator, $MgCl_2$, and KCl. Treatment of the particles with sodium deoxycholate eliminates all the incorporating activity. The addition of a pH 5 fraction prepared from rat liver supernatant restores 40% of the activity originally present in

[19] R. J. Mans and R. Rabson, Abstracts, Federation of American Societies for Experimental Biology (American Society of Biological Chemists), Chicago, Ill., April 11–15, 1960.

the unwashed particles. However, addition of the maize 105,000 × g supernatant to the water-washed or deoxycholate-treated particles results in a complete restoration of activity.

We have initiated studies on fractionation of the maize soluble for the component or components responsible for stimulation of amino acid incorporation. The stimulating activity of the maize soluble remains in the supernatant after precipitation at pH 5, unlike the rat liver material in which the activity is precipitated from the soluble at pH 5. Short-term dialysis of the maize soluble results in an increase in activity, probably owing to the removal of diluting nonlabeled amino acids. Long-term dialysis as well as storage at low temperatures results in rapid inactivation of the maize supernatant. Passing the soluble fraction over Sephadex efficiently removes amino acids and promises to be an effective technique for fractionating the low-molecular-weight components away from the high-speed-soluble fraction.

The kinetics of leucine incorporation indicate that the particles rapidly become saturated with labeled amino acid, at which time further incorporation does not occur. By reseparation of the particles from the soluble materials after incubation of the reaction mixture, we have found that 90% of the radioactivity remains associated with the particle fraction. We do not know whether the inability of the system to incorporate amino acid continuously with a resultant net increase in protein is due to the failure to strip formed protein from the saturated particle sites or if the soluble component is rapidly inactivated during the course of the incubation at 37°.

Summary. — We have developed an amino acid incorporating system derived from maize seedlings consisting of a particle fraction which demonstrates an absolute requirement for soluble materials from maize. We are currently fractionating the soluble materials in order to elucidate the intermediate reactions involved in the net biosynthesis of protein from individual amino acids.

Amino Acid Incorporation Studies in Developing Maize Kernels

R. Rabson R. J. Mans

Introduction. — An earlier report[20] of this investigation described the preparation and some of the properties of a cell-free system isolated from maize kernels which incorporates radioactive amino acids into protein. Preliminary experiments indicated that the isolation of active particles is dependent upon the maturity of the developing maize kernels. Similarly, it is known that the amino acid incorporating activity of germinating maize grain is a function of the maturity of the seedlings.[21] Currently, we are attempting to correlate the change in activity of the incorporation system in the fresh kernel with other physiological events occurring during this period.

Results. — Active preparations have been obtained from fresh kernels of ears ranging in maturity from 16 to 29 days post pollination. We have been able to repeat experiments conducted in 1959 by using fresh kernel material from this year's crop. However, refinements in the isolation procedure and assay system have yielded preparations of considerably higher activity. Kernels, 21 days after pollination, yielded unwashed particles that incorporated more than 1500 counts/min per milligram of protein when incubated for 30 min in the presence of ATP, GTP, $MgCl_2$, KCl, an ATP generating system, and leucine-C^{14} (8.5×10^4 counts/min). This represents about 2.4% incorporation of the added amino acid.

Discussion. — We are pursuing the appearance of amino acid incorporating activity using two approaches. First, we are investigating the degree of maturity of the kernel in which activity can be demonstrated in unwashed particles. Second, the system is being fractionated into a washed particle fraction and a soluble fraction to ascertain if the maturity of the particle or the soluble components or both is involved in the transient appearance of the amino acid incorporating activity. By recombination of

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[20]R. Rabson and G. D. Novelli, Proc. Natl. Acad. Sci. U.S. 45, 484 (1960).

[21]R. J. Mans and R. Rabson, Abstracts, Federation of American Societies for Experimental Biology (American Society of Biological Chemists), Chicago, Ill., April 11–15, 1960.

these components isolated at different stages of development, it is hoped that additional information can be obtained relative to both plant development and protein synthesis.

The Pathway of Sulfate Reduction in Various Microorganisms

H. D. Peck, Jr. T. Deacon

Introduction. — In biological systems sulfate is not directly reduced to sulfite but first must be converted to a nucleotide-bound sulfate. Two pathways of sulfate reduction have been described that involve different sulfur-containing nucleotides. In yeast, sulfate is reduced in the form of 3'-phosphoadenosine 5'-phosphosulfate (PAPS) as shown in Eq. (1).[22]

$$PAPS + TPNH \rightarrow PAP + SO_3^{--} + TPN + H^+ \qquad (1)$$

Sulfate reduction in Desulfovibrio desulfuricans differs from that in yeast, as adenosine 5'-phosphosulfate (APS) rather than PAPS is the form in which sulfate is reduced to sulfite, as shown in Eq. (2).[23]

$$APS + 2e \rightarrow AMP + SO_3^{--} \qquad (2)$$

The role of sulfate in the physiology of these two organisms is quite different. Yeast reduces only small amounts of sulfate in order to supply its nutritional requirements for sulfur, and the process has been termed assimilatory sulfate reduction. Desulfovibrio desulfuricans reduces large amounts of sulfate, which functions in anaerobic respiration as the terminal electron acceptor. This process has been called dissimilatory sulfate reduction. It was therefore of interest to ascertain whether there was a general biochemical difference between assimilatory and dissimilatory sulfate reduction.

Results. — Cell-free extracts of a large number of organisms were prepared, and their ability to reduce both APS and PAPS with TPNH and reduced methyl viologen as electron donors was investigated.

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[22]H. Hilz, M. Kittler, and G. Knape, Biochem. Z. 332, 151 (1959).

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

Dissimilatory sulfate reducers. — Three different "sulfate-reducing bacteria," D. desulfuricans, Clostridium nigrificans, and Vibrio cholinicus, were examined. All reduced APS and showed little or no activity toward PAPS. The activity observed was ten times that observed with any organism on PAPS.

Assimilatory sulfate reducers. — About 25 other organisms which were either assimilatory sulfate reducers or did not reduce sulfate were found to reduce only PAPS or to be inactive toward both substrates. In contrast to yeast, the electron donor for the reduction of PAPS was not always TPNH. Reduced methyl viologen could also serve as electron donor and in some anaerobic organisms was the only electron donor effective for PAPS reduction.

Thiosulfate oxidizers. — A limited number of microorganisms have the capability of oxidizing thiosulfate to sulfate. A photosynthetic organism, Chromatium, and two species of thiobacilli, Thiobacillus thioparus and T. thiooxidans, all grown on thiosulfate, have been examined with respect to their ability to reduce APS and PAPS. Although the Chromatium reduced only APS at a rate considerably slower than that of the sulfate-reducing bacteria, the thiobacilli reduced APS at a rate comparable to that found only in the dissimilatory sulfate reducers. When T. thiooxidans was grown on elemental sulfur, the organism was no longer capable of reducing APS. These results suggest that APS is an intermediate in thiosulfate oxidation as well as sulfate reduction.

Discussion. — These data indicate that dissimilatory sulfate-reducing bacteria reduce sulfate in the form of APS and that assimilatory sulfate reduction involves the PAPS pathway. Thus the pathway of sulfate reduction utilized by a given organism seems to depend upon the role of sulfate in the metabolism of that organism.

