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SEMIANNUAL PROGRESS REPORT

For Period Ending February 15, 1961

Alexander Hollaender, Director

Stanley F. Carson, Assistant Director

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PUBLICATIONS AND LECTURES

Publications. – The total number of papers by members of the Biology Division published and in press for the past six months is 278. This figure compares with 235 at the same time last year. Listed below are the 93 papers already published. Not shown are 25 abstracts, making a total of 118 published items for the period. In press are 118 papers and 42 abstracts.

Radiation Protection and Recovery, edited by Alexander Hollaender, has now been published by Pergamon Press. Nine division members contributed chapters to this book. The proceedings of the Thirteenth Annual Biology Division Research Conference, entitled "Mammalian Genetics and Reproduction," were published as a supplement to the December 1960 *Journal of Cellular and Comparative Physiology*.

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Adler, H. I., and S. D. Haskins	Heterogeneity of Cultures of *Escherichia coli* B/r	*Nature* **188**, 249–51 (1960)
Anderson, N. G.	Approaches to Aging on the Molecular Level	In *The Biology of Aging* (ed. by B. L. Strehler), AIBS, Washington, D.C., 1960, pp 105–12
Anderson, N. G., and W. D. Fisher	Relation of the Physical Properties of Deoxyribonucleoprotein in 1 *M* Sodium Chloride to Nuclear Structure	In *The Cell Nucleus* (ed. by J. S. Mitchell), Butterworths, London, 1960, pp 195–99
Anderson, N. G., W. D. Fisher, and H. E. Bond	Cell Division. V. Molecular Mechanisms	*Ann. N.Y. Acad. Sci.* **90**, 486–99 (1960)
Arnold, W. A.	Life: A Collection of Probable or Improbable Events?	*Radiation Research* (suppl 2), 324–25 (1960)
Bernlohr, R. W., and G. D. Novelli	Uptake of Bacitracin by Sporangia and Its Incorporation into the Spores of *Bacillus licheniformis*	*Biochim. et Biophys. Acta* **41**, 541–43 (1960)
Bollum, F. J.	Calf Thymus Polymerase	*J. Biol. Chem.* **235**, 2399–403 (1960)
Bollum, F. J.	Deoxyribonucleic Acid Synthesis by Mammalian Enzymes: Some Observations on Primers	In *The Cell Nucleus* (ed. by J. S. Mitchell), Butterworths, London, 1960, pp 60–65
Bond, H. E.	A Sex-Associated Protein in Liver Tissues of the Male Rat	*Biochem. Biophys. Research Communs.* **3**, 53–55 (1960)
Christenberry, K. W.	Treatment of Corneal Abrasion with Topical Whole Blood	*Arch. Ophthalmol.* **63**, 948–52 (1960)
Chu, E. H. Y., M. M. Grumbach, and A. Morishima	Karyotypic Analysis of a Male Pseudohermaphrodite with the Syndrome of Feminizing Testes	*J. Clin. Endocrinol. and Metabolism* **XX**, 1608–13 (1960)
Clayton, R. K., and Carolmarie Smith	*Rhodopseudomonas spheroides*: High Catalase and Blue-Green Double Mutants	*Biochem. Biophys. Research Communs.* **3**, 143–45 (1960)
Cole, B. T.	Modification of an Ionization Chamber Gas-Liquid Chromatography Instrument	*Anal. Chem.* **33**, 317–18 (1961)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Cudkowicz, Gustavo, and G. E. Cosgrove	Immunologically Competent Cells in Adult Mouse Liver: Studies with Parent-to-Hybrid Radiation Chimeras	*Proc. Soc. Exptl. Biol. Med.* **105**, 366–71 (1960)
Cudkowicz, Gustavo, and G. E. Cosgrove	Modified Homologous Disease Following Transplantation of Parental Bone Marrow and Recipient Liver into Irradiated F_1 Mice	*Transplant. Bull.* **27**, 90–94 (1961)
Davidson, Douglas	Meristem Initial Cells in Irradiated Roots of *Vicia faba*	*Ann. Botany (London)* **24**, 287–95 (1960)
Davidson, Douglas	Protection and Recovery from Ionizing Radiation: Mechanisms in Seeds and Roots	In *Radiation Protection and Recovery* (ed. by Alexander Hollaender), Pergamon, London, 1960, pp 175–211
Davidson, Douglas, and N. G. Anderson	Chromosome Coiling: Abnormalities Induced by Polyamines	*Exptl. Cell Research* **20**, 610–13 (1960)
Doherty, D. G.	Chemical Protection to Mammals Against Ionizing Radiation	In *Radiation Protection and Recovery* (ed. by Alexander Hollaender), Pergamon, London, 1960, pp 45–86
Dolin, M. I.	The *Streptococcus faecalis* Oxidases for Reduced Diphosphopyridine Nucleotide. VI. Inhibition of the Flavoprotein Peroxidase by Its Electron Donor	*Biochim. et Biophys. Acta* **42**, 61–69 (1960)
Gaulden, Mary E.	The Nucleolus and Mitotic Activity	In *The Cell Nucleus* (ed. by J. S. Mitchell), Butterworths, London, 1960, pp 15–17
Goodman, Joan W.	Bone Marrow Cell Separation Studies	*Exptl. Cell Research* **21**, 88–97 (1960)
Grell, Rhoda F., and E. H. Grell	Correlation of Primary Nondisjunction of X chromosomes with the Segregation of Certain Autosomes in Oocytes of *D. m.*	*Drosophila Inform. Serv.* **33**, 137–39 (1959)
Gude, W. D., and A. C. Upton	Spontaneous Glomerulosclerosis in Aging RF Mice	*J. Gerontol.* **15**, 373–76 (1960)
Hollaender, Alexander	"Die Mitogenetische Strahlung" by A. G. Gurwitsch and L. D. Gurwitsch, Gustav Fischer, Jena, 1959, 308 pages (Manuskript aus dem Russischen ins Deutsch übersetzt von L. S. Kosman, Moskau)	*Quart. Rev. Biol.* **35**, 246–47 (1960) (book review)
Hollaender, Alexander	Introduction	In *Radiation Protection and Recovery* (ed. by Alexander Hollaender), Pergamon, London, 1960, pp 1–2
Hollaender, Alexander (editor)	*Radiation Protection and Recovery*	Pergamon, London, 1960, 392 pages
Hollaender, Alexander	Studies on Microorganisms	*Federation Proc.* **19**, 562–63 (1960)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Hollaender, Alexander	Introduction (for Symposium on Mammalian Genetics and Reproduction)	*J. Cellular Comp. Physiol.* **56** (suppl 1), December 1960, 193 pages
Hollaender, Alexander, and S. F. Carson	*Biology Division Semiannual Progress Report for Period Ending August 15, 1960*	ORNL-2997
Iwamura, Tatsuichi	Distribution of Nucleic Acids Among Subcellular Fractions of *Chlorella*	*Biochim. et Biophys. Acta* **42**, 161–63 (1960)
Jacobson, K. B.	Inhibition of Acetyl Transfer by Amethopterin	*J. Biol. Chem.* **235**, 2713–16 (1960)
Jagger, John	Photoprotection from Ultraviolet Killing in *Escherichia coli* B	*Radiation Research* **13**, 521–39 (1960)
Jagger, John	Photoreactivation	In *Radiation Protection and Recovery* (ed. by Alexander Hollaender), Pergamon, London, 1960, pp 352–77
Jagger, John	Photoreactivation	*Radiation Research* (suppl 2), 75–90 (1960)
Kastenbaum, M. A.	A Note on the Additive Partitioning of Chi-Square in Contingency Tables	*Biometrics* **16**, 416–22 (1960)
Kastenbaum, M. A.	The Separation of Molecular Compounds by Countercurrent Dialysis: A Stochastic Process	*Biometrika* **47**, 69–77 (1960)
Khym, J. X., and W. E. Cohn	Characterizations and Some Chemical Reactions of Periodate-Oxidized Nucleosides	*J. Am. Chem. Soc.* **82**, 6380–86 (1960)
Kimball, A. W.	Estimation of Mortality Intensities in Animal Experiments	*Biometrics* **16**, 505–21 (1960)
Kimball, R. F., L. Vogt-Köhne, and T. O. Caspersson	Quantitative Cytochemical Studies on *Paramecium aurelia*. III. Dry Weight and Ultraviolet Absorption of Isolated Macronuclei During Various Stages of the Interdivision Interval	*Exptl. Cell Research* **20**, 368–77 (1960)
Kondo, Sohei	Neutron Response of Silver-Activated Phosphate Glass	*Health Phys.* **4**, 21–24 (1960)
Kroeger, Heinrich	Hypo- and Hyperdevelopment of the Male Genital Apparatus and the *Bd-M* Combination in *Drosophila melanogaster*	*J. Morphol.* **107**, 227–32 (1960)
Maisin, J. R., and D. G. Doherty	Chemical Protection of Mammalian Tissues	*Federation Proc.* **19**, 564–72 (1960)
Maisin, J. R., G. D. Novelli, D. G. Doherty, and C. C. Congdon	Chemical Protection of the Alimentary Tract of Whole-Body X-Irradiated Mice. I. Changes in Weight, Histology, and Cell Division in Relation to Nucleic Acid and Protein Synthesis	*Intern. J. Radiation Biol.* **2**, 281–93 (1960)
Maisin, J. R., and R. A. Popp	Effect of AET on Sodium, Potassium, and Esterases of the Alimentary Tract of Irradiated Mice	*Am. J. Physiol.* **199**, 251–55 (1960)
Makinodan, Takashi	Advances in Radiation Immunology	*Federation Proc.* **19**, 586–89 (1960)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Makinodan, Takashi, and Nazareth Gengozian	Effect of Radiation on Antibody Formation	In *Radiation Protection and Recovery* (ed. by Alexander Hollaender), Pergamon, London, 1960, pp 316–51
Makinodan, Takashi, Nazareth Gengozian, and R. E. Canning	Demonstration of a Normal Serum Macroglobulin Coprecipitating with the Bovine Serum Albumin (BSA)–Chicken Anti-BSA Precipitate	*J. Immunol.* **85**, 439–46 (1960)
Mans, R. J., and G. D. Novelli	A Convenient, Rapid, and Sensitive Method for Measuring the Incorporation of Radioactive Amino Acids into Protein	*Biochem. Biophys. Research Communs.* **3**, 540–43 (1960)
Moutschen, Jean, and H. K. Ezell, Jr.	Uptake of Tritiated Myleran in Mouse Testicle	*Radiation Research* **13**, 898–902 (1960)
Nicoletti, Benedetto	An Efficient Method for Salivary Gland Chromosome Preparation	*Drosophila Inform. Serv.* **33**, 181–82 (1959)
Novelli, G. D.	Protein Synthesis in Microorganisms	*Ann. Rev. Microbiol.* **14**, 65–82 (1960)
Odell, T. T., Jr., G. E. Cosgrove, and A. C. Upton	Modification of Delayed Somatic Effects of Ionizing Radiation	In *Radiation Protection and Recovery* (ed. by Alexander Hollaender), Pergamon, London, 1960, pp 303–15
Odell, T. T., Jr., and T. P. McDonald	Life Span of Mouse Blood Platelets	*Proc. Soc. Exptl. Biol. Med.* **106**, 107–8 (1961)
Parsons, D. F.	An Efficient Method of Cleaning Platinum Electron Microscope Apertures	*Rev. Sci. Instr.* **31**, 1158–59 (1960)
Peck, H. D., Jr.	Adenosine 5′-Phosphosulfate as an Intermediate in the Oxidation of Thiosulfate by *Thiobacillus thioparus*	*Proc. Natl. Acad. Sci. U.S.* **46**, 1053–57 (1960)
Peck, H. D., Jr.	Evidence for Oxidative Phosphorylation During the Reduction of Sulfate with Hydrogen by *Desulfovibrio desulfuricans*	*J. Biol. Chem.* **235**, 2734–38 (1960)
Phan The Tran and M. A Bender	Factors Affecting the Survival of Mouse Bone Marrow Cells During Freezing and Thawing in Glycerol	*J. Appl. Physiol.* **15**, 939–42 (1960)
Phan The Tran and M. A Bender	Effet de la moelle osseuse de Souris, après congélation en présence de quelques oses et de leurs dérivés polyalcooliques, sur la survie après irradiation par une dose léthale de rayons X	*Compt. rend. soc. biol.* **154**, 1090 (1960)
Phan The Tran and M. A Bender	Survival of Mouse Bone-Marrow Cells Frozen and Thawed in Solutions of Amino Acids	*Exptl. Cell Research* **20**, 651–54 (1960)
Popp, R. A.	Erythrocyte Repopulation in X-Irradiated Recipients of Nucleated, Peripheral Blood Cells of Normal Mice	*Proc. Soc. Exptl. Biol. Med.* **104**, 722–24 (1960)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Popp, R. A., and W. St. Amand	Studies on the Mouse Hemoglobin Locus. I. Identification of Hemoglobin Types and Linkage of Hemoglobin with Albinism	*J. Heredity* **51**, 141–44 (1960)
Randolph, M. L.	Application of Analog Computers to ESR Spectroscopy	*Rev. Sci. Instr.* **31**, 949–52 (1960)
Russell, L. B., and W. L. Russell	Genetic Analysis of Induced Deletions and of Spontaneous Nondisjunction Involving Chromosome 2 of the Mouse	*J. Cellular Comp. Physiol.* **56** (suppl 1), 169–88 (1960)
Russell, W. L.	Recent Advances in Genetics of Mice	In *Molecular Genetics and Human Disease* (ed. by L. I. Gardner), Thomas, Springfield, Ill., 1960, pp 134–52
St. Amand, G. A., N. G. Anderson, and M. E. Gaulden	Cell Division. IV. Acceleration of Mitotic Rate of Grasshopper Neuroblasts by Agamatine	*Exptl. Cell Research* **20**, 71–76 (1960)
Schwartz, Drew	Analysis of a Highly Mutable Gene in Maize. A Molecular Model for Gene Instability	*Genetics* **45**, 1141–52 (1960)
Schwartz, Drew	Deoxyribonucleic Acid and Chromosome Structure	In *The Cell Nucleus* (ed. by J. S. Mitchell), Butterworths, London, 1960, pp 227–30
Schwartz, Drew	Electrophoretic and Immunochemical Studies with Endosperm Proteins of Maize Mutants	*Genetics* **45**, 1419–29 (1960)
Schwartz, Drew	Genetic Studies on Developing Endosperm Proteins	*Maize Genet. Coop. News Letter* **34**, 99 (1960)
Schwartz, Drew	Genetic Studies on Mutant Enzymes in Maize: Synthesis of Hybrid Enzymes by Heterozygotes	*Proc. Natl. Acad. Sci. U.S.* **46**, 1210–15 (1960)
Shekarchi, I. C., and Takashi Makinodan	Electrophoretic Analyses of Sera from Mice Protected from Lethal X Radiation	*Intern. J. Radiation Biol.* **2**, 353–60 (1960)
Sicard, M. A., and Drew Schwartz	Dépendence du mode de conservation après irradiation et des radiolésions produites dans les graines d'orge sêches	*Compt. rend.* **251**, 897–99 (1960)
Smith, L. H., and C. C. Congdon	Experimental Treatment of Acute Whole-Body Radiation Injury in Mammals	In *Radiation Protection and Recovery* (ed. by Alexander Hollaender), Pergamon, London, 1960, pp 242–302
Stapleton, G. E.	Protection and Recovery in Bacteria and Fungi	*Ibid.*, pp 87–116
Stapleton, G. E.	Radiation Microbiology	In *McGraw-Hill Encyclopedia of Science and Technology*, vol 11 (Editor-in-Chief, W. H. Crouse), McGraw-Hill, New York, 1960, pp 236–38
Stapleton, G. E., and M. S. Engel	Cultural Conditions as Determinants of Sensitivity of *Escherichia coli* to Damaging Agents	*J. Bacteriol.* **80**, 544–51 (1960)
Upton, A. C.	Ionizing Radiation and Aging	*Gerontologia* **4**, 162–76 (1960)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Upton, A. C.	Ionizing Radiation and the Aging Process	In *The Biology of Aging* (ed. by B. L. Strehler), AIBS, Washington, D.C., 1960, pp 318–23
Upton, A. C.	Radiation Injury	In *McGraw-Hill Encyclopedia of Science and Technology*, vol 11 (Editor-in-Chief, W. H. Crouse), McGraw-Hill, New York, 1960, pp 225–35
Upton, A. C., A. W. Kimball, Jacob Furth, K. W. Christenberry, and W. H. Benedict	Some Delayed Effects of Atom-Bomb Radiations in Mice	*Cancer Research* 20(8) (part 2), 1–62 (1960)
Upton, A. C., T. T. Odell, Jr., and E. P. Sniffen	Influence of Age at Time of Irradiation on Induction of Leukemia in RF Mice	*Proc. Soc. Exptl. Biol. Med.* 104, 769–72 (1960)
Volkin, Elliot	The Function of RNA in T2-Infected Bacteria	*Proc. Natl. Acad. Sci. U.S.* 46, 1336–49 (1960)
von Borstel, R. C.	Sulla natura della letalità dominante indotta dalle radiazioni	*Atti Assoc. Genet. Ital.* 5, pp 35–50 (1960)
von Borstel, R. C., and P. A. Smith	Haploid Intersexes in the Wasp *Habrobracon*	*J. Heredity* 15, 29–34 (1960)
Wells, Carolyn	The Response of *Tetrahymena pyriformis* to Ionizing Radiation: Strain Specific Radiosensitivities	*J. Cellular Comp. Physiol.* 55, 207–19 (1960)
Wells, Carolyn	*Tetrahymena pyriformis*, Strain HS: Micronucleate or Amicronucleate?	*J. Protozool.* 7, 313–14 (1960)
Whiting, Anna R.	Protection and Recovery of the Cell from Radiation Damage	In *Radiation Protection and Recovery* (ed. by Alexander Hollaender), Pergamon, London, 1960, pp 117–56
Wolf, F. T.	Pteridine Pigments in Microorganisms and Higher Plants	In *Comparative Biochemistry of Photoreactive Systems* (ed. by Mary B. Allen), Academic Press, New York, 1960, pp 53–67
Wolff, Sheldon	Chromosome Aberrations	In *Radiation Protection and Recovery* (ed. by Alexander Hollaender), Pergamon, London, 1960, pp 157–74
Wolff, Sheldon	Problems of Energy Transfer in Radiation-Induced Chromosome Damage	*Radiation Research* (suppl 2), 122–32 (1960)
Wolff, Sheldon, and D. L. Lindsley	Effect of Oxygen Tension on the Induction of Apparent XO Males in Drosophila	*Genetics* 45, 939–47 (1960)

Lectures. – A total of 116 lectures – averaging 20 a month – were given by Biology Division staff members in the period from August 1960 to February 1961. Of these, 60 were made under the Traveling Lecture program and 56 were presented before professional societies, including 6 given abroad. Alexander Hollaender, D. M. Prescott, Takashi Makinodan, T. T. Odell, Jr., R. C. von Borstel, and Miriam Sidran gave lectures in other countries described in more detail in the section "Foreign Travel."

SPEAKER (AND COAUTHORS)	TITLE	PLACE PRESENTED
Anderson, N. G.	Cell Fractionation	Wilson College, Chambersburg, Pa.
Anderson, N. G.	Origin of Life	1. Dickinson College, Carlisle, Pa. 2. Gettysburg College, Gettysburg, Pa. 3. Wilson College, Chambersburg, Pa.
Arnold, W. A.	Hole-Electron Pair Separation in Illuminated Chromatophores	N.A.S. Symposium on Energy-Linked Biological Reactions, Philadelphia, Pa.
Astrachan, Lazarus	Resemblance of Bacterial RNA and DNA	1. Indiana University, Bloomington 2. University of Illinois, Urbana 3. Annual Phage Meeting, Cold Spring Harbor, N.Y.
Bender, M. A	Chromosome Evolution in Primates	Frank Phillips College, Borger, Tex.
Bender, M. A	Effects of Radiation on Cellular Survival	Symposium on Current Topics in Radiobiology, SE Section, Soc. Exptl. Biol. Med., Oak Ridge, Tenn.
Bender, M. A	Radiation-Induced Chromosome Damage in Mammals	1. University of Texas, Austin 2. Stephen F. Austin College, Nacogdoches, Tex.
Bender, M. A	X-Ray-Induced Chromosome Aberrations in the Chinese Hamster in Vivo [*Genetics* **45**, 975 (1960)]	AIBS, Genetics Society, Stillwater, Okla.
Buzzati-Traverso, A. A. (von Borstel, R. C., and A. A. Buzzati-Traverso)	On the Role of Lethal Mutants in the Control of Populations	Colloquium on Radioisotopes and Radiation Entomology, Trombay, India
Chu, E. H. Y.	The Cytogenetics of Mammalian Cells in Vitro	Cell Culture Collection Committee of the Viruses and Cancer Panel, Natl. Adv. Cancer Council, Bethesda, Md.
Chu, E. H. Y. (V. C. Monesi)	Analysis of X-Ray-Induced Chromosome Aberrations in Mouse Somatic Cells in Vitro [*Genetics* **45**, 981 (1960)]	AIBS, Genetics Society, Stillwater, Okla.
Clayton, R. K.	Ionization and Semiconductor Mechanisms in Photosynthesis	Southern Section, Am. Soc. Plant Physiol., Jackson, Miss.

SPEAKER (AND COAUTHORS)	TITLE	PLACE PRESENTED
Cohn, W. E.	Recent Developments in Nucleotide Chemistry	Biochemistry Study Group, Fort Detrick, Md.
Cohn, W. E.	Sequence Determination in Ribonucleic Acids	Yale University Medical School, New Haven, Conn.
Cohn, W. E.	The Use of Nuclear Magnetic Resonance in Nucleotide Structure Investigations	Biochemical Research Department, Lederle Laboratories, New York, N.Y.
Congdon, C. C.	Modification of Radiation Injury: Protection and Recovery	Symposium on Current Topics in Radiobiology, SE Section, Soc. Exptl. Biol. Med., Oak Ridge, Tenn.
Cudkowicz, Gustavo	Evidence for Immunological Reactions by F_1 Hybrid Mice Against Parental Strain Tissues	Tenn. Acad. Sci., Knoxville
Davidson, Douglas	Polyamines and Chromosome Condensation [*Genetics* **45**, 983–84 (1960)]	AIBS, Genetics Society, Stillwater, Okla.
de Serres, F. J.	Allelic Complementation Among *ad-3* Mutants of *Neurospora*	Vanderbilt University, Nashville, Tenn.
de Serres, F. J.	Allelic Complementation Among *ad-3* Mutants of *Neurospora crassa* as Influenced by Mutagenic Origin	1. University of Florida, Gainesville 2. Florida State University, Tallahassee
Doherty, D. G.	Chemical Protection Against Ionizing Radiation	1. Duke University, Durham, N.C. 2. M. D. Anderson Hospital, Houston, Tex. 3. Oklahoma State University, Stillwater 4. Oklahoma Medical School, Oklahoma City
Doherty, D. G.	Enzyme Substrate Complex Formation	1. University of Houston, Houston, Tex. 2. M. D. Anderson Hospital, Houston, Tex. 3. Oklahoma State University, Graduate School, Stillwater
Doria, Gino (J. W. Goodman, Nazareth Gengozian, and C. C. Congdon)	Origin of Antibody-Forming Cells in the Spleen of Long-Term Homologous Bone Marrow and Fetal Liver Chimeras	SE Section, Soc. Exptl. Biol. Med., Oak Ridge, Tenn.
Ehling, U. H.	Strain Differences in the Reproductive Performance of Female Mice After X-Ray Exposure [*Genetics* **45**, 985 (1960)]	AIBS, Genetics Society, Stillwater, Okla.
Engel, M. S. (H. I. Adler)	Studies on the Mechanism of Induced Radioresistance in *Escherichia coli*	Ky.-Tenn. Branch, Soc. Am. Bacteriol., Nashville, Tenn.
Enochs, N. J. (A. H. Haber)	Gibberellin Action on Seedling Growth in the Absence of Photosynthesis	Tenn. Acad. Sci., Knoxville

SPEAKER (AND COAUTHORS)	TITLE	PLACE PRESENTED
Grimm, F. C. (D. G. Doherty)	Beef Heart Malic Dehydrogenase	Am. Chem. Soc., New York, N.Y.
Gude, W. D.	Some Biological Effects of Radiation	Tenn. Junior Acad. Sci., Knoxville
Haber, A. H.	A Critical Examination of the Concepts "Dormancy" and "Germination" in View of Recent Studies with Lettuce Seeds [*Plant Physiol.* **35** (suppl), xvii–xviii (1960)]	AIBS, Am. Soc. Plant Physiol., Stillwater, Okla.
Haber, A. H.	Physiology of Growth and Development	University of Tennessee, Knoxville
Hochman, Benjamin	Isoallelic Competition in Populations of *Drosophila melanogaster* Containing a Genetically Heterogeneous Background	AAAS, Annual Meeting Soc. Study of Evolution, New York, N.Y.
Hollaender, Alexander	Initial Steps in Radiation Damage to Chromosomes and Means of Preventing This Effect	Symposium on Initial Effects of Ionizing Radiations on Living Cells, Moscow, USSR
Jacobson, K. B.	Ribonucleic Acid Biosynthesis and Its Relation to Protein Synthesis	1. College of Medicine, University of Florida, Gainesville 2. Auburn University, Auburn, Ala. 3. University of Georgia, Athens
Jagger, John	Effects of Light on Living Cells	Southern Illinois University, Carbondale
Kimball, R. F.	Effects of Radiation on Genes and Chromosomes	Symposium on Current Topics in Radiobiology, SE Section, Soc. Exptl. Biol. Med., Oak Ridge, Tenn.
Kimball, R. F.	Recent Advances in the Study of the Genetic Effects of Radiation	Meharry Medical College, Nashville, Tenn.
Kimball, R. F.	Studies on the Postirradiation Period in Mutation Induction by Ionizing Radiation	Vanderbilt University, Nashville, Tenn.
Kimball, R. F. (S. W. Perdue)	Autoradiographic Studies of Nucleic Acid Synthesis During Growth of *Paramecium*	Tenn. Acad. Sci., Knoxville
Kimball, R. F. (S. W. Perdue)	The Relation of the Refractory Period for Mutation Induction and Nuclear Synthesis in *Paramecium aurelia*, Syngen 4 [*Genetics* **45**, 996 (1960)]	AIBS, Genetics Society, Stillwater, Okla.
Kirby-Smith, J. S.	The Biophysics of Chromosomal Damage	Sigma Xi, University of Tennessee Medical Units, Memphis
Kirby-Smith, J. S.	Mechanisms Involved in Chromosomal Breakage and Rejoining	Journal Club, University of Tennessee Medical Units, Memphis
Kirby-Smith, J. S.	Primary Processes in the Interaction Between Radiation and Biological Materials	Symposium on Current Topics in Radiobiology, SE Section, Soc. Exptl. Biol. Med., Oak Ridge, Tenn.
Kirby-Smith, J. S.	Radiation Biology	Southwestern College at Memphis, Memphis, Tenn.

SPEAKER (AND COAUTHORS)	TITLE	PLACE PRESENTED
Kirby-Smith, J. S.	Synergistic Action of X-Rays and Ultraviolet Radiation on Chromosomal Breakage in *Tradescantia* Pollen	Symposium on Mutation and Plant Breeding, Cornell University, Ithaca, N.Y.
Kirby-Smith, J. S. (Benedetto Nicoletti and M. L. Gwyn)	The Induction of Chromosomal Aberrations in *Tradescantia* Pollen by Combined X-Ray and Ultraviolet Treatment [*Genetics* **45**, 996 (1960)]	AIBS, Genetics Society, Stillwater, Okla.
Makinodan, Takashi	Antibody Synthesis and Cloning (Panelist)	Antibody Workshop, La Jolla, Calif.
Makinodan, Takashi	Bone Marrow Transplantation	Straub Clinic, Honolulu, Hawaii
Makinodan, Takashi	An Immunological Approach to Study Radiation-Induced Blood Chimeras	VIII International Congress of Hematology, Tokyo, Japan
Makinodan, Takashi	Immunologic Approach to Biologic Problems	National Institute of Genetics, Mishima, Japan
Makinodan, Takashi	Mechanism of Antibody Formation	National Institute of Radiological Sciences, Chiba, Japan
Makinodan, Takashi	Radiation-Induced Blood Chimeras	Kanko Regional Society of Radiology, Japan
Mazur, Peter	The Action of Low Temperatures on Living Cells	Duke University, Durham, N.C.
Mazur, Peter	Effect of the Physical State of Water on the Survival of Yeast Cells at Low Temperatures	AIBS, Botanical Society of America, Stillwater, Okla.
Novelli, G. D.	The Biological Synthesis of Protein	SE Regional Meeting, Am. Chem. Soc., Birmingham, Ala.
Novelli, G. D.	The Cell-Free Synthesis of β-Galactosidase	Rutgers University, New Brunswick, N.J.
Novelli, G. D.	Effects of Radiation on Bio-Chemical Reactions	Symposium on Current Topics in Radiobiology, SE Section, Soc. Exptl. Biol. Med., Oak Ridge, Tenn.
Novelli, G. D.	Mechanisms Involved in the Control of Enzyme Synthesis	15th Annual Symposium on Fundamental Cancer Research, Houston, Tex.
Oakberg, E. F.	Effects of Low Radiation Doses on Mammalian Germ Cells	Argonne National Laboratory, Argonne, Ill.
Oakberg, E. F.	Gamma-Ray Sensitivity of Oocytes of Young Mice [*Anat. Record* **137**, 385–86 (1960)]	AIBS, Am. Soc. Zool., Stillwater, Okla.
Oakberg, E. F.	Radiation Genetics	Symposium on Genetics, Western Reserve University, Cleveland, Ohio
Oakberg, E. F.	The Sensitivity of Oocytes of Young Mice to Acute and Chronic γ Rays	Tenn. Acad. Sci., Knoxville
Odell, T. T., Jr. (Masahide Asano and A. C. Upton)	Effects of Radiomimetic Agents on Blood-Forming Tissues and Homotransplantation	First Pan-American Congress of Biology and Experimental Pathology, Caracas, Venezuela

SPEAKER (AND COAUTHORS)	TITLE	PLACE PRESENTED
Odell, T. T., Jr. (Masahide Asano, T. P. McDonald, and T. T. Odell, Jr.)	Pathological Effects of Nitrogen Mustard on Mouse Tissue; Protection with AET	SE Section, Soc. Exptl. Biol. Med., Oak Ridge, Tenn.
Parsons, D. F.	Mammalian Tumor Viruses	Symposium on Virus-Cancer Interrelationships, SE Section, Soc. Exptl. Biol. Med., Oak Ridge, Tenn.
Parsons, D. F.	Observations on Mammalian Tumor Viruses	Tenn. Acad. Sci., Knoxville
Peck, H. D.	Comparative Metabolism of Inorganic Sulfur Compounds in Microorganisms	Yale University, New Haven, Conn.
Peterson, W. J.	Changes in Population of Antibody-Forming Cells in the Spleen After Primary Sensitization	Tenn. Acad. Sci., Knoxville
Phares, E. F. (M. V. Long and S. F. Carson)	Properties of Methylmalonyl Isomerase of *Propionibacterium pentosaceum*	Am. Chem. Soc., New York, N.Y.
Popp, R. A.	Inheritance of Mouse Hemoglobin Differences: Application to Studies on Cell Differentiation and Radiation Chimeras	1. Louisiana State University, Baton Rouge 2. Spring Hill College, Mobile, Ala.
Popp, R. A.	Mammalian Hemoglobins	Symposium on Methodology of Mammalian Genetics, Am. Genet. Assoc., Bar Harbor, Me.
Prescott, D. M.	The Cell Life Cycle	1. Yale University, New Haven, Conn. 2. Sigma Xi, Auburn, Ala. 3. Tuskegee Inst., Tuskegee, Ala. 4. University of Alabama, Tuscaloosa 5. Beta Beta Beta, Howard College, Birmingham, Ala. 6. Sigma Xi, University of Alabama Medical Center, Birmingham 7. A&M College of Texas, College Station 8. Louisiana State University, Baton Rouge
Prescott, D. M.	Nuclear Function and Nuclear-Cytoplasmic Interactions	1. University of Texas, Austin 2. A&M College of Texas, College Station
Prescott, D. M.	RNA Synthesis in the Nucleus and RNA Transfer to the Cytoplasm in *Tetrahymena pyriformis*	1st Joint Symposium, IUB and IUBS, Stockholm, Sweden
Randolph, M. L.	Saturation Measurements in the Production of Electron Spin Resonances in Crystalline Amino Acids by X Rays	Biophysical Society, St. Louis, Mo.

SPEAKER (AND COAUTHORS)	TITLE	PLACE PRESENTED
Robinson, Martha A.	Antibody-Forming Capacity of Spleen Cells at Varying Times After X-Irradiation	Tenn. Acad. Sci., Knoxville
Russell, Liane B.	The Genetics of Mammalian Sex Chromosomes	AAAS, Am. Soc. Zool., New York, N.Y.
Russell, Liane B. (J. W. Bangham)	Further Analysis of Variegated-Type Position Effects from X-Autosome Translocations in the Mouse [*Genetics* **45**, 1008–9 (1960)]	AIBS, Genetics Society, Stillwater, Okla.
Setlow, Jane K. (R. B. Setlow)	Ultraviolet Action Spectra of Single- and Double-Stranded DNA	Biophysical Society, St. Louis, Mo.
Setlow, R. B. (A. McNeal)	Physical State of Intracellular DNA of Phage ΦX174	Biophysical Society, St. Louis, Mo.
Sidran, Miriam	Electron Spin Resonance Spectra of Riboflavin and Its Complexes	9th AMPERE Conference, Pisa, Italy
Swanson, H. D.	A Model for Differentiation, Based on Gene-Inhibition by Basic Proteins [*Genetics* **45**, 1014 (1960)]	AIBS, Genetics Society, Stillwater, Okla.
Upton, A. C.	Biological Hazards of Radiation in the Modern World	Phillips Academy, Andover, Mass.
Upton, A. C.	Cancer in the World Around Us: Radiations, Natural and Man-Made	4th National Cancer Conference, Minneapolis, Minn.
Upton, A. C.	Changing Concepts Concerning Cancer: Radiation as an Etiologic Factor	4th National Cancer Conference, Minneapolis, Minn.
Upton, A. C.	Effects of Nuclear Radiation on Mammals	George C. Marshall Space Flight Center, Huntsville, Ala.
von Borstel, R. C.	Death of Drosophila Embryos of Exchanges in the Tandem Metacentric X Chromosome [*Genetics* **45**, 1015–16 (1960)]	AIBS, Genetics Society, Stillwater, Okla.
Webber, B. B.	Biochemical and Genetic Studies with Histidine – Three Mutants of *Neurospora crassa*	University of Tennessee, Knoxville
Welshons, W. J.	Pseudoallelism at the Notch locus of *D. melanogaster*	Midwestern Drosophila Geneticists, Bloomington, Ind.
Welshons, W. J. (E. S. Von Halle)	Further Studies of Pseudoallelism at the Notch locus of Drosophila [*Genetics* **45**, 1017 (1960)]	AIBS, Genetics Society, Stillwater, Okla.
Wolff, Sheldon	Biochemical Aspects of Radiation-Induced Genetic Damage (Chromosome Aberrations)	1. Duke University, Durham, N.C. 2. North Carolina State College, Raleigh
Wolff, Sheldon	Biophysical Aspects of Chromosome Aberration Induction	1. Biophysics Committee, University of Chicago, Chicago, Ill. 2. University of Kentucky, Lexington
Wolff, Sheldon	On the Apparent Synergistic Effects of Far-Red and X Rays on the Induction of Chromosome Aberrations	Symposium on Mutation and Plant Breeding, Cornell University, Ithaca, N.Y.

Visiting Lecturers. – During the period covered by this report, 44 lectures have been given on the Biology Seminar program by guest speakers from universities and scientific organizations at home and abroad. Visiting lecturers included scientists from Argentina, Australia, Brazil, Canada, Chile, England, France, Germany, Italy, Japan, the Netherlands, and Scotland.

SPEAKER	AFFILIATION	SUBJECT
J. M. Wiame	Department of Microbiology, New York University College of Medicine, New York	Growth and Repression of Enzyme Synthesis in Bacteria
Crodowaldo Pavan	Department of General Biology, University of São Paulo, São Paulo, Brazil	DNA-RNA and Protein Synthesis in Polytene Chromosomes
Tuneo Yamada	Faculty of Science, Nagoya University, Chikusa, Nagoya, Japan	A Protein Causing Mesodermal Differentiation in the Newt Ectoderm Cultured in Vitro
Louis Siminovitch	The Ontario Cancer Institute, Toronto, Canada	Polyoma Virus Induction of Tumors in Hamsters
P. J. Thung	Institut Neerlandais du Cancer, Laboratorium Bibliotheek, Amsterdam, Netherlands	Relations Between Involution, Hyperplasia, and Tumorigenesis in Old Age
Charles Ray, Jr.	Department of Biology, Emory University, Atlanta, Georgia	Fluorescence Microscopy and Cytogenetics of *Tetrahymena pyriformis*
Herman Klouwen	Institute for Enzyme Research, University of Wisconsin, Madison	The Role of Lipids in Electron-Transport
L. L. Cavalli-Sforza	Istituto di Genetica, Universita di Pavia, Pavia, Italy	Mutation Rate in Man
David Pratt	Virus Laboratories, University of California, Berkeley	The Onset of Mutation and of Stabilization to P^{32} Decay in T4 Infection
Marcello Siniscalco	Institute of Genetics, University of Naples, Naples, Italy	Thalassemia, Glucose-6-Phosphate Dehydrogenase and Malaria in Sardinia
Raymond Latarjet	Laboratoire Pasteur, de l'Institut du Radium, Paris, France	The Quantum Yield at 2537 A for Bacterial and Viral DNA
Roger Weinberg	Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pa.	Genetic Recombination in Bacteria
F. C. Steward	Department of Botany, Cornell University, Ithaca, N.Y.	Growth Induction in Plant Cells: Metabolic and Morphogenetic Considerations
R. M. Klein	Curator, Plant Physiology, The New York Botanical Garden, New York	Interaction of X Rays and Visible Radiation on Plants
Deana Klein	Albert Einstein College of Medicine, Yeshiva University, New York, N.Y.	Interaction of X Rays and Visible Radiation on Plants
H. A. Sober	U.S. Public Health Service, National Cancer Institute, Bethesda, Md.	Chromatography of Nucleic Acids
D. O. Jordan	Department of Chemistry, The University, Adelaide, Australia	The Chemical Basis of Heredity
Charles Greenblatt	National Institutes of Health, Bethesda, Md.	Studies with Human Chromosomes

SPEAKER	AFFILIATION	SUBJECT
G. E. Magni	Istituto di Genetica, Universita di Pavia, Pavia, Italy	Mutations in Yeast
Heinrich Gerhartz	Medical Department, Universität Clinic, Berlin, Germany	Chemical Damage to Bone Marrow
John Cairns	Carnegie Institution of Washington, Cold Spring Harbor, Long Island, N.Y.	Recent Work with the Pox Viruses
Hans Ris	Department of Zoology, University of Wisconsin, Madison	Some Speculations on the Evolution of the Cell
Makio Murayama	National Institutes of Health, Bethesda, Md.	The Specific Chemical Difference in Hemoglobin I
Makio Murayama	National Institutes of Health, Bethesda, Md.	Nature of Mercapto-Mercapto Interactions in Normal Human Hemoglobin
Joseph Albright	Department of Surgery, Medical College of Virginia, Hospital Division, Richmond	Studies of Organization in Model Systems Related to Cytoplasmic Organelles
Richard Young	National Aeronautics and Space Administration, Washington, D.C.	Discussion of Technical Aspects of Space Vehicles
Charlotte Auerbach	Institute of Animal Genetics, University of Edinburgh, Edinburgh, Scotland	Work in Progress at the Mutation Unit in Edinburgh
A. G. DeBusk	Department of Biological Sciences, Florida State University, Tallahassee	Discussion of Results Obtained in Space Research with *Neurospora* Spores
H. Fernandez-Moran	Massachusetts General Hospital, Boston	Electron Microscopy on Biological Materials at Low Temperatures
Rebeca Gerschman	University of Buenos Aires, Buenos Aires, Argentina	A Look at the Inherent Biological Effects of Oxygen
Mary Case	Yale University, New Haven, Conn.	Some Problems with Chemical Mutagenesis in *Neurospora*
R. M. Franklin	The Rockefeller Institute, New York, N.Y.	The Topology of Animal Viria and Animal Virus Multiplication – A Study in Structure and Function
George Hodgson	Section of Biology, University of Chile, Santiago, Chile	Physiology and Biochemistry of Erythropoiesis Stimulating Factor (ESF)
Jerome Gross	Massachusetts General Hospital, Boston	Collagen
H. J. Evans	Department of Biology, Brookhaven National Lab., Upton, Long Island, N.Y.	The Structure, Frequency, and Distribution of Radiation-Induced Chromatid Exchanges
Raymond Schofield	Radiobiology Department, Christie Hospital, Manchester, England	Survival in Primates (Rhesus Monkeys) from Lethal Doses of Radiation by Bone Marrow Transplants
M. J. Bessman	McCollum-Pratt Institute, The Johns Hopkins University, Baltimore, Md.	Enzyme Changes Induced by Phage Infection

SPEAKER	AFFILIATION	SUBJECT
J. J. Jaffe	Department of Pharmacology, Yale University School of Medicine, New Haven, Conn.	The Effect of 6-Azauracil on the Growth of *Trypanosoma equiperdum*
H. F. Blum	Department of Biology, Princeton University, Princeton, N.J.	Hyperplasia and Malignancy
Lawrence Levine	Graduate Department of Biochemistry, Brandeis University, Waltham, Mass.	Immunochemical Characterization of Bacteriophage DNA
Robert Darrow	Mill Hill, London, England	Amylase Synthesis in *Bacillus subtilis*
C. M. Pomerat	Research Director, Pasadena Foundation for Medical Research, Pasadena, Calif.	Recent Observations in Radiobiology with the Use of Cell Cultures
R. P. Perry, Jr.	The Institute for Cancer Research, Fox Chase, Philadelphia, Pa.	On Ribonucleic Acid Synthesis and Transport Within an Intact Cell
C. C. Li	Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pa.	Population Genetics

Speakers at Professional Meetings, Spring–Summer 1961. – Fifty-nine speakers on Biology Division research are currently scheduled to present papers or participate in symposia before 12 professional societies during the period between this and the next semiannual report. As usual it is anticipated that additional speeches will be scheduled later in the period. For the corresponding time last year, March–August 1960, 56 talks were originally listed; 71 were given. Listed below in calendar order are the societies meeting during the coming period, followed by the names of the speakers:

Southeastern Chapter, Society of Nuclear Medicine – R. J. Mans.

Fifth Southeastern Developmental Biology Conference – Douglas Davidson.

139th National Meeting of the American Chemical Society – J. X. Khym.

South Carolina Academy of Science – B. T. Cole.

Fourteenth Annual Biology Research Conference – H. I. Adler, R. F. Kimball (coauthor, S. Wolff), J. S. Kirby-Smith (coauthor, M. L. Randolph), G. D. Novelli (coauthor, T. Kameyama).

52nd Annual Meeting of the American Association for Cancer Research – A. C. Upton (coauthors, J. W. Conklin and M. L. Randolph), Gustavo Cudkowicz.

Federation of American Societies for Experimental Biology – N. G. Anderson (coauthors, M. L. Barber and Sister Francis Clare Ladd), Lazarus Astrachan (coauthor, Thelma Fisher), R. R. Becker (coauthors, O. A. Darling and R. G. Ona), A. Bezkorovainy (coauthor, D. G. Doherty), E. E. Capalbo (coauthors, Paul Urso and W. D. Gude), Franco Celada (coauthor, Rachel Carter), R. K. Clayton, W. E. Cohn (coauthor, J. X. Khym), C. C. Congdon, Gustavo Cudkowicz, Gino Doria (coauthors, J. W. Goodman, N. Gengozian, and C. C. Congdon), F. J. Finamore (coauthor, Elliot Volkin), H. D. Peck (coauthor, Earl Fisher), Wallace Friedberg (coauthor, V. K. Jenkins), J. W. Goodman, A. L. Kretchmar (coauthor, C. C. Congdon), Charlotte Lea (coauthor, G. D. Novelli), Rusty J. Mans, T. P. McDonald (coauthors, M. Asano and D. G. Doherty), T. T. Odell, Jr. (coauthor, T. P. McDonald), E. H. Perkins (coauthor, Martha A. Robinson), and Paul Urso (coauthor, T. Makinodan).

Symposium of the Society of General Microbiology (London, England) – F. J. de Serres.

Association of Southeastern Biologists – B. T. Cole (coauthor, N. G. Anderson).

Society of American Bacteriologists – M. I. Dolin (coauthors, E. Godiniaux and M. Grunberg-Manago), J. M. Eisenstadt (coauthor, G. D. Novelli), M. S. Engel (coauthor, G. E. Stapleton), Peter Mazur, H. D. Peck, S. F. Carson (E. F. Phares and S. F. Carson), M. B. Taylor (coauthor, G. D. Novelli), and W. H. Taylor (coauthor, G. D. Novelli). Also taking part in a special symposium on Autotrophy: R. K. Clayton.

American Society of Human Genetics – Margery W. Shaw (coauthor, E. H. Y. Chu).

Radiation Research Society – H. I. Adler (coauthor, E. G. Copeland), M. A Bender (coauthor, Carolyn Gooch), Franco Celada (coauthors, N. G. Anderson and Takashi Makinodan), Franco Celada (coauthor, W. J. Welshons), C. C. Congdon (coauthors, R. D. Owen, G. D. Novelli, and C. J. Wust), G. E. Cosgrove (coauthors, R. L. DeHart and A. C. Upton), H. L. Cromroy, Gustavo Cudkowicz, M. S. Engel, John Jagger (coauthor, R. S. Stafford), R. A. Popp, Jane Setlow, A. C. Upton, R. C. von Borstel, and Sheldon Wolff.

Foreign Travel. – Alexander Hollaender made a trip to Russia in October 1960 under the sponsorship of the United Nations Educational, Scientific and Cultural Organization and the International Atomic Energy Agency. He gave an invited paper in Moscow as part of the Symposium on Primary and Elementary Processes in Living Cells Initiated by Ionizing Radiations. His talk was entitled "Initial Steps in Radiation Damage to Chromosomes and Means of Preventing This Effect."

Additionally, Dr. Hollaender reviewed the work of the Biology Division before the Institute of Radiation and Physico-Chemical Biology at Moscow. Also in that city, he participated in a seminar on radiobiology at the Kurchatov Institute for Nuclear Research. He also spent a few days in Poland where he visited with scientific personnel. At Warsaw, he conducted a discussion before the Institute of Hygiene on radiation protection work being done in the Biology Division of the Oak Ridge National Laboratory.

In September, W. L. Russell served by invitation as an advisor to the United States delegation at the Eighth Session of the United Nations Scientific Committee on the Effects of Atomic Radiation. The meeting was held in Geneva.

D. M. Prescott presented an invited paper at the First Joint Symposium of the International Union of Biochemistry and the International Union of Biological Sciences at Stockholm, Sweden, last September. He also attended the Tenth Congress of the International Society for Cell Biology in Paris.

C. C. Congdon attended the Symposium on Diagnosis and Treatment of Radiation Injury last October in Geneva, Switzerland. He led a discussion on "Problems for Future Study."

R. C. von Borstel was coauthor of a paper given by A. A. Buzzati-Traverso at a Colloquium on Radioisotopes and Radiation Entomology at Trombay, India. Miriam Sidran, a research participant in the summer of 1959, presented a paper at the Ninth A.M.P.E.R.E. Conference at Pisa, Italy, on her work at the Laboratory.

Takashi Makinodan gave a paper entitled "An Immunological Approach to the Study of Radiation-Induced Blood Chimeras," at the Eighth International Congress of Hematology, Tokyo, Japan. On his return to continental United States, Dr. Makinodan stopped on Hawaii, where he visited the University of Hawaii and presented two lectures.

In October, T. T. Odell, Jr., spoke at the First Pan-American Congress of Biology and Experimental Pathology in Caracas, Venezuela. At Puerto Rico he consulted with AEC officials and faculty members of the Biology Department of the University of Puerto Rico.

Fourteenth Annual Biology Research Conference. – The 1961 Spring Conference convened at the Mountain View Hotel in Gatlinburg, Tennessee, on April 3 for a four-day session. Supported by the Atomic Energy Commission, the conference was comprised of invited papers and open discussions on the general topic "Recovery of Cells from Injury." The conference considered various ways in which cells recover from or repair radiation injury, with attention to related biochemical and biophysical problems. The committee was composed of H. I. Adler, N. G. Anderson, C. C. Congdon, R. F. Kimball (chairman), G. D. Novelli, and Sheldon Wolff. The program was as follows:

MONDAY, APRIL 3

MORNING

Postirradiation Processes in Dry Material

Chairman – L. H. Gray, Mount Vernon Hospital and Radium Institute, Northwood, England

Modification of radiation-induced electron spin resonances – J. S. Kirby-Smith and M. L. Randolph, Biology Division, Oak Ridge National Laboratory

Reversible and irreversible radiation injury in dry biological systems – E. L. Powers, Argonne National Laboratory, Argonne, Ill.

Special Discussant – A. D. Conger, University of Florida, Gainesville

AFTERNOON

Renaturation and Reactivation of DNA

Chairman – Peter Alexander, Chester Beatty Research Institute, London, England

The effect of ultraviolet light on the biological and physical-chemical properties of DNA – Julius Marmur, Brandeis University, Waltham, Mass.

Repair of ultraviolet damage in cellular DNA – C. S. Rupert, The Johns Hopkins University, Baltimore, Md.

EVENING

Smoker – Host: Union Carbide Corporation

TUESDAY, APRIL 4

MORNING

Reactivation of Bacteriophage

Chairman – Herbert Marcovich, Institut du Radium, Laboratoire Pasteur, Paris, France

Gene controlled reactivation of ultraviolet-inactivated bacteriophage – Walter Harm, Institut für Genetik, Universität Köln, Köln, Germany

The role of recombination in phage reactivation – A. H. Doermann, Vanderbilt University, Nashville, Tenn.

AFTERNOON

Excursion: Great Smoky Mountains

EVENING

Reception and dinner

WEDNESDAY, APRIL 5

MORNING

Reversible Processes in Cell Inactivation

Chairman – Alexander Hollaender, Biology Division, Oak Ridge National Laboratory

Factors influencing bacterial survival after ionizing radiation – H. I. Adler, Biology Division, Oak Ridge National Laboratory

Special Discussant – Herbert Marcovich

Postirradiation kinetics of surviving mammalian cells grown in culture – M. M. Elkind, National Cancer Institute, Bethesda, Md.

AFTERNOON

Postirradiation Processes in the Induction of Mutation

Chairman – C. P. Swanson, The Johns Hopkins University, Baltimore, Md.

The terminal event in mutagenesis initiated by ultraviolet light – Evelyn M. Witkin, State University of New York, Brooklyn

Special Discussant – C. O. Doudney, M. D. Anderson Hospital, Houston, Tex.

Postirradiation processes in the induction of genetic changes by ionizing radiation – R. F. Kimball and Sheldon Wolff, Biology Division, Oak Ridge National Laboratory

EVENING

Round-Table Discussion: Evidence for Recovery Processes in Germ Cells

Chairman – Charlotte Auerbach, Institute of Animal Genetics, Edinburgh, Scotland

Participants: K. G. Lüning, Institute of Genetics, University of Stockholm, Sweden
E. F. Oakberg, Biology Division, Oak Ridge National Laboratory
I. I. Oster, The Institute for Cancer Research, Philadelphia, Pa.
F. H. Sobels, University of Leiden, Leiden, Netherlands
W. L. Russell, Biology Division, Oak Ridge National Laboratory

THURSDAY, APRIL 6

MORNING

Biochemical and Physiological Aspects

Chairman – Stanfield Rogers, University of Tennessee Memorial Research Center, Knoxville

Biochemical processes in injured cells in relation to cell recovery – Maurice Errera, Université Libre de Bruxelles, Bruxelles, Belgium

The effect of ultraviolet light on an enzyme forming system – G. D. Novelli, Tadenori Kameyama, and J. M. Eisenstadt, Biology Division, Oak Ridge National Laboratory

Annual Information Meeting. – The ORNL Advisory Committee for Biology made its annual visit to the division November 2, 3, and 4, 1960. Present were Drs. Erwin Chargaff, Curt Stern, E. L. Tatum, and P. C. Zamecnik. Group leaders and their associates presented a review of research studies then under way in sessions attended by all staff members. The Advisory Committee also visited individual

laboratories and participated in informal discussions. Union Carbide Corporation invited the committee and Biology Division staff to dinner November 2 at the Holiday Inn where Alexander Hollaender, who had just returned from Russia and Poland, spoke about biological research and living conditions in those two countries.

AET–Bone Marrow Conferences. – The fall 1960 Chemical Protection and Bone Marrow Conference was held November 18 and 19 at the Cancer Institute of the New England Deaconess Hospital in Boston. Drs. Shields Warren of that hospital and William Dameshek of the New England Medical Center organized the program which emphasized clinical research problems.

"Chemical Protection Against Ionizing Radiation" was the subject of the conference held March 16 and 17, 1961, at Rochester, New York. This program was arranged by Dr. Victor Di Stefano of the University of Rochester.

For April, Dr. L. M. Tocantins of Jefferson Medical College planned a round-table conference on new research in bone marrow transplantation and chemical protection at the time of the Federation Meetings in Atlantic City.

A series of specialized as well as general conferences is being planned for 1961 and 1962. Among them is a transplantation conference in Chile in late summer, 1961, to which C. C. Congdon and other Biology Division members have been invited.

Education. – In addition to participation in the Traveling Lecture and Small College programs, members of the Biology Division have presented a number of lectures both in the area and at schools throughout the country. At the request of the University of Georgia, S. F. Carson and G. D. Novelli gave lectures in connection with a United States Public Health Service Program. Sheldon Wolff presented a seminar entitled "Biophysical Aspects of Chromosome Aberration Induction" at the University of Chicago and participated in a one-week biophysics course at Harvard University. W. L. Russell and A. C. Upton spoke at the Twelfth Annual Nuclear Science Seminar, sponsored by the office of Naval Research Reserve in cooperation with the Atomic Energy Commission and the Oak Ridge National Laboratory. Dr. Upton gave two lectures in a six-week Radiation Safety Training Program conducted by ORNL for Radiation Control Officers. Additionally, he took part in three ORINS educational activities–the 77th session of the Radioisotopes Techniques School, the 12th Navy Research Seminar, and the Seventh Seminar for Pathologists: Diagnostic Uses of Radioisotopes. In February, Dr. Upton addressed an ORINS training group on the subject "Biological Effects of Radiation" and a Physics Division Seminar on the topic "Biological Hazards of Radiation." In January, E. F. Oakberg presented a seminar at Argonne National Laboratory on "Effects of Low Radiation Doses on Mammalian Germ Cells."

Along with the Physics and Chemistry Divisions, the Biology Division has been cooperating since January 1961 in a daily series of television lectures to seventh and eighth grade science students. G. D. Novelli, H. I. Adler, D. G. Doherty, Bruce Jacobson, and N. G. Anderson each gave a lecture in the "General Science" winter series.

Division members spoke to local high school students on "Career Day" and lectured before "The Traveling Science Teachers" program sponsored by ORINS.

Two courses for staff members were given in the six-months period being reported. J. S. Kirby-Smith and M. L. Randolph taught a ten-week course in Radiological Physics last fall. Just before the Annual Spring Conference at Gatlinburg, R. F. Kimball, chairman of the organizing committee for the conference, conducted a course that previewed the papers presented there. Division members who spoke at Gatlinburg (names listed with the program) lectured, as well as John Jagger and D. R. Krieg who summarized the background work of the speakers from the other laboratories.

The Biology Division is cooperating with ORINS in the preparation of a radiobiology course for their summer institute of 1961 and with the Health Physics Division in the preparation of a similar series of lectures.

Applications for the Summer Student Trainee program have been reviewed and 18 students selected to participate in 1961. Lecturers H. I. Adler, N. G. Anderson, and C. J. Wust have visited or are planning visits to undergraduate colleges in the southeast. Undergraduate groups have visited the Biology Division and other visits are scheduled for the spring months.

CYTOLOGY AND GENETICS

Paramecium

R. F. Kimball
Stella W. Perdue
H. Marine Scandlyn

Tradescantia, Vicia, Hordeum

Sheldon Wolff
Carlos García-Benítez[1]
H. E. Luippold
Sister Augustine Mattingly[2]

Maize and Phage

Drew Schwartz[3]
D. R. Krieg
Jane K. Setlow[4]
Rea M. Fulkerson
C. B. Kincaid
Kathrine H. McGrath

Neurospora

F. J. deSerres
H. E. Brockman[5]
H. G. Kølmark[6]
B. B. Webber[4]
Ida R. Cox
Arlee P. Teasley

Cell Growth and Reproduction

D. M. Prescott
R. C. von Borstel
Douglas Davidson[6]
J. G. Carlson[4]
Mary E. Gaulden[4]
E. A. Löbbecke[6]
Anna R. Whiting[4]
Romance F. Carrier
P. E. Eide
Catherine H. Shappert

Drosophila

W. J. Welshons[7]
D. L. Lindsley[8]
E. H. Grell
Rhoda F. Grell[4]
Benjamin Hochman[9]
C. G. Mead[2]
P. E. Thompson[5]
Guthrie T. Pratt
Mary L. Rekemeyer
Ruby D. Wilkerson

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[1]Loanee.

[2]USPHS Fellow.

[3]Leave of absence, NSF Senior Postdoctoral Fellowship, Jerusalem, Israel.

[4]Consultant.

[5]Research associate.

[6]Visiting investigator from abroad.

[7]Temporary *Drosophila* group leader.

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

[9]Research participant.

Induction of Mutations in *Paramecium aurelia* by 5-Bromodeoxyuridine and Its Relation to Radiation-Induced Mutation

R. F. Kimball Stella W. Perdue H. Marine Scandlyn

Introduction. – At least ten times as many mutations are produced in *Paramecium aurelia* by irradiation during the G1 (interphase before duplication) as in the G2 (interphase after duplication) period.[10,11] The most plausible hypothesis to account for the low rate of induction in G2 is that the doubleness of the chromosomes favors efficient repair of premutational chromosomal lesions.[11] An alternative hypothesis might be considered, however, if almost all of the mutations resulted from incorporation of radiation-altered precursors into DNA. In this case, altered precursors produced by irradiation during G1 might remain in an intracellular pool available for incorporation during S, the period of DNA synthesis. Altered precursors formed during G2 might either be eliminated rapidly during this period or at some subsequent period, such as cell division, and consequently be lost before the next S period. It seemed desirable to test this hypothesis, although on a priori grounds it seemed improbable.

In the absence of any information about the hypothetical radiation-altered precursors, it was decided to use the thymidine analog, 5-bromouracil deoxyriboside (BrUDR), which is known from work with microorganisms[12] to be incorporated into DNA and to be mutagenic. It would be anticipated that mutations induced by this analog would follow the same time pattern as those induced by radiation if the latter were due to altered precursors with similar properties to BrUDR.

Results. – Paramecia of known age since division were exposed for approximately $1\frac{1}{2}$ hr to 7.4 mg/ml of BrUDR in culture medium. Three age groups were used, 0.1 hr old, 1.6 hr old, and 3.1 hr old at the start of the exposure. The S period is short in *Paramecium aurelia*, requiring no more than a half hour around 2.5 hr after division. Thus the 0.1-hr group was entirely in G1 during exposure; the 1.6-hr group was in G1, S, and G2; and the 3.1-hr group was in G2 and perhaps the early part of prophase, which also has a low mutation rate with x rays. The amount of mutation (arbitrary units) in these three groups was 0.25 ± 0.04, 0.62 ± 0.07, and 0.32 ± 0.04, respectively. The untreated control had a rather high spontaneous rate, 0.23 ± 0.04. Thus there is no question that BrUDR is mutagenic for *Paramecium* when it is present during the S period, but there is no clear evidence for the induction of any mutations when it is present at other times.

Conclusions. – The results are those expected for an analog that induces mutation by incorporation into DNA and is not retained in an appreciable amount within an intracellular precursor pool.

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[10] R. F. Kimball, N. T. Gaither, and S. W. Perdue, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1960*, ORNL-2913, pp 29–30.

[11] R. F. Kimball and S. W. Perdue, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, pp 31–33.

[12] R. M. Litman and A. B. Pardee, *Biochim. et Biophys. Acta* **42**, 117–30 (1959).

They are not at all what would be expected if most of the mutations induced by radiation were produced by incorporation of radiation-altered precursors into DNA. Radiation induces at least 75% as many mutations in early G1 as it does in late G1. If BrUDR and x rays induced mutations in similar ways, the 0.1-hr group should have given a mutation value of 0.52, which is clearly significantly higher than the 0.25 actually found. Thus altered precursors behaving like BrUDR cannot be responsible for any large part of the radiation-induced mutation.

Autoradiographic Studies on RNA Synthesis in the Macronucleus of *Paramecium aurelia*

R. F. Kimball Stella W. Perdue H. Marine Scandlyn

Introduction. – It has been shown[13] by microspectrophotometric procedures that the amount of RNA in the *Paramecium* macronucleus is constant during the first half of the interdivision interval and increases only at the same time that DNA increases during the second half of the interval. Does this mean that RNA is not synthesized except when DNA is also being synthesized or does it mean that RNA is synthesized in and lost from the nucleus at the same rate during the first half of the interval? The latter alternative seems much more likely on the basis of other work. (See, for example, the work on the *Euplotes* macronucleus in another section of this present report.) To answer this question, the rate of formation of RNA was measured by the rate of incorporation of tritiated cytidine and uridine as judged by autoradiographs.

Results. – Preliminary experiments showed that tritiated cytidine and uridine were both incorporated specifically into RNA and not DNA, since all label is removed by ribonuclease digestion even when labeling was done during the period of DNA synthesis. Apparently cytidine is not utilized for DNA synthesis in *Paramecium*, unlike the situation in some other organisms.

The paramecia were placed in medium containing the labeled precursor for 10 to 15 min and then either immediately dried on slides or washed into unlabeled medium and incubated for a time before drying. The labeled precursors were incorporated at approximately the same rate in paramecia exposed in the early part of the interdivision interval as in those exposed in the later part. Most of the label was in the macronucleus when the paramecia were dried immediately after a brief labeling period. The amount of label in the macronucleus was less and there was considerable label in the cytoplasm when the paramecia were incubated for an hour in unlabeled medium.

Conclusions. – The data show that RNA synthesis in the macronucleus occurs at a nearly equal rate throughout the interdivision interval. Thus the absence of a net increase during the first half of the interdivision interval must mean that RNA is being lost from the nucleus as rapidly as it is formed. The net increase in RNA during the second half of the interdivision interval is associated with DNA synthesis and probably means that the amount of RNA retained in the nucleus at any one time is proportional to the amount of chromosomal material. The results are in good

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[13] R. F. Kimball, L. Vogt-Köhne, and T. O. Caspersson, *Exptl. Cell Research* 20, 368–77 (1960).

general agreement with work on *Tetrahymena*[14] and are consistent with the hypothesis that RNA is synthesized in the nucleus and then transferred to the cytoplasm.

On the Stage Sensitivity of Radiation-Induced Chromosome Aberrations

Sheldon Wolff H. E. Luippold

Introduction. – If cells are irradiated in various stages of cell division and then allowed to proceed to metaphase where induced chromosome aberrations can be observed, it then appears as though chromosomes in the different stages are differentially sensitive to radiation. The causes for such changes in sensitivity are actually unknown, although at one time or another it has been speculated that such changes are correlated with differences in coiling, DNA content, water content, and even other factors[15] at the molecular level.

Our recent studies on the biophysical aspects of the induction of chromosome aberrations by x rays have led to the conclusions that only breaks that are within 0.2 μ of one another can rejoin to give visible exchange aberrations and that there are only a small number of places in the nucleus where the chromosomes are within this distance over which they can rejoin. Breaks that do not have another break this close to them can only restitute, that is, rejoin in the original configuration. A consideration of the types of aberrations observed indicates that with the exception of the very small group of terminal deletions all aberrations come from the reunion of breaks. It now appears that the differential sensitivity of chromosomes at the different stages of cell division can be accounted for on the basis of the availability of another break within the distance over which an exchange can take place.

Results. – Table 1 shows the results of scoring for aberrations induced during a sensitive stage, when the chromosome reacts to the radiation as though double (chromatid aberrations), and during a relatively insensitive stage, when the chromosome reacts as though single (chromosome aberrations). It may be seen that the numbers of exchanges induced are about the same. The same is probably the case with the numbers of terminal deletions that come from breaks that are basically unrejoinable. The large increase in aberrations observed in the sensitive stage comes from the isochromatid aberrations that arise when breaks from two sister strands rejoin with one another.

Conclusion. – Within the nucleus of the cell there are very few sites where the chromosomes are close enough to one another so that breaks can rejoin to form aberrations. Breaks that are not in these places can only restitute, and the vast majority of primary breaks, therefore, do not give rise to visible aberrations. After chromosome duplication, however, the chromatid has a sister chromatid strand within the rejoining distance all along its length. Breaks, therefore, that would

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[14] D. M. Prescott and C. H. Shappert, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, pp 63–64.

[15] C. P. Swanson, in *Cytology and Cytogenetics*, Prentice-Hall, Englewood Cliffs, N.J., 1957.

Table 1. The Percentage of Various Types of Aberrations Induced in *Tradescantia* Microspores at a Sensitive Period (Chromatid Aberrations Observed) and in a Relatively Insensitive Period (Chromosome Aberrations Observed)

500 cells scored in each period

	Per Cent
Chromatid aberrations	
Deletions*	9.2
Isochromatid deletions	22.6
Exchanges	13.2
Total	45.0
Chromosome aberrations	
Terminal deletions	3.4
Interstitial deletions	9.2
Exchanges	11.8
Total	24.4

*The aberrations listed as chromatid deletions are a heterogeneous group composed of true deletions plus some achromatic lesions that are probably not true breaks.

not ordinarily be at a site where they would be close enough to another strand now automatically have a strand nearby. If this, too, is broken near the original break, the breaks now do not necessarily restitute but can form isochromatid aberrations. The increased sensitivity of chromosomes in different stages of cell division, therefore, does not seem to reflect a difference in sensitivity at the molecular level, but it is only a result of there being more opportunity for a break to rejoin by reuniting with another rather than restituting.

On the Increase of Sites for Chromosome Exchange Formation After Chromosome Duplication

Carlos García-Benítez Sheldon Wolff

Introduction. – Although most chromosome aberrations are distributed within the cell according to a Poisson distribution, it has been observed that chromosome exchanges, as a rule, do not fit such a distribution. Generally, the number of cells observed showing multiple exchanges is smaller than the number expected if this type of aberration were randomly distributed.[16] Such is the case with *Vicia faba* lateral roots and *Tradescantia paludosa* microspores when irradiated with x rays.

It has been shown that for chromosome exchanges to occur, the breaks must be produced within a certain distance h. This distance was originally calculated by Lea to be about 1 μ, but recently[16] it has been shown that this distance is actually 0.3 μ or may be even as small as 0.1 μ.

[16] S. Wolff *et al.*, *J. Biophys. Biochem. Cytol.* 4, 365 (1958).

Thus one can well figure that there will be a limited number of sites for the interphase chromosomes to lie within this calculated distance h. The average number of sites per cell at interphase for *Vicia* has been found to be 2 and for *Tradescantia* 4.

Chromatid exchanges in *Tradescantia*, as a rule, fit a Poisson distribution. Since chromatid exchanges occur after the chromosomes have duplicated, does this mean that the average site number per cell just doubles, or is it greatly increased? If it only doubles, then a site number of 8 would be proper for *Tradescantia*. With such a site number, the distribution of the data would be such that it would be impossible to distinguish between a Poisson and a binomial distribution.

However, in *Vicia*, where the average site number per cell is 2 for the interphase chromosomes, if this number only doubled and became 4 after chromosome duplication, the distribution of chromatid aberrations would not be expected to follow a Poisson distribution but would fit the binomial to the fourth power.

Results. – Table 2 shows the test for the goodness of fit with a Poisson distribution of the allotment of chromatid aberrations induced by x rays in *Tradescantia* microspores and in *Vicia faba* lateral roots.

Table 2. Number of Cells Showing 0, 1, 2, or 3 Chromatid Exchanges

Material	Radiation and Dose		Number of Cells with Indicated Number of Aberrations per Cell				χ^2 Test
			0	1	2	3	
Tradescantia	X rays, 120 r	Observed	208	76	14	2	$\chi^2 = 0.04786$, $n = 1$, $P = 0.5$
		Expected	209.4	75.3	15.3		
Vicia	X rays, 200 r	Observed	149	88	19	6	$\chi^2 = 0.908$, $n = 2$, $P = 0.62$
		Expected	149.6	85.6	23.3	5.5	

Conclusions. – Since the numbers of chromatid exchanges induced in *Vicia faba* lateral roots are distributed according to a Poisson distribution, it is apparent that, after chromosome duplication, there are many sites within the nucleus where the chromosomes lie within the exchange distance h. Therefore, after chromosome doubling, the site number which was 2 does not merely double. It appears that now other regions of the doubled chromosomes may come to lie within the calculated distance h of one another. Thus when breaks are produced by the radiation, there are enough places for exchanges to occur so that a random distribution of exchanges per cell will be obtained.

Interallelic Competition in Enzyme Synthesis in Maize

Drew Schwartz Kathrine H. McGrath

Earlier investigations on the genetic control of a basic esterase in maize have established that heterozygous combinations of any two of the alleles E^F, E^N, or E^S show both parental-type enzymes and in addition a new hybrid enzyme with an intermediate migration rate in starch gel electrophoresis.[17] An exceptional case has been studied recently in heterozygotes resulting from crosses of W-23 $(E^N/E^N)^{♀} \times$ M-14 (E^F/E^F). The F_1 endosperms contained only the N type enzyme rather than the expected N, NF, and F enzyme types. The reciprocal cross was not available, and plants are being grown for this test. In contrast to the situation in the endosperm, F_1 seedlings from the same cross showed all three enzyme types, N, NF, and F. It was possible to conduct electrophoretic analyses on extracts from single immature embryos in kernels harvested 16 days after pollination. The immature F_1 embryos were found to contain all three enzyme types, while the endosperms from the same kernels contained only the N enzyme type, lacking NF and F. M-14 inbred plants homozygous for E^F produced normal amounts of F type enzyme in the endosperm. It thus appears that whereas the E^F allele of M-14 can function normally, it is a "weak" or recessive allele in that it cannot compete against E^N.

A similar situation is found in the hybridization of maize with teosinte. The strain of teosinte employed in these studies was obtained from A. Shlomi of Israel and when tested was found to be segregating for two E alleles specifying basic esterase types exhibiting slow and normal migration rates. Teosinte seedlings heterozygous for the two alleles show an intermediate migrating hybrid enzyme type in addition to the two parental enzymes. However, the teosinte alleles are not identical to the E^S and E^N alleles in maize. Electrophoretic analyses of mixtures of maize and teosinte extracts, as well as crossing experiments, have established that the S allele of teosinte (E^{S^t}) and the N allele of teosinte (E^{N^t}) control the synthesis of esterase types which migrate at a faster rate than the esterases synthesized by E^S and E^N of maize respectively. These differences persist even when the teosinte alleles are introduced into maize cytoplasm, in the F_1 (maize $\times$ teosinte) and in the backcross [maize$^{♀}$ $\times$ (maize$^{♀}$ $\times$ teosinte)]. Thus five alleles of this esterase gene are now available: E^S, E^N, E^F, E^{S^t}, and E^{N^t}. In heterozygous condition the alleles from maize and teosinte interact to produce a hybrid enzyme type. E^F/E^{S^t} heterozygotes contain F, FS^t, and S^t enzymes.

F_1 seedlings resulting from crosses of maize single-cross 203 $\times$ R_4 $(E^F/E^F)^{♀}$ by teosinte (segregating E^{S^t} and E^{N^t}) should all be either E^F/E^{S^t} or E^F/E^{N^t} in genotype. However, out of 22 F_1 seedlings tested only 9 showed the presence of the esterase specified by the teosinte alleles (8 seedlings contained F, FS^t, and S^t enzyme types, and 1 had F and N^t). The remaining 13 seedlings contained only the F esterase type specified by the E^F allele from the maize parent. The results suggest that either the E^{N^t} allele cannot compete well against the E^F allele of maize or that

[17] D. Schwartz, *Proc. Natl. Acad. Sci. U.S.* **46**, 1210–15 (1960).

E^{N^t} does not function well in maize cytoplasm. Reciprocal crosses are being made to test whether or not the reverse situation is found when teosinte is used as the female parent in the cross.

Since this basic esterase represents only a small fraction of the total proteins synthesized, it is quite unlikely that the competition between the alleles is for available amino acids. If substrate competition is involved, the substrate would have to be something other than amino acids, such as peptides or some other limited subunits which are specifically involved in the synthesis of these enzymes. An alternative to substrate competition is repression either at the gene or ribosome level.

The Genetic Specificity of Ethyl Methane Sulfonate-Induced Mutations

D. R. Krieg Rea M. Fulkerson

Introduction. – The mutagenic action of DNA base analogs has contributed greatly to our understanding of gene mutation, and the *r*II mutants of bacteriophage T4 have been intensively studied in this regard. Such mutations have been interpreted as changes of individual nucleotide pairs within a portion of DNA. One class of changes, the so-called transitions, is the replacement of a guanine-cytosine pair by an adenine-thymine pair, or vice versa. Mutants induced by an analog are generally revertible by the same and other analogs; hence each of these mutagens is postulated to induce both kinds of transitions. Different analog-revertible mutants have been found to differ greatly in the magnitude of their response to the same mutagen, and they did not fit readily into two distinct classes. This failure to distinguish unambiguously the two types of transitions deprived the "transition theory" of one test of internal consistency but did not necessarily invalidate it, since one could assume that a mutagen induces the two transitions with highly variable rates dependent on the particular mutant examined. However, the interpretation would be greatly strengthened by the finding of a mutagen which induces only one of the transitions, or both but at clearly distinct rates not subject to such variability. Some observations of Green, in this laboratory, suggested that ethyl methane sulfonate (EMS) may be such a mutagen. EMS-induced mutants seemed generally revertible by analogs, and some analog-induced mutants were revertible by EMS, but he found no detectable EMS-induced reversion from EMS-induced mutants. Experiments are now in progress to substantiate and extend his tentative conclusions. Four sets of bacteriophage *r*II mutants are being investigated. They were induced from wild-type T4 by EMS, by the analogs 2-aminopurine and 5-bromouracil, and by proflavine – a different type of mutagen whose mutants are not generally revertible by analogs.

Methods. – The frequency of revertants is measured among the progeny of EMS-treated and control phage. A selective plating is utilized only after the potential revertants have had an opportunity to be expressed, and plaques due to so-called partial revertants can often be distinguished from typical wild-type plaques. Since "partial revertants" may be due to mutations at sites other than the one originally mutated, they may occur as exceptions to the pattern of revertibility expected for "true back-mutations."

Results and Discussion. – Seventeen mutants have now been tested in at least one experiment each. Three 2-aminopurine-induced mutants have been found to give apparent wild-type revertants

at a high level (50–125 per million progeny); one of these also yields partial revertants at a comparable level. One proflavine-induced mutant yields induced partial revertants only. The other mutants either yield no induced revertants or do so at a rate of one per million or less. The survey is planned to cover more than 40 mutants. If wild-type revertants are not induced from any of the proflavine set, we can infer that EMS-induced mutations are distinct from those induced by that agent; and if all of the EMS-induced mutants and some of the analog-induced mutants do not yield EMS-induced wild-type revertants, we can infer that only one of the two possible kinds of transitions is induced by EMS. In addition to supporting the transition concept of mutagenesis, such results would permit the distinguishing of guanine-cytosine and adenine-thymine sites, and these could help in testing the specificity of radiation and other mutagens.