The Oxidation of Thiosulfate by Extracts of Thiobacillus thioparus

E. Fisher, Jr. H. D. Peck, Jr.

Introduction. — Orthophosphate is required for the complete oxidation of thiosulfate to sulfate by whole cells of the chemoautotroph Thiobacillus

thioparus, and arsenate can replace phosphate in this process.[24] Santer[25] observed that during the oxidation of thiosulfate in the presence of O^{18}-labeled orthophosphate O^{18} is transferred to the sulfate produced in the oxidation and this transfer is insensitive to 2,4-dinitrophenol. These observations, plus the fact that extracts of this organism reduce adenosine 5'-phosphosulfate (APS) and not 3'-phosphoadenosine 5'-phosphosulfate (PAPS), suggest that the pathway for the oxidation of thiosulfate involves the sulfur-containing nucleotide APS as an intermediate.

Results. — The activities of enzymes known to be involved with the metabolism of sulfate in other organisms were examined in extracts of T. thioparus. Thiosulfate reductase, Eq. (1), sulfide oxidase, Eq. (2) APS reductase, Eq. (3), ADP sulfurylase, Eq. (4), and adenylate kinase, Eq. (5), were found to be present in high activity. The sum of Eqs. (1)–(5) represents a possible mechanism for the oxidation of thiosulfate to sulfate and elemental sulfur as occurs with whole cells.

$$4H^+ + 4e + 2S_2O_3^{--} \xrightarrow{\text{thiosulfate reductase}} 2SO_3^{--} + 2H_2S \qquad (1)$$

$$2H_2S + O_2 \xrightarrow{\text{sulfide oxidase}} 2S^0 + 2H_2O \qquad (2)$$

$$2SO_3^{--} + 2AMP \xrightarrow{\text{APS reductase}} 2APS + 4e \qquad (3)$$

$$2APS + 2P_i \xrightarrow{\text{ADP sulfurylase}} 2ADP + 2SO_4^{--} \qquad (4)$$

$$2ADP \xrightarrow{\text{adenylate kinase}} AMP + ATP \qquad (5)$$

$$2S_2O_3^{--} + O_2 + AMP + 2P_i + 4H^+ \rightarrow 2S^0 + 2SO_4^{--} + ATP + 2H_2O \qquad (6)$$

This reaction sequence explains the phosphate requirement for thiosulfate oxidation and the substitution of arsenate for phosphate in this oxidation. It also supplies an explanation for the O^{18} data of Santer.[25] When cell-free extracts of T. thioparus are supplemented with fluoride, Versene, and reduced glutathione, oxygen is consumed in the presence of thiosulfate. Glutathione is only active in substrate amounts and is probably required for the reductive cleavage of thiosulfate to sulfite and sulfide, Eq. (1).[26]

[24] W. Vishniac and M. Santer, Bacteriol. Revs. 21, 195 (1957).

[25] M. Santer, Biochem. Biophys. Research Communs. 1, 9 (1959).

[26] A. Kaji and W. D. McElroy, J. Bacteriol. 77, 630 (1959).

Optimum conditions for cell breakage, pH, fluoride concentration, and Versene concentration have been determined. As indicated by Eq. (6), the disappearance of orthophosphate has been observed. When thiosulfate is oxidized in the presence of $P^{32}O_4^{---}$, radioactivity is incorporated into a compound that is adsorbed on charcoal and not extracted from water by an isobutanol-benzene reagent.[27] After elution of this material from charcoal and electrophoresis, the radioactive material was identified as ADP^{32}. The occurrence of radioactivity in ADP probably indicates that adenylate kinase is inhibited by the fluoride present in the reaction mixture. This esterification of phosphate is probably not the result of oxidative phosphorylation as it is insensitive to 2,4-dinitrophenol. For each micromole of thiosulfate oxidized, 1 micromole each of orthophosphate and oxygen are utilized and 1 micromole of sulfate is produced. This stoichiometry agrees with Eqs. (1)–(5) when glutathione is employed as reducing agent. After dialysis for 24hr, extracts still retain their ability to oxidize thiosulfate; however, when extracts are treated with charcoal, it is possible to demonstrate a partial requirement for AMP with the utilization of oxygen and orthophosphate and the production of sulfate. It has also been possible to observe oxygen utilization, sulfate production, and phosphate esterification when the putative intermediate, sulfite, is employed as substrate.

Discussion. – All the data obtained thus far are consistent with the mechanism of thiosulfate oxidation proposed in Eqs. (1)–(5). Isotope and protein fractionation studies are now in progress which should supply further evidence in support of this mechanism of thiosulfate oxidation.

The Reversibility of Adenosine 5'-Phosphosulfate Reduction in *Desulfovibrio desulfuricans* and *Thiobacillus thioparus*

W. J. Payne H. D. Peck, Jr.

Introduction. – The form in which sulfate is reduced to sulfite (acid-volatile sulfur) by extracts of *Desulfovibrio desulfuricans* is adenosine 5'-phosphosulfate, and the enzyme responsible for this reduction has been called APS reductase.[28] The observation that extracts of *Thiobacillus*

[27] S. O. Nielsen and A. L. Lehninger, J. Biol. Chem. 215, 555 (1955).

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

thioparus, grown on thiosulfate, contain large amounts of APS reductase indicates that APS is an intermediate in the oxidation of thiosulfate and implies that APS reductase catalyzes a reversible oxidation-reduction system. Thus, in the reversal of this reaction, Eq. (1), a high-energy sulfate is produced which can be exchanged for phosphate by ADP sulfurylase, thereby forming a high-energy phosphate bond.

$$SO_3^{--} + AMP \rightarrow APS + 2e \qquad (1)$$

This reaction is the first known instance of a substrate sulfurylation, and it is only appropriate that this reaction has been found in the thiobacilli which grow chemoautotrophically on inorganic compounds of sulfur.

Results. – Partially purified APS reductase preparations from D. desulfuricans have been shown to exchange $S^{35}O_3^{--}$ and AMP-8-C^{14} into APS [see Eq. (1)]. The labeled APS was identified by paper electrophoresis, hydrolysis, and lack of acid volatility. These preparations were free of ATP sulfurylase and ADP sulfurylase which might have exchanged sulfate, a contaminant of sulfite, into APS. The APS reductase of D. desulfuricans does not incorporate appreciable amounts of S^{35} into sulfite in the absence of the electron donor, reduced methyl viologen. In contrast, APS reductase preparations from T. thioparus rapidly incorporate the S^{35} of APS^{35} into sulfite in the absence of added electron donor. This reaction depends upon the presence of added sulfite. It was possible to demonstrate the incorporation of added $S^{35}O_3^{--}$ in APS, and this indicated that the reaction was an exchange reaction and not a net oxidation-reduction reaction. Upon fractionation of the APS reductase from T. thioparus, the ability to incorporate S^{35} from APS^{35} into sulfite disappeared, although the ability to produce acid-volatile sulfur in the presence of reduced methyl viologen was still evident. Boiled extracts and known cofactors did not restore the ability of extract to catalyze the exchange reaction. The addition of extract which had been treated at room temperature with ethanol markedly stimulated the exchange reaction but not activity with reduced methyl viologen.

Discussion. – The chemical nature of this factor is unknown; however, the data suggest that it is the natural electron acceptor for the oxidation of sulfite by APS reductase. The further characterization of this

factor will aid in demonstrating a net reversal of APS reductase and is necessary for further studies on the mechanism of action of this enzyme.

Acetylation Reactions in Higher Animals

K. B. Jacobson

Introduction. — The acetyl transfer reaction previously discussed[29] became more interesting because of the diversity of substrates with which the enzyme reacted. An opportunity arose to study the influence that para substituents had on the transfer of acetyl groups from an acetanilide-type molecule to an aromatic amine acceptor. It was possible to interpret the results in relation to the electrophilic effects of these para substituents since the enzyme has a rather broad substrate specificity. The substrate binding site is probably at the amide bond, and variation in the position para to it quite likely exerts no influence on the enzyme except through its influence on the electronic configuration of the amide bond.

Results and Discussion. — The following acetyl donors, p-nitroacetanilide, p-chloroacetanilide, p-bromoacetanilide, acetanilide, and p-methylacetanilide, were compared kinetically to determine the initial rate at various concentrations and the saturating concentration. The relative decreasing order of the rate using these substrates was nitro > chloro = bromo > H > CH_3. This series is also one of decreasing electronegativity and therefore leads to the suggestion that the para substituents on the acetanilide derivatives are exerting their effect on the reaction rate by attracting electrons from the amide bond. Such a mechanism is reasonable since it would deprive the carbonyl carbon of the acetyl group of its full share of the covalent bond electrons. Since the acetyl is transferred as a carbonium ion the mechanism suggested would facilitate the release of the acetyl group.

Previous studies on the inhibitors of acetyl transfer, amethopterin and related compounds, and their mechanism of action will appear in the Journal of Biological Chemistry in August 1960.

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

Studies on the Mechanism of Glycogen Synthesis and Breakdown in Tetrahymena pyriformis

M. P. Stulberg

Introduction. — The multinuclear ciliate Tetrahymena pyriformis HSM has been studied as a system for examining control mechanisms in the synthesis and degradation enzymes.

Since this ciliate accumulates large amounts of glycogen during growth (up to 22% of its dry weight at stationary phase of growth), it was deemed feasible to examine enzymes responsible for glycogen synthesis and breakdown.

Results and Discussion. — A glycogen stain technique using a combination of periodic acid and Feulgen fuchsin was developed by modification of a published method and used for scrutinizing whole organisms for their glycogen content. In the course of this study, it was noted that glycogen is uniformly distributed throughout the cell rather than in discrete areas as previously indicated by the literature. The qualitative stain determination of glycogen within cells agreed remarkably well with actual glycogen isolation from the cells followed by quantitative determination.

It was found that when cells were harvested at or near stationary phase of growth, then placed in a starvation medium under conditions of anaerobiosis, glycogen was rapidly degraded within the cells. Furthermore, upon placing cells depleted in glycogen in fresh starvation media and exposing them to air, the glycogen was rapidly resynthesized and stored within the cells.

A study of phosphorylase, during this cycle of degradation and resynthesis of glycogen, revealed no significant changes in the activity per cell of the enzyme, whereas total protein per cell gradually disappeared during the cycle.

Phosphorylase from Tetrahymena is not affected by cysteine but is activated approximately 20% by AMP. Cyclic 3'-5'-AMP plus ATP does not affect the phosphorylase activity in homogenates of Tetrahymena.

It was interesting to note that cells in a concentrated suspension in water were apparently 100% broken by freezing in liquid nitrogen and then thawing at 37°C. However, the addition of fluoride to 0.1 M (to protect

phosphorylase from inactivation) before freezing apparently fixes the cells and prevents breakage.

Further studies on the nature of the control mechanism responsible for glycogen synthesis and degradation in these organisms are contemplated.

Enzymes of Starch Biosynthesis in Maize: UDP-D-Glucose D-Fructose Transglucosylase (Saccharese)

A. N. Best G. D. Novelli

Introduction. — This is a continuing and cooperative project with the Maize Genetics Group[30] that is concerned with the mechanism of gene action at the molecular level. The enzymes involved in starch biosynthesis have been selected for study because there are a large number of mutants in maize that affect the carbohydrate composition of the endosperm. It seems to be a reasonable assumption that some of the mutations may alter the enzymes in starch biosynthesis.

It had previously been assumed that the main enzyme in starch synthesis was phosphorylase. The reaction catalyzed by phosphorylase is:

$$\text{glucose-1-phosphate} + \text{"primer"} \rightleftharpoons \text{starch}$$

and a similar reaction was believed to be involved in the mammalian synthesis of glycogen. Recently, however, it has been found that glycogen is synthesized by an enzyme that is quite different from phosphorylase.[31] This enzyme is called UDPG-glycogen transferase and catalyzes the following reaction:

$$\text{UDP-D-glucose} + \text{"primer"} \rightarrow \text{glycogen} + \text{UDP}$$

The discovery of this reaction raised the possibility that a similar reaction might be involved in starch formation in plants. Since sucrose is the transport form of carbohydrate in plants and since the above reaction involves UDPG, the first problem is then the conversion of sucrose to UDPG. Cardini et al.[32] had previously reported on the presence in plants

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[30] D. Schwartz et al., Biol. Semiann. Prog. Rep. Feb. 15, 1959, ORNL-2702, p 46–48.

[31] L. F. Leloir and C. E. Cardini, J. Am. Chem. Soc. 79, 6340 (1957).

[32] C. E. Cardini, L. F. Leloir, and J. Chiriboga, J. Biol. Chem. 214, 149 (1955).

of an enzyme catalyzing the following reaction:

$$\text{UDPG} + \text{fructose} \rightleftharpoons \text{sucrose} + \text{UDP}$$

Since the reaction is reversible, although the equilibirum is largely to the right, it seems possible that this enzyme might indeed be the first step in the conversion of sucrose to starch. Another interesting possibility concerning this enzyme derives from the observation of Laughnan[33] that the mutant gene shrunken-2 (Sh_2) leads to a large accumulation of sucrose and a corresponding decrease in starch formation. If the pathway of starch synthesis is assumed to follow the pathway

$$\text{sucrose} \rightarrow \text{UDPG} + \text{primer} \rightarrow \text{starch}$$

then an alteration of the enzyme, saccharese, involved in the conversion of sucrose to UDPG might be responsible for the sh_2 phenotype. Accordingly we have begun to study this enzyme in maize endosperm.

Results and Discussion. — Using the assay described by Cardini et al.[32] we have indeed detected the presence of this enzyme in waxy maize endosperm. Because of a large amount of reducing substances in crude endosperm extracts, it is not possible to measure the enzyme accurately unless the protein is first precipitated with saturated ammonium sulfate. With this procedure we found that waxy endosperm has this enzyme with a specific activity of 2.5 units per milligram of protein. This is a surprisingly high specific activity for crude material, since Cardini et al.[32] purified this enzyme 50 fold from wheat germ and reached a final specific activity of 2.4. Thus waxy maize endosperm is some 50 times more enriched for this enzyme than wheat germ.