Ultraviolet Action Spectra of Single- and Double-Stranded DNA

Jane K. Setlow R. B. Setlow

Introduction and Methods. – It has been postulated that single- and double-stranded DNA have different ultraviolet action spectra,[18] although the absorption spectra are similar, and that the action spectra criterion may be used to diagnose DNA strandedness in biological systems. This criterion has been used to provide evidence that T2 DNA has a single-stranded stage during replication.[19] A critical test of the action spectra hypothesis has been made by irradiating with monochromatic ultraviolet light biologically active DNA, *Hemophilus influenzae* transforming principle, under two conditions: (1) at 85°C, where the DNA is in native, two-stranded state, and (2) at 98°C, where hydrogen bonds are broken and the DNA may be considered single-stranded. The ability of the irradiated DNA to transform cells to streptomycin resistance was used as the measure of its biological activity.

Results. – The survival of irradiated transforming principle at each wavelength is given by the relation $\sqrt{N_0/N} = 1 + CD$, where N is the number of transformed cells after a dose D to the DNA and N_0 is the number after no dose. The constant C was taken as the measure of sensitivity at each wavelength. The sensitivity was plotted against wavelength to form an action spectrum. The shapes of the action spectra are different for the 85 and 98°C irradiations. The minimum in the action spectrum of the two-stranded DNA is at 2350 A, and that of single-stranded DNA is shifted over to 2375 A, similar to the change in action spectrum of T2 during intracellular development, which has been interpreted to be a change in DNA strandedness.

Conclusion. – The results indicate that the action spectra of single- and double-stranded DNA differ as previously postulated.

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[18] R. B. Setlow, *Biochim. et Biophys. Acta* **39**, 180 (1960).

[19] J. K. Setlow and R. B. Setlow, *Proc. Natl. Acad. Sci. U.S.* **46**, 791 (1960).

The Mechanism of Ultraviolet Inactivation of Transformation Principle

Jane K. Setlow

Introduction and Methods. – Data from ultraviolet inactivation of *Hemophilus influenzae* transforming principle (t.p.) may be fitted to an equation for a straight line, $\sqrt{N_0/N} = 1 + CD$, where N_0 is the number of bacteria transformed by unirradiated t.p., N is the number transformed by t.p. irradiated with dose D, and the proportionality constant C is a measure of sensitivity.[20] In an attempt to investigate the mechanism underlying this dose-survival relationship, experiments have been done combining ultraviolet inactivation of streptomycin-resistance-marked *Hemophilus influenzae* t.p. with the two other types of inactivation, given either before or after ultraviolet treatment: (1) sonication, and (2) heat above the melting point of the DNA.

Results. – It has been shown that ultrasonic treatment breaks DNA molecules.[21] Rupert and Goodgal's equation, with dose represented by time of sonication, also fits the sonication inactivation data. Previous sonication treatment decreases the uv sensitivity, and successive doses of the two types of inactivation appear to be additive. Heating above the melting point, with subsequent cooling, also decreases the uv sensitivity, as does heating after uv.

Discussion. – These data are in accord with a model like that discussed for cross-reactivation in bacteriophage.[22] Transformation by a marker requires genetic recombination within two intervals of the t.p. molecule, from the marker to its ends or nearest uv lesions, and the number of transformants decreases as the intervals shorten. The shorter the intervals, the larger the additional uv dose required to produce lesions within the intervals, since the "target" is smaller. Sonication decreases the amount of transformation by breaking molecules and thus shortening the intervals, and this type of inactivation and uv inactivation are additive, implying that the number of transformants depends only on the size of the intervals, not on whether the intervals are terminated by breaks in the molecule or by uv lesions. The heat data may also be explained with the aid of the model by assuming that the t.p. molecules with biological activity which have reconstituted after heating above the melting point are on the average shorter than before heating.

Complexities in the Induction of Colonial Morphology by Sorbose in *Neurospora crassa*

F. J. deSerres H. G. Kølmark H. E. Brockman

Introduction. – L-Sorbose has been used extensively to induce the colonial growth form in strains of *Neurospora crassa* that are not genetically colonial since the discovery of its colonizing effect by Tatum *et al.*[23] One per cent sorbose and 0.1% sucrose has been generally applicable,

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[20] C. S. Rupert and S. H. Goodgal, *Nature* 185, 556 (1960).

[21] P. Doty, B. B. McGill, and S. A. Rice, *Proc. Natl. Acad. Sci. U.S.* 44, 432 (1958).

[22] F. Stahl, pp 353–85 in *The Viruses*, ed. by F. M. Burnet and W. M. Stanley, Academic Press, New York, 1959.

[23] E. L. Tatum, R. W. Barratt, and J. M. Cutter, Jr., *Science* 109, 509–11 (1949).

but in forward-mutation experiments[24] utilizing these concentrations, a distinct colonial morphology was not always obtained. As a uniform and reproducible colonial morphology is essential for the determination of forward mutation rates at the *ad-3* locus, experiments were initiated to determine the basis of the observed variations.

Results. – As sorbose-sucrose solutions darken during autoclaving, it was suspected that filter-sterilized sorbose and/or sucrose might give more uniform colonial morphology. Surprisingly, when a filter-sterilized solution of sorbose and sucrose was tested, the viability of a conidial population of the wild-type strain 74A dropped to 1.7%. This result suggested that sucrose could not be efficiently utilized by *Neurospora* and that autoclaving yielded the hydrolysis products, fructose and glucose, which could be utilized as an energy source. The results shown in Table 3 supported the

Table 3. Viability of *Neurospora* Conidia

Autoclaved Solutions		Filter-Sterilized Solutions		
Autoclaving Time of Sucrose and Sorbose (min)	Per Cent Viability	Per Cent Fructose + Glucose	Per Cent Sucrose	Per Cent Viability
0	1.7	0	0.10	4.9
5	50.6	0.0002	0.0998	9.4
10	66.3	0.002	0.098	53.2
15	68.2	0.02	0.08	58.5
20	73.7	0.04	0.06	75.1
		0.06	0.04	70.9
		0.08	0.02	76.8
		0.10	0	78.3

validity of this reasoning. Viability increased as a function of increased autoclaving time of the sorbose-sucrose solutions and also increased as a function of the percentage of fructose + glucose present in a filter-sterilized mixture of sucrose, fructose, and glucose. However, when the sucrose-fructose-glucose mixtures were tested in the absence of sorbose in liquid media and on solid media, the growth rate was identical for all values tested from 0.1% sucrose + 0% fructose to 0% sucrose + 0.05% fructose + 0.05% glucose. These observations suggested that filter-sterilized sucrose could be utilized for growth as efficiently as fructose and glucose, but that in the presence of sorbose + filter-sterilized sucrose, only a small fraction of the total population could survive. By varying the concentration of sorbose from 0 to 3% in combination with 0.1% filter-sterilized sucrose, it was demonstrated that the viability decreased rapidly with increasing sorbose concentrations (Table 4). In order to determine if viability was influenced by previous culture conditions, conidia were harvested from cultures grown on media containing filter-sterilized sucrose,

[24] F. J. deSerres and H. G. Kølmark, *Nature* 182, 1249–50 (1958).

Table 4. Survival of *Neurospora* Conidia at Different Sorbose Concentrations

Per Cent Sorbose	Per Cent Viability
0	95.0
0.2	71.4
0.4	67.0
0.6	56.8
0.8	42.9
1.0	32.9
2.0	13.2
3.0	5.0

autoclaved sucrose, filter-sterilized glucose, filter-sterilized fructose, or filter-sterilized glycerol, and plated on media containing various concentrations of sorbose and 0.1% of either autoclaved or filter-sterilized sucrose. No significant differences in viability due to previous culture conditions were found.

Discussion. – It has been determined that two main factors: (1) the time that sucrose is autoclaved and (2) the concentration of sorbose influence the viability of *Neurospora* conidia and the morphology of colonies formed from viable conidia when sorbose is used as a colonizing agent in the presence of sucrose. As a result of these observations, it has been possible to determine that the most favorable conditions for colony formation in the forward-mutation experiments are to autoclave the sorbose and sucrose solutions separately in water for 15 min before adding to the autoclaved, cooled media. Experiments are in progress to determine if the difficulties encountered in the use of sorbose + sucrose to induce colonial growth might be more easily circumvented by utilizing sorbose + glucose or sorbose + fructose. Preliminary results indicate that fructose may be useful because the sorbose:fructose ratio which induces distinct colonial morphology is less than the sorbose:sucrose ratio of 10 which is generally used.

CELL GROWTH AND REPRODUCTION

Synthesis of RNA, DNA, and Protein in the Replicating Nucleus of the Ciliated Protozoan, *Euplotes*

D. M. Prescott R. F. Kimball Catherine H. Shappert

Introduction. – As a repository of genetic specificity, DNA must perform two functions: the dissemination of information to the surrounding metabolic system and its own self-replication during growth. It has been suggested that only double-helix DNA can transmit information to the cell, possibly with RNA as the intermediate carrier of specificity, and that only single-strand DNA is capable of self-replication. The macronucleus of *Euplotes* has some unique features that make it possible to study this problem through an examination of the relations between RNA, DNA, and

protein syntheses during replication. During the last half of interphase two reorganization bands travel through this very elongated nucleus, and Gall[25] has demonstrated that these bands represent waves of DNA synthesis. The experiments presented here deal with DNA, RNA, and protein syntheses in the nucleus during this replication.

Results. – The individual reorganization band consists of two zones. At the advancing border of the forward zone the granularly organized DNA is transformed into a homogeneously dispersed state. The latter is converted into a highly dilute form at the rear border of the forward zone, and the rear zone contains DNA in this highly diluted form only. Autoradiography with tritium thymidine shows that all DNA synthesis is restricted to this dilute zone. Histidine incorporation into protein is heavy only at this point. Staining and autoradiography with tritium uridine have shown that RNA is present and is synthesized throughout the nucleus except in the bands. Virtually all RNA is absent from both zones of the band, so that RNA is either destroyed or ejected from the nucleus into the cytoplasm at the advancing border of the band. No detectable synthesis of RNA takes place in the band itself. At the rear border of the rear band, the granular organization of DNA is re-established and RNA synthesis is resumed.

Discussion. – Mutual exclusiveness of RNA and DNA synthesis occurs in the *Euplotes* macronucleus. This result supports the hypothesis that DNA involved in its own replication is unable to serve any role, template or otherwise, in the synthesis of RNA. This separation of RNA and DNA syntheses may possibly reside in the two known configurations of DNA, double helix for release of information and single strand for self-replication. Although there is no direct evidence of single-strand DNA in *Euplotes* nuclei, there are two dramatic reorganizations of DNA at the microscopic level prior to DNA replication, and one of these might represent the formation of single-stranded molecules.

Protein synthesis occurs in very close association with DNA synthesis, although at what point of the molecular events of DNA synthesis protein is made cannot be decided here. Since RNA synthesis is not resumed until the chromatin protein has been synthesized, this protein possibly may have some role in permitting the release of information from DNA.

Differential Reactions of Native and Melted DNA Films with Dyes

R. C. von Borstel P. E. Eide

Introduction. – It is known that single-stranded DNA is required to prime DNA synthesis in vitro. Two methods are described here that distinguish between single- and double-stranded DNA.

Results. – The DNA is used at a dilution of 2 mg per milliliter of 0.02 *M* NH_4HCO_3. The single-stranded DNA is prepared by melting this concentration of DNA in a boiling water bath for 10 min. Three-tenths of a milliliter is pipetted onto glass or quartz strips, and the strips are vacuum dried.

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[25] J. G. Gall, *J. Biophys. Biochem. Cytol.* 5, 295 (1959).

The Feulgen method: During normal staining procedure, where the strips are passed from solution to solution, the melted DNA is readily soluble in 30% alcohol or water but the native DNA is not; consequently the strips with the double-stranded DNA are colored the characteristic bright crimson, whereas the strips on which the single-stranded DNA has been placed are blank. When acid hydrolysis and staining are carried out in solutions containing 50% or more alcohol, then both single-stranded and double-stranded stain the characteristic color.

Methyl green, pyronin: Staining of the films of DNA in a standard solution of methyl green and pyronin mixture dramatically demonstrates that single-stranded DNA differentially binds with the red pyronin dye, and the double-stranded DNA binds with the methyl green dye.

Discussion. – These two methods for demonstrating the presence of single- or double-stranded DNA are complementary in their usefulness. RNA also binds with pyronin, but RNA is susceptible to ribonuclease and single-stranded DNA is not. If it is suspected that single-stranded DNA is present initially but goes into solution during incubation of the material in ribonuclease, then the Feulgen method can be used as a supplementary and definitive test.

When macronuclei of *Euplotes* are stained with methyl green and pyronin, the homogeneous band stains with the characteristic bluish green of methyl green, confirming the discovery of Prescott, Kimball, and Shappert[26] that RNA is not present in this region. The zone of DNA synthesis, directly behind the bluish-green homogeneous band, appears as a thin red line, indicating that DNA is single-stranded in this zone, but this conclusion cannot be considered as definitive at this time.

RNA Synthesis and Mitosis

Douglas Davidson

Introduction. – In *Tetrahymena*[27] and *Vicia*[28] RNA synthesis, as measured by the incorporation of tritium-labeled cytidine, occurs primarily, if not exclusively, in the nucleus. The experiments to be reported here were carried out to test the ability of cells to synthesize RNA while the chromosomes were in various stages of mitotic condensation.

Results. – Grains were counted in autoradiographs of lateral roots of *Vicia* that had been exposed to tritiated cytidine for 30 and 60 min. (1) After 30 min exposure there was no detectable incorporation of labeled compound in cells at metaphase, anaphase, or telophase; the number of grains over these cells was not greater than background grain counts. Prophase cells incorporated some labeled precursor, though less than interphase cells. In both prophase and interphase cells the incorporation was greater in the nucleus than in the cytoplasm. (2) After 60 min exposure, metaphase-telophase cells again showed no incorporation of precursor. Marked incorporation had, however, taken place in the prophase and interphase cells; the nucleus/cytoplasm ratio was almost 2:1.

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[26] D. M. Prescott, R. F. Kimball, and C. H. Shappert, preceding paper in this report.

[27] D. M. Prescott and C. H. Shappert, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, pp 63–64.

[28] P. S. Woods, *Brookhaven Symposia in Biol.* No. 12, 153–74 (1959).

Discussion. – Incorporation of labeled precursor was greatest in interphase cells. It fell with the onset of prophase and could not be detected in metaphase-telophase cells. The evidence that the nucleus is the sole site of RNA synthesis suggests the following interpretation of these results. Only DNA in the dispersed state (interphase) is capable of supporting RNA synthesis. As the chromosomes begin to undergo mitotic condensation, RNA synthesis in the nucleus is shut off. Tritiated cytidine offered to cells in mitotic stages cannot enter nuclear RNA and presumably is not utilized by the cytoplasm because the cytoplasm has no independent capacity to synthesize RNA at any stage of the cell cycle.

DROSOPHILA

The Infertility of Sons of Irradiated Males in *Drosophila melanogaster*[29]

D. L. Lindsley S. A. Toledo[30] M. E. Lyrio[30] C. S. Camba[30]

As early as 1928 Muller[31] noted that the sons of irradiated males in *Drosophila* exhibit a much higher incidence of sterility than the sons of control males. Since such males are generally X/Y*; A/A* in constitution (where the asterisk indicates paternal derivation and A represents chromosomes 2, 3, and 4) the induced sterility may be attributed to changes in the Y chromosome or to autosomal dominant sterility mutations. Induced loss of either X or Y chromosome is an additional source of sterile males. Prabhu[32] showed that autosomal dominant mutations, at least those whose action is limited to males, do not contribute greatly to the sterility of X/Y*; A/A* males. Prabhu concludes, therefore, that Y chromosome mutation and/or sex chromosome loss play an important role in induced sterility, although he does not rule out a contribution of autosomal dominants that are not sex-limited in their action. Berg[33] and Prabhu[32] have demonstrated that the X chromosome is also subject to frequent male-sterilizing mutations.

The experiments described here were designed to estimate the incidence of X and Y chromosome sterility mutations (excluding sex chromosome loss) and autosomal dominant sterility mutations of both sex-limited and non-sex-limited types. Crosses were used that produce males of the same genotype but that had inherited their X, their Y, both their X and Y, or neither X nor Y chromosome from their irradiated father (see Table 5). The males used in the crosses were irradiated with doses of 0, 1, 2, 3, or 4 kr of Co^{60} gamma rays at a rate of 22 r/min. The fertility of their y w/y^+Y sons was determined by mating them with RM, y^2 *su-w*^{a} w^a *bb*/B^SY females and scoring for the production of larvae. The use of y^+Y avoids the inclusion of XO males among those tested.

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[29] Work performed at University of São Paulo, Brazil.

[30] Associated with University of São Paulo, Brazil.

[31] H. J. Muller, *Z. induktive Abstammungs- u. Vererbungslehre*, suppl 1, 234–60 (1928).

[32] S. S. Prabhu, *J. Genet.* 38, 177–91 (1939).

[33] R. L. Berg, *Genetics* 22, 240–48 (1937).

Table 5. Crosses Used to Produce Males of Identical Genotype but with Different Combinations of Irradiated Elements

An asterisk represents an irradiated element

P_1 ♀	Irradiated P_1 ♂	y w/y^+Y F_1 ♂
y w/y w	y/y^+Y	X/Y*; A/A*
y^2 *su-w*^{a} w^a *bb*/y^+Y	y w/B^SY	X*/Y; A/A*
y^2 *su-w*^{a} w^a *bb*/O	y w/B^SY/y^+Y	X*/Y*; A/A*
y w/y w/y^+Y	Y^SX·Y^L, In(1)EN, y B/O	X/Y; A/A*

The results of these experiments are presented in Table 6 and Fig. 1. It can be seen that in the case where autosomes alone are derived from the irradiated father (i.e., X/Y; A/A*) there is no evidence of an induced increase in the incidence of sterile males. Therefore we have failed to find evidence of any autosomal dominant male-sterilizing mutations. When the X chromosome as well as the autosomes is irradiated (i.e., X*/Y; A/A*), however, there is evidence of a small but definite increase of sterility with dose; this sterility is attributable to X chromosome mutations. Similarly, X/Y*; A/A* males provide ample evidence of Y-chromosome-linked sterility mutations. These conclusions are corroborated by the data from the X*/Y*; A/A* males.

The testes of over 200 of these sterile males were examined microscopically; they were dissected in a drop of saline, covered with a coverslip, and examined at 400X. The lesions of sterility may be subdivided into three general classes: prespermatogenic, spermatogenic, and postspermatogenic. Prespermatogenic sterility is characterized by complete acellularity of the testes; the testis walls are present, but the testes are small empty sacs. It seems possible that this phenotype is the consequence of failure of pole cell migration and would not therefore be expected to be limited to males. This type of sterility was not observed to increase with dose; surprisingly, however, it was observed in the X*/Y*; A/A* males with about ten times the frequency that it was observed in the other three types of males. This increased incidence must represent some type of parental effect. Spermatogenic sterility is the type most frequently encountered (162 of 212 testes examined) and is characterized by the failure to produce motile sperm. In general the seminal vesicles are empty, and the most mature spermatozoans in the testis often show signs of necrosis; meiosis and the initial stages of spermiogenesis appear normal. The high correlation observed between empty seminal vesicles and nonmotility may indicate that sperm enter the seminal vesicles by virtue of their own motility. This type of lesion showed a marked increase with dose and was most pronounced in Y-bearing sperm (50/60 sterile X/Y*; A/A* males examined from 4 kr). It is also produced as a consequence of irradiating the X chromosome, but to a lesser extent. Postspermatogenic sterility is characterized by the production of motile sperm. The seminal vesicles are full of motile sperm, yet the male upon repeated

Table 6. The Fertility of the Sons of Irradiated Males as a Function of Dose of Irradiation and of the Elements Inherited from the Irradiated Father

Dose (kr)	Experiment	X/Y ; A/A*		X*/Y ; A/A*		X/Y* ; A/A*		X*/Y* ; A/A*	
		Fertile	Sterile	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
0	1	43	1	57	3	79	0	20	1
	2	134	4					60	1
	3	226	0	177	2	295	4	6	0
	Total	403	5	234	5	374	4	86	2
1	1	52	0	73	1	55	3	28	2
	2	140	3			223	7	59	1
	3	187	2	125	5	347	10	4	1
	Total	379	5	198	6	625	20	91	4
2	1	57	1	74	3	72	8	24	2
	2	189	1			189	11	51	6
	3	223	7	173	14	479	37	7	0
	Total	469	9	247	17	640	56	82	8
3	1	50	0	65	1	70	8	13	0
	2	407	2					27	4
	3	191	2	4	0	349	43	5	4
	Total	648	4	69	1	419	51	45	8
4	1	33	1	34	2	68	16	4	2
	2	529	8			170	22	17	3
	3	256	3	25	2	311	59	3	2
	Total	818	12	59	4	549	97	24	7

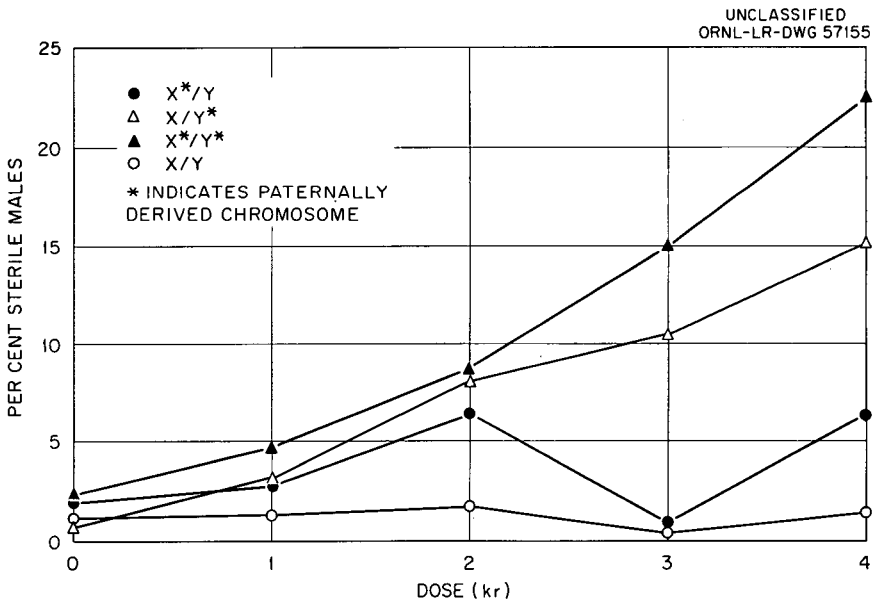

Fig. 1. Fertility of Sons of Irradiated Males as a Function of Dose of Irradiation to and Elements Inherited from Father.

crossing proves to be sterile. The dissection of numerous mates of such males revealed that in no case was there transfer of sperm from male to female. This type of sterility shows some increase in frequency with dose, but the data do not suggest what its chromosomal nature might be. Its incidence seems to be about equal in X*/Y, X/Y*, and X*/Y* males, in which the frequency seems to be considerably higher than in X/Y males. The equality of X*/Y, X/Y*, and X*/Y* males suggests an autosomal basis for postspermatogenic sterility, but the lower incidence in X/Y males and the failure of X/Y males to show evidence of induced sterility belies this suggestion. It should be emphasized here that each sterile male studied represents an independently occurring sterilizing event; consequently there is no reason why a given morphological class of sterility should be homogeneous either with respect to the lesion involved or to chromosomal basis. The present data provide only a rough idea of the composition and chromosomal basis of induced male sterility.

Cytological Analysis of Death of *Drosophila* Embryos Carrying a Recombinant $Y^SXY^S{\cdot}Y^L$ Chromosome

Mary L. Rekemeyer D. L. Lindsley

Introduction. – It has been reported[34,35] that the single recombinant $Y^SX(\cdot Y^L)$ is recovered from *Drosophila* females of the constitution K^S y cv v f $\cdot/y^2$ su-w^a w^a $(\cdot K^L)$ y^+ with a very low frequency, approaching zero in some cases, whereas the remaining products of the tetrad are recovered with normal frequency. Since failure of this strand to be included in the egg nucleus could be ruled out, it was concluded that inclusion of this recombinant in the egg nucleus resulted in mortality of the consequent zygote. It was suggested that the inviability of the $Y^SX(\cdot Y^L)$ chromosomes is associated with their inability to function properly in cell division. The observation of von Borstel[36] that mitotic delay of the fusion nucleus leads to early death of the *Habrobracon* zygote led us to the supposition that similar mitotic delay might be responsible for the death of recombinant-bearing zygotes. Consequently a series of observations on the early embryological stages of the progeny of $Y^SX\cdot/XY^S{\cdot}Y^L$ females was undertaken.

Results. – In the first experiment a 24-hr egg sample was collected from $XY^S{\cdot}Y^L$, y^2 su-w^a w^a $K^S{\cdot}K^L$ $y^+/Y^SX\cdot$, K^S y cv v f $\cdot$ y^+ females crossed with Canton S males. After an appropriate interval the proportion of eggs that had hatched was determined, and a sample of the unhatched eggs was fixed and stained for observation. The embryos that had hatched were allowed to continue development to adulthood and their genotypes determined; the progeny scored agreed well with previous genetic data on the same cross. Twenty-five per cent (217/867) of the eggs failed

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[34] D. L. Lindsley, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1955*, ORNL-1953, pp 40–43.

[35] D. L. Lindsley and E. S. Von Halle, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1956*, ORNL-2060, pp 23–24.

[36] R. C. von Borstel, *Atti assoc. genet. ital.* 5, 35–50 (1960).

to hatch, which is consistent with the expectation based on the genetic results of about 20% mortality. The distribution of the stages of development reached by the embryos that failed to hatch is given in Table 7; it can be seen that the eggs die in many different stages rather than at the very specific time resulting from mitotic delay observed in *Habrobracon*. Those dying before blastoderm formation are typical of the death from mitotic delay.

Table 7. Stage of Death of Unhatched Embryos

Stage of Development	Number of Eggs Examined	Per Cent of Total
<32 nuclei	24	8
>32 nuclei	20	7
Blastoderm	0	0
Differentiated embryo		
Early	14	5
Late	19	6

The second experiment involved taking 1-hr egg samples from the same cross as before and fixing and staining the embryos after various times of development. The distribution of stages reached by the embryos at various times after development is presented in Table 8. Although these data are small, there is no evidence of a fraction of eggs that is developing at a rate that is appreciably lower than the remainder; rather it seems as if the embryos that cease development reach their ultimate stage of development at the same time as do those whose development continues.

Table 8. Developmental Stage Reached by Embryos Fixed at Different Times After Oviposition

Numbers of eggs examined

Age When Fixed (hr)	<32 Nuclei	>32 Nuclei	Blastoderm	Differentiated Embryo		Total
				Early	Late	
1–2	6	8	8	2	10	34
3–4	2	1	10	6	7	26
5–6	3	0	2	15	22	42
7–9	6	3	0	12	59	80

Discussion. – Two expected results of mitotic delay of the zygote nucleus, based on von Borstel's observations on *Habrobracon*, are a rather uniform time of death and evidence of decreased rate of development. Neither of these expectations is fulfilled by the present observations, although some do die during very early development. It may be that a delay that is so short as to be undemonstrable is sufficient to upset the balance of the developing embryo, and that death

caused by this unbalance occurs at various times; alternatively, mitotic delay may have nothing to do with the death of $Y^SXY^S \cdot Y^L$ bearing embryos.

The Effect of Plasma-Cell Tumor X5563 on Antibody Production in $C_3H \times 101$ Mice

Guthrie T. Pratt D. L. Lindsley

Introduction. – The cell selection theory of antibody formation[37,38] postulates the existence of a pool of undifferentiated cells with a highly mutable nucleotide sequence for globulin synthesis. Within this pool at any given time exists the capacity for the production of an immense array of globulin molecules that are in turn complementary to a vast array of molecules. Stimulation by antigen is postulated to result in the selection from this pool of cells capable of producing complementary globulins and in the stabilization of their globulin-synthesizing mechanism as well as an increase in their number. Some plasma-cell tumors involve hyperplasia of globulin-producing cells. The fact that they produce globulin that is much more uniform than that produced by the globulin synthetic system as a whole indicates that these cells are not versatile in their synthetic abilities. This indication is strengthened by the observation in man that these cells cannot be induced to produce antibodies; in fact, the antibody-producing capacity of myeloma patients is definitely subnormal. This negative correlation between stability of the globulin-synthesizing system and the ability to produce antibody may not be general; rather, both attributes may be pathological effects of the neoplastic condition of the cells. However, such a correlation accords well with expectations based on the cell selection theory of antibody production. Consequently, a more detailed study of the relation between plasma-cell tumor growth and antibody production has been undertaken in the mouse.

Four groups of approximately 80 $C_3H \times 101$ mice of both sexes were used in the experiment. The antigen dose in all cases was 1 ml of a suspension of formalin-killed *Salmonella choleraesuis* which contained approximately 1×10^8 organisms/ml.

To test the effect of the tumor on the primary antibody response, 0.5 ml of a minced X5563 plasma-cell tumor tissue suspension was injected subcutaneously into 12-week-old mice. One week later, antigen was administered. Normal 13-week-old mice receiving the same antigen dose served as controls. For the effect of the tumor on the secondary antibody response, two groups of normal 6-week-old mice were given antigen. At 12 weeks, one of these groups received tumor tissue as in the primary response study, and at 13 weeks both groups were given the secondary antigen dose. In all four groups, the mice were sacrificed by decapitation; the serum was collected, frozen, and later tested by flocculation tests for serum antibody levels. Ten mice per point were bled at 3, 6, 12, 18, 24, and 30 days, respectively, after the last (or only) dose of antigen. In the groups bearing tumors, the tumor was carefully removed at the time of sacrifice and its weight recorded.

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

[38] J. Lederberg, *J. Cellular Comp. Physiol.* **52**, suppl 1, 383–401 (1958).

Results. – Primary response in mice bearing tumors was somewhat more erratic (possibly due to the sensitivity of the test at low antibody levels), but, nevertheless, significantly lower than the normal controls (Fig. 2). At 6 days, when the tumor was still very small, the response was no different from normal. After this point, however, the titer never reached the level of the normal response, and by 30 days there was a fivefold difference in titers.

In the secondary response study, the mice bearing tumors showed approximately the same response as normal mice through 12 days; then the titer dropped rapidly, so that by 30 days there was approximately a sixfold difference in response (Fig. 3).

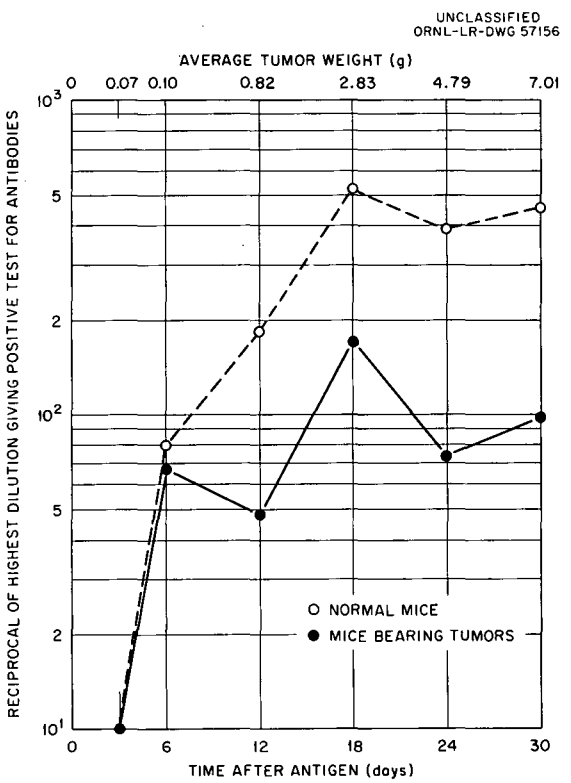

Fig. 2. Primary Response.

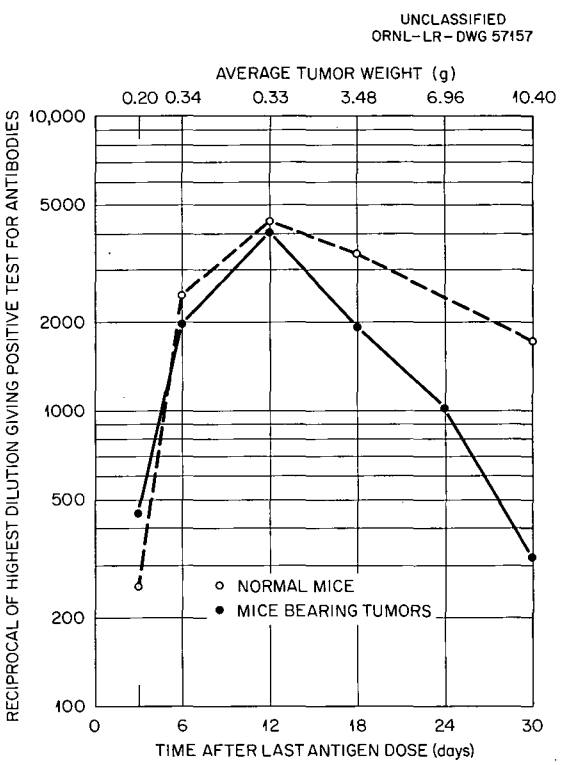

Fig. 3. Secondary Response.

Discussion. – Smith *et al.*,[39] testing hemolysin titers after administration of sheep RBC in C_3H mice injected with X5563 plasma-cell tumor, also found that there was a lowered response to a single antigen injection in the tumor-bearing mice, as compared with normal. They found very little difference in the secondary response between tumor-bearing and normal mice. They postulate that the tumor causes depletion of the number of antibody-producing cells participating in the response, but that it does not abolish the primary response, since the secondary response in tumor-bearing mice appears near normal. These measurements were made through only 7 days after the

[39] F. Smith, M. M. Grenan, and J. Owens, *J. Natl. Cancer Inst.* 25, 803–12 (1960).

administration of antigen. Our results do not refute his findings, but extend over a longer period and do show as great a difference in the secondary response as in the primary response at 30 days after the last dose of antigen, when the tumor has reached considerable size. Nathans *et al.*[40] have shown that this plasma-cell tumor produces an abnormally high serum gamma globulin concentration which is directly correlated with tumor size. This being the case, it seems likely that as the tumor grows, the materials needed for protein synthesis, whether it be antibody or any other protein, could be used preferentially by the tumor cells, leaving less material for use in antibody production. Other observations of Smith *et al.*[39] indicate that there may also be a more rapid degradation of antibody protein in these mice, since antibodies injected into tumor-bearing mice disappear from the serum more rapidly than those injected into normal mice. They further postulate that hypervolemia, which occurs in mice bearing this tumor, could account for the difference they observed. By using Cr^{51}-labeled isologous erythrocytes injected intra-arterially, they found an increase in blood volume of 35% in mice with tumors. This is no doubt an important factor in serum antibody levels, but would not account for all the difference we observed. A more appealing explanation is the combination of all these factors – depletion of the amino acid pool by the rapidly growing tumor cells, degradation of antibody protein, and hypervolemia accompanying tumor growth.

Further studies being carried out on this problem include electrophoretic analysis of serum from these experiments so that comparison of increase in tumor size, relative gamma globulin concentration, and antibody concentration can be made. Antibody response in mice having a relatively large tumor at the time of antigen administration is also being studied.

Preferential Segregation of Second Chromosomes in Triploid Females of *Drosophila melanogaster*

E. H. Grell

Preferential segregation in a triploid (or trisome) indicates the situation when the three homologous chromosomes are not equivalent at the first meiotic division and the inclusion of certain homologous chromosomes into haploid and diploid (or monosomic and disomic) gametes is not at random. For example, if segregation is preferential in a triploid, the homologous chromosomes A_x, A_y, and A_z are distributed at meiosis so that the three segregations A_x/A_yA_z, A_y/A_xA_z, and A_z/A_xA_y do not take place with equal frequencies. If segregation is nonpreferential the frequencies of these segregations are equal. That an inversion-bearing chromosome when present with two normal homologs tends to be preferentially distributed to the diploid gametes produced by triploid females has been reported.[41–43] The data presented below are taken from a study into the nature of this type of preferential segregation.

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[40] D. Nathans, J. L. Fahey, and M. Potter, *J. Exptl. Med.* 108, 121–30 (1958).

[41] B. T. Kozhevnikov, *Bull. biol. méd. exptl. U.R.S.S.* 9, 13–14 (1940).

[42] J. Schultz and H. Redfield, *Cold Spring Harbor Symposia Quant. Biol.* 16, 175–97 (1951).

[43] C. G. Doyle, *Science* 130, 1415 (1959).

Methods. – The four rearranged second chromosomes that were used form a series with respect to the differences between them and a normal chromosome. In(2L)Cy has an inversion in the left arm of the second chromosome. Ins(2L + 2R)Cy has In(2L)Cy plus an inversion in the right arm of the chromosome. SM1 has a large pericentric inversion superimposed upon Ins(2L + 2R)Cy. SM5 is the most complex rearrangement of the series. It was derived by irradiation of SM1 and has several breaks superimposed upon those of SM1. All of the rearrangements are marked by the dominant mutant, Cy.

Triploid females of the following genotypes were produced: In(2L)Cy/+/+, Ins(2L + 2R)Cy/+/+, SM1/+/+, and SM5/+/+ (+ represents a normal-sequence chromosome). These females were mated to wild-type males and their progeny examined. The progeny were scored as to whether they were derived from a maternal gamete with one or two second chromosomes and whether or not one of those chromosomes was the rearrangement.

Results. – The inversion-bearing chromosomes were found to be present in diploid and a normal chromosome present in haploid gametes more often than if segregation were nonpreferential. In Table 9 are the frequencies of the two types of segregations.

Table 9. Segregation Frequencies of Second Chromosomes in Triploids

Chromosomes of Mothers	Segregation 1, In/+ and + Gametes	Segregation 2, In and +/+ Gametes
In(2L)Cy/+/+	0.73	0.27
Ins(2L + 2R)Cy/+/+	0.79	0.21
SM1/+/+	0.90	0.10
SM5/+/+	0.97	0.03
If nonpreferential	0.67	0.33

Discussion. – The results indicate that the two normal chromosomes pass to opposite poles of the first meiotic division in excess of nonpreferential expectation. The preferential segregation is believed to be preceded by preferential pairing of chromosomes. A rearrangement is less likely to pair with a normal chromosome than two normal chromosomes are to pair with one another.

Furthermore, the tendency for a rearrangement not to pair appears to increase with increasing deviation of the rearrangement from normal sequence. In(2L)Cy pairs nearly as often as a normal chromosome, but the complex SM5 pairs much less.

Preferential segregation has undoubtedly been of importance in the evolution of polyploid species and is also of interest in plant breeding. It indicates that stable tetraploids may be produced as long as homologs occur as structurally different pairs, even if the four homologs are genetically identical. The two members of each pair will form bivalents at meiosis, and balanced gametes will be produced.

Aging Effect on Nondisjunction in Translocation-Bearing Oocytes

P. E. Thompson

Introduction. – Recent evidence of the presence of translocations in human families or individuals manifesting mongolism has suggested that the influence of maternal age on the occurrence of this disorder may be an effect on nondisjunction in the translocation system. The aging of oocytes, which is an invariable feature of human oogenesis, can be simulated in females of *Drosophila* by placing unmated females together in crowded conditions. These females produce eggs but retain them under such conditions for as long as eight days, at which time the eggs degenerate.

Accordingly, two groups of females were isolated from lines heterozygous for reciprocal translocations of chromosomes III and IV. Heterozygotes for these translocations regularly produce some haplo-IV and triplo-IV offspring which arise by spontaneous nondisjunction. One group of females was mated immediately after eclosion to wild-type males. The other group was aged without mating for seven days and was mated to wild-type males after that interval. Both groups were allowed to deposit eggs for two days, and the frequency of haplo-IV progeny was ascertained.

Results. – Translocation 86D yielded 19.4% haplo-IV offspring from unaged females and 17.3% from aged females, with $P = 0.50$. It appears that the control here gives free nondisjunction, with a maximum of somewhat less than 25% haplo-IV possible (due to lower viability of this type). Translocation 89E, which has a different disposition of translocation breaks, yielded 5.1% haplo-IV progeny from unaged females and 8.1% haplo-IV from aged females ($P < 0.05$).

Discussion. – It appears that in favorable tests the aging of oocytes increases nondisjunction within a translocation system. This may be due to a shift in the relative strength of homologous and nonhomologous pairing forces. Other effective variables are being sought.

MAMMALIAN GENETICS AND DEVELOPMENT

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Mutation Frequency and Radiation Intensity

W. L. Russell
Elizabeth M. Kelly

In the most recent semiannual progress report[4] we presented the results of an experiment in which specific locus mutation frequency was measured in spermatogonia of mice irradiated at a dose rate of 48 r/hr for a total dose of 600 r (Cs^{137} source). This was one of a series of experiments started to explore the implications of our finding that a given dose of chronic irradiation produces fewer mutations than does the same dose of acute irradiation. The intensity of 48 r/hr

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[1]Consultant.
[2]Visiting investigator from abroad.
[3]Loanee.
[4]W. L. Russell and E. M. Kelly, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, p 77.

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 was found that the mutation frequency at 48 r/hr was in close agreement with that obtained at 90 r/week and significantly below the frequencies obtained at 90 r/min and 24 r/min. Thus, most or all of the intensity effect in males occurs within the thirtyfold dose-rate range from 48 r/hr to 24 r/min.

In the experiment discussed above, the mutation frequency was measured in irradiated spermatogonia. A parallel experiment, to be described here, was made in females. The germ cells irradiated were in dictyate oocyte stage. The total dose delivered at the 48-r/hr intensity was 400 r. In all other respects the experiment was similar to that on males.

To date, 13 specific locus mutations have been observed in 36,083 offspring of the irradiated females. This mutation frequency is between one-third and one-quarter of that obtained in an earlier experiment in which females were exposed to 400 r of 90-r/min x irradiation.[5] The difference is significant ($P < 0.001$). Thus, a dose-rate effect on mutation frequency is demonstrated which is similar to that found for males, in that it occurs within the same range of radiation intensity. (The mutation frequency in the new data on females is not significantly different from that obtained in an earlier experiment at 90 r/week.[5])

Results for females have an importance over and above the mere confirmation of the effect in males. They are not subject to the possible complications that could arise in males from the fact that spermatogonia are rapidly dividing cells. The intensity effect in males might conceivably result from differential selective action of the two intensities on the variety of cell division stages present, if, in turn, these stages should have differential mutational sensitivity. Since the intensity effect in females cannot possibly be explained on such a basis, it seemed likely that the effect in both sexes could be attributed to a more direct and, therefore, more interesting mechanism. This point has been emphasized before.[5-8] The present data provide further information on the nature of the mechanism. This will now be explained.

In our earlier work on females, a dose rate of 90 r/week was used for comparison with the 90-r/min data. The lower dose rate necessitated three weeks of exposure for the dose given, and the animals were fertile for an almost normal length of reproductive life. The exposure at the higher dose rate took only a few minutes, and the females became sterile after producing only one litter or, at most, two. Thus, it could be argued that if the mutational sensitivity of the oocytes increased as the ovarian follicles matured, then the 90-r/min irradiation experiment would have involved only the most mutationally sensitive oocytes, while the 90-r/week experiment would have involved more resistant oocytes. There were various arguments against such an interpretation.

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[5] W. L. Russell, L. B. Russell, and E. M. Kelly (Symposium on "Immediate and Low Level Effects of Ionizing Radiation," Venice, 1959), *Intern. J. Radiation Biol.*, suppl, 311–20 (1960).

[6] W. L. Russell *et al.*, *Proc. Natl. Acad. Sci. U.S.* **44**, 901–5 (1958).

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

[8] W. L. Russell, L. B. Russell, and M. B. Cupp, *Proc. Natl. Acad. Sci. U.S.* **45**, 18–23 (1959).

The most cogent of these was the demonstration that restriction of the 90-r/week data to the first litter conceived after irradiation still showed a significantly lower mutation frequency than in the 90-r/min experiment. However, it was pointed out that even this argument was not completely watertight. Thus, it could be counterargued that the oocytes might become mutationally sensitive only in the last few days before ovulation. In such a case, even those oocytes which contributed the first postirradiation litter would have received a large part of the three-week 90-r/week irradiation when they were still insensitive.

The present experiment excludes any such interpretation. The exposure time was only 8 hr, and the females, which, like those in the 90-r/min experiment, were placed with males on the day after irradiation, produced only one litter or, at most, two before becoming sterile. Thus, the follicle stages of the oocytes at the time of irradiation must have been essentially identical for the 48-r/hr and the 90-r/min experiments. The fact that these two experiments do, nevertheless, give significantly different mutation frequencies indicates that it is truly the mutational process within the cell which is dose-rate dependent.

An XXY Male in the Mouse

Liane B. Russell E. H. Y. Chu

Chromatin-positive Klinefelter males, presumably XXY, occur relatively frequently in human populations.[9] On the other hand, the XXY type had not been found in the course of the original XO experiments in the mouse, even though the markers used in crosses for the detection of XO would, in most cases, also have led to detection of XXY.[10] When, for some time thereafter, subsequent experiments also failed to yield this type, we concluded that either XXY mice occurred very rarely or that they were, for one of several reasons, undetectable. Thus, XXY might be female, or a female-like intersex; or XXY might be inviable; or the expression of the sex-linked markers used might be altered by the presence of the Y.

Doubts concerning detectability have now been dispelled by our recent finding of an XXY mouse in crosses marked by sex-linked genes. The XXY constitution, detected by genetic means, has been verified by cytological studies. The exceptional animal is viable, of normal size, and of male phenotype, though apparently sterile. Its genotype is $+/Ta/Y$, and the expression of the marker Ta (= Tabby) is not affected by the Y. The animal was derived from a cross of $+\ sf/+\ +$ ♀ × $Ta\ +/Y$♂.

Conclusions. – The finding of a mouse which has a clear XXY karyotype and for which the genetic evidence shows that it must be of an $X^M X^P Y$ constitution (X^M and X^P being the maternally and paternally derived X's, respectively) has a number of implications.

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[9] P. E. Polani, pp 153–78 in *Molecular Genetics and Human Disease* (L. T. Gardner, ed.), Charles C. Thomas, Springfield, Ill., 1960.

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

It may be concluded that XXY in the mouse is viable and of normal size. The fact that XXY is male confirms the male-determining properties of the Y. The sterility of the animal does not necessarily lead to the conclusion of female determiners on the X. Thus, it could be due to dosage phenomena of genes not necessarily directly concerned with sex determination. Or, though less likely, it is not impossible that X and Y have a homologous region and that trisomy for this region leads to certain defects which result in infertility.

The lack of effect of the Y on the *Ta*/+ phenotype is in good accord with independent studies on V-type position effects in the mouse, where it has been shown that the Y is not the cause of the sex difference in phenotype.[11,12] Further, if Tabby can be considered to be a dosage-compensated gene, then the phenotypic similarity of +/*Ta* and +/*Ta*/Y confirms Stern's conclusion[13] that "dosage compensation is founded on modifiers in the X chromosome which act independently of the sex-developmental system."

Since both X^{Ta} and Y are known to have been contributed by the father, the exceptional $X^{+}X^{Ta}Y$ mouse is actually the only mammal reported to date in which the stage of nondisjunction can have been no other than the first meiotic division in a presumably normal male. In man, cases of Klinefelter's syndrome in which the derivation of the X's is marked by the gene for color blindness are of the $X^{M}X^{M}Y$ type,[9] with both X^{M} chromosomes bearing the same marker. Such individuals can be the result of nondisjunction at any one of three stages: in the second meiotic division of the mother; in the first meiotic division combined with a crossover; and in the first cleavage.

Frequency relations are discussed elsewhere in this volume.[14]

Induction of Abnormal Sex-Chromosome Number

Liane B. Russell Clyde L. Saylors

Evidences of nonrandomness in the original XO data in the mouse,[10,15] for example, the finding of XO littermates, suggested that cleavage events might be very important in the spontaneous origin of XO and led us to attempt to increase the frequency of XO by irradiation during cleavage stages. Results of this experiment are summarized in Table 10. A preliminary report was presented earlier.[16]

Using appropriate sex-linked markers, both cases of maternal and paternal sex-chromosome loss could be detected. We were able to show that XO frequency could be significantly increased by irradiating on the day of fertilization ("day 0" in Table 10). Since, in the mouse, almost a full

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[11] L. B. Russell and J. W. Bangham, *Genetics* **45**, 1008–9 (1960).

[12] L. B. Russell and J. W. Bangham, *Genetics* **46**, in press (1961).

[13] C. Stern, *Can. J. Genet. and Cytol.* **2**, 105–18 (1960).

[14] L. B. Russell, this report.

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

[16] L. B. Russell and C. L. Saylors, *Science* **131**, 1321–22 (1960).

Table 10. Frequencies of XO Individuals Following Irradiation at Various Stages Postfertilization

P– and M– designate lack of paternal and maternal sex chromosome (i.e., X^MO and OX^P types, respectively)

Stage Irradiated		Number Observed			Corrected Percentage* of P– plus M–	
Day	Hour	Total Animals	P–	M–		
0	11:00 AM	425	14	4	5.2	4.6
	12:30–2:00 PM	44	2	0	4.5	
	3:30 PM	70	1	0	1.4	
1½–13½		269	1	0	0.4	1.1
Control		362	6	0	1.7	

*Estimated frequency of occurrence, calculated by taking account of the fact that only one-half of all losses of maternal X are detectable.

day elapses between fertilization and the first cleavage, it was possible to test the effect of irradiation at various times during that day. The frequency of XO was highest following irradiation at the earliest time tested (11:00 AM) and fell off thereafter. Irradiation during later cleavages (days 1½ to 4½) or at postcleavage stages was ineffective in increasing the XO frequency. One point of considerable interest was that irradiation at 11:00 AM, the stage that produces the highest total frequency of induced XO, yielded some animals lacking the *maternal* X, an event which has not yet been observed to occur spontaneously[14] (see also Table 12) or from irradiation at other stages.

None of the XO animals obtained in this experiment gave the appearance of mosaicism. Moreover, the XO's, as well as many phenotypically nonexceptional animals, were progeny-tested for gonadic mosaicism, but failed to reveal any. We thus conclude tentatively that we have induced chromosome loss prior to the first cleavage. Although four, and in some cases five, autosomes were also marked, no autosome losses were detected. It may be assumed that such losses are cell lethal.

Spontaneous Occurrence of Mice with Abnormal Numbers of Sex Chromosomes

Liane B. Russell

Results. – Comparisons of spontaneous frequencies of various sex-chromosome anomalies found in the mouse have been made, using data from 17 crosses, involving five sex-linked markers in various combinations and in XO × XY as well as XX × XY matings. The two main comparisons are: (*a*) between the frequency of lacking a paternal sex chromosome (% P–) and that of having an extra paternal sex chromosome (% P+), and (*b*) between the frequency of lacking a maternal sex chromosome (% M–) and that of lacking a paternal one (% P–).

Table 11 summarizes results from all crosses in which *simultaneous* detection of P– and P+ events was possible. The estimated frequency of occurrence (far-right columns) has been calculated by taking account of the fact that detectability of one or the other event is only one-half in some crosses (left-hand columns). The single P+ event is the $X^{+}X^{Ta}Y$ male discussed in another section of this report.[17] Unfortunately for the clarity of Table 11, this animal occurred in an experiment in which postfertilization stages were irradiated.[18] However, its chromosome constitution rules out radiation as cause of the XXY type. (Naturally, the 17 P– events in this experiment, which could have been the result of irradiation, were not included in the calculation of the frequency of spontaneous occurrence of P– shown at the bottom of Table 11.) While it cannot be stated with any degree of certainty that the ratio of P– to P+ is actually 38 (a value based on only one occurrence of P+), it is quite clear that this ratio is, indeed, quite high.

Table 11. Frequencies of Mice Lacking a Paternal Sex Chromosome (% P–) and of Those Having an Extra Paternal Sex Chromosome (% P+)

Combined data from 16 different types of crosses

Detectability		Number Observed			Corrected Percentage[a]	
P–	P+	Total Animals	P–	P+	P–	P+
		Spontaneous Occurrences				
1	1	1819	17	0	0.93	0
½	½	3660	11	0	0.60	0
½	1	350	1	0	0.57	0
		Radiation Experiment[b]				
1	1	539[c]	17[d]	1		
			Totals			
		5829	29		0.76	
		6368		1		0.02

[a]Estimated frequency of occurrence calculated by taking account of detectability which, in some crosses, is only ½.

[b]Irradiation on day 0 postfertilization (see Table 10).

[c]Excluded from calculation of total spontaneous P– frequency, but included in spontaneous P+ frequency (see text).

[d]Excluded from calculation of total P– frequency.

Table 12 summarizes data from 8 crosses in which genetic markers were present for the detection of both M– and P– events. In a population in which 13 spontaneous occurrences of P– could be detected there were no cases of M–. For comparison, data from the radiation experiment[18] are also shown in Table 12 (bottom line).

[17]L. B. Russell and E. H. Y. Chu, this report.

[18]L. B. Russell and C. L. Saylors, this report.

Table 12. Frequencies of Mice Lacking a Paternal Sex Chromosome (% P–) and a Maternal Sex Chromosome (% M–)

Combined data from 8 different types of crosses

Detectability		Number Observed			Corrected Percentage*	
P–	M–	Total Animals	P–	M–	P–	M–
		Spontaneous Occurrences				
1	½	1271	13	0	1.02	0
½	½	67	0	0	0	0
	Total	1338	13	0	0.99	0
		Radiation Experiment**				
1	½	425	14	4	3.29	1.88

*Estimated frequency of occurrence calculated by taking account of detectability which, in some crosses, is only ½.

**Irradiation at 11:00 AM on day 0 postfertilization (see Table 10).

Conclusions. – That nondisjunction of sex chromosomes *can* occur in meiotic divisions, or at least in the first meiotic division of the male, was shown by our discovery of an $X^+X^{Ta}Y$ animal from a $X^+X^+ \times X^{Ta}Y$ mating.[17] However, the frequency comparisons suggest that this event is rare in the mouse in comparison with others that produce abnormal numbers of sex chromosomes.

Specifically, the results lead to the conclusion that events which produce *equal* frequencies of X^MX^PY and X^MO – namely, those leading to nondisjunction at the first meiotic division – are very much rarer than events that yield X^MO without X^MX^PY. (The superscripts M and P designate maternal and paternal derivation, respectively, of the X.) Events yielding X^MO but not X^MX^PY can occur during meiosis (e.g., anaphase lagging; or nondisjunction in the second division, which would also produce presumably nondetectable XX and YY gametes); or they can occur at the first cleavage. Cleavage as a relatively frequent source of XO's in the mouse was already suggested by nonrandomness of the original XO data and has now been implicated even more by the demonstration that the frequency of XO can be increased by irradiation shortly after fertilization.[18] Cytological evidence also suggests that nondisjunction may be more frequent at cleavage.[19]

As has been shown, not only is X^MO much more frequent than X^MX^PY, but X^MO is also much more frequent than OX^P. A hypothesis that would fit *both* of these results is that there exists a relatively high probability of loss of the paternally contributed sex chromosome sometime between fertilization and completion of the first cleavage. Such a hypothesis is not unreasonable in the light of the observation[20] that, during that interval, the nuclear material contributed by the male

[19] S. Ohno, W. D. Kaplan, and R. Kinosita, *Exptl. Cell Research* 18, 382–84 (1959).

[20] C. R. Austin, *Australian J. Sci. Research, Ser. B* 5, 354–65 (1952).

undergoes considerably more changes than that contributed by the female in such factors as size of the pronucleus, number of nucleoli, and total nucleolar volume. It is, of course, not inconceivable that autosomes as well as the sex chromosome contributed by the father have an increased probability of being lost at this stage. Such an event, however, would probably lead to death of the individual, unless the maternally contributed homolog were present, or came to be present, in double dose – an explanation which has been invoked to account for a certain class of spontaneous mutants in the mouse.[21]

Radiation-Induced Genetic Damage of the Skeleton in Mice

U. H. Ehling M. L. Randolph

Introduction. – One approach in determining genetic effects of radiation is to make empirical measures of over-all damage that cannot be estimated accurately from information on a limited number of genes. The effect on survival to weaning age of offspring derived from x-irradiated spermatogonia[22] and shortening of life in the offspring of male mice exposed to neutrons[23] have already been reported. The present experiment was designed to test the feasibility of measuring part of the over-all damage by means of skeletal studies in the F_1 generation.

Method. – Different stages of spermatogenesis were irradiated, and different sources were used for the exposure. The age of the irradiated 101 males was 16–20 weeks. One group of males was exposed to 600 r of x rays (80 r/min); another group of 12 males was irradiated with monoenergetic 3-Mev neutrons at about 5 rads/hr at 15 cm from the target of a Cockcroft-Walton accelerator. Immediately after irradiation the males were mated to adult C3H females. Single-pair mating was used in the presterile period for the group of males exposed to x rays and for the contemporary control (groups *a* and *b* in Table 13). In all other groups, one male was mated to three adult C3H females. For presterile period matings the males were transferred to a fresh set of C3H females 7, 14, and 21 days after irradiation (groups *b*, *d*). Nonirradiated control males were handled and mated in the same way (groups *a*, *c*). In all groups, litters were recorded at birth and were sacrificed at the age of 26–28 days. Skeletal preparations were made, using Alizarin-red staining. The whole skeleton was examined in detail.

Results and Conclusion. – The results of this experiment are summarized in Table 13. The frequency of different abnormalities was used as the basis for classification. In class 1 are abnormalities which occurred only once in the whole experiment; in class 2, abnormalities that were found in two or more animals. On a morphological basis, class 1 is further subdivided into major and minor abnormalities; class 2 into dyssymphysis of vertebrae and sternal abnormalities.

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[21] L. B. Russell and W. L. Russell, *J. Cellular Comp. Physiol.* **56**, suppl 1, 169–88 (1960).

[22] W. L. Russell and L. B. Russell, *Progr. in Nuclear Energy, Ser. VI* **2**, 179–88, Pergamon Press, London, 1959.

[23] W. L. Russell, *Proc. Natl. Acad. Sci. U.S.* **43**, 324–29 (1957).

Table 13. Incidence of Skeletal Anomalies in F_1 Generation Derived from Irradiated and Nonirradiated Males

Source	Period	Dose (r)	Group	n	Percentage Frequency of Animals with Class-1 Abnormalities		Percentage Frequency of Animals with Class-2 Abnormalities	
					Major Abnormality	Minor Abnormality	Sternal Abnormality	Dyssymphysis*
X-ray,	Presterile,	0	*a*	102		1.0	1.0	1.0
80 r/min	0–21 days	600	*b*	49	2.0	6.1		2.0
Neutron,	Presterile,	0	*c*	63			3.1	3.2
3.0 Mev	0–21 days	80	*d*	134	0.8	1.5	3.0	
X-ray,	Poststerile	0	*e*	73			2.7	1.4
80 r/min		600	*f*	79	1.3	1.3		1.3
Total from nonirradiated males				238		0.4	2.1	1.7
Total from irradiated males				262	1.1	2.3	1.5	0.8

*Dyssymphysis of thoracal vertebra X, which is very common, has been excluded.

Class-1 major abnormalities were as follows. The designations indicate group in Table 13 and mating period (7 = 0–7 days, 14 = 8–14 days, 21 = 15–21 days).

1. *b*-7, male. Fusion of lumbar vertebrae (V + VI); deformation of distopia tubercoli anterioris (left); articulation of rib VIII with the sternum (left); dyssymphysis of thoracal vertebra X.
2. *d*-7, female. Dyssymphysis of atlas and thoracal vertebrae I + II; processus spinosus shifted onto Th III; 14 thoracal vertebrae; 14 ribs left, 13 ribs right; articulation of rib VIII with the sternum (left); 6 sternal plates.
3. *f*, male. 14 thoracal, 6 lumbar, 5 sacral, and 30 caudal vertebrae; 14 ribs; articulation of rib VIII with the sternum; articulation of the ribs is irregular.

Class-1 minor abnormalities were as follows:

1. *a*-7, female. Fracture of caudal vertebra XIX.
2. *b*-7, male. Asymmetrical axis.
3. *b*-7, male. Deformation of processus spinosus (Th II).
4. *b*-7, male. Short tail (23 caudal vertebrae).
5. *d*-7, female. Abnormal articulation of ischium and pubis; ischium larger than normal.
6. *d*-7, female. Fusion of caudal vertebrae (XXVI + XXVII).
7. *f*, male. Fusion of lumbar vertebrae (IV + V).

Class-2 abnormalities were as follows: Dyssymphysis of cervical vertebra VII and thoracal vertebrae I + II. The incidence of dyssymphysis of thoracal vertebra X is very high and was,

therefore, not included in Table 13. Characteristics for the sternal abnormalities are fusions and bridges between two sternal plates.

The results of this pilot experiment indicate that the frequency of class-1 abnormalities may be increased by irradiation and therefore that it may be possible to use skeletal studies as a means of measuring part of the over-all genetic damage. An additional 468 skeletons are now being examined in the same way to see whether the present trend persists. After the pattern of normal variation is well established, it may be possible to shorten and simplify the skeletal examination so that large-scale experiments could be done without a prohibitive amount of labor.

Survival of Spermatogonia of the Mouse at Different Dose Rates

E. F. Oakberg Evelyn Clark W. L. Russell

In the previous semiannual progress report, Russell and Kelly[24] presented results from an experiment on mutation frequency in spermatogonia of mice irradiated at an intensity of 48 r/hr that agreed closely with the frequency obtained at 90 r/week and was significantly lower than the rate observed at ~90 r/min. Unpublished data on these mice indicate that reduction of the dose rate to 48 r/hr had little effect on the time at which fertility was regained after the period of temporary sterility. This suggested that while reduction of the dose rate from ~90 r/min to 48 r/hr had an effect on mutation frequency, cell killing might be unaffected.

It was important to check this possibility by direct quantitative histological measurement since its confirmation would clinch the earlier interpretation that the dose-rate effect on mutation frequency is not dependent on differential cell killing. Male mice were exposed to the total doses given in Table 14 at dose rates of ~86 r/min and 9.3 r/min for x rays and at 48 r/hr for Cs^{137}

[24] W. L. Russell and E. M. Kelly, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, p 77.

Table 14. Experimental/Control Ratios for Number of Type *A* Spermatogonia After Exposure at Different Dose Rates

Dose (r)	Time After Irradiation (days)	Irradiation		
		Cs^{137} Gamma, 48 r/hr	X Ray, 9.3 r/min	X Ray, 86 r/min
11	3	0.68	0.73*	0.77**
21	3	0.57	0.62	0.63
100	3	0.20	0.22	0.23
300	3	0.14	0.15	0.12
300	5	0.11	0.08	0.08
600	3	0.09	0.08	0.10
600	5	0.04	0.03	0.03

*Based on 2 mice.
**Based on 3 mice.

gamma rays. Quantitative histological procedures were the same as those used previously. The data in Table 14 are experimental / control ratios of the number of type *A* spermatogonia counted in 50 tubule cross sections per mouse.

The data in Table 14 are incomplete, since scoring of only half the tubules to be counted per mouse has been completed, and one control, two mice in the 11-r treatment at 9.3 r/min, and one mouse in the 11-r group at 86 r/min are not included. Nevertheless, the data are remarkably uniform, with closely similar survival at all three dose rates.

These results are of great interest both in respect to the basis for radiation-induced cell death of spermatogonia and for interpretation of the lower mutation rate obtained at 48 r/hr.[24] No increased repair (or prevention) of radiation-induced damage to cellular systems involved in cell death appears to occur as the dose rate is reduced from ~90 r/min to 48 r/hr, yet reduction in frequency of mutations in the same cells has been observed in this dose range.[24] This suggests that cell death and mutation induction are dependent upon different mechanisms. It also greatly strengthens the earlier conclusion of Russell *et al.*[25,26] that the effect of dose rate on mutation frequency is not dependent on selection in the spermatogonial population.

DNA Synthesis and Mitotic Cycle in Spermatogonia of Mouse Testis

Valerio Monesi

The present research has been carried out in association with experiments aimed to investigate the relation of DNA synthesis and mitotic cycle to x-ray sensitivity in the spermatogonia of mouse testis. Its purpose was to determine the generation time, the duration of DNA synthesis, and its position in the mitotic cycle in the spermatogonia of the mouse.

Ninety-two male mice, 10–13 weeks old, ranging in weight between 21 and 24 g, were injected intraperitoneally with 15 μc of H^3-thymidine per mouse (specific activity 1.9 curies/millimole) and killed at intervals ranging from 30 min to 57 hr after injection. Autoradiographs of 5-μ sections were prepared, and the number of unlabeled and labeled interphases, early and late prophases, metaphase-anaphases, and telophases of type *A*, intermediate, and type *B* spermatogonia was scored in 100 seminiferous tubules per mouse. Only cells containing more than four grains per nucleus were scored as labeled. Each type *A* spermatogonium undergoes three to four divisions before differentiating into intermediate spermatogonia.[27] The mitotic cycle and DNA synthesis have been studied separately for each of these divisions.

The percentage of labeled nuclei in each of the cell types undergoing DNA synthesis fluctuates to some extent in the different time intervals from the injection of thymidine. It is relatively

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

[26] W. L. Russell, L. B. Russell, and E. M. Kelly (Symposium on "Immediate and Low Level Effects of Ionizing Radiation," Venice, 1959), *Intern. J. Radiation Biol.*, suppl, 311–20 (1960).

[27] E. F. Oakberg, *Am. J. Anat.* 99, 391–413 (1956).

low at 30 min after injection, reaches a maximum at 1 hr, then decreases slightly at 2 to 3 hr, and remains constant in later times.

The curves of the percentages of labeled metaphase-anaphase figures plotted against time after injection of H^3-thymidine give precise information as to the average generation time and the maximum, minimum, and average post-DNA synthetic time, that is, from the end of DNA synthesis to metaphase. The average generation time is estimated by measuring the interval between two successive peaks of labeled metaphase-anaphases. The minimum post-DNA synthetic time is the time between injection of thymidine and the appearance of the first labeled metaphases; the maximum time between end of DNA synthesis and metaphase is the time when practically 100% of metaphase-anaphases are labeled.[28,29]

The percentage of labeled cells after a short contact with H^3-thymidine is a direct measure of the fraction of the generation time during which DNA is synthesized. Therefore, by knowing the average generation time and the percentage of labeled cells at 1 hr following injection of H^3-thymidine it is possible to determine the average duration of DNA synthesis. The average pre-DNA synthetic time, from metaphase to the onset of next DNA synthesis, is estimated by subtracting the average post-DNA synthetic time and the average DNA synthetic time from the average generation time.

The average generation time is about the same in all cell types studied, or about 28–32 hr. A remarkable difference exists, however, in the average DNA synthetic time and post-DNA synthetic time.

The average DNA synthetic time is about 10–11 hr for intermediate and type *B* spermatogonia and much shorter for type *A* spermatogonia. Among type *A* spermatogonia, however, it varies greatly from one division to another. It is very short, about 4 hr, in the first type *A* mitosis and increases gradually in the successive mitoses. In the last type *A* division before differentiation into intermediate spermatogonia, the average DNA synthetic time is not very different from the times for intermediate and type *B* spermatogonia.

The average post-DNA synthetic time is very short in type *B* spermatogonia (5, 6 hr), a little longer in intermediate spermatogonia (6, 7 hr), and gradually longer from the fourth to the first type *A* mitosis. The first type *A* mitosis has a post-DNA synthetic time of about 13 hr.

The average pre-DNA synthetic time is about the same in all types of spermatogonia, ranging between 11 and 14 hr.

The analysis of the shape of the rising and downward curves of the percentages of labeled metaphase-anaphases gives indications as to the degree of variability of the DNA synthetic time and post-DNA synthetic time.

The difference in the duration of DNA synthesis and post-DNA synthetic time between intermediate-type *B* spermatogonia and type *A* spermatogonia can be evaluated in an attempt to elucidate the basis of the different x-ray sensitivity of these cells.

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[28] R. B. Painter and R. M. Drew, *Lab. Invest.* 8, 278–85 (1959).

[29] J. H. Taylor, *J. Biophys. Biochem. Cytol.* 7, 455–64 (1960).

Distribution of Offspring of XO Female Mice

W. J. Welshons Bobbie J. Scandlyn

Hemizygous female mice of the constitution *Ta*/O and +/O are currently in use. The *Ta*/O females mated to normal males produce +/O and *Ta*/+ females and *Ta*/Y males, while parental +/O females produce *Ta*/O and *Ta*/+ females and +/Y males. One might expect to find among the progeny of X/O females a ratio of 1 X/X : 1 X/O : 1 X/Y, but this expectation is not realized due to the failure of some of the X/O females to survive.

On the basis of 1140 classified offspring of *Ta*/O and +/O females, the distribution of X/X, X/O, and X/Y offspring obtained from the phenotypically different parents has been compared and found to be remarkably homogeneous in spite of the fact that the X/O female and X/Y male progeny have different phenotypes in the two crosses. Because of the homogeneity in these tests, it is possible to estimate the frequency of X/X, X/O, and X/Y progeny from both *Ta*/O and +/O parents on the basis of the 1140 offspring: The observed values are 39.4% X/X females, 21.4% X/O females, and 39.2% X/Y males.

Since both the X/X females and X/Y males have 40 chromosomes while the X/O females have 39, one can make chromosome counts on "litters of embryos" at 12 days of pregnancy and determine if the shortage of X/O females is due to a loss of these animals between 12 days *in utero* and 8 to 10 days after birth, which is the age at which the offspring have been classified. While both the X/X female and X/Y male embryos will have chromosome counts of 40, it is still possible to distinguish between the two on the basis of the recognition of the Y chromosome in the male and the determination of its absence in the female; this aspect of the analysis has not progressed far enough to merit consideration at this time. On the other hand, one can still compare in a less precise manner the frequencies of embryos with 40 chromosomes (X/X females plus X/Y males) and those with 39 chromosomes (X/O females). Implicit in this comparison is the assumption that the Y/O type is eliminated at an even earlier stage of development. On the basis of the data presented above one would expect 78.6% of the embryos to have 40 chromosomes and 21.4% to have 39.

With the cooperation of E. H. Y. Chu it has been possible to date to make chromosome counts on the tissues of 49 twelve-day-old embryos, of which 41 (83.7%) had 40 chromosomes and 8 had 39 (16.3%). The reduction in the number of 39-chromosome counts compared with the expected value of 21.4% is not significant; hence it would appear that the loss of X/O females occurs before 12 days *in utero*; however, since the data are small, the conclusion must remain tentative.

"Chromosome-21 Anomalies" in Man

E. H. Y. Chu

Introduction. – The chromosome analysis of human subjects with various types of congenital disorders has been continued. Two families with cases of mongolism have been selected for this report since the findings have led to certain conclusions concerning the cytogenetic basis for the etiology of this syndrome.

Mongolism is now known to be associated with 47 chromosomes and a trisomic condition for the small acrocentric autosome 21. Although a chromosome number of 46 has been reported in exceptional cases of typical mongolism, chromosomal translocations were demonstrable in such patients, so that they also probably had triplicate quantities of chromosome 21. In addition, recent evidence also indicates that abnormalities involving chromosome 21 are related to the occurrence of acute leukemia.

Results and Discussion. – In a sibship of six children that we have examined, four boys showed incomplete and atypical features of mongolism, including borderline dermatoglyphics. Both parents were mentally and physically normal. The maternal grandparents, the father, and two normal sibs had normal karyotypes. The mother and all the four abnormal children had 46 chromosomes including normal sex chromosomes, but one member of the smallest acrocentric pair 22 apparently had a somewhat enlarged arm. The simplest interpretation is that the mother was a 21/22 translocation heterozygote and that the abnormal children have a duplication for a small segment of 21 which was responsible for a phenotype showing some resemblance to mongolism.

A second family attracted interest because a young mother had given birth to two mongol children, one at age 23 and the second at age 25. The first child, a son aged 4, was normal. The second, a daughter aged 3, and the third, a son aged 14 months, were typical mongols with 47 chromosomes involving trisomy-21. The father's chromosomes were normal in number and configuration. The mother had 46 chromosomes but showed "15/21" translocation (duplication for a segment of 21). This cytological interpretation is corroborated clinically by the fact that the mother's physical appearance suggested certain mongoloid features although her intelligence was probably within the normal range.

Trisomy can result from nondisjunction at meiosis or during cleavage. The risk of giving birth to a mongol is closely related to increasing maternal age. It appears that the probability of nondisjunction increases with the changed physiological conditions in females near the end of the reproductive period. Perhaps an increased rate of nondisjunction is also a possibility in a young mother carrying a duplication, as in the case of the second family just described.

On the basis of these and other studies, it can also be concluded that the satellited end of chromosome 21 is probably not involved in mongolism. Trisomy for a whole chromosome or a major portion of the long arm of 21 is responsible for a complete and typical manifestation of this syndrome, whereas triplication for a small terminal piece of the long arm of 21 is responsible for an incomplete and atypical manifestation. These studies have raised the possibility of systematically exploring the phenotypic effects of different degrees of triplication of chromosome 21.

Finally, it has been established that mongols have a heightened probability of developing acute leukemia. Recent reports[30,31] of partial deletion of one chromosome, presumably 21, in several cases of acute leukemia add further hope for a study on dosage effect and cytological mapping of this chromosome.

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[30] D. A. Hungerford *et al.*, *Am. J. Human Genet.* 11, 215 (1959).

[31] A. G. Baikie *et al.*, *Nature* 188, 1165 (1960).

Chromosomes of Lemurs[32]

E. H. Y. Chu Barbara A. Swomley

Introduction. – Chromosome studies utilizing cell-culture techniques have been made of a number of species and subspecies of lemurine lemurs, hitherto unknown cytologically. A wide variation in chromosome number and morphology was observed, which suggests interesting evolutionary relationships.

Results and Discussion. – The diploid chromosome numbers of the group of lemurs so far examined are as follows: *Hapalemur griseus olivaceus*, ♂, 2n = 58; *H. griseus griseus*, ♂, ♀, 2n = 54; *Lemur mongoz*, 2♂♂, 3♀♀, 2n = 60; *L. fulvus rufus*, 2♀♀, 2n = 60; *L. fulvus albifrons*, ♀, 2n = 60; *L. catta*, 2♂♂, 2n = 56; *L. fulvus*, nov. subsp., ♂, 2n = 52; *L. fulvus fulvus*, ♂, ♀, 2n = 48; *L. variegatus*, ♂, 2n = 46; *L. macaco*, ♂, ♀, 2n = 44.

Detailed karyotypic comparisons indicate similarities between chromosomes of different species or subspecies. A Robertsonian type of chromosome evolution (involving structural rearrangement) has probably played a significant role in this group of primates. A tentative phylogeny has been proposed.

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[32] E. H. Y. Chu and B. A. Swomley, *Science* (in press).

RADIATION MICROBIOLOGY

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Effect of Sodium Bromide on the Radiation Sensitivity of Microorganisms

Alexander Hollaender A. Marie McCarthy

Introduction. – When spores of *Aspergillus terreus* were treated before and during x irradiation with *S*,*β*-aminoethylisothiuronium (AET)•Br•HBr and AET•Cl•HCl (rearranged at pH 7.0 to form 2-mercaptoethylguanidine), differences were observed in the results obtained with the two compounds. AET•Br•HBr showed toxicity at concentrations above 0.01 *M*, as evidenced both by a decrease in the number of survivors and an increase in the number of mutations produced. Such toxicity was not seen when AET•Cl•HCl was employed.[3] It was suggested that the differences might be explained by the nature of the inorganic ion present. The present study is an attempt to explore this possibility.