The next experiment was to compare the activity of saccharese in extracts from waxy endosperm with extracts from sh_2. If the theoretical pathway for starch synthesis as postulated in the introduction is correct, then we should expect to see less activity of saccharese in sh_2 extracts. When these measurements were made, we found to our surprise that the specific activity of saccharese in sh_2 was 4.6, or 1.8 times more active

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[33]J. R. Laughnan, Genetics 38, 485 (1953).

than in waxy extracts. Thus it is clear that a decreased activity of this enzyme is not responsible for the sh_3 phenotype.

We are presently purifying this enzyme from waxy endosperm.

Studies of Polysaccharide Synthesis by a Soil Diphtheroid

M. L. Taylor G. D. Novelli

Introduction. — It has been known for many years that bacteria produce extracellular polysaccharide, either as slime excreted into the medium or in the form of capsules. In the genera Pneumococcus and Klebsiella the capsular polysaccharides have been shown to be associated with virulence. Although there is much known about the structure and mode of synthesis of homopolysaccharides, there is almost nothing known of the mechanisms by which heteropolysaccharides are synthesized.

Since the composition of the heteropolysaccharide of a given organism is constant regardless of the carbon and energy source used for growth, and is genetically determined, the mode of synthesis of heteropolysaccharides offers another system in which biological specificity can be studied.

Results and Discussion. — The heteropolysaccharide, obtained by Waring blender agitation of a cell suspension of a soil diphtheroid, has been found to be complexed with protein. Fifteen amino acids were identified after hydrolysis in 6 N HCl for 16 hr at 105°. Alanine was found to represent 27% of the amino acids found.

The polysaccharide-protein complex has a strong negative charge and moves toward the positive pole during electrophoresis at pH 4.1, 7.8, and 9.1. The polysaccharide-protein complex cannot be separated into two components by electrophoresis, alcohol precipitation, or ultracentrifugation.

No sulfur-containing amino acids, or derivatives of sulfur-containing amino acids, were found after hydrolysis of the complex. The polysaccharide-protein complex was found to contain 3.5% ash. Since preliminary evidence suggested that a portion of the ash might be sulfate, the polysaccharide-protein complex was isolated from organisms grown on a medium containing $S^{35}O_4^{--}$. Experiments are now in progress using material obtained from growth on S^{35} medium to determine if SO_4^{--} is present.

Polysaccharide-protein complex labeled with C^{14} has been obtained from cells grown on radioactive sodium acetate. Experiments are now in progress

using this material to look for minor components present after hydrolysis of the complex.

The ultracentrifugation pattern of the protein-polysaccharide complex showed only one major peak. This peak was an extremely sharp, symmetrical spike, giving an S_{20} of 9.6.

Future work on this problem will attempt to show cell-free synthesis of the polysaccharide either by use of cell-free extracts or protoplasts. The role or necessity of the cell wall for the synthesis of external polysaccharide will be investigated.

Studies on Antibody Synthesis

C. J. Wust

Introduction. — Experiments describing the in vitro synthesis of antibody have been reported in recent literature.[34] In almost all instances, tissue slices, tissue fragments, or cell suspensions were used in short-term tissue culture procedures. In the classical sense, these investigations have been concerned with in vitro systems. It is the purpose of this report to describe initial experiments designed to develop a cell-free system of antibody synthesis.

Results. — Particles sedimentable by centrifugation at 105,000 × g were prepared by established procedures from various tissues of mice and rabbits in order to obtain starting material. These preparations were made from liver, spleen, lung, and lymphoid tissue such as lymph nodes and granulomae developing as the result of the injection of water-in-oil emulsion (adjuvant of Freund). Cell suspensions of the latter three tissues have been shown to produce antibody by Askonas and Humphrey.[35] These tissues were treated as described by Rendi and Hultin,[36] with the exception of spleen. Spleen was ground in a loose-fitting, all-glass tissue grinder and centrifuged at 1500 rpm as a brei layered on a 35% sucrose solution. The top layer, containing few if any erythrocytes, connective tissue fragments, or whole cells, was reground in a tight-fitting tissue grinder and

[34] J. H. Vaughan, J. Immunol. 84, 258 (1960).

[35] B. A. Askonas and J. H. Humphrey, Biochem. J. 70, 212 (1958).

[36] R. Rendi and T. Hultin, Exptl. Cell Research 19, 253 (1960).

centrifuged at 20,000 × g. The resulting turbid supernatant was centrifuged at 105,000 × g to obtain particles and a hemolysis-free supernatant. These particle preparations were then tested for their ability to agglutinate with antigen or species-specific antiglobulin serum.

Animals have been hyperimmunized with bovine serum albumin, β-galactosidase obtained from Escherichia coli, and triose phosphate dehydrogenase obtained from rabbit muscle. To overcome individual variation, the mouse has been the species of choice although rabbits and chicks have been and will be studied. On the basis of previous reports,[37] the spleen was presumed to be the organ of choice. Initial experiments have indicated, however, that liver particles obtained from immunized animals give higher titrations with antigen. With particles obtained from both immunized and normal mice, a serological reactivity was found with rabbit anti-mouse γ-globulin.

Discussion. — The ability of a mammal to produce antibody assumes that certain cellular types can be induced with antigen to make a specifically reacting protein called antibody. Since cellular proteins are made on submicroscopic particles of endoplasmic reticulum, antibody also can be assumed to be manufactured on these particles. The presence of immunologically reactive material on these particles obtained from immunized animals indicates that this is probably true. The role of antigen as inducer and the implication of the production of antibody by the liver are being studied.

Properties of Substances Producing Histological Changes in Spleens of Lethally Irradiated Mice

C. J. Wust C. C. Congdon[38] G. D. Novelli

Introduction. — This is a continuation of a joint effort of the Enzymology and Mammalian Recovery Groups to isolate and characterize substances producing a characteristic histological change in the germinal centers of the white pulp of the spleen.

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[37]W. H. Taliaferro, J. Cellular Comp. Physiol. 50, Suppl. 1, 1 (1957).

[38]Mammalian Recovery Group.

Results and Discussion. — Water homogenates of mouse spleen centrifuged at 12,000 × g have been observed to form three distinct layers. The bottom pellet consists of cellular debris; the top layer comprises a reddish-brown supernatant; and the middle layer is of a mucoid nature. This mucoid material is homogeneous and cell-free and accounts for approximately 20% of the total volume of the homogenate. In the homologous irradiated animal, this mucoid layer administered either intraperitoneally or intravenously gave a positive but slight histological change in the germinal centers of the recipients' spleens. Once the mucoid material was isolated, it appeared to be inactivated by the enzyme neuraminidase, supporting previous observations that heterologous material from rats causing the histological response contain the sialic acid neuraminic acid. In our experiences with this system, this is the first time a positive result has been observed with cell-free preparations of homologous origin. This mucoid material also appears to be inactivated with freezing and thawing, although whole frozen spleens have been used for the initial isolation.

Mouse erythrocyte stroma from several inbred lines did not engender the histological change in the $(C3H \times 101)F_1$ hybrid.

MICROBIOLOGY

Enzyme and Tracer Studies on Bacterial Metabolism

S. F. Carson
R. K. Clayton
M. I. Dolin[1]
E. F. Phares
M. V. Long
C. Smith
D. B. George[2]
C. G. Jones[2]

Induced Synthesis of Catalase in *Rhodopseudomonas spheroides*

R. K. Clayton C. G. Jones

The following facts have now been established regarding the induced synthesis of catalase in *Rhodopseudomonas spheroides*:[3-6] Aeration produces intracellular H_2O_2. The peroxide then promotes an energy-dependent formation of "internal inducer," an unidentified substance or condition within the cells that is stable at 0°C in darkness. This step does not require a reaction between H_2O_2 and catalase. The internal inducer then promotes a *de novo* synthesis of catalase. Dissipation of internal inducer is not related stoichiometrically to the formation of new catalase. The different responses of young and stationary cultures of *R. spheroides*[3] are accounted for by differences in the lifetime of internal inducer. Strong light potentiates the inducing action of traces of O_2 by promoting an efficient conversion of O_2 to H_2O_2.

Carotenoids and Photooxidative Killing in *Rhodopseudomonas spheroides*

R. K. Clayton C. Smith

Mutants of *Rhodopseudomonas spheroides* that lack colored carotenoids are killed by the combination of light and air.[7] The protection afforded

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[1]Leave of absence, Guggenheim Fellowship, Paris, France.

[2]Student trainee.

[3]R. K. Clayton, Biochim. Biophys. Acta *37*, 503 (1960).

[4]R. K. Clayton, J. Biol. Chem. *235*, 405 (1960).

[5]R. K. Clayton, J. Cellular Comp. Physiol. *55*, 1 (1960).

[6]R. K. Clayton, J. Cellular Comp. Physiol. *55*, 9 (1960).

[7]W. R. Sistrom, M. Griffiths, and R. Y. Stanier, J. Cellular Comp. Physiol. *48*, 473 (1956).

by colored carotenoids probably rests on their ability to act as preferential substrates for photooxidation.[8]

Because we have shown that strong light potentiates the conversion of O_2 to H_2O_2 in R. spheroides, it was natural to inquire whether H_2O_2 is involved in photooxidative killing of this organism. To this end mutants of R. spheroides have been obtained having an abnormally high catalase content (as high as 25% of the total protein) and lacking colored carotenoids.[9] These were studied in comparison with a low-catalase carotenoidless mutant. It was found that catalase affords no protection against photooxidative killing in light strong enough to promote rapid H_2O_2 formation. The killing action of H_2O_2 on R. spheroides, both the high-catalase mutant and the wild type, is now being studied in collaboration with H. I. Adler of the Radiation Protection Group.

High-Catalase Mutants of Rhodopseudomonas spheroides

R. K. Clayton

Our high-catalase mutants of Rhodopseudomonas spheroides, in which one-fourth of the protein is catalase, are undoubtedly the richest sources of catalase in existence. The ability of an organism to apply its protein-forming machinery so heavily to the synthesis of a single enzyme poses interesting biochemical and genetic questions, such as: "Is there a vast number of catalase-forming templates, or are these templates unusually efficient?" At present the catalases of mutant and wild-type R. spheroides are being purified and characterized exhaustively.

Carotenoid Biosynthesis and Carotenoid-Bacteriochlorophyll Interactions

R. K. Clayton C. Smith
C. G. Jones

Genetic[10] and biochemical[11] evidence supports the following pathway for carotenoid biosynthesis in Rhodopseudomonas spheroides:

phytoene → phytofluene → ζ-carotene → neurosporene → Y → R

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[8]M. Dworkin, Nature 184, 1891 (1959).

[9]R. K. Clayton and C. Smith, Biochem. Biophys. Research Communs. (1960), in press.

[10]M. Griffiths and R. Y. Stanier, J. Gen. Microbiol. 14, 698 (1956).

[11]S. L. Jensen et al., Biochim. Biophys. Acta 29, 477 (1958).

where Y and R are the yellow and red carotenoids of wild-type R. spheroides, and phytoene and phytofluene are colorless polyenes that absorb in the ultraviolet region of the spectrum. Each step in the pathway is an oxidation involving an increase in the number of conjugated double bonds.

We have obtained several abnormally pigmented mutants of R. spheroides that support this pathway: one accumulating phytoene, one accumulating phytoene and phytofluene, several that accumulate neurosporene, and four that accumulate none of these polyenes. These last four are being examined for the accumulation of squalene precursors, in collaboration with J. W. Porter of the University of Wisconsin.

Colored carotenoids interact with bacteriochlorophyll, in vivo, in three observable ways: (1) Carotenoids protect cells against photooxidative killing sensitized by bacteriochlorophyll. (2) When light is absorbed by bacteriochlorophyll, the absorption spectrum of the carotenoids changes slightly. (3) The presence of carotenoids causes a profound change in the infrared absorption spectrum of the bacteriochlorophyll. The last two of these interactions appear to require precise structural relationships between carotenoid and chlorophyll molecules.[12,13] By all three of these criteria, interaction with bacteriochlorophyll is maximal for the Y and R carotenoids, is a little less for neurosporene, and is nil for phytoene and phytofluene. This constitutes preliminary evidence that the integration of carotenoids into a precise structural arrangement, within the chromatophores of R. spheroides, occurs mainly at the level of ζ-carotene and/or neurosporene.

The Cobamide Coenzyme of Propionibacterium pentosaceum

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

A key step in propionate formation by propionic acid bacteria is the isomerization of succinyl-CoA to methylmalonyl-CoA. This reaction requires a cobamide coenzyme[14] (a vitamin B_{12} derivative). We studied the methylmalonyl isomerase of Propionibacterium pentosaceum by direct assay of succinate-1,4,-C^{14} formed from methylmalonyl-1,4-C^{14}-CoA. Optimum conditions,

[12]R. K. Clayton and W. Arnold, Biochim. Biophys. Acta, in press.

[13]R. C. Fuller and J. A. Bergeron, Proc. Intern. Photobiol. Congr., 3rd, Copenhagen, 1960.

[14]H. A. Barker, J. Biol. Chem. 235, 480 (1960).

inhibitors, and activators for the isolated reaction have been determined.

The apoenzyme of methylmalonyl isomerase was prepared from P. pentosaceum by treatment of a cell extract with protamine and charcoal followed by dialysis and aging. Residual activity in the tracer assay was negligible. Activity could be restored by the addition of boiled juices or purified preparations. The dimethylbenzimidazolyl cobamide (furnished by H. A. Barker) had an apparent K_m of about 5×10^{-5} M. Purified preparations were prepared[14] from lyophilized P. pentosaceum by chromatography of alcohol extracts on Dowex 50 (Na^+) and on Dowex 2 (OH^-), extraction with phenol, and a final column purification on Dowex 50 (pH 3). The main coenzyme peak was found to be quite heterogeneous with respect to both spectrum and coenzyme activity. The first half had low coenzyme activity and showed mainly 260 mμ absorption. The second half was fully active in the enzyme assay system (K_m, 1×10^{-6} M), and exhibited mainly the characteristic adenylcobamide absorption peak at 460 mμ. The tail of the peak was devoid of absorption in the visible region but displayed coenzyme activity comparable to that of the adenylcobamide; no vitamin B_{12} coenzyme with this absorption has been found until now, as both the benzimidazolyl and adenyl compounds have absorption peaks in the visible region. The P. pentosaceum coenzyme exhibits the characteristic vitamin B_{12} coenzyme 370 mμ peak and loss of activity when exposed to strong light.