Methods and Results. – In addition to spores of *A. terreus*, suspensions of *Escherichia coli* B and B/r were treated with NaBr in concentrations varying from 0.01 to 0.3 *M*. Treatment was carried out before and during x irradiation. All three organisms showed a very sharp decrease in survival levels whenever the NaBr concentration was in excess of 0.1 *M*. With *A. terreus* this decrease in survival was always accompanied by an increase in the number of mutations produced. However, organisms grown in the presence of 0.3 *M* NaBr but washed prior to irradiation showed no appreciable change in x-ray sensitivity, making it apparent that the NaBr must be present during the time of x-ray treatment in order for an increase in the lethal effect to occur. It was also found that the presence of NaBr had no effect on the ultraviolet sensitivity of *E. coli* B/r.

Finally, *E. coli* B/r was subjected to gamma radiation both in the presence and in the absence of NaBr. In this case, the presence of NaBr did not affect the radiation sensitivity of the organisms when they were subjected to doses up to 30 kr. However, when higher doses were employed the rate of killing was very markedly increased in the NaBr-treated samples.

It was suggested that the bromide ion might somehow become incorporated into the DNA of the cells during x-ray treatment. Preliminary to testing this proposal, uracil was treated with varying x-ray doses both in the presence and in the absence of NaBr. The degree of destruction of uracil

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[1]Leave of absence, IAEA technical assistance appointment, Buenos Aires, Argentina.

[2]Visiting investigator from abroad.

[3]A. Hollaender and A. M. McCarthy, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1960*, ORNL-2913, p 64.

was determined by spectrophotometric analysis. If uracil was x-rayed alone, 42% was found to be destroyed by a dose of 25 kr, 82% by 50 kr, and 100% by 75 kr. When 0.3 *M* NaBr was present during x-ray treatment, only 14% of the uracil was destroyed at 25 kr, and, as the dose was increased, the uracil appeared to brominate, as evidenced by a shift toward the longer wavelengths in the absorption spectrum. Raising the pH from 5.5 to 11.2 caused a further shift in the same direction – increasing evidence of the possibility that the uracil was brominating under these conditions.

Discussion. – These experiments indicate that results obtained with AET • Br • HBr must be interpreted with considerable care because of the observed effects of the bromide ion on radiation sensitivity. Further experiments are being considered to determine the explanation of the bromide-ion effects.

Cultural Conditions That Enhance Resistance to Ionizing Radiation

H. I. Adler

Previous work from this group has established the existence of two distinct but related factors of preirradiation growth which affect the sensitivity of *Escherichia coli* to ionizing radiation. These are growth in glucose and growth under anaerobic conditions. Both result in an enhanced resistance to ionizing radiation.

The bacterium *E. coli* H_7 has certain unique properties that allow us to study these effects in greater detail. It can be grown as a catalase-positive, cytochrome-positive organism (H_7+) or a catalase-negative, cytochrome-negative one (H_7–). Thus, the relationship of this *E. coli* strain to oxygen and glucose utilization is under experimental control. Using this bacterium we have established that the glucose-pH effect described by Stapleton and Engel[4] can only occur in the catalase-positive, cytochrome-positive cells. Likewise, the anaerobic effect described by Hollaender *et al.*[5] occurs only in catalase-positive, cytochrome-positive cells. Apparently, the increase in resistance to ionizing radiation observed as a result of these growth conditions depends on the organism's having a functioning catalase and cytochrome system. The way in which these particular systems are involved is currently under study, and preliminary results show that catalase activity per cell is enhanced by anaerobic growth but suppressed by growth in glucose. This last statement applies to both H_7+ and *E. coli* B/r ORNL.

Physiological Control of Radiation Damage in *Escherichia coli*

M. S. Engel G. E. Stapleton

Introduction. – It has been shown that cells of *Escherichia coli* grown aerobically in peptone-glucose-phosphate are more resistant to ionizing radiation than those cultured in peptone alone.

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[4]G. E. Stapleton and M. S. Engel, *J. Bacteriol.* **80**, 544–51 (1960).

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

This effect has been related to the final pH of the growth medium.[4] A number of explanations for the resistance phenomenon have been investigated. The resistant and sensitive cells both show a normal oxygen effect, and their growth rates after irradiation are similar. Microscopic examination showed that the resistance was not due to clumping or multicellular forms. However, the glucose-grown cells were found to be uniformly larger.

Results and Discussion. – Size distribution studies were made with an electronic cell counter, and it was established that the glucose-grown cells have twice the cell volume of the peptone-grown cells. This effect was found in several strains of *E. coli*. The volume ratio of 2 for resistant to sensitive cells correlated well with deoxyribonucleic acid, ribonucleic acid, protein, dry-weight, and packed-cell-volume ratios.

In an attempt to find the requirements for resistance, a synthetic medium was devised which simulated a commercial peptone. Cells grown in this synthetic medium (which contained 17 amino acids) showed the glucose effect described above. However, cells grown in a synthetic medium without amino acids produced a low final pH but were sensitive and small. Attempts were then made to find out which amino acids are required for the induction of resistance. The involvement of methionine and glutamate or aspartate in the resistance phenomenon has been established, while a number of other amino acids appear not to be involved. It has been established that the resistant cells have twice the volume and nucleic acid content of the sensitive cells and that this resistance has certain requirements involving substrates, cofactors, and possibly the presence of certain enzymes.

Further Studies of the Effect of Organic Peroxides on Yeast

J. H. Fränz

As we have demonstrated in previous experiments,[6] some water-soluble compounds of ultraviolet-irradiated methyl linolenate, probably hydroperoxides, cause a delay of the first cell division in *Saccharomyces cerevisiae*. Furthermore, it was found that the endogenous respiration is increased remarkably, although the exogenous respiration is not affected.

In the present experiments it was demonstrated that the observed effects depend on the inoculum of yeast cells as well as on the concentration of the peroxides. A decrease of the inoculum from 30×10^5 to 7×10^5 cells per unit volume of culture at a constant concentration of linolenic peroxide extends the lag phase of the growth curve for about 6 hr. One can get the same effect by increasing the peroxide concentration.

Because the ultraviolet oxidation products are not definitely established, it was desirable to study the effect of an organic compound with a well-defined hydroperoxide or peroxide group. Recent experiments with unirradiated disuccinyl peroxide, whose active compound in an aqueous solution is the succinic peracid, have shown effects in yeast similar to those observed after treatment with the ultraviolet-irradiation products of methyl linolenate. A concentration of 0.01% disuccinyl

[6] J. H. Fränz and B. T. Cole, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, p 92.

peroxide at an inoculum of 17×10^5 cells delays the first cell division for about 6 to 8 hr. Whether the effect of organic peroxides is independent of the constitution of the peroxide group (peroxide, hydroperoxide, etc.) and of the position of this group in the molecule has to be studied in other organic compounds with a peroxide group.

Protection of Three Strains of *Escherichia coli* by β-Mercaptoethylamine

H. L. Cromroy

The protective effect of β-mercaptoethylamine has been studied for three strains of *Escherichia coli:* B/r ORNL, B ORNL, and B_s Hill. These three organisms, although very closely related, have very different radiation sensitivities. For example, at a dose of 20 kr in air, the percentage survivals are: B/r ORNL, 18.5%; B ORNL, 0.178%; B_s Hill, 0.0024%. Complete survival curves for these organisms have been run in air-saturated suspensions, nitrogen-saturated suspensions, and β-mercaptoethylamine-treated suspensions. For all three organisms β-mercaptoethylamine was more protective than oxygen removal by nitrogen bubbling. However, the magnitude of the oxygen effect decreased with increasing radiation sensitivity of the strains. In the case of B_s Hill it is barely observable under our experimental conditions. Further investigation of this problem is in progress.

MAMMALIAN RECOVERY

Hematopoietic Recovery

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Bone Marrow Physiology

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The Primary Histologic Events in Lymphatic Tissue After Bone Marrow Transplantation

C. C. Congdon

Pathologic studies on lethally irradiated mice treated with foreign bone marrow pointed to the lymphatic tissues of these animals as the primary site of the immune disease that limits the usefulness of foreign marrow as a practical treatment for acute radiation sickness.

To understand the pathologic anatomy of these remarkable changes in radiation chimeras, it became necessary to investigate the effects of foreign marrow on the lymphatic apparatus of normal mice. Ordinary antigenic materials, such as sheep red blood cells and bovine serum albumin, were also studied in normal mice for comparison with the antigenic materials in bone marrow. A sequential pattern of cellular changes in the lymphatic apparatus, apparently common to all antigenic materials including foreign bone marrow, was observed.

The cellular changes in the spleen white pulp after intravenous injection of rat marrow were well established at 24 hr after injection. There was loss of nearly all organized germinal centers and the tissue lymphocytes. Many antibody (Ab) forming cells were present with migration into the spleen red pulp. In a few days there was reorganization of the germinal center tissue, and the spleen gradually resumed its normal appearance. With homologous marrow all changes were exceedingly drawn out in time.

In the present work, changes that took place during the first 24 hr in the mouse spleen after an intravenous injection of rat bone marrow were studied. Figure 4 shows schematically the histologic anatomy of the tissue where changes were observed.[4]

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[1]Consultant.

[2]Visiting investigator from abroad.

[3]Leave of absence, NSF Senior Postdoctoral Fellowship, Rijswijk, The Netherlands.

[4]C. C. Congdon and P. G. Delker, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, pp 97–99.

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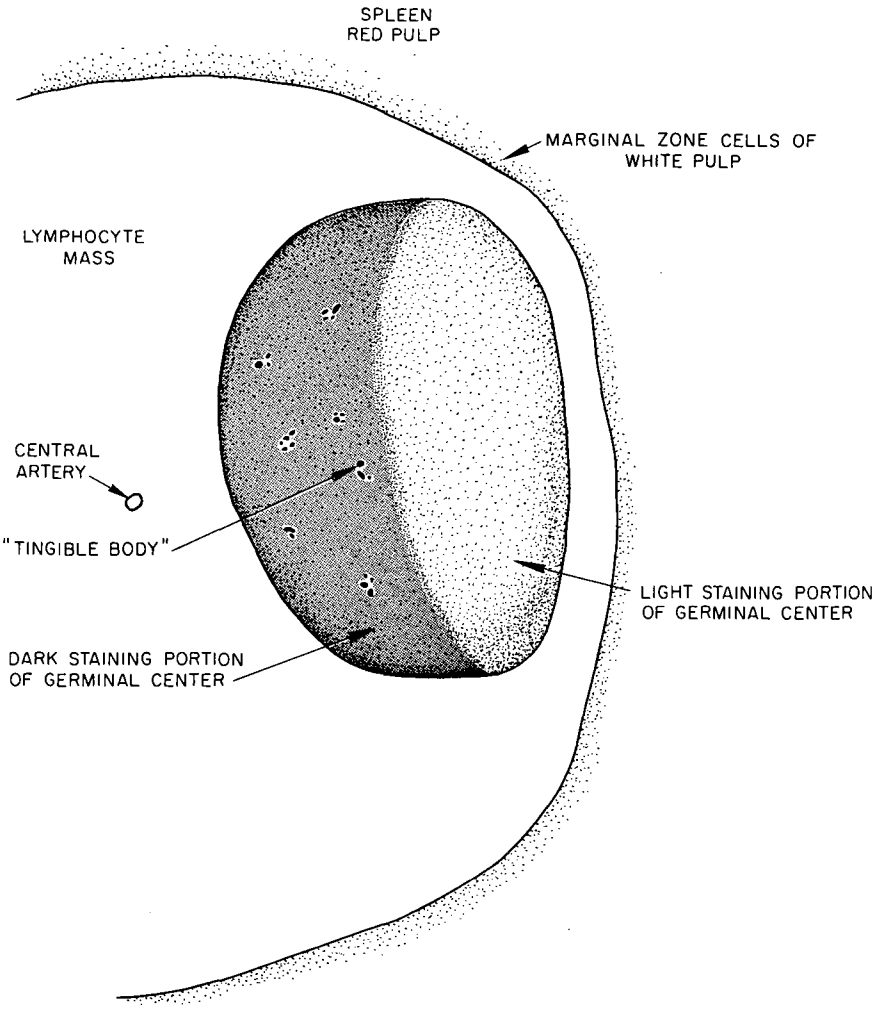

Fig. 4. Schematic Drawing of Histologic Anatomy of White Pulp of Spleen.

Within 1 hr after marrow administration, the marginal zone cells were swollen and irregularly arranged. The most striking change at about 4 to 5 hr after injection was the infiltration of granulocytes into the germinal centers of the white pulp. These cells apparently undergo necrosis in the "tingible body," contributing to the great increase in cellular debris in these structures at this time interval. By 9 hr the germinal center cells (dark staining portion) near the "tingible bodies" had formed a dissociated proliferating and migrating mass of Ab-forming cells that then filled the white pulp by the end of the first 24 hr after antigen injection. Quite similar changes were found at the same intervals after sheep red blood cell or egg albumen injection.

Summary. – These new findings point to the potential importance of the marginal zone cells, the granulocytes, and the "tingible bodies" in understanding the recognition mechanism of the antibody-forming system. Apparently the "tingible body" is a place where granulocytes are destroyed. Its

very intimate relation to the germinal center Ab-forming cells suggests that it might be involved in transferring information about the antigen to the germinal center cells. The presence of granulocytes suggests a means for getting antigenic information to the sensitive area.

The Immune Tissue Reaction in Irradiated Mouse Spleen

Patricia G. Delker

Introduction. – A temporary immune tissue reaction was present in the lymphatic tissues of lethally irradiated mice given rat bone marrow.[5]

The reaction was present during the first three days after treatment, and was thought to be the residual immune response to transplantation antigens. Spleen weight changes after irradiation appeared to be an indication of the presence of the reaction, and since this particular immune tissue reaction has a potential use as an assay method for transplantation antigens, a more systematic study of the spleen weight after rat bone marrow injection was carried out. The more cumbersome histologic technique was previously used to determine the presence or absence of the reaction.

Results and Discussion. – Figure 5 shows the results on male (C3H × C57BL)F_1 mice. During the time that the histologic reaction is most evident there was a significant difference between the spleen weights of the mice receiving radiation alone and those also given rat bone marrow in addition to the radiation. There was also a rough correlation between the amount of rat marrow injected and the spleen weight ratios at the different intervals.

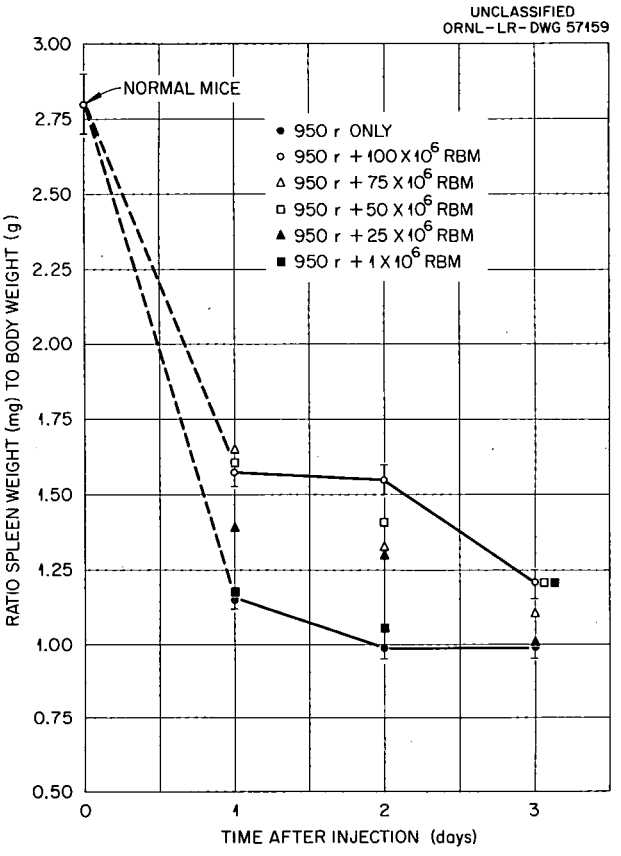

Fig. 5. Spleen/Body Weight Ratio in Lethally Irradiated Mice Given Different Doses of Rat Bone Marrow Cells (RBM) Intravenously. Each point represents the average of ten mice.

The data suggest that spleen weight alone probably could be used in this assay system for transplantation antigens.

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[5] C. C. Congdon *et al.*, *Rev. arg. cancerol.* 2, 3–13 (1960).

Identification of Antibody-Forming Cells in Heterologous Chimeras

Gino Doria

A study is being made of the relative immune capabilities of host and donor cells in the spleen of mouse-rat heterologous chimeras sensitized against sheep RBC. Lethally irradiated test mice are used as a culture medium for a known number of spleen cells from donors previously injected with sheep RBC. The irradiated recipients are injected with the test antigen at the time of the spleen cell transfer and bled six days later for serum titration of anti-sheep-RBC agglutinins. The titer is due to the secondary response of the transferred spleen cells; if the irradiated recipients have been preimmunized to the donor, no antibody activity can be detected.

Heterologous chimeras four to five months old and having only donor-type red cells in the peripheral blood are injected intraperitoneally with sheep RBC. Twelve days later the sensitized chimeras are bled, and blood is individually collected for serum titration of anti-sheep-RBC agglutinins. Chimeras showing a positive titer are sacrificed, and their spleen cells are injected intravenously into two groups of irradiated test recipients (one preimmunized against the host and the other against the donor) which also receive sheep RBC intraperitoneally. The sera of the test recipients sacrificed six days after the spleen cell transfer are titrated for anti-sheep-RBC agglutinins. Preliminary results show evidence for a predominant activity of host-type antibody-forming cells. If this is confirmed by repeating experiments now in progress, the immune system of heterologous chimeras should be considered completely different from that of homologous chimeras, in which only donor-type immune activity was found.

Amino Acid Changes in Mice with Grafted Homologous Bone Marrow

A. L. Kretchmar[6]

Lethally irradiated mice protected by foreign-strain bone-marrow cells develop a secondary syndrome that apparently alters protein metabolism. Accordingly, amino acids of liver, plasma, and muscle were studied in such animals. Mice were given 900 r and 40×10^6 bone-marrow cells (isologous or homologous), and liver, muscle, and plasma were sampled 7, 12, 21, and 35 days later. The procedure of Tallan *et al.*[7] for tissues and of Spackman *et al.*[8] for analysis of amino acids was used. Irradiated mice and those starved three days had lower threonine, serine, tyrosine, and phenylalanine in liver and muscle; glycine was reduced one-third, muscle proline elevated, and liver glutamine decreased. X ray but not starvation resulted in increased arginine and glutamine in muscle. Injection of marrow cells allowed recovery of irradiated mice, and the time course of changes was followed. Glutamine and glutathione in liver, low initially, showed recovery after the 12th day. Mice given homologous cells showed a secondary decrease, however, and glycine and serine were

[6]Medical Division, Oak Ridge Institute of Nuclear Studies, in collaboration with Mammalian Recovery Group.

[7]H. H. Tallan, S. Moore, and W. H. Stein, *J. Biol. Chem.* 211, 927 (1954).

[8]D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.* 30, 1190 (1958).

low in their liver and plasma through 35 days. Changes found only in irradiated-treated mice were an increase in liver β-alanine and β-aminoisobutyric acid (12 days) and (in mice given homologous cells) a persistent increase in aspartic acid of liver. Except for aspartic acid, no change occurring only in homologous chimeras was found.

Effect of Leukocyte Injections on Peripheral Leukocyte Count

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

Introduction and Methods. – Alteration in the quality of hematopoietic tissue can be achieved by several methods. For example, the intravenous injection of compatible red cells into animals suppresses the erythrocytic series of bone marrow, whereas withdrawal of red cells stimulates erythrocyte formation. The number of circulating erythrocytes, therefore, influences red cell production. The fact that large numbers of leukocytes can be obtained from blood of mice that bear a particular transplantable squamous cell carcinoma led us to consider the effects that injection of these cells would have on the dynamics of leukocyte formation. Our first approach to the problem was to determine whether a significant alteration in peripheral leukocyte count could be achieved by serial injection of isologous leukemoid leukocytes.[9] Each day for four days, normal (C57BL × A)F_1 mice were given leukemoid leukocytes separated from whole blood by dextran sedimentation of red cells. Twenty-four hours after the last injection, peripheral leukocyte counts were made and compared with preinjection counts. Saline-injected mice served as controls. Injection volume was 0.5 ml.

Results and Discussion. – Preinjection leukocyte counts in peripheral blood averaged $14 \times 10^3/mm^3$. Counts in mice injected with 800×10^6 leukocytes over a period of four days averaged $5.1 \times 10^3/mm^3$, while saline control value was $8.6 \times 10^3/mm^3$. Although injection of either leukocytes or saline lowered the peripheral count, the difference between control and experimental levels was not significant. Perhaps the decrease is a matter of dilution, but within 24 hr after injection of a 0.5-ml volume, readjustment of circulating blood volume to normal would be expected to have occurred. Assuming a peripheral leukocyte count of $15 \times 10^6/ml$ and a blood volume of 2.5 ml, the total circulating cells would number about 40×10^6. Injection of 800×10^6 leukocytes, therefore, would represent a 20-fold increase. However, the number of circulating leukocytes probably represents but a small fraction of total body leukocytes. Thus, it is not possible to determine accurately the magnitude of overloading represented by 800×10^6 leukocytes. An effect may be observed in hematopoietic tissues or in differential blood counts (the majority of leukemoid leukocytes are, morphologically, normal-looking granulocytes). Tissues and terminal blood smears were obtained on all mice, but the material has not been examined.

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

Repopulation of Bone Marrow and Lymph Nodes of Irradiated Parent Mice by F_1 Leukemoid Blood

Joan W. Goodman Paulette G. Lankford

Introduction. – The protective effect of leukemoid blood (from mice carrying the transplantable carcinoma A-280) given to lethally irradiated isologous mice was reported by Congdon *et al.*[10] That the blood, which contains almost all the same nucleated cell types as bone marrow, affords protection by repopulation of the irradiated recipients' hemopoietic organs has been deduced from hematological and histopathological study of the recovering mice. Smith *et al.*,[11] making use of a hemoglobin difference between two very closely related strains, showed in three animals that donor-type red cells were formed in such chimeras.

Leukemoid blood has regularly failed to protect irradiated homologous mice, and it is inferred from other studies[12] that this failure results from killing of the recipient by immunologically competent cells in the donor leukemoid blood.

The present work was designed to test this inference and to add substantial and technically different information to earlier repopulation data.

Methods. – Parent mice (and in some cases other strains) were lethally x-irradiated and then transfused with 1 ml of pooled, heparinized heart blood taken from F_1 hybrids carrying ulcerated leukemoid tumors. The nucleated cell dose per recipient varied from 30 to 135 million in different experiments. Mortality was recorded in all cases. Chimeras were killed at various times, and lymph node and bone-marrow cell suspensions were typed by the cytotoxic method of Gorer and O'Gorman.[13] Specific antisera were prepared in each parent strain against the other parent. Since the F_1 hybrid tissues reacted with both sera and each parent with only one, identification of donor (F_1) cells could be made with confidence.

Results and Discussion. – In two homologous cases (B6D2F_1 and CAF_1 recipients of LAF_1 blood), none survived beyond 20 days after irradiation. In one homologous case, in which the two strains were very closely related (B10D2 recipient of B6D2F_1 blood), 20-day survival was excellent, and five of the nine mice were alive after 90 days. One of these mice was tested and found to have lymphoid and bone-marrow cells serologically identical with the blood donors.

When F_1 leukemoid blood (either B6D2F_1 or LAF_1) was given to lethally irradiated appropriate parent strains, 44 out of 81 animals survived the period of hemopoietic death, and more than half of these survivors appeared to be in good health three months later. This result is consistent with the presence in the leukemoid blood of an immunologically competent cell that is capable of reacting

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[10] C. C. Congdon *et al.*, *Radiation Research* 4, 424 (1956).

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

[12] J. W. Goodman and C. C. Congdon, *A.M.A. Arch. Pathol.* (in press).

[13] P. A. Gorer and P. O'Gorman, *Transplantation Bull.* 3, 142 (1956).

against and killing an irradiated homologous host, despite the blood's capacity to repopulate damaged bone marrow. When the capacity to recognize a difference is removed (as in the case of the F_1 blood in the parent recipient), repopulation is successful, and survival results.

Tissues from nine leukemoid blood chimeras were tested cytotoxically, and positive identification of donor-type cells was made in the marrow suspensions of eight, and in lymph node suspensions of six. The results of the remainder were not negative but were uninterpretable because of the high cell death rate in normal serum. This condition often is seen in cell suspensions of reactive chimeric lymph nodes and spleens.

Somatic Chromosome Abnormalities in Irradiated Humans

M. A Bender P. Carolyn Gooch

Introduction. – Previous investigations of radiation-induced chromosome aberrations in human cells[14,15] have been limited to cells irradiated in vitro. We have now examined the chromosomes of eight normal men who received excessive acute exposure of the whole body to mixed gamma and fission irradiation. These men received doses estimated to range between 22.8 and 461 rems of mixed gamma rays and fission neutrons. Five of them received doses estimated to exceed 298 rems; three others received lower doses.

Method. – Blood samples were drawn between October 18, 1960, and January 20, 1961, roughly 29 months after the exposure occurred. In addition, cultures were examined from three normal unirradiated controls. The culture method was modified from that of Moorhead *et al.*[16] The serum and leukocytes were separated from the blood samples and incubated at 37°C for three days in a commercial tissue culture medium. Before fixation the cultures were treated with colchicine and hypotonic saline. Since few if any mitoses are seen in cultures fixed earlier than three days, the cells scored were mainly in their first in vitro mitosis. In scoring, cells were selected at low magnification and then scored at 900X. The number of chromosomes, and the presence of chromatid aberrations, ring and dicentric chromosomes, minute chromosomes, and chromosomes of otherwise abnormal form were recorded. In addition, the easily identified pairs of chromosomes (Nos. 1, 2, 3, 13, 14, 15, 21, 22, and the Y) were checked. Detailed karyotypic analyses have also been carried out on a few cells from several individuals.

Results. – The results of our investigation are presented in Table 15. It will be seen that, even after two and one-half years, most of the irradiated individuals show abnormalities in both number and form of their chromosomes. Two of the "low-dose" cases (under 100 rems) show only a

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

[15] M. A Bender, pp 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, London, 1960.

[16] P. S. Moorhead *et al.*, *Exptl. Cell Research* 20, 613 (1960).

Table 15. Chromosome Analysis of Irradiated Humans and Normal Controls

Case	Age*	Estimated Dose		Cells Scored	Chromatid Aberrations (%)	Chromosome Aberrations						Total Abnormal Cells (%)
		Rads	Rems			Cells with $2n \neq 46$ (per 100 cells)	Minutes per 100 Cells	Rings per 100 Cells	Dicentrics per 100 Cells	Others** per 100 Cells	Total Abnormal Chromosomes (%)	
						Irradiated						
A	40	365	461	100	2	14	5	0	1	1	10	18
B	32	270	341	100	1	20	0	0	1	1	2	20
C	39	339	428	142	1	23.2	2.4	3.2	13.4	1.6	19.7	28.2
D	51	327	413	100	0	14	5	1	3	1	9	19
E	35	236	298	75	1.3	6.7	0	0	1.3	0	1.3	6.7
F	41	68.5	86.5	48	0	8	0	0	0	0	0	16.7
G	56	68.5	86.5	33	0	2	0	0	0	1	3	6
H	25	22.8	22.8	98	2.0	4	0	0	0	0	0	4
						Control						
P.C.G. (female)	25			100	1	0	0	0	0	0	0	0
E.H.Y.C.	33			100	5	1	0	0	0	0	0	1
M.A B.	31			50	0	0	0	0	0	0	0	0

*At the time of exposure for the irradiated humans.

**Chromosomes of abnormal length or centromere position.

greater numerical variation than the controls, but all of the "high-dose" cases also show one or more abnormal chromosomes. Detailed karyotype analyses have been made on nine grossly normal cells from two of the "high-dose" individuals. Seven of these show additional abnormalities in the form of inverted or deleted chromosomes. In a number of cases, the same abnormal karyotype has been found in several cells of the same individual.

Discussion. – The persistence of dicentric chromosomes is of great interest, since one would expect them to be at a selective disadvantage due to anaphase bridge formation. The persistence of ring chromosomes in several of the cases is also of interest, since somatic crossing over, if it occurs, should put them at a similar selective disadvantage. The survival of abnormal cell types in the present cases demonstrates that chromosome analysis may be of considerable value in dose estimation. Early chromosome aberration analysis, particularly in the period yielding chromatid-type aberrations immediately following irradiation, would be of great clinical value in deciding on the therapeutic measures to be taken. If made shortly after irradiation, such analyses would be free from the lack of quantitative relationship to dose which is seen in the present investigation, doubtless caused by cell selection in the long interval between irradiation and examination.

RADIATION IMMUNOLOGY

Takashi Makinodan
E. H. Perkins
E. E. Capalbo[1]
Franco Celada[1]
R. D. Owen[2]
Isabel C. Shekarchi[2]
Rachel R. Carter
W. J. Peterson
Martha A. Robinson
Patsy F. Lincoln[2]
Paul Urso[3]

Further Studies of the Radiosensitive Nature of Antibody-Forming Cells

Takashi Makinodan M. A. Kastenbaum W. J. Peterson

Introduction. – Previously[4] we were able to demonstrate that the radiosensitivities of primary and secondary antibody-forming cells are comparable. These data should, therefore, permit us to determine the radiation inactivation curve and compare the "extrapolation number" and the "target" size with those for cell survival in other mammalian systems.

Results. – As shown in Fig. 6, it can be seen that the fitted regression $C_m/C_k = 2.64\ e^{-0.0142x}$ with an intercept of 2.64 is very much like the curve $C_m/C_k = 1 - (1 - e^{-0.0142x})^{2.72}$ with an extrapolation number of 2.72 and a "D_{37} value" of 130 r. The diameter of the radiosensitive site can be estimated to be 0.4 μ if the following assumptions are made: (1) the "target" theory is applicable to this system; (2) there are approximately two radiosensitive sites, each of which requires 65 r for inactivation; (3) the site is a sphere with a density of 1.0; and (4) there is an average energy loss of about 100 ev in a primary ionization (p.i.).

Discussion. – We have concluded that cell division is a necessary phase of the antibody-forming mechanism because of the following observations: (1) The antibody-forming activity is a function of cell number. (2) The slopes of the regression

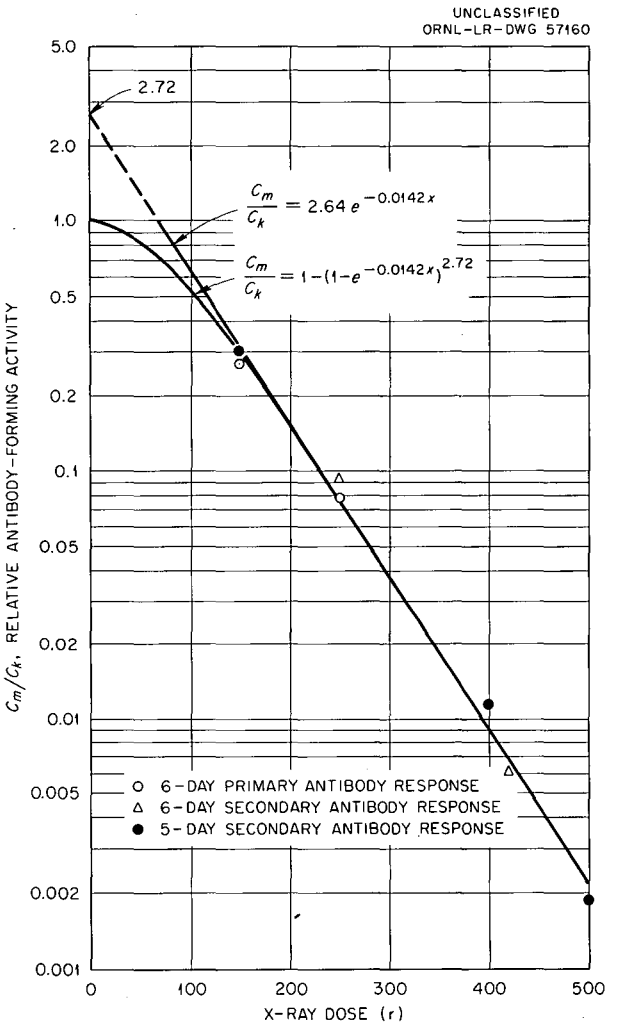

Fig. 6. X-Ray Inactivation of Antibody-Forming Capacity of Spleen Cells.

[1]Visiting investigator from abroad.

[2]Consultant.

[3]ORINS Fellow.

[4]T. Makinodan and W. J. Peterson, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, pp 114–15.

lines (activity by cell number) are not significantly different (1.0) for primary and secondary antibody responses before and after x-ray treatments. (3) The radiosensitivity of the proliferative capacity of cells is comparable with that of antibody-forming activity. (4) The extrapolated "2.7-hit" value shown here is comparable with those reported for cell survival. (5) The estimated "D_{37} value" reported here is comparable with those for normal and malignant cells cultured in vitro and in vivo. The significance of the target site to the role of cellular differentiation must await precise immunohistological studies.

Use of Diffusion Chambers for Agglutinin Response by Spleen Cells

E. E. Capalbo

Introduction. – It is known that spleen cells can proliferate and produce antibodies against the test antigen when injected in in vivo tissue cultures (heavily irradiated recipients).

To obtain information on the morphological changes which these cells undergo during antibody formation, it is desirable to keep them isolated from the recipient but yet in an environment similar to the in vivo tissue culture. To do this, cells are placed in a Lucite chamber enclosed by two porous membranes (diffusion chamber) which is inserted intraperitoneally into recipient mice.

Results. – To test the diffusibility of cells through the porous membranes of the chambers, empty diffusion chambers made with Millipore filters of different porosity were inserted intraperitoneally into normal mice. We found that cells passed through filters of 1.2- and 0.45-μ porosity but did not pass through those of 0.1 or less.

We then investigated the possibility that normal recipients could react against cells placed in the diffusion chambers. Diffusion chambers with varied porosity containing sheep RBC antigen were implanted into the peritoneal cavity of normal mice. Antibody response against the test antigen was obtained even with chambers of 0.1- and 0.01-μ porosity. From these results it seemed that the entire sheep red blood cell was not necessary to provoke an antibody response. Consequently it was necessary to depress the immune response of the recipient by radiation to prevent its response against test cells placed in diffusion chambers. Thus, x-rayed (500 r) mice implanted with chambers of 0.1-μ porosity containing $3–9 \times 10^6$ RBC failed to respond against this antigen. Knowing this, we tried to obtain a primary antibody response against sheep RBC by spleen cells. Diffusion chambers of 0.1-μ porosity containing 12×10^6 spleen cells from nonimmunized isologous (BDF_1), homologous $(C3H \times 101)F_1$, or heterologous (rat) donors labeled in vivo with H^3-thymidine and 6×10^6 sheep RBC antigen were implanted intraperitoneally into 500-r x-rayed BDF_1 recipients. Figure 7 shows that a primary antibody response was obtained even in a heterologous system. In accordance with the antibody titer levels, the viability of the cells was better with the isologous than with the homologous and heterologous cells.

Discussion. – The standard diffusion-chamber technique was modified here by using Millipore filters of 0.1-μ porosity, which are impermeable to cells, and by implanting chambers into x-rayed recipient mice. The dose of radiation was sufficient to depress their immune system.

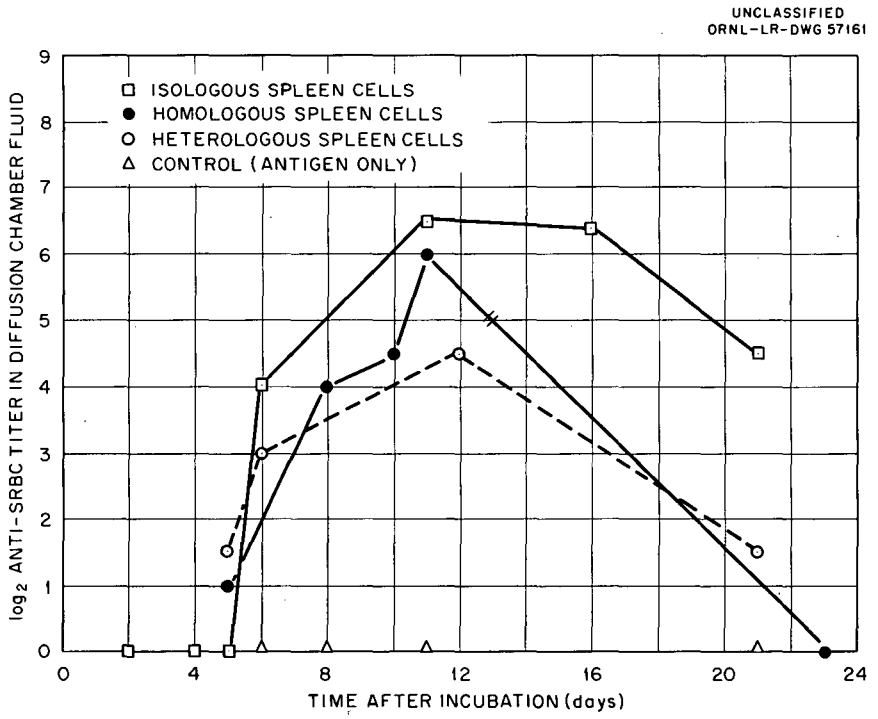

Fig. 7. Primary Antibody Response Against Sheep Red Blood Cells from Normal Spleen Cells in Diffusion Chambers.

The results show that this modified diffusion-chamber technique can be useful in studying systematically the cellular changes which accompany antibody response, especially the role of division and maturation vectors during the induction of antibody formation.