Synthesis of Methylmalonate-1-C^{14}

E. F. Phares M. V. Long D. B. George

The starting material for preparing the C^{14}-labeled substrate for the methylmalonyl isomerase reaction is methylmalonyl-1-C^{14}. Methylmalonate-1-C^{14} was prepared in a trial run to determine the suitability of the method for small-scale synthesis of this compound at high specific activities. The alpha-carbonation of esters with sodium triphenylmethide as the condensing agent (Hauser et al.) has not been well utilized as a synthetic method for C^{14}-labeled malonic acid derivatives. A study of the individual steps of the synthesis is being made in order to improve the over-all yield of product from barium carbonate-C^{14}.

PLANT PHYSIOLOGY AND PHOTOSYNTHESIS

Plant Physiology

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Photosynthesis

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Action of Maleic Hydrazide on Dormancy, Cell Division, and Cell Expansion

A. H. Haber J. D. White

The following paragraphs are taken from a paper bearing the above title that has been published in Plant Physiology 35, 495–99 (1960):

Introduction. – Maleic hydrazide (MH) is of practical and theoretical interest as a growth-regulating chemical that can inhibit plant growth without causing obvious morphological abnormalities.[2,3] After recent reports of growth interactions between MH and gibberellic acid (GA),[4,5] Brian and Hemming called attention to the many growth effects for which MH has actions opposite to those of GA.[6] From studies of interactions of MH and GA on pea stem extension, these authors concluded that "MH prevents the response to GA of GA-sensitive plants" and that it is therefore likely that "MH inhibits growth by blocking some essential reaction at a stage preceding that where GA normally exerts its effect."[6] In this paper, we have

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††Student trainee, summer 1959.
[1]On official leave, summer 1960.
[2]A. W. Naylor and E. A. Davis, Botan. Gaz. 112, 112 (1950).
[3]D. L. Schoene and O. L. Hoffman, Science 109, 588 (1949).
[4]M. J. Bukovac and S. H. Wittwer, Mich. State Univ., Agr. Expt. Sta., Quart. Bull. 39, 307 (1956).
[5]J. Kato, Science 123, 1132 (1956).
[6]P. W. Brian and H. G. Hemming, Ann. Appl. Biol. 45, 489 (1957).

studied the effects of MH and compared them with those of GA on three systems: lettuce seed germination, mitosis in the absence of growth by expansion in dormant lettuce seeds,[7,8] and growth by cell expansion in the absence of mitosis in γ-irradiated wheat.[9]

Summary. — MH can inhibit cell division in dormant lettuce seeds in which cell division occurs in the absence of growth by cell expansion. Concentrations of MH that inhibit normal growth of wheat seedlings by 85–90% have no effect on the growth of wheat from irradiated grain, which grows without any cell division. Thus MH affects mitosis in a system where GA does not, and MH has no effect on cell expansion in a system where GA is active. When wheat is treated with a combination of MH and GA, the two chemicals apparently act independently on growth. All these findings suggest that the effect of MH on seedling growth can be largely or entirely attributed to an inhibition of cell division and not to any appreciable effect on cell expansion. MH and GA do not appear to give specific interactions on lettuce seed germination, thereby providing further evidence against the theory that MH and GA affect growth through common mechanisms. A number of similarities between effects of MH and of ionizing radiation are discussed, including the capacity to permit germination and limited seedling growth of wheat without cell division.

Action of 6-(Substituted) Purines on Mitotic Activity in "Dormant" Lettuce Seeds

A. H. Haber H. Luippold

The following paragraphs are taken from a paper bearing the above title that has been published in Physiologia Plantarum 13, 298–307 (1960):

Introduction. — Although kinetin (6-furfurylaminopurine, "furfuryladenine") has diverse effects on plant growth, it has probably attracted greatest attention as a cell-division factor.[10,11] Investigations of the

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[7]A. H. Haber and H. J. Luippold, Plant Physiol. 35, 168 (1960).

[8]A. H. Haber and H. J. Luippold, Plant Physiol. 35, 486 (1960).

[9]A. H. Haber and H. J. Luippold, Am. J. Botany 47, 140 (1960).

[10]F. Skoog and C. Miller, Symposia Soc. Exptl. Biol., No. 11, 118 (1957).

[11]F. M. Strong, Topics in Microbial Chemistry: Antimycin, Coenzyme A, Kinetin and Kinins, John Wiley and Sons, Inc., New York, 1958.

action of kinetin in cell division have proceeded along two lines: (a) comparisons of the chemical structure of kinetin analogs with their biological activity and (b) cytological examinations of kinetin-treated tissues. Experiments in which the first approach was used have suggested that the function of the side chain substituted in the 6-amino group of adenine "... is to endow adenine with the right physical properties — possibly the right solubility — to reach some critical spot within the cell."[11] Experiments in which the second approach was used suggested that kinetin functions in cell division by inducing "... premature prophase initiation" and by certain "... effects on the coiling cycle of the chromosomes."[12] Nongerminated lettuce seeds should be favorable material for such studies because effects on cell division are uncomplicated by other growth effects[7,8] and because lettuce seeds have been reported to respond (in respects other than cell division) to kinetin analogs other than 6-(substituted) aminopurines.[13]

Summary. — At a concentration of 5×10^{-5} M, 6-benzylthiopurine, 6-benzylaminopurine, and kinetin accelerate mitosis and cell division in radicles of nongerminated lettuce seed. The same concentration of adenine or 6-mercaptopurine has no effect on mitotic activity, and 10^{-3} M 6-mercaptopurine may be slightly inhibitory. Under conditions for which kinetin and its analogs stimulate mitotic divisions in the nongerminated seeds, they do not alter the relative frequencies of figures observed in prophase, metaphase, anaphase, and telophase and do not cause cytological abnormalities. The yield of radiation-induced chromosomal aberrations in seedling root tips was unaffected by sowing γ-irradiated seeds on kinetin. These results indicate that kinetin cannot accelerate cell division by serving as a source of adenine to sites within the cell, and that it seems to "trigger" the onset of mitotic division in interphase cells but does not affect the course of mitosis once it has begun or otherwise act directly on the chromosomes.

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[12]R. Guttman, Chromosoma 8, 341 (1956).

[13]C. G. Skinner et al., Plant Physiol. 32, 117 (1957).

Photosynthesis

W. A. Arnold R. K. Clayton
E. Gassner

The first step in photosynthesis, involving the interaction of light quanta with electrons, has been studied using chromatophores (photosynthetically active subcellular particles) of photosynthetic bacteria.