Role of Cellular Division and Maturation During the Precipitin Response

Paul Urso

Introduction. – It is generally agreed that the cellular reaction to antigenic stimulus involves the maturation of primitive reticular cells through a series of changes culminating in the mature antibody-forming plasma cell or lymphocyte. Most investigators also agree that associated with the maturation vector there is a proliferation of the competent cells. However, two basic questions remain unanswered. These are: (1) Does cellular division precede the appearance of antibody-forming cells? (2) Are cellular division and maturation integral parts of the productive phase of antibody-forming mechanism? To provide information on these points, the cellular reaction to antigen was studied by using the immunofluorescent technique and radioactive markers.

Results. – In the first experiment H^3-thymidine labeled lymphoid cells from preimmunized rabbits were incubated with bovine serum albumin in diffusion chambers of 0.1-μ porosity and implanted intraperitoneally into 450-r rabbits and 600-r mice. Results were as follows: (1) The quantity of tritiated immune cells increased 1.75 times within 24 hr, or 2.5 times higher than the

nonimmune cells. (2) Mitotic index reached a peak at 2–3 days (0.36%) and then slowly decreased to 0.15% at seven days. (3) Blast cells and transitional cells reached a peak at 2–3 days. Proplasma cells and plasma cells increased to 18% at five days. (4) Antibody-containing cells were first detected at about three days and reached a peak at 6–7 days (by immunofluorescence). (5) About 3.5% of the antibody-containing cells were either binucleated or in mitosis.

In the second experiment recipient mice with chambers containing immunologically competent cells were injected with colchicine intraperitoneally on days 5, 6, and 7 after implantation of the chambers. The mitotic indices of the total population were 1.87, 1.68, and 1.62 respectively. On the other hand, the percentage of antibody-containing cells in mitosis was 6.2, 7.0, and 6.1.

In the last experiment recipient mice received multiple injections of tritiated thymidine intraperitoneally from day 3–5. The percentage of H^3-labeled cells on days 5, 6, and 7 was 32, 45, and 47, respectively. The percentage of antibody-containing cells that were labeled with H^3 was 63, 88, and 95 at the same time intervals.

Discussion. – The results reported here show that mitosis is closely associated with the secondary immune response in both the inductive phase (from the time of antigen uptake to appearance of antibody) and the productive phase of antibody formation. Furthermore, antibody-containing cells preferentially divide and take up DNA precursor during the productive phase of the immune response. Therefore, it appears that mitosis, in conjunction with maturation, is an integral part of the immune response. The fact that there exists an induction phase during the 24–48 hr prior to productive phase substantiates the idea that the antibody response begins in an undifferentiated cell which proliferates and matures into antibody-synthesizing cells upon antigenic stimulation.

A Quantitative Study of the Male Antigens in Mice

Franco Celada W. J. Welshons

Introduction. – Studies on the Eichwald-Silmser effect (immune reaction of the female against isologous male tissues) have been limited by the use of skin grafting to a qualitative or semiquantitative evaluation, although evidence of the presence of the male antigens in hemopoietic cells has been submitted. Lacking, however, is definitive evidence that the "male antigen" is due solely to the presence of the Y chromosome and not to a "single X" condition, as could be alternatively suggested.

Results and Discussion. – The present investigation was performed by utilizing the quantitative method for the assay of transplantation antigens described by Celada and Makinodan,[5] based on spleen-cell transfer into preimmunized recipients. Normal males and females of various strain combinations and XO females were used as starting material. Our preliminary data indicate the following: (1) The male antigen(s) is clearly linked to the Y chromosome. (2) The potency of the Y chromosome antigen is much less than that of the H_2 homologous antigens. For example:

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

(*a*) The radiation dose required to prevent a primary response by the recipient against the test graft is ~700 r in the former and ~850 r in the latter case; (*b*) the preimmunizing antigen dose required to stimulate the recipient to reject 50% of the grafted cells is ~ 10^7 cells in the former and ~ 10^5 in the latter case. (3) Although the Y chromosome antigens of different mouse strains cross-react with each other to a great extent, their relative immunizing potencies seem to differ. In the course of this study we also observed a "hybrid-toward-parent" type of reaction when a C57BL parent is involved. This reaction is of the same order of magnitude as that of the female-toward-male type but is not related to it. These results show that the method is a reliable tool to investigate minor immunogenetic specificities in the mouse.

Homograft-Rejecting Capacity of Isolated Spleen Cells

Franco Celada Rachel R. Carter

Introduction. – Our previous studies[6] have shown that in intact animals the mechanism of homograft rejection is less radiosensitive than that responsible for titratable antibody formation, although the radiosensitivity of the former varies with the relative strength of the antigen involved (H2 linked, Y-chromosome linked, or recessive like in the hybrid-parent reaction). Two explanations can be offered for this phenomenon: (1) Distinct types of somatic cells are involved, and (2) one cell type is involved in both mechanisms, but the total number of potentially competent cells is different. To test these possibilities it is necessary to use isolated cells as active material and compare the percentage decrease of activity following x rays in either type of reaction, so that the difference in sensitivity of the assays is circumvented.

Results. – We developed a new model which appears to be quantitative and versatile. Heavily irradiated F_1 hybrid mice were used as in vivo test tubes, in which the homograft-rejecting activity of P_1 (1st parent) cells against P_2 (2d parent) cells was evaluated by measuring the decrease of agglutinin formation by the latter cells. The killing or inhibition of these P_2 cells is shown to be a linear function of the number of P_1 cells present, and the data are expressed in decrease of titer, with reference to the controls (Fig. 8). Both primary

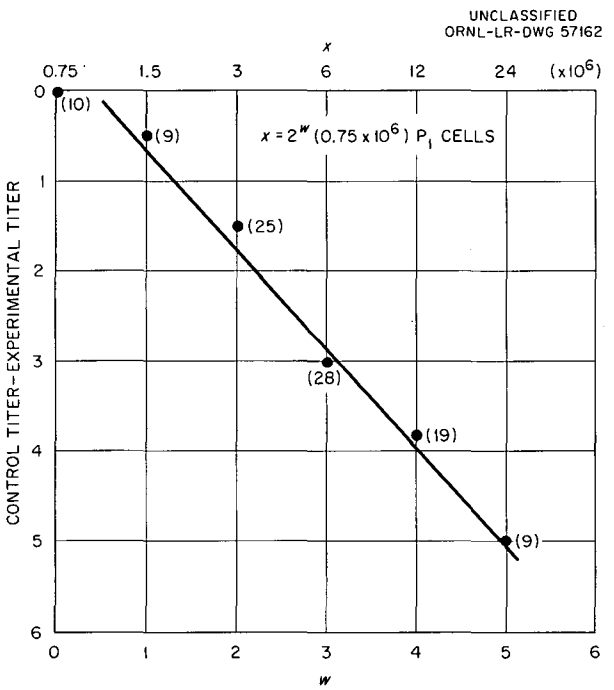

Fig. 8. Log_2 Titer Decrease in Carrier Mice as a Function of Number of Active (P_1) Spleen Cells. Numbers in parentheses represent mice used for each point.

[6] F. Celada and T. Makinodan, *J. Immunol.* (in press).

and secondary response are being studied in addition to the effect of in vitro irradiation.

Discussion. – The present model seems to fulfill the requirements of quantitation and reliability for this investigation and will be easily applied to the comparative study of homograft-rejecting capacity of different lines of cells, different stages of embryonic development, and conditions such as tolerance, immune paralysis, and chimerism.

Effect of Time of Antigen Injection on Antibody Response by Transplanted Spleen Cells

E. H. Perkins Martha A. Robinson

Introduction. – Our past studies have shown that spleen cells infused into lethally irradiated isologous recipients will proliferate, that the antibody-forming activity of these cells is directly related to the number of cells, and that the antibody-forming cells are capable of dividing. According to the clonal selection theory of antibody formation, pre-existing clones of antibody-forming cells should always be available to react with the specific antigen. One would therefore expect to find an increase in the immunologically competent cells during proliferation of infused spleen cells in lethally irradiated isologous mice. To test this, we have injected the test antigen (SRBC) at varying time intervals after cell infusion.

Results. – Spleen cells (0.25 or 10×10^6) from preimmunized donors showed no change in antibody-forming activity when antigen was injected immediately, 1, 2, or 5 days after cell infusion. In contrast, the antibody-forming activity of 20×10^6 spleen cells from *nonimmunized* donors decreased progressively with time. The mean $\log_2$ titer of 3.6, observed when antigen was injected immediately after spleen cell infusion, fell to 2.5 when antigen was injected one day later, to 0.8 two days later, and to 0.3 five days later. However, if isologous bone marrow cells (10×10^6) were given six days prior to, or simultaneously with, 20×10^6 *nonimmunized* spleen cells, no decrease in antibody-forming activity was observed. Similarly, when large numbers (200×10^6) of *nonimmunized* spleen cells were infused, no change in antibody-forming activity was noted with time.

Discussion. – With spleen cells from *preimmunized* donors, the expected rise in antibody activity associated with cell division was not observed. To explain this in accordance with the clonal selection theory, two possibilities exist if antibody-forming cells were dividing during this period: (*a*) The death rate must be equal to the division rate, or (*b*) in division an immunologically competent cell gives rise to one immunologically competent daughter cell and one incompetent daughter cell. With spleen cells from *nonimmunized* donors, the antibody-forming activity decreased with time, unless isologous bone marrow was added or large numbers of spleen cells infused. This observation would necessitate further modification of the clonal selection theory.

Analysis of Immune Precipitate by Serum-Agar Diffusion

Nazareth Gengozian Takashi Makinodan Rachel R. Carter

Introduction. – When bovine serum albumin (BSA) and chicken anti-BSA serum were reacted in recent experiments, the immune precipitate contained a coprecipitating protein in addition to the antibody gamma globulin and BSA antigen.[7] This coprecipitating protein was then shown to be a macroglobulin with an $S_{20,w}$ 20, mobility and salting-out properties of a gamma or beta globulin, and present in normal as well as in immune chicken serum. Also, it failed to react against rabbit anti-chicken gamma globulin with anti-BSA activity. The present study was undertaken to determine whether this coprecipitating phenomenon occurred in other species by analyzing BSA-anti-BSA serum precipitates in Ouchterlony serum-agar diffusion plates. Anti-BSA sera were obtained from chickens, guinea fowls, turkeys, pheasants, ducks, rabbits, rats, mice, and guinea pigs.

Results. – The results are summarized in Table 16. Except for the guinea fowl and rabbit systems, it shows that an additional protein besides the antibody gamma globulin and BSA antigen

Table 16. Analysis of Immune Sera from Avian and Mammalian Species by Quantitative Precipitin and Serum-Agar Diffusion Tests

Immune Serum (species)	Antibody Nitrogen (μg/ml)	Number of Serum Proteins in Antigen-Antibody Complex by Serum-Agar Diffusion Test
Avian		
Chicken	566	2
Guinea fowl	224	1
Pheasant	164	2
Duck	1120	2
Turkey	72*	2
Mammalian		
Rabbit	340	1
Rat	134	2
Mouse	168	2
Guinea pig	500	2

*Estimated from equivalence-point determination.

was present in the immune aggregate. This coprecipitating phenomenon seems to be independent of the antibody content of the various sera.

Discussion. – These results clearly show that the coprecipitating phenomenon previously seen in the chicken system is widely distributed throughout the different species. The biologic significance of this phenomenon must await further studies.

[7] T. Makinodan *et al.*, *J. Immunol.* 85, 439 (1960).

PATHOLOGY AND PHYSIOLOGY

Section Chief – A. C. Upton

Pathological Effects of Neutrons and High-Energy Radiation in Mammals

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J. W. Conklin
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Physiological and Chemical Effects of Radiation

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Late Effects of Radiostrontium in RF Male Mice

G. E. Cosgrove W. D. Gude

Introduction. – The RF mouse has been studied intensively for various delayed somatic effects, notably myeloid leukemia, following various forms of irradiation. It seemed appropriate, therefore, to use this animal to study the effects of radiostrontium on the incidence of myeloid leukemia.

Results. – Young adult RF male mice were injected intravenously with radioactive strontium as follows: group I, 0.2 $\mu c/g$ of Sr^{90} after splenectomy; group II, 0.2 $\mu c/g$ of Sr^{90} after sham splenectomy; group III, 0.51 $\mu c/g$ of Sr^{89}; group IV, 0.04 $\mu c/g$ of Sr^{90} ($\mu c/g$ of Sr^{90} means μc of Sr^{90} per gram of body weight of mouse). Splenectomy was carried out to remove the large foci of extramedullary myelopoiesis which are normally present in the mouse spleen. Effects on the incidence of leukemia

[1] Visiting investigator from abroad.
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[3] Research associate.

and lymphoma were limited to an increased incidence of thymic lymphosarcoma following 0.2 $\mu c/g$ of Sr^{90}, with or without splenectomy (Table 17). Tumors of bone were noted in 1–6% of injected animals, whereas only three malignant bone tumors were observed in 258 controls. (The skeletal lesions were studied radiographically by Miriam P. Finkel, Argonne National Laboratory). Effects on the survival time were variable.

Table 17. Mean Survival Time and Major Lesions in RF Male Mice Receiving Sr^{89} and Sr^{90}

Treatment:	Splenectomy, Sr^{90} i.v., 0.2 $\mu c/g$	Sham splenectomy, Sr^{90} i.v., 0.2 $\mu c/g$	Sr^{89} i.v., 0.51 $\mu c/g$	Sr^{90} i.v., 0.04 $\mu c/g$	Controls
Number of mice	51	57	75	75	67
Mean survival time, months	18.9	17.7	19.2	17.6	19.8
Thymic lymphosarcoma, %	16	13	5	5	1.5
Myeloid leukemia, %	0	6	0	0	8
Bone tumor, %					
Malignant	4	0	0	1	0
Benign	2	4	1	1	0

Discussion. – The effects of radiostrontium on bone tumor and lymphoma incidence are consistent with earlier observations in other strains. The failure of radiostrontium to induce myeloid leukemia seems surprising, since the bone marrow is relatively heavily irradiated by this radionuclide. Although the significance of this effect remains to be elucidated, it recalls the fact that myeloid leukemia has not been a major complication of radium intoxication in man. This observation and the contrasting induction of lymphomas warrant further study.

Immunological F_1-Hybrid-Antiparent Reaction in Radiation Chimeras

Gustavo Cudkowicz

Introduction. – General experience in tissue homotransplantation has led to the assumption that all alleles governing histocompatibility are codominant and that F_1 hybrids from two inbred strains of mice produce all the antigens of both parents. There is, however, growing evidence that F_1 hybrids may lack parental specificities[4–6] and therefore react immunologically to transplantation antigens of grafted parental cells. The following experiments were undertaken to evaluate whether presensitization of F_1 hybrid mice to parental spleen cells would interfere with their subsequent acceptance of marrow from the same parental strain.

[4] G. Cudkowicz and G. E. Cosgrove, *Proc. Soc. Exptl. Biol. Med.* 105, 366 (1960).

[5] G. Cudkowicz and G. E. Cosgrove, *Transplantation Bull.* 27, 90 (1961).

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

Results. – Young adult (C57BL × 101)F_1, (C3H × 101)F_1, and (BALB/c × A)F_1 mice were injected intravenously with 20×10^6 viable spleen cells of parental strain donors, that is, C57BL, C3Hf, BALB/c, and A mice, respectively. Four weeks later the sensitized F_1 mice were given an $LD_{100}/30$ of x rays followed by 10×10^6 bone marrow cells of the donor strain to which they had been sensitized. Nonsensitized mice of corresponding strains were submitted to analogous procedures, and another series of control mice were sensitized to parental transplantation antigens but grafted with isologous F_1 marrow. Spleen and marrow cells were derived from adult donors killed by bleeding. The *sensitized* (C57BL × 101)F_1 and (C3H × 101)F_1 recipients of parental marrow showed an increased mortality 20 to 90 days after grafting, as compared with nonsensitized recipients or sensitized recipients given isologous F_1 marrow (Fig. 9). Sensitization of the (BALB/c × A)F_1 hybrid with BALB/c or A spleen cells did not, however, affect survival.

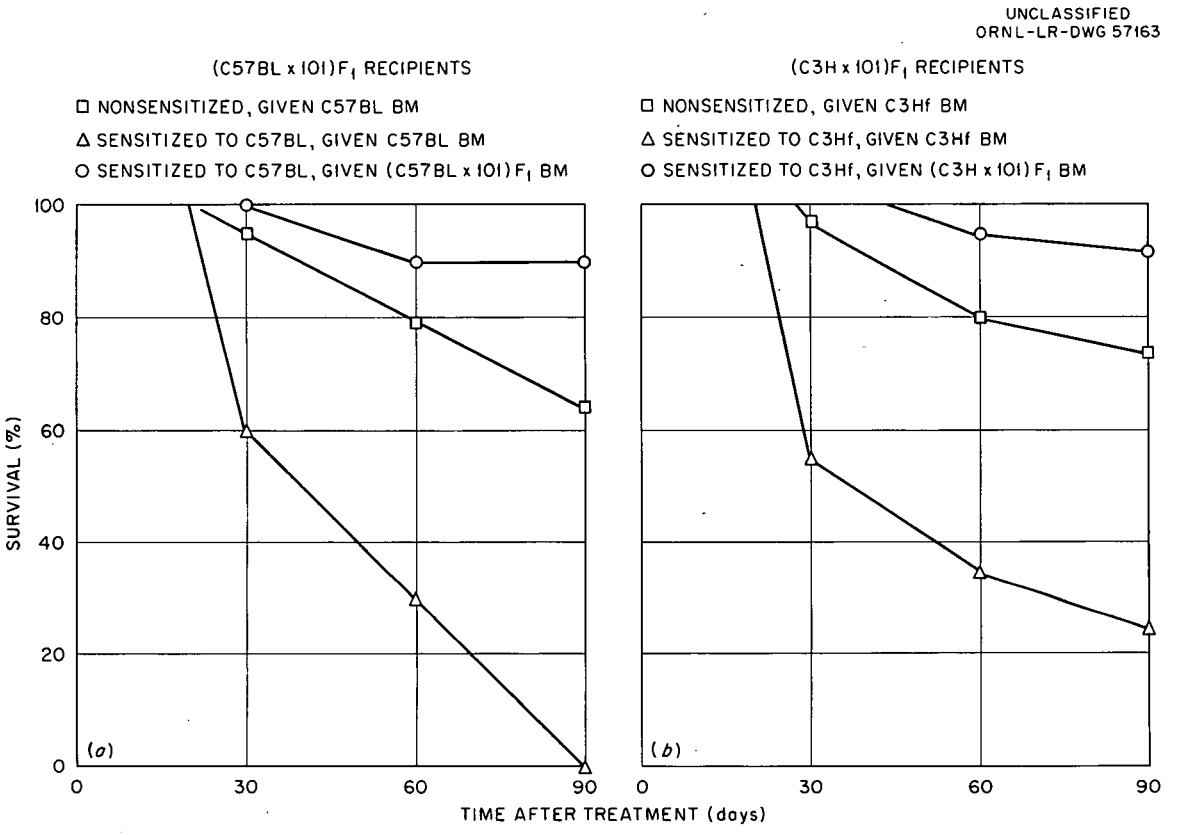

Fig. 9. Survival of 900 r Irradiated F_1 Hybrid Mice Given Parental Bone Marrow (BM).

Discussion. – The higher mortality among irradiated (C57BL × 101)F_1 recipients of C57BL marrow and (C3H × 101)F_1 recipients of C3Hf marrow that were previously sensitized to the respective parental donor strains is ascribed to an immunological F_1-antiparent reaction. The lack of a similar reaction in (BALB/c × A)F_1 recipients may possibly be explained by the fact that there is relatively little immunogenetic difference between the two parental strains, that is, the A

strain (H-2^{dk}) and the BALB/c strain (H-2^{d}). These results suggest that parental histocompatibility genes are not necessarily expressed as codominants in F_1 hybrid mice and that a host-vs-graft reaction may contribute to homologous disease when it occurs in parent-to-hybrid radiation chimeras.

Chemical Protection Against Sublethal Doses of X Radiation

Gustavo Cudkowicz D. G. Doherty[7]

Introduction. – Chemical protective compounds with optimal activity reduce acute radiation damage in mice by a factor of 1.5–2, as measured by 30-day survival. However, all survival tests carried out thus far have been at lethal radiation levels. In this experiment *S*,2-aminoethylisothiourea (AET) and *S*,3-aminopropyl-*N'*-methylisothiourea (APMT) have been tested as protective agents against radiation damage resulting from 200 to 400 r of x radiation.

Methods and Results. – The procedure used is as follows: 24 hr after an initial x-ray dose given to female RF mice with or without chemical protection, the $LD_{50}/60$ to x rays was determined by a second exposure without protection. Under these conditions differences of $LD_{50}/60$ values between protected and unprotected mice will indicate the degree of protection conferred. Both compounds, as seen in Fig. 10, maintained their protective activity in the 200 to 400 r dose range, reducing the radiation effect by the same factor as when the tests are run in the lethal dose range.

Discussion. – Chemical protection by AET and APMT is maintained in the sublethal dose range as well as in the lethal one. The main difference between the two compounds resulting from lethal dose studies is that the dose reduction factor is higher for AET than for APMT; the latter compound, however, is less toxic at effective dosage than AET.[8]

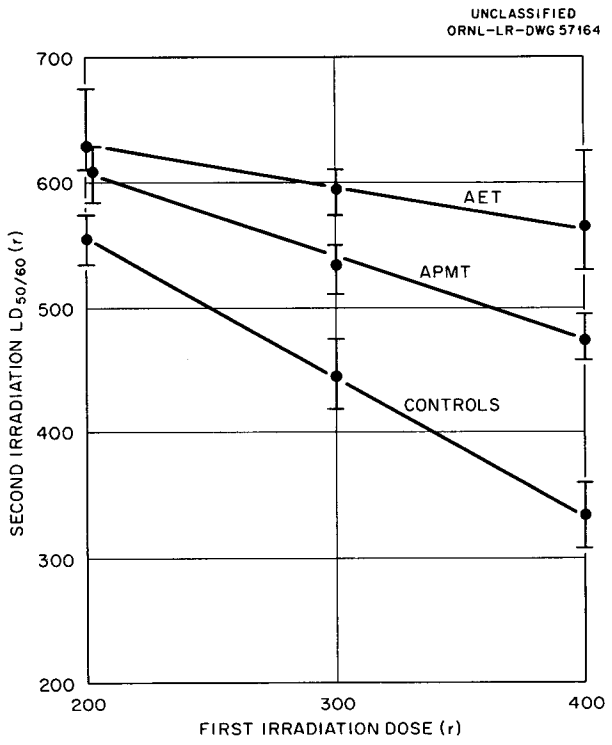

Fig. 10. Values for the $LD_{50}/60$ of Female RF Mice Exposed to Paired Doses of X Rays. Interval between doses, 24 hr. AET, 8.8 mg per mouse intraperitoneally 20 min before irradiation; APMT, 16 mg per mouse perorally 30 min before irradiation.

[7] Chemical Protection and Enzyme Catalysis Group.

[8] D. G. Doherty, "Chemical Protection to Mammals Against Ionizing Radiation" in *Radiation Protection and Recovery*, ed. by A. Hollaender, Pergamon Press, 1960.

Delayed Somatic Effects of Radiomimetic Agents and X Rays

J. W. Conklin T. P. McDonald K. W. Christenberry W. D. Gude

Introduction. – Nitrogen mustard derivatives are similar to x rays in their biological action, but little information is available concerning the delayed somatic effects of such agents. In an attempt to elucidate the life-shortening action of ionizing radiation, methyl bis(β-chloroethyl)amine (HN2), triethylene melamine (TEM), and x rays were administered to mice, and their effects on the life span investigated.

Results. – Ten-week-old RF female mice surviving midlethal doses of HN2, TEM, and x rays exhibited varying degrees of life shortening (Fig. 11), tumorigenic response (Table 18), and lens opacification (Fig. 12).

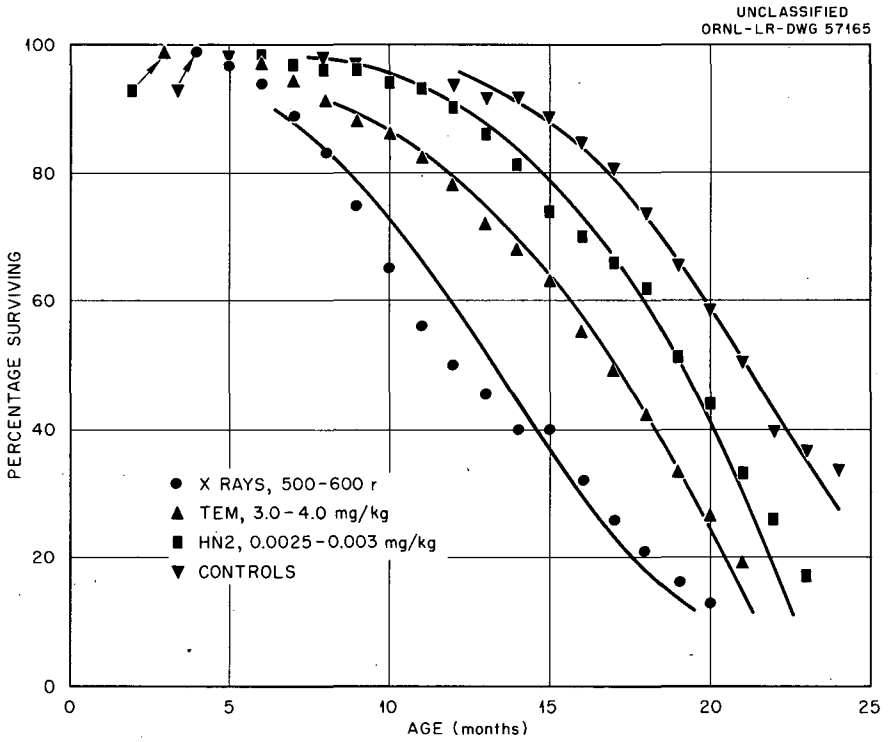

Fig. 11. Survival of Mice Treated with Midlethal Doses of X Rays, TEM, and HN2.

Table 18. Effects of HN2, TEM, and X Rays on the Incidence of Neoplasms in RF Female Mice

Treatment	Surviving 30 Days		Incidence of Neoplasms (%)			
			Leukemia			
	Fraction	Percentage	Myeloid	Thymic Lymphoma	Lung	Ovary
HN2	160/270	59	8	5	49	9
TEM	157/360	44	4	14	43	48
X rays	248/360	69	7	25	7	33
Control	130/130	100	2	5	15	5

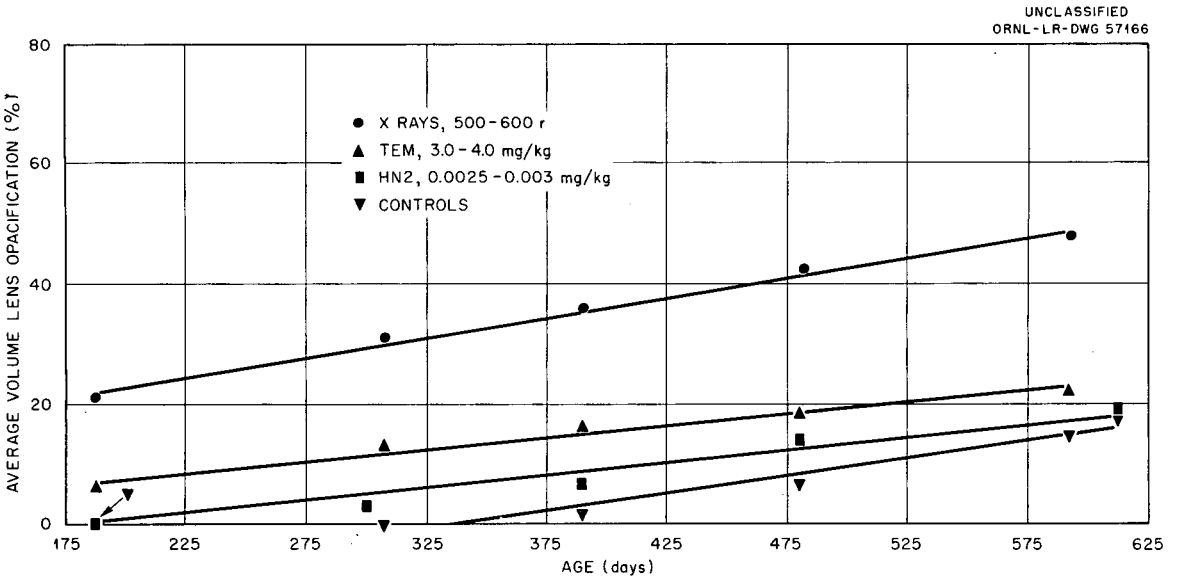

Fig. 12. Lens Opacities in Mice Treated with X Rays, TEM, and HN2.

Discussion. – HN2 and TEM resembled x rays in shortening the life span, inducing neoplasia, and causing opacification of the lens. It is clear, however, that the "radiomimetic" activity of the two compounds varied with the agent and effect in question and that for most effects the chemicals were less potent than x rays at the dose levels tested. It is tempting to ascribe the greater effectiveness of TEM, as compared with HN2, to its relatively greater chromosome-breaking activity, but further interpretation of these results must await additional study.

Relative Biological Effectiveness of Fast Neutrons and Gamma Rays as Influenced by Dose Rate

J. W. Conklin W. D. Gude

Introduction. – Although it has been noted previously that the relative biological effectiveness (RBE) of fast neutrons for delayed somatic effects increases with decreasing dose rate, there are few quantitative data on the subject. The following experiments with RF mice were designed to elucidate this question.

Results. – Female RF mice were exposed in groups of about 100 to whole-body Co^{60} gamma rays and to fast neutrons from the ORNL 86-in. cyclotron (1-Mev average energy), from a Po-Be source (4-Mev average energy), and from the Biology Division Cockcroft-Walton linear accelerator (14-Mev average energy). The mice were irradiated at varying dose rates, beginning at ten weeks of age, until a predetermined dose had been accumulated or until 50% of the animals had died. From preliminary results with several doses and dose rates (Figs. 13–20), the radiations exhibit variations in relative effectiveness (Table 19), depending on the effect and dose rate in question.

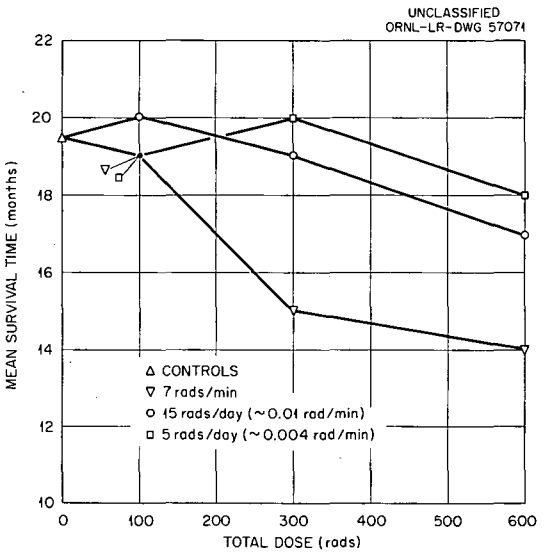

Fig. 13. Mean Survival Time in Relation to Dose and Dose Rate of Gamma Radiation.

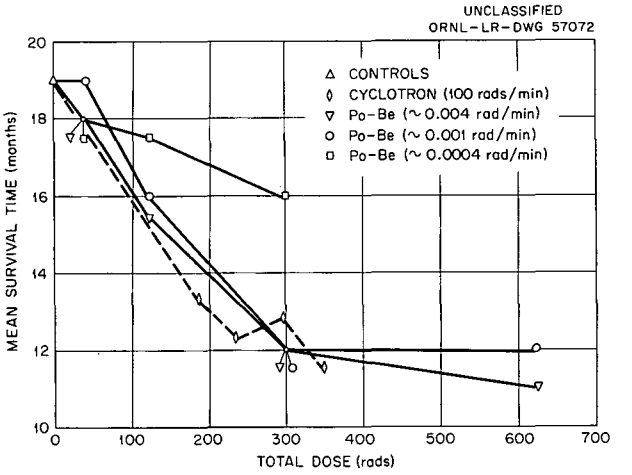

Fig. 14. Mean Survival Time in Relation to Dose and Dose Rate of Fast Neutrons.

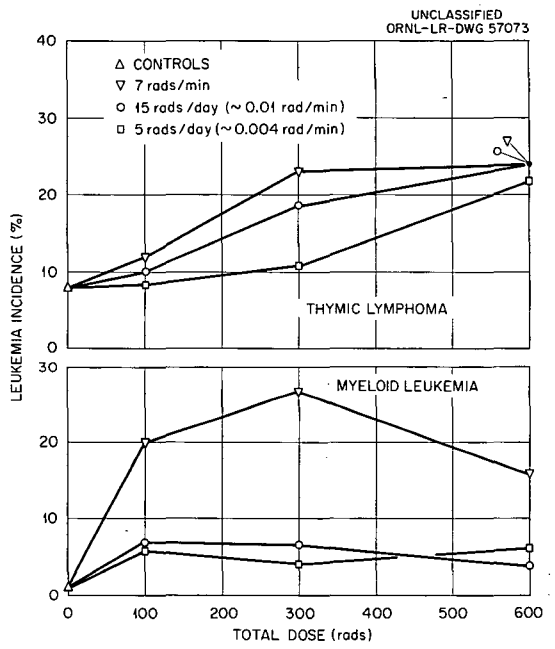

Fig. 15. Leukemia Incidence in Relation to Dose and Dose Rate of Gamma Radiation.

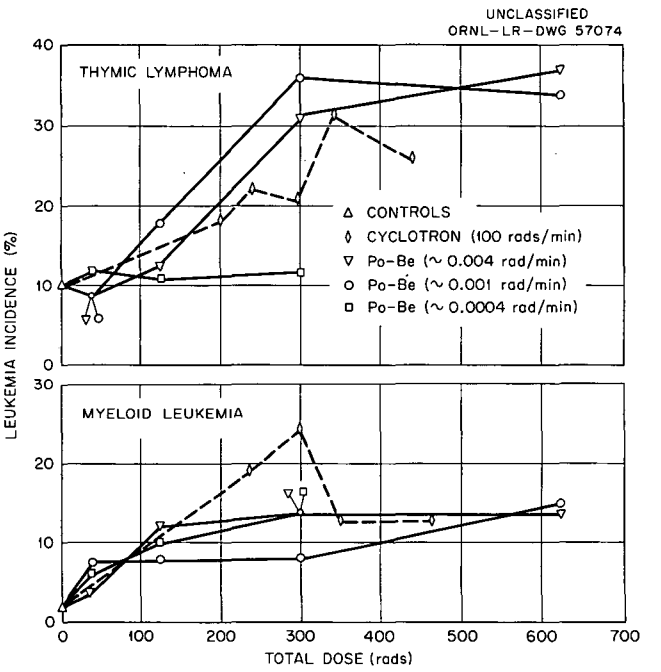

Fig. 16. Leukemia Incidence in Relation to Dose and Dose Rate of Fast-Neutron Radiation.

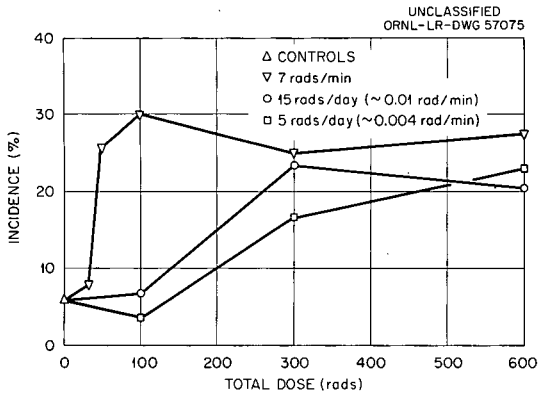

Fig. 17. Incidence of Ovarian Tumors in Relation to Dose and Dose Rate of Gamma Radiation.

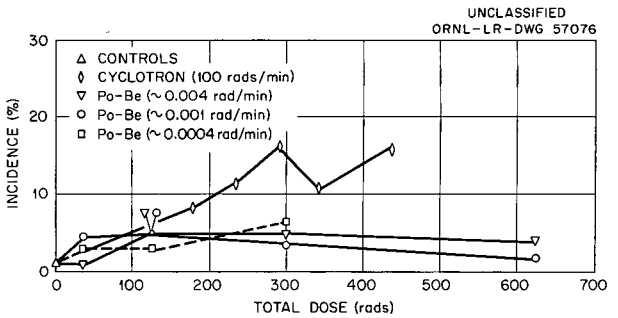

Fig. 18. Incidence of Ovarian Tumors in Relation to Dose and Dose Rate of Fast-Neutron Radiation.

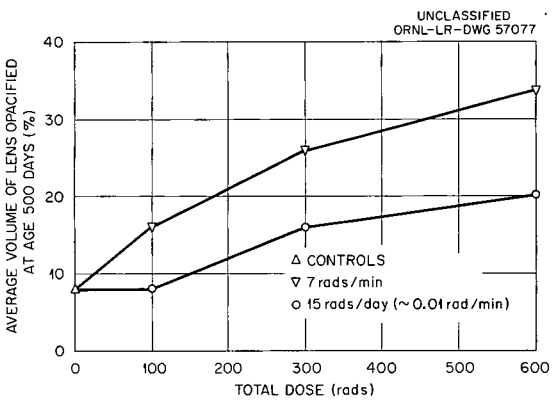

Fig. 19. Severity of Lens Opacities in Relation to Dose and Dose Rate of Gamma Radiation.

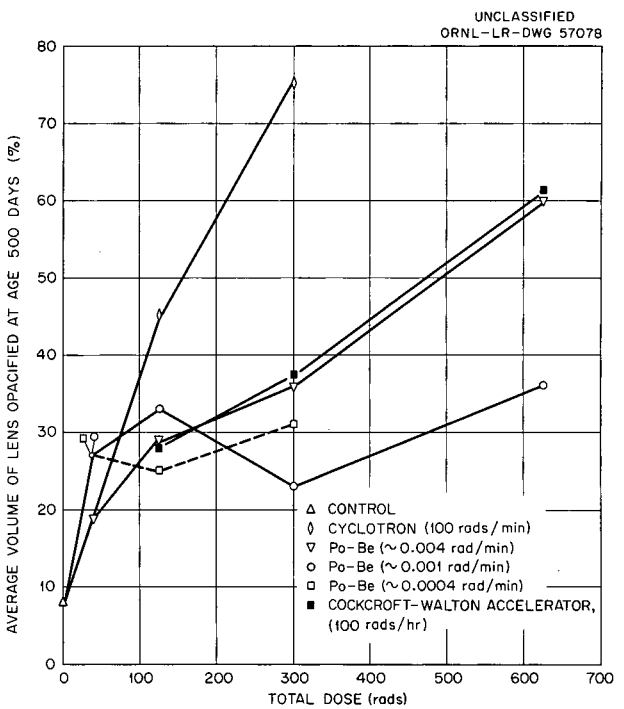

Fig. 20. Severity of Lens Opacities in Relation to Dose and Dose Rate of Fast-Neutron Radiation.

Discussion. – The foregoing analysis confirms and extends the earlier observations by Henshaw *et al.*[9] and Evans.[10] The RBE of neutrons, although generally high, is relatively intensity-independent, whereas the RBE of gamma rays varies inversely with intensity by a factor of 3–5. The values in Table 19 for any one intensity must be interpreted with reservation, however, since the dose-response curves for the effects in question are not necessarily linear, and the figures may therefore differ with the degree of effect under consideration. The influence of neutron energy (or LET) on the RBE of neutrons is also uncertain and requires further investigation.

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[9] P. S. Henshaw, E. F. Riley, and G. E. Stapleton, *Radiology* **49**, 349–60 (1947).

[10] T. C. Evans, pp 393–413 in *Symposium on Radiobiology*, J. J. Nickson (ed.), Wiley, New York, 1952.

Table 19. Biological Effectiveness of Gamma Rays and Fast Neutrons at Different Dose Rates

Radiation:	Gamma Rays		Fast Neutrons		
Source:	Co^{60}	Co^{60}	Cyclotron	Po-Be	Linear Accelerator
Mean energy:	1.2 Mev	1.2 Mev	1 Mev	4 Mev	14 Mev
Dose rate:	7 rads/min	5 rads/day[a]	100 rads/min	5 rads/day[a]	100 rads/hr
Effect:					
Life shortening[b]					
Average per cent per rad	0.054	0.016	0.14	0.13	
RBE	1.0	0.30	2.6	2.4	
Myeloid leukemia					
Average per cent per rad	0.094	0.018	0.07	0.053	
RBE	1.0	0.19	0.75	0.57	
Thymic lymphoma					
Average per cent per rad	0.039	0.013	0.050	0.045	
RBE	1.0	0.33	1.3	1.2	
Ovarian tumor					
Average per cent per rad	0.113	0.022	0.050	0.019	
RBE	1.0	0.19	0.44	0.17	
Lens opacification					
Average per cent of volume[c] per rad	0.061	0.016[d]	0.29	0.13	0.13
RBE	1.0	0.26	4.8	2.1	2.1

[a] Exposure for 23 hr daily.
[b] Shortening of mean after-survival time.
[c] Average volume of lens opacified at 500 days of age.
[d] Dose rate: 15 rads/day.

Stimulation of Platelet Production by Serum of Bled Rats

T. T. Odell, Jr. T. P. McDonald T. C. Detwiler

Introduction. – Reports in the literature have suggested the presence of blood-platelet regulatory factors in plasma or tissue extracts, and, indeed, experience with other biological systems forecasts the presence of a regulatory mechanism. It seems logical then that under suitable circumstances regulatory factors might be found in the circulating blood. These experiments were undertaken to determine whether a platelet-stimulating factor could be found in the serum of rats in which the platelet count had been reduced by bleeding.

Methods. – Blood was removed from male Sprague-Dawley donor rats by cardiac puncture in two steps. In the morning about 40% of the blood volume (calculated as 5% of body weight) was withdrawn, and on the afternoon of the same day, an additional 25%. Each time, saline, equal to one-half the volume of blood removed, was injected intraperitoneally to replace fluid loss. Approximately 24 hr after the first withdrawal, the rats were exsanguinated from the dorsal aorta, and the blood was allowed to clot. The serum was collected and kept frozen until used. Assay of this serum from bled rats was carried out by injection twice a day for two days (four injections) subcutaneously into untreated recipient Sprague-Dawley rats in quantities of 1 to 12 ml per injection. Peripheral platelet counts were taken at intervals for several days (Fig. 21).

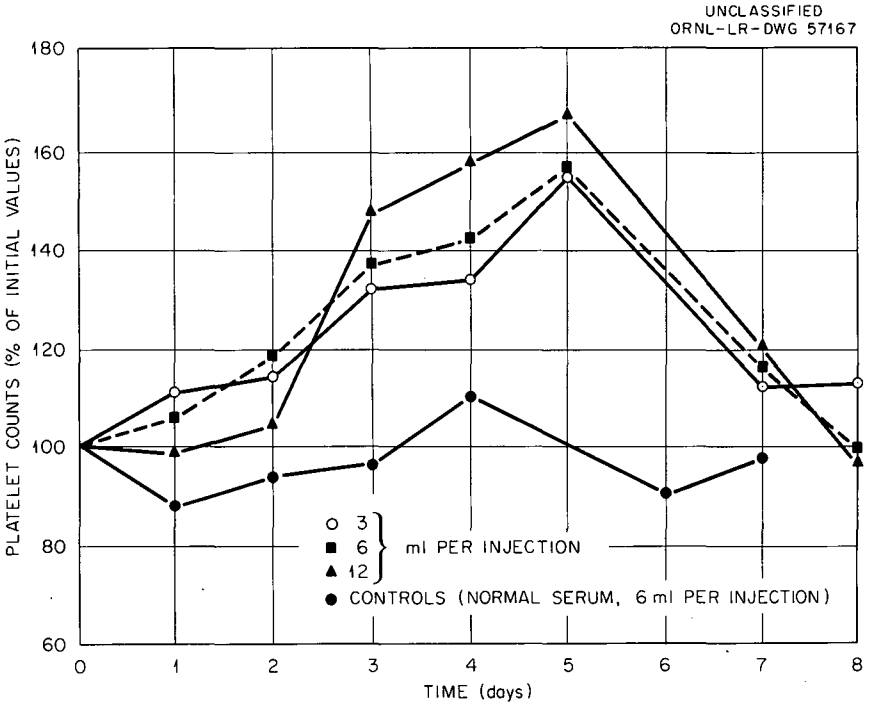

Fig. 21. Response of Peripheral Platelet Counts of Rats to Subcutaneous Injections of Serum for Bled Donors. Platelet counts were plotted as a percentage of their initial values. Serum was injected twice a day for two days.

Results and Discussion. – Relatively little change in platelet count was seen during the two days following the initial injection. Thereafter the platelet count rose rapidly to a peak, on the fifth day, of 150 to 160% of the original count, falling off thereafter and reaching normal levels around the eighth day. The responses described here and shown in Fig. 21 were observed after injection of "active" serum in total amounts of 12, 24, and 48 ml. No response was observed after injection of 4 ml (4 × 1 ml) of serum from bled rats nor after as much as 40 ml of serum from nonbled, untreated donors. In Fig. 21, the peripheral platelet count of control rats that received four injections of 6 ml each of normal rat serum on the same schedule as rats receiving "active" serum is also plotted.

These results demonstrate that serum collected from bled rats contains some agent(s) that is able to bring about an increase in the number of blood platelets in the peripheral circulation. It seems fairly clear that the mechanism does not involve simply a release of stored platelets, since several days are required for development of the response. Rather, it seems that the agent is in some way able to stimulate the production of blood platelets from megakaryocytes in the bone marrow. The nature of the agent and its mechanism of action are under study.

Competence of Retransplanted Homologous Marrow Cells in Relation to Time After Original Transplantation into Irradiated Mice

R. A. Popp

Introduction. – Irradiated recipients of homologous bone marrow cells in many donor-recipient combinations die of a wasting disease within 30–60 days after treatment.[11–13] Such deaths have been attributed to immunological incompatibility between recipient and donor tissues; however, some recipients may survive and retain a graft of the infused homologous marrow for many months. This study was undertaken in an attempt to evaluate the immunological competence of such grafted cells over a period of time in which they should be reacting against the host.

Methods and Results. – C57BL and 101 parental bone marrow cells that were grown for periods of 15 to 64 days in lethally irradiated (C57BL × 101)F_1 recipients were retransplanted into secondary F_1 recipients. When either type of parental bone marrow was allowed to grow in primary recipients less than 30 days and subsequently transplanted into secondary recipients, the survival of secondary recipients was inferior to that of primary recipients (Table 20). However, when parental bone marrow remained in primary recipients more than 30 days, the survival of secondary recipients was superior to that of primary recipients (Table 20). The erythrocytes in all the secondary recipients, as judged by red cell markers,[14] were of donor type.

Discussion. – These effects may be ascribed to alterations of the immunological competence of the donor marrow rather than to its failure to take or to its rejection. These results are consistent with the idea that sensitization of the donor marrow[15] may have been exhibited when the marrow was retransplanted within 30 days after the initial transfer, but immunological paralysis, exhaustion, partial tolerance, or mutual adaptation between donor cells and recipient was exhibited when the marrow was retransplanted after periods of 30 days or longer.

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

[12] J. J. Trentin, *Proc. Soc. Exptl. Biol. Med.* 96, 139–44 (1957).

[13] D. Uphoff, *J. Natl. Cancer Inst.* 19, 123–30 (1957).

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

[15] G. E. Cosgrove, Jr., *et al.*, *Proc. Soc. Exptl. Biol. Med.* 100, 417–20 (1959).

Table 20. Time-Dependent Effects of Retransplanted Bone Marrow on Survival of Irradiated Recipients

Original Donor	Irradiated Recipient 1	Period of Growth in Recipient 1 (days)	Irradiated Recipient 2	Number of Mice	Days After Treatment 8	15	20	28	60
					Survival of Recipient 2 (%)				
C57BL	(C57BL × 101)F_1	15	(C57BL × 101)F_1	29	100	90	83	68	24
		22		25	100	96	96	40	4
		29		27	100	67	52	33	22
		36		22	100	73	63	50	50
		43		22	100	95	95	95	91
		50		10	100	90	90	80	50
		57		12	100	92	92	92	75
					Survival of Recipient 2 (%)				
101	(C57BL × 101)F_1	15	(C57BL × 101)F_1	30	100	100	97	80	13
		22		25	100	96	80	52	32
		29		24	100	92	88	71	50
		36		21	100	95	95	95	80
		43		9	100	89	89	89	89
		50		10	100	90	90	90	60
		57		10	100	90	90	90	90
		64		10	100	100	100	90	90
					Survival of Recipient 1 (%)				
C57BL	(C57BL × 101)F_1			139	100	86	79	76	67
101	(C57BL × 101)F_1			98	100	93	93	93	78

A Second Locus Affecting the Properties of Mouse Hemoglobin

R. A. Popp Diana M. Popp

Introduction. – The hemoglobin locus (*Hb*) in linkage group I of the mouse governs the molecular characteristics which cause the electrophoretic patterns of hemoglobins to be either single (Hb^1) or diffuse (Hb^2) (ref 16). Several other physicochemical differences between single and diffuse hemoglobins have also been noted, for example, rate of alkali denaturation,[17] solubility and crystal formation in phosphate buffer,[18] and composition of peptides released during tryptic digestion of hemoglobin.[19] This study was done to determine whether such differences are controlled autonomously

[16] R. A. Popp and W. St. Amand, *J. Heredity* **51**, 141–44 (1960).

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

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

[19] R. A. Popp, "Mammalian Hemoglobins" in *Symposium on Methodology in Mammalian Genetics, Bar Harbor, September 1960.*

by alleles of *Hb* or whether some of these physicochemical differences result from the action of other loci that also affect the molecular characteristics of mouse hemoglobins.

Materials and Results. – Strain C57BL ($aaBBCHb^1/CHb^1DSe/DSe$) and SeC ($aabbc^{cb}Hb^{SeC}/c^{cb}Hb^{SeC}dse/dse$) mice have electrophoretically indistinguishable, single-type hemoglobins; however, the solubility of C57BL hemoglobin in phosphate buffer is greater than that of SeC hemoglobin.[16] The coat-color markers (C and c^{cb}) are closely linked with *Hb*; thus, the coat-color markers and the solubility differences between C57BL and SeC hemoglobins should be linked if the solubility traits are governed by alleles of *Hb*. The results show that the coat color and solubility traits segregate independently (Table 21); therefore, the hemoglobin locus in linkage group I does not govern the molecular characteristics that determine the solubility properties of these hemoglobins.

Table 21. Progeny of Genetic Tests to Determine the Relationship of *Hb* and *Sol*

Phenotype of Progeny	Matings	
	SeC × (C57BL × SeC)F_1 (number)	(C57BL × SeC)F_1 × (C57BL × SeC)F_1 (number)
$c^{cb}c^{cb}Sol^{C57BL}Sol^{C57BL}$		19
$c^{cb}c^{cb}Sol^{C57BL}Sol^{SeC}$	76	37
$c^{cb}c^{cb}Sol^{SeC}Sol^{SeC}$	74	15
$C - Sol^{C57BL}Sol^{C57BL}$		67
$C - Sol^{C57BL}Sol^{SeC}$	88	116
$C - Sol^{SeC}Sol^{SeC}$	72	62

Discussion. – A second locus affecting the molecular characteristics of mouse hemoglobins has been demonstrated through genetic analysis. The new locus has been designated by the symbol *Sol*. Its position in the mouse genome remains to be established, but it is not linked with *c*, *dse*, *b*, or the sex chromosome. It will be interesting to determine how the loci *Hb* and *Sol* affect the amino acid sequence in the polypeptide chains of the hemoglobin molecule.

Techniques for the Improved Preservation of Tissues in Electron Microscope Preparations

D. F. Parsons E. B. Darden, Jr.

Introduction. – Preliminary electron microscope observations of young oocytes of the mouse after irradiation with low doses of x rays (50 r) indicated that not only do changes occur in the nucleus[20] but also in the cytoplasm. The most frequent cytoplasmic effect encountered was a breakage

[20] E. F. Oakberg, *Proc. Intern. Congr. Genet., 10th, Montreal, 1958* 2, 207 (1958).

of the outer wall of mitochondria and a disorganization of the inner cristae. Interpretation of these cytoplasmic changes was hindered by the frequent occurrence of the artifact of polymerization damage[21] in both control and irradiated material. Polymerization damage results from an uneven polymerization and contraction of the plastic in which the tissues are embedded for electron microscopy. Strains are set up, and membranes of the cell are broken or distorted. These artifacts were very similar to some of the radiation effects observed. Methods are described here for their elimination.

Results. – Mouse testes, mouse ovary, and mouse heart muscle fixed in osmic acid and permanganate, and *Rhodopseudomonas spheroides* bacteria (kindly supplied by R. K. Clayton) were selected for the experiments because of their special sensitivity to polymerization damage. These tissues after dehydration were embedded in various methacrylate and epoxy resin mixtures. It was demonstrated that most polymerization damage occurred when temperatures in the range 40 to 70°C were used to polymerize the methacrylate. Polymerization damage was almost entirely eliminated when curing was effected at room temperature with a high-intensity ultraviolet lamp (UVIARC, 250 w) emitting both short and long wavelengths. Polymerization with low-intensity sources (15–30 w) or with those emitting only the longer wavelengths were not effective in eliminating polymerization damage.

Treatment of the fixed and dehydrated tissues with alcoholic solutions of ferric chloride or chromic nitrate was found also to markedly reduce polymerization damage. Investigation of this effect indicated that polymerization of the plastic inside the cells was being inhibited by reduction products of the osmic acid and permanganate fixatives. Apparently, the intense ultraviolet source counteracted these inhibitors by the initiation of polymerization by a high concentration of free radicals.

Discussion. – A combination of treatment with ferric chloride or chromic nitrate in alcohol and polymerization by the high-intensity ultraviolet source have been shown to give methacrylate preparations entirely free of polymerization damage. With these techniques, tissues could be satisfactorily preserved after only 5 min of fixation in osmic acid or only 2 min of fixation in potassium permanganate. Such short fixation times are believed to be preferable for the cytochemical demonstration of enzymes with the electron microscope, using specific, electron-dense reagents. When methacrylate embedding is used, the above treatments have been found essential for the electron-microscope study of changes produced by radiation in tissues.

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[21] S. B. Newman, E. Borysko, and M. Swerdlow, *J. Research Natl. Bur. Standards* **43**, 183 (1949).

CELL PHYSIOLOGY

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Separation System for Nucleotides, Nucleosides, and Bases

N. G. Anderson Sister Frances Clare Ladd

Introduction. – The purpose of the present work is to develop a general method for the quantitative determination of nucleotides obtained from RNA hydrolysates, of nucleotides, nucleosides, and bases released from RNA by ionizing and ultraviolet radiations, and of similar substances found in acid extracts of cell fractions. Methods have not previously been available for separating bases, nucleosides, and 2′ and 3′ isomers of ribonucleotides on a single column.

Experimental. – In an extended series of studies the effects of resin particle size, buffer composition, ionic strength, temperature, and flow rate on the separation of known mixtures were examined. The column effluent was analyzed every 5 sec alternating between 2600 and 2800 A using a recording system developed in the Biology Division. Hydraulic fractionation of Dowex 1, 8% crosslinked, using tap water was found to give very poor separation because very fine beads tended to adhere to large ones. Reasonable fractionation could only be achieved in dilute HCl solutions. A particle size was chosen which allowed a flow of 0.82 ml/min at 20°C at 70 psi pressure. It was found that quantitative analyses of the mixture shown in Fig. 22 could be achieved using a linear gradient at pH 4.4 and temperatures of 20 and 45°C. Commercial samples labeled 2′ and 3′ uridylic acid were found to contain negligible amounts of the 2′ isomer. The position of this isomer was determined in separate experiments using alkaline hydrolysates of RNA. The method is currently being used to analyze samples of liver soluble RNA.

Discussion. – A reproducible quantitative analytical system for bases, ribonucleosides, and ribonucleotides has been devised. In the case of uridine, adenosine, and guanosine, the nucleoside preceded the comparable base. In the case of cytosine and cytidine, however, the base preceded the nucleoside.

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[1]Research associate.

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[4]ORINS Fellow.

[5]Loanee.

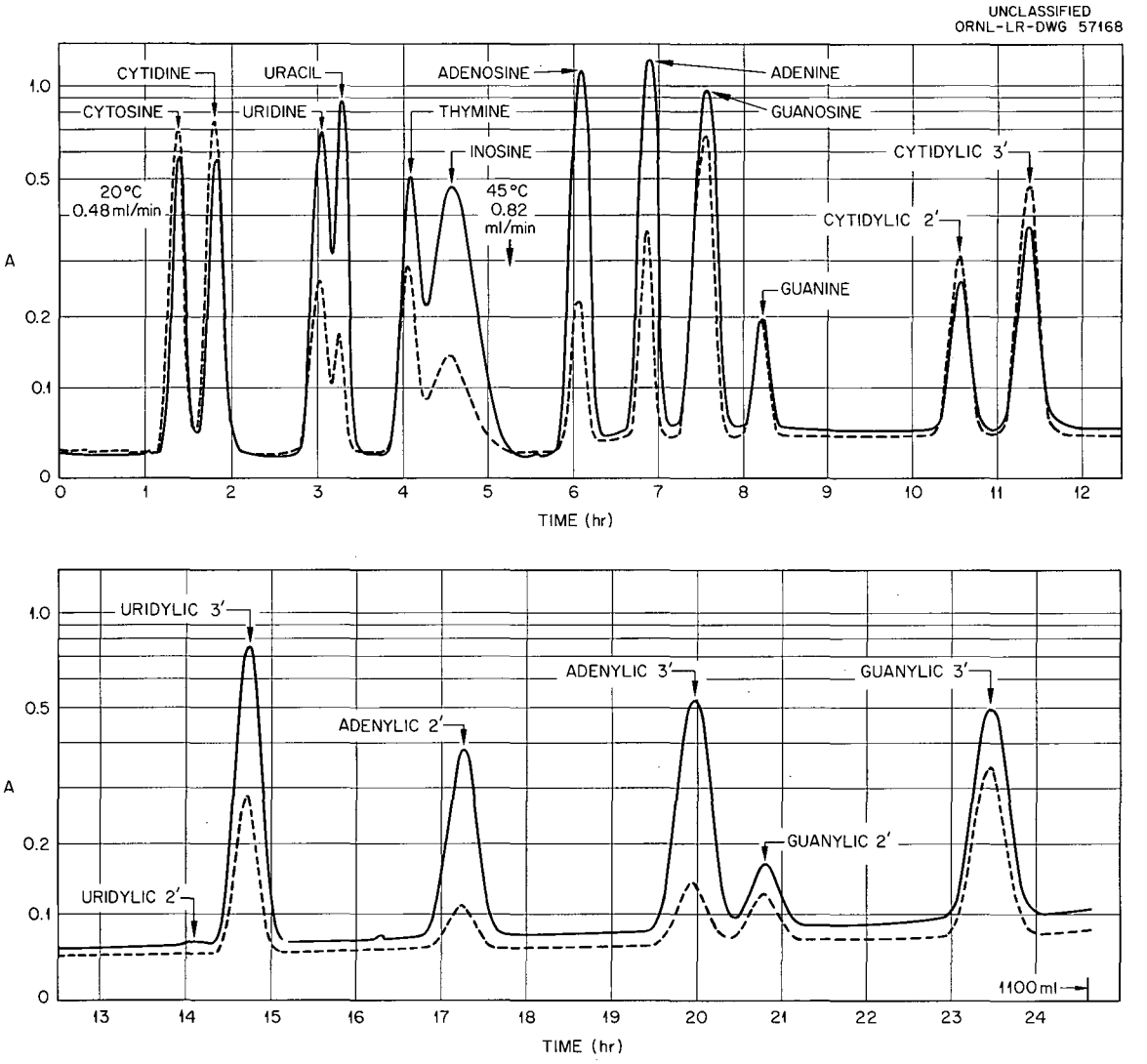

Fig. 22. Separation of a Standard Mixture of Ribonucleotides, Nucleosides, and Bases by Anion Exchange. Exchanger: Dowex 1-X8 acetate, hydraulically sized from through 400 mesh material, 0.9 × 150 cm; eluting solution: 0.6 *M* sodium acetate grading linearly to 2 *M* sodium acetate, all at pH 4.4; flow and temperature: 0.48 ml/min at 20°C changing to 0.82 ml/min at 45°C; recording: optical density at 260 and 280 mμ recorded at 5-sec intervals using 1-cm quartz flow cells and a recording system developed in the Biology Division.

Polypeptidyl-α-chymotrypsin Derivatives

R. R. Becker

Introduction. – The modification of proteins by reaction with N-carboxyamino acid anhydrides[6–8] provides a method of preparing a variety of protein derivatives useful in studies of both

[6]R. R. Becker and M. A. Stahmann, *J. Biol. Chem.* **204**, 745 (1953).

[7]A. Stracher and R. R. Becker, *J. Am. Chem. Soc.* **81**, 1432 (1959).

[8]M. Sela and E. Katchalski, *Advances in Protein Chem.* **14**, 391 (1959).

physical and biological properties. Studies with a series of chymotrypsin derivatives have been continued with special emphasis on the influence of the hydrocarbon side chains of valine, isoleucine, and *t*-leucine on the susceptibility of these protein derivatives toward acid hydrolysis. Experiments concerning enzymatic activities of the derivatives toward a series of synthetic substrates have been completed, as have end group studies to determine the number of sites in the protein that have been acylated. The analytical data on the amino acid composition of these polypeptidyl-proteins will be reported here.

Poly-L-valyl-ribonuclease has been prepared for the purpose of physical studies with special reference to the problem of hydrophobic bonding.[9]

Results. – The polypeptidyl-enzyme derivatives listed in Table 22 were hydrolyzed in constant boiling HCl at 110°C and then analyzed by the automatic technique of Spackman, Moore, and Stein.[10] The data, as yet incomplete, indicate that a hydrolytic period of 120 hr is enough to cleave all of the peptide bonds only in the polyglycyl- and polyalanyl-proteins. Analytical data on several of the other amino acids (not shown here), which also increase with increasing time, indicate the presence of peptides in the shorter term hydrolysates of the valyl-, isoleucyl-, and *t*-leucyl-proteins. This is in agreement with earlier work in which DNP-peptides were isolated from 16-hr hydrolysates of DNP-polyvalyl-proteins.[11]

Table 22. Polypeptidyl-α-chymotrypsin

Moles of added amino acid found per mole of protein

Modified Protein	Time of Hydrolysis			
	24 hr	48 hr	72 hr	120 hr
Poly-gly-chtr	7		7	
Poly-DL-ala-chtr	15		15	
Poly-D-val-chtr	21	23	29	
Poly-L-val-chtr				
Poly-DL-val-chtr	23	26	29	30
Poly-DL-ileu-chtr	12	20	25	28
Poly-DL-*t*-leu-chtr	10	12	13	14

Discussion. – The number of sites acylated per mole of modified enzyme, obtained by end group analysis, together with the analytical data reported here, permits the calculation of an average chain length for the peptides added. Such information is necessary for the interpretation of changes noted in enzymatic activity. The increased resistance to acid hydrolysis of those proteins modified with

[9] W. Kauzmann, *Advances in Protein Chem.* **14**, 37 (1959).

[10] D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.* **30**, 1190 (1958).

[11] A. Stracher, W. H. Konigsberg, and R. R. Becker, *Biochim. et Biophys. Acta* **20**, 595 (1956).

amino acid residues containing hydrophobic groups on the α carbon was anticipated on the basis of earlier results with DNP-proteins.

Ribonuclease has been chosen for future study since it will be possible with this protein to obtain complete information regarding the structure of the modified product.

The Isolation and Characterization of Tissue Proteins

H. E. Bond N. G. Anderson

Introduction. – Investigations described in previous reports involving soluble proteins of the rat liver have continued. Emphasis has been placed upon studying a sex-associated protein which occurs in the liver tissues of the male rat. In addition, chromatographic methods have been developed for use in isolating tissue transplantation antigens and specific serum antibodies.

Results. – The main effort has been expended in the attempt to obtain enough sex-associated protein in a highly purified form to conduct definitive chemical and physical studies upon it. Chromatography upon DEAE-cellulose followed by rechromatography upon CM-cellulose promises to provide the ultimate purification of the material. At present, however, the proper conditions have not been found to do this without extensive losses due to instability of the protein. Recently, the method of chromatographing upon DEAE-cellulose has been modified to provide better initial separation and the use of preparative columns. The improvement in technique should speed progress in the purification of the protein.

The relationships of the male and female hormones to the sex-associated protein were investigated using permutations of estradiol dipropionate and testosterone phenylacetate with normal male and female rats and with castrated male rats. The protein was reduced in amount in the castrated male and apparently eliminated entirely in the castrate treated with the estradiol dipropionate. The protein was eliminated also from the intact male treated with estradiol dipropionate. The castrated male treated with testosterone phenylacetate had nearly normal amounts of the sex-associated protein, and the female treated with the same material has approximately half the amount of protein found in the normal male controls. The hormones were administered at levels judged to be about maximum consistent with the health of the animals. The experiments are being repeated in a manner to obtain dose-response effects.

The versatility of the cellulose ion exchange materials has been incorporated into two projects involving the close collaboration of T. Makinodan and F. Celada of the Radiation Immunology Group. Considerable progress has been made in separating specific serum antibodies. Investigations are currently being made to determine the feasibility of isolating transplantation antigens using cellulose ion exchange chromatography.

Discussion. – The possibility that the protein may be associated with the histocompatibility locus of the Y chromosome was postulated in the previous report, and is still being investigated. The effects of hormones upon the occurrence of the protein do not necessarily argue against the

hypothesis that it is associated with the Y-histocompatibility locus, since the sex hormones can complicate the results of male to female skin grafting.[12]

Research on the sex-associated protein has provided an avenue for studying both a specific hormonal effect and the synthesis of an easily identifiable protein.

Effects of Subzero Temperatures on Yeast Cells

Peter Mazur

Introduction. – Experiments reported in the preceding semiannual report[13] indicated that cells of the yeast *Saccharomyces cerevisiae* not only were killed by exposure to –30°C but also underwent marked changes in morphology. The changes noted included (1) a reduction in cell volume to one-half that of normal cells, (2) an increase in cell density, (3) the loss of the large vacuole in normal yeast, (4) an increase in the *concentration* of intracellular solids, and (5) a decrease in *total* intracellular solids resulting from the escape of solids into the surrounding medium. These findings have now been extended and confirmed.

In our experiments, a living cell has been defined as one able to reproduce itself sufficiently to form a colony on a specific agar medium. It was of some significance to determine whether the percentage of cells surviving a given low-temperature treatment was dependent on the definition used. Another question of interest was whether the survivors were genetically more resistant to the stresses induced by low-temperature exposure.

Results and Discussion. – Quantitative data on morphological changes in yeast cells after exposure to –30°C are summarized in Table 23. The escape of small-molecular-weight solids

[12]E. J. Eichwald *et al.*, *Ann. N.Y. Acad. Sci.* **64**, 737 (1957).

[13]P. Mazur, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, pp 149–50.

Table 23. Alterations in Yeast Cells Exposed to Subzero Temperatures

Factor	Method	Untreated Control Cells	Cells Cooled Rapidly to –30°C, Thawed Slowly
Survival	Colony counts	100%	0.01%
	Micrometry	117 μ^3	63 μ^3
Volume	Sedimentation rate and Stokes' law	114 μ^3	59 μ^3
Specific gravity	Sedimentation rate and Stokes' law	1.14	1.25
Concentration of solids in cell	Interference microscopy	21 g/100 ml	28 g/100 ml
Total solids in cell	Interference microscopy	25 $\mu\mu$g	17 $\mu\mu$g
Concentration of solids in solution lost from cell	Interference microscopy		13–16 g/100 ml
	Refractometry of external medium		11 g/100 ml
Size of solids leaving cell	Dialysis through Visking tubing		<Raffinose (mol. wt 600)

from the cell suggests that freezing and thawing has damaged the plasma membrane. The retention of larger molecules within the cells indicates either that the plasma membrane continues to exist as a partial barrier or else that the residual permeability barrier is the cell wall. The loss of a large quantity of small-molecular-weight solutes would produce a considerable decrease in the osmotic pressure within the cell. A decrease in osmotic pressure would decrease the tendency for water to enter the cell and, hence, decrease the turgor pressure against the elastic cell wall of the yeast cell. The lowered turgor pressure, in turn, would permit the cell wall to shrink and so produce the observed decrease in volume. It is the decreased volume, of course, which accounts for the fact that both the concentration of intracellular solids and the specific gravity of the cells increased even though the total quantity of intracellular solids decreased.

The reduction in volume and the appearance of solids in the external suspending medium had already occurred within a few seconds after thawing of the suspensions. Such rapidity suggests (although it does not prove) that the alterations were a direct result of events occurring at subzero temperatures rather than, say, of post-mortem catabolic reactions. Our hypothesis is that they resulted from intracellular freezing.[14]

The standard assay for survival was to count the number of colonies appearing on a primarily synthetic agar medium. These numbers were not changed significantly by substituting highly enriched plating media made from yeast extract–malt extract–peptone, potato-dextrose, Difco wort agar, or Difco yeast morphology agar. Moreover, the percentage of dead cells based on a plate count assay was similar to the percentage of cells stained by methylene blue. Finally, suspensions exposed to −30°C not only contained fewer than one cell in 10,000 capable of producing colonies on agar media, but also showed no measurable endogenous or exogenous (glucose added) respiration by Warburg manometry.[15] All these facts indicate that death from low-temperature exposure involved considerably more than loss of ability to reproduce and grow. On the other hand, death was not accompanied by gross cellular disintegration. The progeny of the few cells that did survive were not more resistant to subsequent low-temperature exposure than the cells of the original untreated population.

Yolk Proteins of the Amphibian Egg

R. A. Wallace

Introduction. – The yolk of amphibian eggs exists in the form of discrete platelets. The platelets are surrounded by a membrane and contain protein,[16] display a strong birefringence,[17] can

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[14] P. Mazur, *Biophys. J.* 1, 247–64 (1961).

[15] Experiments performed in collaboration with J. H. Franz.

[16] P. R. Gross and L. I. Gilbert, *Trans. N.Y. Acad. Sci.* 19, 108 (1956).

[17] J. Holtfreter, *J. Expt. Zool.* 103, 81 (1946).

be "split" or "cracked" by micromanipulation,[16] and have an interior with a crystalline structure.[18] They are derived apparently from the mitochondria of the developing oocyte since, in *Rana pipiens*, a hexagonal structure with regular fringe systems gradually fills out the interior of the mitochondrion during early oogenesis.[19] It is suggested here that phosphorylation of a soluble protein within the mitochondria provides a mechanism for the formation of the crystalline complex. A study of the proteins of the yolk platelets has been undertaken to further elucidate these relationships.

Results. – Methods have been developed for the isolation of yolk platelets in very pure form as determined microscopically and by the uv absorption of the dissolved proteins (280/260 ratio $\geqq$ 1.72). Platelets can be rapidly dissolved at low ionic strengths ($\Gamma/2 = 0.02$) below pH 5 and also at higher ionic strengths ($\Gamma/2 = 0.10$) above pH 10, leaving behind a "ghost" or membrane. As the ionic strength is increased, less basic solutions are needed for dissolution until at $\Gamma/2 = 0.50$ the platelets dissolve above pH 4, but become insoluble below this pH (i.e., salt out). When the platelets are dissolved in Veronal buffer at pH 9.0 ($\Gamma/2 = 0.2$), two components (other than the membranes) are found in the ultracentrifuge, in agreement with Barth and Barth[20] and Schjeide *et al.*[21] but not with Gross and Gilbert.[16] The larger and faster-sedimenting component ($s = 10.4$, uncorrected) accounts for 88% of the total protein (278-mμ absorption), whereas the smaller component ($s = 6.3$, uncorrected) is presumably identical with the phosphoprotein of Barth and Barth[20] and Flickinger and Schjeide.[22] This latter component is relatively unstable, changing to a substance with a sedimentation rate of $s = 4.5$ (uncorrected) upon incubation at pH 9 and at 25° for 5 hr. This new component will not complex with the larger component to form an insoluble precipitate at neutral pH and low ionic strength. Presumably, at least the phosphate group(s) and concomitant acidic properties have been lost to the protein. The larger component is a basic protein (isoelectric point around pH 8.9) with a yellow color. The pigment can be extracted from the protein by methanol and displays the absorption spectrum of a carotenoid in various solvents. The carotenoid chromatographs as a single component on weakened neutral alumina and is eluted by 8% acetone in petroleum ether. Hypophasic solubility properties and the weak inflection of the 450-mμ band (in CS_2) indicate that it is apparently an unesterified xanthophyll with two β-ionone rings.

Discussion. – Amphibian yolk platelets provide a system for the study of the genesis, disposition, interaction, and final utilization of two relatively pure proteins. It is hoped to characterize the component proteins of the yolk of *R. pipiens* eggs as completely as possible with this end in

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[18] S. Karasaki, *Embryologia* 4, 247 (1959).

[19] R. Ward, personal communication, 1960.

[20] L. G. Barth and L. J. Barth, *The Energetics of Development*, Columbia University Press, New York, 1954.

[21] O. A. Schjeide, E. Levi, and R. A. Flickinger, *Growth* 19, 297 (1955).

[22] R. A. Flickinger and O. A. Schjeide, *Exptl. Cell Research* 13, 312 (1957).

view. The platelets of various urodele and anuran species also display definite geometric shapes according to species, perhaps related to the differences in their component proteins. This will also be the subject for future investigation.

Rat Serum Macroglobulin

R. E. Canning

Introduction. – As reported in the last biology progress report,[23] it was planned to make a large ammonium sulfate (AmS) fraction of rat serum in order to obtain a quantity of macroglobulin sufficient for determining the partial specific volume, diffusion coefficient, and amino acid analysis.

Results. – One hundred Sprague-Dawley rats were exsanguinated and the blood obtained was defibrinated. The resulting serum was twice precipitated with AmS to give a 45% concentration. The precipitate was taken up in 0.15 *M* NaCl, dialyzed against 0.16 *M* Tris-phosphate buffer, pH 8.0, and placed on a 4.5 × 31 cm diethylaminoethyl cellulose (DEAE) column. A 0.16 *M* Tris-phosphate buffer, pH 8.0, followed by a 0.30 *M* Tris-phosphate buffer was used to elute the protein. The protein concentration was monitored at 280 mμ, using a 1.0-cm cell and recording system developed in the group. The tubes containing the macroglobulin component were pooled, concentrated, dialyzed against 0.05 *M* acetate, pH 5.0 buffer, and placed on a 1.9 × 26 cm carboxymethyl cellulose (CM) column. The column was eluted with 0.05 *M* acetate, pH 5.0 buffer of increasing ionic strengths. Individual tubes from the fraction collector were checked for macroglobulin using the agar plate diffusion method.

Discussion. – It is felt now that a sufficient quantity of pure rat macroglobulin (S20) has been obtained. It is now planned to determine the physical properties of this protein by electrophoresis, ultracentrifugation, amino acid analysis, and the partial specific volume.

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[23] R. E. Canning, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, p 147.