A film of dried chromatophores changes reversibly in three ways when irradiated with bacteriochlorophyll-absorbed light. Its absorption spectrum is altered, its electric conductivity and dielectric constant increase, and it emits delayed light. The change in absorption spectrum corresponds to a lowering of the ground-state energy level of the bacteriochlorophyll; this change occurs at 1°K as well as at room temperature. All three phenomena are related kinetically; they appear to reflect the light-induced separation of electrons and holes in a chlorophyll-protein crystal having properties of a semiconductor.[14] The spectral changes are absent in healthy intact photosynthetic bacteria; poisoning the cells with hydroxylamine suppresses photosynthetic metabolism and concomitantly brings on the spectral changes seen in the chromatophores. We conclude that the separated electrons and holes do not accumulate when the enzymatic machinery of photosynthesis is operating. The first step in photosynthesis appears therefore to be a separation of electrons and holes in a chlorophyll semiconductor. This separation occurs when electrons of the chlorophyll are excited by light quanta. The separated reducing and oxidizing power can then be carried into metabolic channels by electron-transport enzymes.

Evidence for this mechanism is currently being sought in higher plants and algae. Dried subcellular particles from blue-green algae show spectral changes when illuminated that may be analogous to those seen in photosynthetic bacteria.

It is well known that in photosynthesis the quantum efficiency of far-red light is abnormally low, but can be enhanced by an admixture of blue light. This "Emerson effect" is now being examined in the light of the foregoing semiconductor mechanism.

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[14]W. Arnold and R. K. Clayton, Proc. Natl. Acad. Sci. U.S. 46, 769 (1960).

Studies of carotenoid-chlorophyll interactions, performed mainly under the aegis of the Microbiology Group, are described under "Carotenoid Biosynthesis and Carotenoid-Bacteriochlorophyll Interactions."

BIOPHYSICS

J. S. Kirby-Smith
J. Jagger
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Induction of Chromosomal Aberrations in Tradescantia Pollen by Combined X-Ray and Ultraviolet Treatment

J. S. Kirby-Smith B. Nicoletti[3] M. L. Gwyn

Introduction. — Investigation of the strong synergism reported previously[4] for the combined action of ionizing and ultraviolet radiation has continued. Explorative investigations of the phenomenon's sensitivity, its variation with humidity, and its dependence on the time intervals between exposure to x rays and ultraviolet radiation, as well as on the interval between treatments and sowing of pollen, have been completed. With this general background information we are now able to commence more complex studies of the synergistic effect, such as its dependence on LET and its ultraviolet action spectrum.

Results. — The effect is clearly demonstrated with an x-ray dose of 0.25 rad in combination with 0.01×10^6 ergs/cm^2 surface exposure of 2650-A ultraviolet radiation. Using 1 rad of x rays in combination with this ultraviolet exposure (a figure less than one-tenth the UV exposure required to give a detectable aberration frequency when given alone) the observed aberration frequencies are roughly equal to those resulting from 50 to 100 rads of x rays given alone. The magnitude of the phenomenon is dependent in a complex manner on the time intervals between ultraviolet and x-ray exposures for both pre- and posttreatment with the ultraviolet radiation, and also on the interval between exposure and sowing of the pollen. In the time ranges studied, from 10 min to 2 hr, the effect increases to a maximum at an interval of 30 min and subsequently decreases to its initial (10 to 15 min) value after 1 hr. Consequently, in order to permit reproducible

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[1]Research participant.

[2]Graduate student, University of Tennessee.

[3]Drosophila Cytology and Genetics Group.

[4]J. S. Kirby-Smith, B. Nicoletti, and M. L. Gwyn, Biol. Semiann. Prog. Rep. Feb. 15, 1960, ORNL-2913, p 131.

and quantitative studies all experiments of action spectra and LET dependence now in progress are being carried out with a standard 15-min interval between ultraviolet and x-ray exposure and between termination of exposure and sowing.

The magnitude of the synergistic effect (and also the sensitivity of pollen to ultraviolet radiation acting alone) has been found to be inversely proportional to the water content of the pollen. All comparative studies are now being carried out at 50% humidity to standardize conditions.

A small although significant photoreactivation has been demonstrated for pollen exposed to ultraviolet in the dry state but given the photoreactivating light in the wet growing condition.

Discussion. — From considerations of the sensitivity of the phenomenon, it seems unlikely that radiation inactivation of chromosomal repair mechanisms can be an important factor in the effect. The production by either activation or ionization of long-lived lesions or activated sites in the chromosomal strands which can interact irreversibly to produce a permanent break appears far more likely, particularly since the synergism is much greater in the dry state than in the wet. It is expected that studies now in progress on the LET dependence of the effect, as well as the presence or absence of the effect in other wet and dry systems, will shed some light on this question.

Modification of the Frequency of Recessive Lethals Induced by X Rays in *Drosophila*. Use of Ultraviolet Radiation

B. Nicoletti[3] W. J. Welshons[5]

Introduction. — Studies on the combined action of ionizing and non-ionizing radiation have been extended to the induction of recessive lethals in mature sperms of *Drosophila melanogaster*. Mackenzie and Muller[6] reviewed the induction of recessive lethals in mature sperms of *Drosophila* by ultraviolet irradiation and concluded that doses of the order of 2 ×

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[5]Temporary Drosophila Group Leader, and Mammalian Genetics and Development Group.

[6]K. Mackenzie and H. J. Muller, Proc. Roy. Soc. (London) B129, 491–517 (1940).

ORNL-2997
Errata

The section "Modification of the Frequency of Recessive Lethals Induced by X Rays in *Drosophila*," by Benedetto Nicoletti and W. J. Welshons, which appears on page 198, correctly belongs with the section "*Drosophila* Cytology and Genetics."

10^{10} ergs/cm^2, wavelength of 280–320 mμ, can induce lethal mutations with the approximate frequency of 3%. The irradiation is delivered through the male abdomen, and only a small percentage of the total dose reaches the sperms after penetrating chitin and muscles. Kaufmann and Hollaender,[7] on the other hand, reported in 1946 that a posttreatment of UV radiation after 4000 r delivered to adult males of Drosophila could decrease the number of salivary chromosome aberrations detectable in the F_1 larvae. The small dose used (1.8×10^5 ergs/cm^2) and the less penetrating wavelength (2537 A) suggested a great effectiveness of the UV posttreatment, in spite of the fact that the decrease observed was not very large. Our recent results obtained with Tradescantia pollen,[8] where a remarkable synergism in the production of chromosomal aberrations was observed after combined treatment of x and UV rays, suggested the repetition of similar experiments with Drosophila in order to gain more information. The M5 technique for scoring recessive lethals in Drosophila was substituted for the examination of F_1 chromosomal aberrations since we wanted to enlarge the sample of chromosomes treated and make more significant the results of the combined irradiations.

Results. – Adult males of the Oregon-R strain were irradiated a few hours after emergence with a dose of x rays that was supposed to be of 3000 r (due to malfunction of the x-ray machine in the first experiment the dose was lower as indicated by the lethals induced with the single x-ray treatment). Half of the males were then exposed to 3×10^6 ergs/cm^2 of UV rays (3000 A), and the irradiation was completed within 2 hr from the time of x-ray treatment using the same dispositive described in the Kaufmann and Hollaender[7] paper. The other half represented the control for the x ray alone. A similar number of males of the same age received only the UV irradiation. The treated males were then mated to M5 virgin females, and in order to test only mature sperms the parents were discarded after four days. Using the M5 technique to score for recessive le-

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[7]B. P. Kaufmann and A. Hollaender, Genetics 31, 368–76 (1946).

[8]J. S. Kirby-Smith, B. Nicoletti, and M. L. Gwyn, this report.

thals, a sample of chromosomes for each group was tested, and the results are presented in Table 19.

Table 19. Induction of Recessive Lethals in Drosophila

Experiment No.	Treatment	Chromosomes Tested	Lethals	Per Cent Lethals
1	X rays	1100	40	3.64
	UV	817	3	0.37
	X + UV rays	1324	84	6.34
	Controls	926	1	0.11
2	X rays	641	47	7.33
	UV	970	6	0.62
	X + UV rays	472	47	9.96

Discussion. — These first results show that there is a clear indication that UV irradiation following x rays can significantly increase the amount of recessive lethals induced by the x rays. At the UV dose applied the effect is more than additive, suggesting also in this case a synergistic action between the two radiations. Since the recessive lethals are not a direct measure of the chromosomal rearrangements induced by the x rays, one might suggest that our increase in recessive lethals is mainly due to production of point mutation lethals. This interpretation would be consistent with the data of Kaufmann and Hollaender,[7] and future experiments will discriminate between the two possible alternatives.

Production and Decay of Free Radicals Induced by X Irradiation of Dry Lettuce Seeds

M. L. Randolph A. H. Haber[9]

Introduction. — The study of whole dry seeds by techniques of electron spin resonance (ESR) offers the possibility of a rather direct measurement of free radicals in vivo. Hence, by correlations with biological

[9]Plant Physiology and Photosynthesis Group.

end points such as germination, possibilities exist for testing various theories of the action of free radicals in seeds subjected to various treatments such as irradiation, variations in moisture, temperature, and oxygen, or combinations of such treatments. Our present work is partly an extension of previous more basic ESR studies on physical radiation effects[10,11] and studies on ground wheat embryos.[12]

Results. – The dose-response curve for the production of free radicals in dry lettuce seeds shows a saturation limit. At low doses about 300 ev are dissipated in the material per radical formed, most of which are in the embryo. Some radicals are present in unirradiated seeds. The decay of radiation-induced radicals is markedly influenced by moisture and heat and empirically seems to fit best the formula (suggested to us by the work of Henriksen[13])

$$N(t) = N(1) - \alpha \ln t \quad , \qquad (1)$$

where $N(t)$ is the concentration of resonances present at time t and α is a constant. The inhibition of germination of seeds given 500 kr irradiation and germinated in 10^{-3} M gibberellic acid was partly reversed by preirradiation treatment with 10^{-3} M AET. The same AET treatment did not influence inhibition of germination by the radiomimetic chemical maleic hydrazide. The concentrations of free radicals immediately after irradiation and their rates of decay both increased with concentration of AET (see Fig. 27).

Discussion. – The initial production efficiency, saturation limit, and decay of resonances are qualitatively similar to the effects previously found for wheat embryo.[12] The empirical formula to which decay data were fitted (see Fig. 27) is unsatisfactory at both very short and very

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[10]J. S. Kirby-Smith and M. L. Randolph, p 11 in Immediate and Low Level Effects of Ionizing Radiations, published as a supplement to Intern. J. Radiation Biol. (1960).

[11]M. L. Randolph, to be published in the proceedings of Stanford Symposium on Free Radicals in Biological Systems (Palo Alto, California, March 1960).

[12]A. D. Conger and M. L. Randolph, Radiation Research 11, 54 (1959).

[13]T. Henriksen and A. Pihl, Nature 185, 307 (1960).

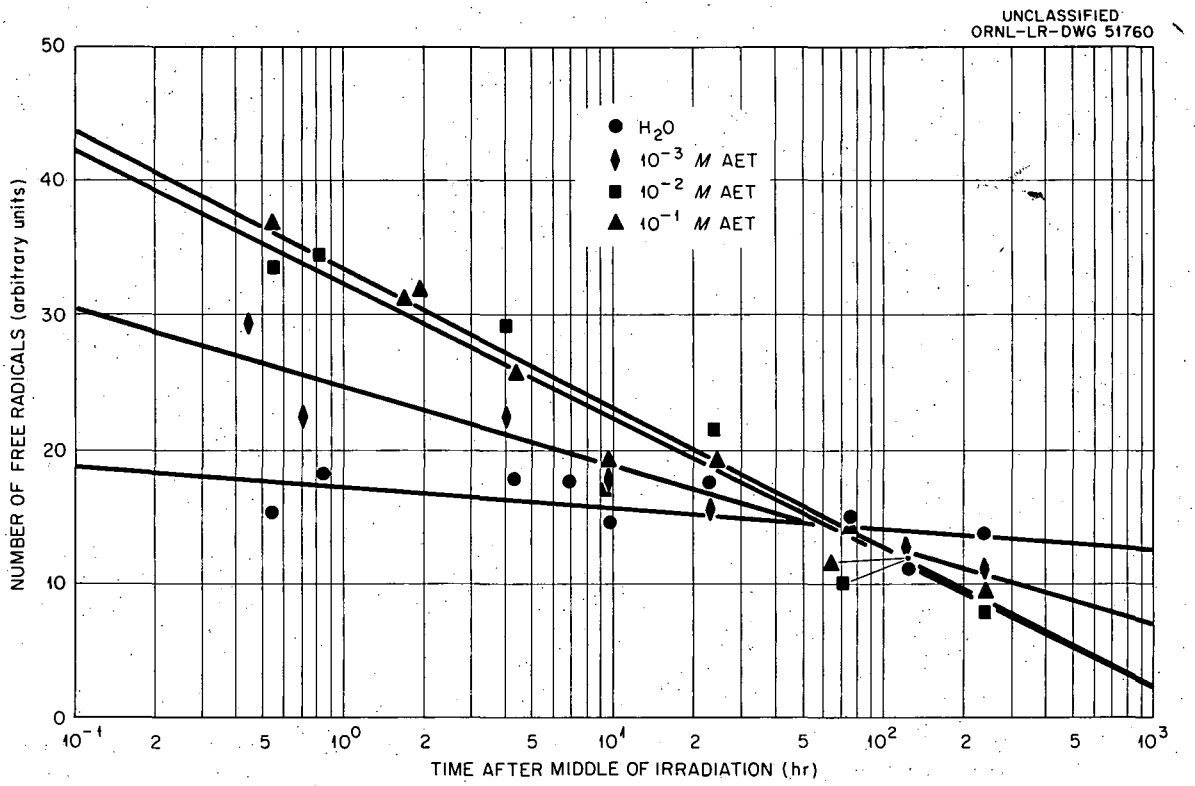

Fig. 27. Decay of Radiation-Induced Free Radicals in Seeds Pretreated with Water and Various Concentrations of AET. Lines are least-squares fits to the data.

long times, and we lack a physical model to account for such decay. Similar decay curves over limited ranges can be produced by assuming the observations represent the sum of several exponentials, but our data seem too few and variable to justify the number of empirically determined coefficients necessary to improve the fit. Assuming that the observed radicals are biologically significant, we are presently considering mechanisms to correlate these effects of AET treatment on free radical concentration, decay, and germination.

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