NUCLEIC ACID CHEMISTRY

W. E. Cohn
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N. B. Furlong, Jr.
J. X. Khym
L. O. Frøholm[1]
A. Jeannine Bandy
Patricia A. Williams

The Nucleoside Elimination Reaction

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

The opening of the ribose ring by periodate oxidation, a classic reaction in sugar chemistry, has long been a primary step for the specific degradation of nucleoside 5′-phosphates. This oxidation does not of itself introduce specific labilities that may be turned to advantage, but the dialdehyde compound thus produced may undergo complex reactions in alkaline solutions. However, the products formed by the reactions of the aldehyde groups with amino compounds do appear to have properties of considerable interest with regard to specific bond weakening. We are concerned with the nature of the reaction with amines and alkaline agents, the characterization of the addition compounds produced by the amines, and the conditions under which these addition compounds may be degraded.[2]

This reaction, which has recently been discovered in two other laboratories, is of considerable significance and utility in problems of nucleic acid structure and nucleotide chemistry. For maximum usefulness in these problems, precise knowledge of the intermediates and exact control of the course of the reaction is necessary. Characterizations of these are in progress, involving the preparation and purification of intermediates and the investigation of various parameters of the addition and cleavage reactions.

Reactions of Periodate-Oxidized Nucleotides

J. X. Khym

Introduction. – In a previous report[2] it was mentioned that the release of inorganic phosphate from periodate-oxidized 5′ nucleotides seems to occur by a more complex mechanism than reports in the literature have indicated. More thorough experiments have now shown that the nature of base determines the rate and pH at which phosphate is released from these periodate-oxidized nucleotides.

Results. – In the pH range 12 to 13, nonamino bases catalyze the release of phosphate in a few hours, but in the pH range of 8 to 11 this reaction is slowed considerably and is incomplete even after 24 hr. In the presence of amino bases such as ammonia, primary amines, and certain amino acids, inorganic phosphate is slowly released in the pH range 8 to 11, but rapidly and quantitatively, depending upon the amino compound used, when the solutions are held at pH's below 8.

[1] Visiting investigator from abroad.

[2] J. X. Khym, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, pp 156–57.

When primary amines are used at pH values above 8, presumably Schiff base formation occurs, and the phosphate bond in the oxidized nucleotides can be stabilized by reduction of the Schiff bases with sodium borohydride.

Discussion. – The reactions studied seem to indicate that the amino bases react with the aldehyde groups of the periodate-oxidized nucleotides to form α-hydroxy amine complexes of the type described in several reviews. The formation of these complexes results in the labilization of the phosphate bond, which becomes extremely sensitive to very mild acidic conditions (i.e., pH below 8). Reduction of Schiff bases should yield a secondary amine, and such compounds are not labile in acid solutions, thus explaining the stabilization of phosphate after the oxidized nucleotides are treated with sodium borohydride.

Structure of Products Formed from Oligodeoxyribonucleotide Primers

F. J. Bollum

Structural examination of the compounds formed from deoxyribonucleoside triphosphates and synthetic oligodeoxyribonucleotides[3] in the presence of an enzyme isolated from calf thymus gland that synthesizes DNA has now been completed. Synthetic and degradation studies show that the compounds are formed by the addition of deoxyribonucleoside triphosphate monomers to the 3′-hydroxyl group of the pre-existing oligodeoxyribonucleotide chain:[4]

$$(pX)_n + ppp^{32}Z \longrightarrow (pX)_n p^{32}Z + pp\ ,$$

$$(pX)_n p^{32}Z + ppp^{32}Z \longrightarrow (pX)_n (p^{32}Z)_2 + pp,\ \text{etc.}$$

The synthetic experiments consisted in a survey of compounds for their ability to serve as "primer" in the enzyme-catalyzed reaction. Compounds of the general structure $(pX)_n$, that is, having a free 3′-hydroxyl, with n greater than 2, all have priming ability. Acylation of the 3′-hydroxyl resulted in the loss of priming ability. Compounds of the general structure $(Xp)_n$, having a free 5′-hydroxyl on the opposite end of the chain and a phosphoryl group blocking the 3′-hydroxyl group, were found to be inactive as primers. The synthetic experiments indicate a requirement for the free 3′-hydroxyl function, and it was deduced that the monomers were adding there.

The degradative experiments were designed to prove the site of addition by specific enzymic hydrolysis. The four reaction mixtures of Table 24 were set up and the products formed were isolated by chromatography on DEAE-paper. Each product was then treated with alkaline phosphatase

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[3] F. J. Bollum, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1960*, ORNL-2913, pp 108–9.

[4] pX = a mononucleotide bearing 5′-phosphate and free 3′-hydroxyl; Xp = a mononucleotide bearing 3′-phosphate and free 5′-hydroxyl; $(pX)_n$ and $(Xp)_n$ are polymers of the above monomers resulting from formation of 3′-5′-diester bonds.

Table 24. Degradation of Synthetic Oligodeoxyribonucleotides

Reaction Mixture[a]	Presumed Structure of Products[b]	P^{32} Distribution		
		3′-Mononucleotide	Required	Found
$(pT)_4$ + ppp*T	pTpTpTpTp*T	Tp*	1.00	0.98
$(pT)_4$ + ppp*C	pTpTpTpTp*C	Tp*	1.00	>0.90
	pTpTpTpTp*Cp*C	Tp*	0.50	0.51
		Cp*	0.50	0.49
$(pT)_4$ + ppp*A	pTpTpTpTp*A	Tp*	1.00	0.97
	pTpTpTpTp*Ap*A	Tp*	0.50	0.58
		Ap*	0.50	0.42
$(pT)_4$ + ppp*G	pTpTpTpTp*G	Tp*	1.00	0.89
	pTpTpTpTp*Gp*G	Tp*	0.50	0.50
		Gp*	0.50	0.50
	pTpTpTpTp*Gp*Gp*G	Tp*	0.33	0.33
		Gp*	0.67	0.63

[a]T = thymidylyl, C = cytidylyl, A = adenylyl, G = guanylyl.

[b]Other products, presumably higher polymers, were formed in every case. The compounds listed above were the major products and the only ones formed in yield sufficient for subsequent degradation.

(*E. coli*) and the dephosphorylated products were purified on DEAE-cellulose. Each dephosphorylated product was then hydrolyzed with spleen diesterase, resulting in the formation of 3′-deoxyribomononucleotides. The 3′-mononucleotides were then separated and the distribution of radioactivity in the individual mononucleotides was examined. The radioactivity from the incoming 5′-p^{32}-monomer is transferred to its nearest neighbor in the specific diesterase degradation and permits a decision as to the nucleotide to which the monomer was attached. The quantitative determination of label in the 3′-nucleotides (Table 24) shows that the presumed structure of the products is correct, and the structures are therefore proved.

Effect of Primer Length on Priming Function of Polydeoxynucleotides

N. B. Furlong, Jr.

Calf thymus polymerase catalyzes two types of reactions: (1) the addition of individual nucleotide units to pre-existing chains, and (2) the replication of new DNA chains. DNA molecules can serve as primers for either reaction, but oligodeoxyribonucleotides, three to seven units long, will prime only the first reaction. Measurements of the addition to long and small chain primers and of replication with large polymers have been made to compare reaction characteristics. Several differences were found: (1) a concentration of KCl that depresses replication by 80% gives only 10 to 25% inhibition of the addition reactions; (2) addition appears to be less sensitive to the substitution of Mn^{2+} for Mg^{2+}; (3) an indication was obtained that the pH optima for these reactions are different.

The addition and replication reactions have been performed using calf thymus DNA partially degraded by DNase I as primers. The rate of incorporation of radioactive triphosphate was maximal at 20 to 30% hydrolysis for replication and 50 to 60% hydrolysis for addition. In all cases the replication reaction proceeds at a rate at least ten times greater than addition of single substrates.

Experiments are now in progress in which DNA degraded by spleen DNase will be tested as primers. These products will not be capable of the addition reactions since they have no free 3′-hydroxyl group. From experiments of this nature we hope to obtain an estimate of the lower limit of the primer size required for replication.

The Biosynthesis of Pseudouridylic Acid in Rat-Liver RNA

L. O. Frøholm

Orotic acid has previously been shown to act as a precursor for the uracil moiety of pseudouridine in rat-liver RNA. It was thought that a careful analysis of the relative specific activities of cytidylic, uridylic, and pseudouridylic acid in the various RNA fractions at different time intervals might give valuable information as to the route of biosynthesis for the compound in question. Such a time course study was started at the McArdle Memorial Laboratory under direction of V. R. Potter, and it has been continued here. Most of the earlier work was done by counting of the radioactive samples in windowless gas flow counters. This met with many difficulties because of the small amount of material available and also very low levels of activity in some samples. Liquid scintillation counting seems very promising and will be used in the future.

Method. – Groups of male albino rats have been intraperitoneally injected with from 20 to 80 μc of orotic acid-6-C^{14} and sacrificed from 50 min to 20 hr later. Following fractionation of the pooled livers by differential centrifugation of a sucrose homogenate, the RNA was isolated from the pH 5 precipitates of the high-speed supernatant, the microsomes, and the nuclei. After alkaline hydrolysis the nucleotides were separated by column and paper chromatography.

Results. – The results indicate that the small amount of pseudouridylic acid found in microsomal RNA is not merely a result of trace contamination with adsorbed soluble RNA, because the specific activities are somewhat different. Pseudouridine is also a constitutent of nuclear RNA. At the shortest time intervals and especially in nuclear RNA – but also in the other RNA fractions – pseudouridine has a significantly lower specific activity than uridine. At the longer time intervals, however, it has at least as high a specific activity as uridine. Cytidine is always considerably less radioactive than the other pyrimidine nucleosides.

Discussion. – These results might be interpreted as a closer precursor-product relationship between pseudouridine and uridine than between either of these and cytidine, but it is difficult to tell how different pool sizes and other factors might interfere. With the present methods of RNA fractionation and with this rather inefficient incorporation system, it does not seem promising to continue far along these lines, but some experiments with different precursors at the same time intervals are planned.

NUCLEIC ACID ENZYMOLOGY

Elliot Volkin
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Nucleic Acids and Nucleotide Derivatives of Undeveloped Eggs

F. J. Finamore
Elliot Volkin
M. Helen Jones

In view of the demonstration by Finamore and Volkin[2] that the RNA's of undeveloped amphibian eggs possess unique chemical characteristics, studies were made to elucidate further the chemical and physical properties of egg nucleic acids.

Results and Discussion. – *Amphibian Egg RNA's.* – It has been found that the solubilities in cold sodium chloride solution of ribonucleoproteins from mature ovarian eggs of *Rana catesbieana* differ markedly from those exhibited by the ribonucleoproteins of frog liver and mammalian tissue. Whereas virtually all the ribonucleoprotein of differentiated tissue is extracted in cold 0.17 *M* NaCl, less than 5% of the ribonucleoprotein from the eggs is solubilized in this solution. The remainder of the ovarian egg ribonucleoprotein is extracted along with deoxyribonucleoprotein in cold 1.0 *M* NaCl. The RNA isolated from this fraction by detergent and purified by alcohol precipitation is unique in that about half is soluble in cold 0.5 *M* $HClO_4$ (RNA_1), the remainder being insoluble in the acid (RNA_2). The RNA isolated from the ribonucleoprotein present in the 0.17 *M* NaCl extract, using the phenol procedure and purified by alcohol precipitation, is completely soluble in acid (RNA_3). The mononucleotide compositions of the three different RNA's are distinct, characteristic of each type, and reproducible (Table 25).

Thus more recently we have found that at least *two* types of acid-soluble RNA exist in mature ovarian eggs.

Table 25. Nucleotide Compositions of Ovarian Egg RNA's

Average of five determinations each

Acid	RNA_1 (%)	RNA_2 (%)	RNA_3 (%)
Cytidylic	29.6	30.0	23.4
Adenylic	20.2	19.3	32.4
Uridylic	20.1	12.3	12.5
Guanylic	30.1	38.4	31.7

Amphibian Egg DNA. – The cold NaCl–detergent procedure has been found to be a most satisfactory method for the preparation of undegraded, highly viscous DNA from amphibian eggs. It is significant, however, that only in eggs is a major portion of the RNA extracted with the DNA. Attempts to rid the RNA from the DNA by repeated extraction and precipitation have failed, and, characteristically, the final preparations always contain about 67% DNA and 33% RNA. There apparently is an intimate association between the DNA and a significant amount of RNA in amphibian eggs. Evidence that this

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[2]F. J. Finamore and E. Volkin, *J. Biol. Chem.* (in press).

association is unique comes from the fact that DNA, completely free of RNA, is routinely prepared from frog liver using the same procedure. Experiments designed to elucidate the nature of this association in amphibian eggs are under way.

Brine Shrimp Egg Nucleic Acids. – We have observed that the ribonucleoproteins of the undeveloped eggs of *Artemia salina* exhibit solubility characteristics similar to those of amphibian eggs. Although the brine shrimp RNA fractions contain a large quantity of an acid-soluble component, it appears to be distinct from the acid-soluble RNA found in amphibian eggs. This component is not a free mononucleotide although it has ultraviolet absorption characteristics similar to guanylic acid. It contains labile phosphorus, resists alkaline and ribonuclease hydrolyses, and no inorganic phosphorus is released by human prostatic phosphatase or snake venom phosphodiesterase. Inorganic phosphorus is liberated, however, by phosphodiesterase hydrolysis followed by prostatic phosphatase treatment. Guanosine 5′-phosphate is formed by hydrolysis of the component with snake venom phosphodiesterase.

Experiments designed to elucidate the structure of this component and its role in nucleic acid metabolism are in progress.

T2-Specific RNA

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Experiments are under way in an attempt to purify the fraction of RNA in T2-infected bacteria whose apparent composition resembles T2 DNA. These include: (1) density gradient separation of bacterial particles, (2) $CsCl_2$ centrifugation of purified nucleic acid preparations, (3) differential enzymic hydrolysis of the RNA, (4) attempts at reformation of T2 DNA and T2-specific RNA, and (5) chromatographic separations with the use of such cellulose ion exchangers as ECTEOLA and DEAE.

Results. – Experiments with ECTEOLA chromatography demonstrate that reproducible separations of various RNA species from T2-infected *E. coli* B can be obtained. Although some purification of highly labeled RNA can be effected, it is evident from the mononucleotide composition of this fraction that it is still contaminated with normal bacterial RNA. Conditions for more refined separations are being investigated.

CHEMICAL PROTECTION AND ENZYME CATALYSIS

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Cynthia K. Myers

Chemical Protection

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

Introduction. – The importance of spleen protection to the survival of lethally irradiated mice has been amply documented in the past. However, there have been few attempts, other than weight response, to quantitate spleen protection or recovery in mice given chemical protection prior to irradiation. In view of the findings of Van der Meer and van Bekkum[2] and Distefano and Leary[3] that some protective agents produced hypoxia in the spleen, we believed that information on the spleen response would be pertinent to an understanding of the mechanism of chemical protection. Therefore, an investigation was initiated to develop a quantitative method, similar to that used to measure bone marrow recovery,[4] and to use it to evaluate the effects of various chemical protective agents on the spleen.

Results and Discussion. – A technique was devised to obtain a suspension of spleen cells, which were stained and counted as previously described for bone marrow.[4] Although there was a wide variation in spleen weights of the mice, the cell count per milligram of spleen weight for a series of normal animals was relatively constant. Lethal (900-r) irradiation precipitated a rapid fall in both spleen weight and cell count by the fourth day with no evidence of recovery. In animals treated with AET (as MEG) prior to 900-r irradiation, the weight loss was modified, and recovery of both weight and cell count occurred after the fourth day, reaching normal between the 14th and 20th day. The recovery obtained was roughly comparable with that seen in untreated 450-r irradiated mice. Lethally irradiated (900 r) animals pretreated with *S*,β-aminopropyl-*N*′-methyl isothiourea diHBr (APMT) showed a spleen response similar to that observed for the bone marrow with this compound in that (1) doubling the protective dose had no effect on the recovery response, (2) the initial drop was not modified, and (3) the recovery process was delayed until the 12th day, and then was very rapid, reaching normal cell count values by the 18th day. The spleens of the APMT-treated animals were considerably enlarged at this time, returning to normal size by the 35th day. Histological examination (C. C. Congdon) revealed that the increase in mass was confined to the red pulp of the spleen. These results correlate spleen recovery quantitatively with cell population, and the technique should prove useful for the evaluation of spleen response to protective compounds.

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[1]Research associate.

[2]C. Van der Meer and D. W. van Bekkum, *Intern. J. Radiation Biol.* 1, 5–23 (1959).

[3]V. Di Stefano and D. Leary, personal communication.

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

Enzyme Substrate Complex Formation

F. C. Grimm D. G. Doherty

Introduction. – Mitochondrial malic dehydrogenase purified 230-fold from beef heart has been partially characterized, and equilibrium dialysis studies have been initiated in an attempt to elucidate the mechanism of the enzymic reaction.

Results. – This beef heart enzyme was found to have a molecular weight of about 65,000 – 19,000 moles of DPN or 32,000 moles of DPNH being converted per minute per mole of enzyme (at pH 9). The reduced coenzyme ($K_m = 2.5 \times 10^{-5}$) is more firmly bound to the enzyme than the oxidized form ($K_m = 5.4 \times 10^{-4}$). The amino acid analysis reveals that tryptophan is either present in low quantities or absent from the pure enzyme. Complete inhibition by iodoacetamide (5×10^{-3} M) indicates that one or more of the six half-cystine residues present (from amino acid analysis on the automatic analyzer) possess "free sulfhydryl" groups essential for activity.

The equilibrium dialysis studies, using a technique similar to that previously reported from this laboratory,[5] have supported the kinetic data which indicate a firm binding of the reduced coenzymes to the enzyme; at pH 7.4, 23°C, the free energy of binding of DPNH to the enzyme is –6500 cal. Of the conditions thus far tested, only dialysis vs 40–60% ethanol will effectively dissociate the reduced coenzyme from the enzyme. Two moles of coenzyme have been observed to bind per mole of enzyme.

Conclusions. – Contrary to a previous report by Pfleiderer and Hohnholtz[6] that only one mole of DPNH was bound per mole of enzyme, the results obtained indicate that mammalian heart malic dehydrogenase binds two moles of DPNH per mole of enzyme. This result is in accord with the results obtained with other dehydrogenases where two moles of DPNH are bound per mole of enzyme.[7] The binding studies also suggest a role of water in the structure of the active site of the enzyme. Further work is in progress on the thermodynamics of coenzyme and substrate binding.

Blood Platelet Glycoproteins

Anatoly Bezkorovainy D. G. Doherty

Introduction. – Blood platelets have been shown to be of major importance in the formation of blood clots and clot retraction, and many disorders in the process of blood coagulation have been ascribed to a deficiency or abnormality of platelets. Moreover, platelets have been shown to be very fragile and to lose their biological activity very easily. It is, therefore, of practical as well as theoretical interest to obtain information on the chemical structure of blood platelets and to relate this information to the peculiar properties of these particles.

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[5] D. G. Doherty and F. Vaslow, *J. Am. Chem. Soc.* **74**, 931 (1952).

[6] G. Pfleiderer and E. Hohnholtz, *Biochem. Z.* **331**, 245 (1959).

[7] J. Van Eys *et al.*, *Biochim. et Biophys. Acta* **27**, 63 (1958).

Experimental and Results. – Bovine blood was collected immediately after death and was mixed with 0.1 volume of 5% EDTA in 0.9% NaCl. Platelets were separated from plasma and the other formed elements by differential centrifugation. Generally, the platelet preparation was free of erythrocytes and leukocytes. Platelets were then lysed by grinding in a glass homogenizer, followed by exhaustive dialysis against water. The insoluble material, termed platelet ghosts, was isolated by centrifugation. Usually 200–300 mg of ghosts was obtained from 18 liters of blood. The platelet ghosts were found to consist mainly of protein (84%) with protein-bound carbohydrate. The carbohydrates consisted of hexose 3%, hexosamine 1%, pentose 1%, sialic acid 0.5%, uronic acid 0.7%. Oxidation of the platelet ghosts with periodate results in periodate uptake, with the destruction of some 60% of sialic acid and uronic acid, but with no change in hexose, hexosamine, or pentose content. It might, therefore, be inferred that these sugars occupy a predominantly terminal position in the poly(oligo)saccharide chain(s) present. Dinitrophenylation of platelet ghosts results in an uptake of 0.6 to 0.7 micromole of fluorodinitrobenzene per milligram of ghosts. The DNP-platelet ghosts, when treated with base (Veronal buffer pH 8.6), can be separated into a soluble DNP-protein fraction and an insoluble one. The ratio solubles : insolubles is 2 : 3. The soluble material appeared to be homogeneous electrophoretically but showed three peaks in the ultracentrifuge, with one of the peaks accounting for some 60–70% of the total material. End group analysis of the soluble and insoluble DNP-protein fractions revealed serine to be the predominating *N*-terminal group, with minor traces of glutamic and aspartic acids in both cases. The carbohydrate composition was almost identical in both fractions and was comparable with that of the intact platelet ghosts. It was found that the DNP-protein fractions, as well as the intact ghosts, were extremely attracted to surfaces of all kinds, such as glass, paper, and synthetic resins. It was, therefore, found to be impossible to purify the heterogeneous soluble DNP fraction on any solid medium by paper electrophoresis, ion exchange, etc.

Work is now in progress for the purpose of further characterizing the carbohydrate components of platelet ghosts, the modes of linkages involved, and the amino acid composition and sequence.

ENZYMOLOGY

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The Effect of X Irradiation on the Induction of β-Galactosidase in *Escherichia coli*

J. M. Eisenstadt G. D. Novelli

Introduction. – Previous studies have indicated that ionizing radiations have no effect on subsequent enzyme induction. In fact, as much as 380 kr, a dose which kills 99% of yeast cells, has no demonstrable effect on the cells' ability to form maltozymase upon induction.[6]

In vivo experiments have indicated that ionizing radiations inhibit DNA synthesis,[7] while P^{32} decay experiments demonstrate the apparent necessity of intact DNA for enzyme induction.[8] In a cell-free system, β-galactosidase synthesis requires the presence of inducer as well as intact DNA.[9] In spite of the previous negative result of ionizing radiation on enzyme induction, it was of interest to examine the effect of low doses (up to 30 kr) of x rays on β-galactosidase synthesis.

Results. – Preliminary studies utilizing *E. coli* B revealed that with a dose in which less than 1% of the cells were able to form colonies, induction of β-galactosidase is inhibited approximately 80–90% (as compared with an untreated control). At this dosage, the synthesis of ornithine transcarbamylase (OTC) as well as general protein synthesis, as measured by the Folin-Ciocalteu method, is inhibited only 10–15% (compared with the untreated control). A dose of 2 to 20 kr leads to an exponential decrease in the ability to form β-galactosidase. Exposure of cells to x irradiation at various times after

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[6]L. S. Baron, S. Spiegelman, and H. Quastler, *J. Gen. Physiol.* **36**, 361 (1953).

[7]A. Howard, "Ionizing Radiations and Cell Metabolism" in *Ciba Foundation Symposium*, Churchill, London, 1956.

[8]E. McFall, A. Pardee, and G. Stent, *Biochim. et Biophys. Acta* **27**, 282 (1958).

[9]T. Kameyama and G. D. Novelli, *Biochem. Biophys. Research Communs.* **2**, 393 (1960).

induction to form β-galactosidase indicates an apparent increase in resistance to the inhibitory action of the x rays the longer induction proceeds.

Measurement of the effect of x rays on OTC synthesis, when the enzyme has been released from repression, reveals that its formation is now just as sensitive as β-galactosidase. (One interpretation of these results is that proteins which are being newly formed are susceptible to inhibition by x irradiation, while those previously synthesized are not.)

Another aspect of these investigations was the examination of the sensitivity of β-galactosidase and protein synthesis in strains of *Escherichia coli* which have been shown to behave differently on x irradiation. Experiments with *E. coli* B/R ORNL have indicated that in this organism β-galactosidase and viability are equally sensitive to irradiation, while general protein synthesis is resistant. By contrast, in *E. coli* B/S Hill at doses in which less than 0.1% of the cells are viable, the ability to form β-galactosidase is inhibited to the same extent as is general protein synthesis.

These studies would seem to support the hypothesis that nucleic acid metabolism is involved in some manner in the formation of proteins. These studies, therefore, will be continued and expanded by utilizing the cell-free synthetic system in an attempt to evaluate the role of the nucleic acids in protein synthesis.

Factors Affecting the Synthesis of β-Galactosidase by Cell-Free Extracts of *Escherichia coli*

J. M. Eisenstadt G. D. Novelli

In recent years it has become well established that ribonucleic acid is involved in protein synthesis, at least on the amino acid level. Other investigations (transduction, transformation, P^{32} decay) have indicated that the integrity of the hereditary material, DNA, is necessary for protein synthesis, but, as yet, there is no direct evidence that DNA is implicitly concerned.

For many years, the synthesis of inducible enzymes in microorganisms has been a major tool in attacking these problems. In the case of β-galactosidase in *E. coli*, enzyme induction involves the *de novo* synthesis of a specific, enzymatically active protein from its constitutive amino acids. Recently β-galactosidase synthesis has been shown to occur in cell-free preparations.[9] In the system described, the particulate and the supernatant fractions, as well as the inducer and a source of energy, are required for enzyme synthesis. The reaction is stimulated upon the addition of amino acids and di- and triphosphate nucleosides. Of great interest is the observation that ribonuclease and deoxyribonuclease markedly inhibit the synthesis of the enzyme. This would indicate that the integrity of the DNA molecule is necessary for the induction of β-galactosidase.

Recently, ultraviolet and x-ray irradiation of *E. coli* cells have indicated that the inducibility of β-galactosidase is severely inhibited. Preliminary experiments have also indicated that β-galactosidase induction is more sensitive than constitutive cellular protein synthesis after x irradation. In fact, an x-ray dose (20 kr) which causes only 10–15% inhibition of general protein synthesis inhibits β-galactosidase formation 80–90% as compared with the untreated control.

Experiments were therefore undertaken to study directly the effect of x rays on the synthesis of β-galactosidase, utilizing the cell-free system. Preliminary experiments established that cell-free preparations obtained from cells irradiated with an x-ray dose which inhibited in vivo β-galactosidase biosynthesis were 90% inactive for in vitro synthesis. Mixing the 105,000 $\times$ g supernatants and particles from normal and irradiated cell extracts established that the inactive portion was the supernatant. Normal supernatant (S_n) and x-irradiated particles (P_x) synthesize approximately 75% as much β-galactosidase in 60 min as the completely normal (S_n, P_n) system. By contrast, normal particles (P_n) and x-irradiated supernatant (S_x) produce only approximately 10% as much as the complete normal extract.

A nucleic acid extract was prepared from the 105,000 $\times$ g supernatant of untreated cells by streptomycin precipitation. This extract, containing approximately 2.6 mg of deoxyribonucleic acid per milliliter, was added to the reaction mixtures described above. The results indicate that this extract is able to restore the ability of the x-irradiated supernatant (S_x) to catalyze enzyme synthesis in the cell-free system. In fact, even the completely normal system is stimulated approximately 10–15% by this extract. DNase but not RNase treatment of the nucleic acid extract completely eliminates the ability of the extract to reverse the x-ray inhibition. Examination of the nucleic acid extract by ultraviolet spectrophotometry and chemical analysis supports the contention that DNA is the active material in the extract. The x-ray-damaged DNA is not able to function in the formation of β-galactosidase in cell-free preparations. Future experimentation will be directed to the elucidation of the role of DNA in protein synthesis.

Induction of Tyrosine-α-Ketoglutarate Transaminase in Rat Liver: Immunochemical Analysis

F. T. Kenney

Introduction. – Recent experiments have demonstrated[10] that the adrenal-steroid-requiring induction of rat liver tyrosine-α-ketoglutarate transaminase is due to an activation rather than to *de novo* synthesis from amino acids. Using the quantitative precipitin technique, in which the total protein precipitated by antibody is measured, it was found that this parameter remained independent of activity changes due to induction, suggesting that an immunologically competent but enzymatically inactive precursor was present in noninduced extracts. Further analysis of this precursor-enzyme relationship required a reliable antitransaminase, which in turn required an extensive purification of the enzyme. This purification and the results of analysis using a more specific immunochemical assay are reported here.

Results and Discussion. – The enzyme was purified from liver extracts from rats that had been induced by administration of tyrosine, about 4 mg of enzyme purified approximately a thousandfold being obtained from 600 livers. As a final purification step, the preparation was chromatographed on hydroxylapatite columns, a procedure which indicated an extensive heterogeneity of the highly purified enzyme. It is not known whether this heterogeneity is also typical of the normal enzyme or arises as a

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

consequence of induction; this point may be critical to understanding the mechanism of induction and will be pursued further.

An active antiserum was prepared from rabbits treated with the enzyme in Freund's adjuvant. Analysis by double diffusion in agar gel indicated a single sharp band for the reaction between the purified enzyme and antibody, but at least two reacting components in crude extracts which appeared unrelated to the enzyme. It is thus apparent that precipitin assays of crude extracts are not sufficiently specific for the demonstration and assay of precursor. Accordingly, a competitive assay was developed, in which the capacity of a crude liver extract to remove antibody to the enzyme from the antiserum is measured. If a cross-reacting precursor is present in noninduced extracts, these extracts should remove much more antitransaminase than would be expected from enzyme activity measurements. The results of these measurements establish conclusively that a cross-reacting precursor is not present, indicating that the presumed protransaminase lacks immunological as well as catalytic sites which are present on the active enzyme. This is in accord with the observation that antibody inhibits the enzyme, suggesting identity of immunological and enzymic sites.

Induction of Tyrosine-α-Ketoglutarate Transaminase in Rat Liver: Studies on the Role of Substrate and of Adrenal Steroids

F. T. Kenney

Introduction. – In the original description of the induction of rat liver tyrosine transaminase,[11] Lin and Knox demonstrated that tyrosine is fully effective in intact rats but ineffective in adrenalectomized animals unless small amounts of hydrocortisone are also given. This result was interpreted as indicating two modes of control, one hormonal and the other a specific substrate induction. Rats at birth undergo a rapid increase in hepatic tyrosine transaminase similar to the induced response in adults in requiring intact adrenals;[12] however, this change was unaffected by tyrosine but stimulated by methionine.[13] This observation prompted the present reinvestigation of the requirements for inductive activity, in the hope of extending our understanding of the role of substrate and adrenal steroids in the control of this enzyme.

Results and Discussion. – Eleven amino acids and derivatives, chosen for structural similarity to tyrosine and as representatives of the various types of amino acids, were tested for ability to induce the tyrosine transaminase in intact rats. Under conditions known to be optimal for induction by tyrosine, it was found that tryptophan was more effective than tyrosine and that phenylalanine, methionine, leucine, and glutathione were partially effective. The role of the adrenal in this phenomenon was emphasized by the finding that comparable amounts of Celite or bentonite are also capable of fully inducing the transaminase, these materials being chosen as nonspecific stressers of the pituitary-adrenal

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

[12] F. Serini, F. T. Kenney, and N. Kretchmer, *J. Biol. Chem.* **234**, 609 (1959).

[13] F. T. Kenney, unpublished observations.

axis. It is thus apparent that little information of value can be obtained by using the intact rat as test animal, and, accordingly, subsequent experiments were carried out by using rats that had been adrenalectomized 10–12 days prior to assay. The results of these experiments can be summarized as follows: Hydrocortisone in large doses is fully effective as transaminase inducer; lower doses cause a partial response. The effect of lower doses of steroid can be made maximal by simultaneous administration of tyrosine, tryptophan, or methionine – other amino acids were not tested since the results as stated establish that the effect of tyrosine is not related to its role as substrate of the enzyme. Experiments are in progress in which the possibility is being investigated that these compounds inhibit the degradative metabolism of hydrocortisone.

Tryptophan has long been recognized[14] as the inducer of hepatic tryptophan pyrrolase, the classical example of a substrate-induced enzyme in animal tissues. The present experiments demonstrate that under conditions suitable for pyrrolase induction, tryptophan also induces the tyrosine transaminase, and thus suggest that something other than a true "substrate induction" may be operative in this system as well as in the transaminase induction.

Amino Acid Incorporation into Particles Isolated from Maize Seedlings

R. J. Mans

Introduction. – Studies are being continued on the fractionation of the high-speed supernatant of the cell-free amino acid incorporating system from maize seedlings. Both the particle and the soluble components have been stabilized by storage in liquid nitrogen immediately after preparation. A rapid kinetic assay of incorporation has been developed to facilitate fractionation studies.[15]

Results. – As reported previously,[16] washing the maize microsomal pellet with sodium deoxycholate removes the ability of the particle to incorporate amino acids in the absence of the soluble component. Deoxycholate treatment also permits storage of the particles at –180°C without significant losses in their ability to incorporate amino acids when incubated with supernatant and appropriate supplements. The soluble component likewise can be stored without significant loss in activity by freezing in liquid nitrogen immediately after preparation. At temperatures above 0°C, increases occur in the RNase activity of the supernatant. It has not been possible, however, to directly correlate this with the loss of incorporating activity, although added RNase does inhibit incorporation. The addition of RNase inhibitors to the reaction mixture during incubation has not been effective in enhancing incorporation nor in protecting the supernatant activity at 0°C.

A rapid method for the measurement of incorporation has been developed to facilitate fractionation studies. The filter-paper technique of Bollum[17] has been modified to include several washings and extractions required to remove all materials other than the labeled protein from the sample. In addition,

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[14] W. E. Knox, *Brit. J. Exptl. Pathol.* 32, 462 (1951).

[15] R. J. Mans and G. D. Novelli, *Biochem. Biophys. Research Communs.* 3, 540 (1960).

[16] R. J. Mans and G. D. Novelli, *Biochim. et Biophys. Acta* (in press).

[17] F. J. Bollum, *J. Biol. Chem.* 234, 2733 (1959).

the filter paper disks are counted directly in the liquid scintillation spectrometer with greater precision and sensitivity than obtained previously with samples dissolved in formic acid.[15]

Discussion. – The stabilization of the particle component of the maize incorporating system and the development of a rapid method for measuring incorporation should greatly facilitate the fractionation of the soluble component. Studies are now in progress to determine the nature of the labile factor(s) in the soluble component as well as to attempt to stabilize this fraction at temperatures above 0°C.

Amino Acid Incorporation Studies in Developing Maize Kernels

Robert Rabson R. J. Mans

Introduction. – Previous studies involving the incorporation of radioactive amino acids into microsomal particles from developing maize kernels indicated that the isolation of active preparations is dependent on the maturity of the developing kernel.[18] We undertook to confirm this observation and, by limited fractionation, to determine the component(s) responsible for the observed effect.[19]

Results and Discussion. – Unwashed microsomes were prepared from fresh kernels of developing waxy maize at various times subsequent to hand pollination of the ears. The ability of these preparations to incorporate labeled leucine when incubated with a source of energy, GTP (guanosine triphosphate) and Mg^{2+}, was determined. Pollination was found to be essential for the isolation of active preparations. No incorporating activity was detected in preparations from kernels until eight days after pollination. However, from the eighth to fifteenth day after the pollination, the level of activity increased linearly and then plateaued for an additional five to ten days. At this stage of maturation the bulk of the activity was found associated with the endosperm. A gradual decline in activity was observed as maturation of the kernel continued, with a shift in activity from the endosperm to the embryo. The changes in activity of the two portions of the grain are correlated with the morphological development of the maize kernel.

To determine the component(s) responsible for the maturation of incorporating activity, we prepared a washed ribosomal and a supernatant fraction from homogenates of developing kernels. By appropriate mixing of immature with mature components we found that both the immature supernatant and immature particles lack the full capacity for incorporation present in the mature components. Although the immature supernatant was found to contain three times as much endogenous leucine as the mature supernatant, this dilution of the added labeled leucine could not account for the difference in activity of the two preparations. The total amounts of ribosomal protein obtained in the two cases were comparable; however, the RNA-to-protein ratio of the mature preparations was higher than that in the immature, suggesting a qualitative difference between the ribosomal preparations. Furthermore, the mature ribosomes were found to be less heterogeneous than the immature preparations on examination of ultracentrifuged material.

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[18] R. Rabson and G. D. Novelli, *Proc. Natl. Acad. Sci. U.S.* **46**, 484 (1960).

[19] R. Rabson, R. J. Mans, and G. D. Novelli, *Arch. Biochem. Biophys.* (in press).

Studies on an Amino Acid Incorporating System from Mouse Liver

Charlotte R. Lea G. D. Novelli

Introduction. – Investigations on the amino acid incorporating system previously described[20] are continuing.

Results and Discussion. – The component(s) of the soluble system previously found to be labile can be stabilized to storage at –20°C by the presence of 0.01 *M* glutathione (GSH). The presence of GSH in the reaction mixture greatly enhances the incorporating ability of the system. In the presence of GSH the reaction continues at a reduced rate for a period of hours rather than ceasing abruptly, as noted previously. Under these conditions further increase in incorporation cannot be obtained by adding more supernatant after 15 to 30 min as had been possible in the absence of GSH. Both particles and supernatant can be separately preincubated at 37°C for 30 min in the presence of the complete reaction mixture without significant loss of activity when subsequently tested in combination. The incorporated radioactivity remains associated with the particles, and there is no evidence of any release of TCA-insoluble labeled material in the soluble fraction. It would seem from these results and information presented previously that the limiting factor in this incorporating reaction is the availability of leucine-binding sites on the particles.

Studies on the Transfer of Activated Amino Acid to Carrier RNA

Maria D. Garcia-Pineda G. D. Novelli

Introduction. – One of the problems which exists in studies on protein synthesis is concerned with the nature, role, and specificity of S-RNA.[21] This soluble ribonucleic acid is known to be involved in the transfer of activated amino acids to microsomal fractions and can be demonstrated as being charged with amino acids on isolation. The present studies were undertaken in order to attempt to answer some of the questions regarding this transfer S-RNA.

Results and Discussion. – *Fractionation of Amino Acid Activating Enzymes.* – Extracts of *E. coli* were prepared, following disruption with glass beads. All the DNA and part of the RNA were removed by precipitation following the addition to the extract of streptomycin sulfate. In order to remove the remaining RNA the resultant supernatant was incubated. It was found that the RNA was degraded to its nucleotides, probably by the action of endogenous ribonuclease. These nucleotides were flocculated at 55°C, centrifuged off, and discarded. During the heat treatment, addition of stabilizing components was made to preserve the activation enzymes. The enzymes were precipitated with $(NH_4)_2SO_4$ and then dissolved in potassium phosphate buffer, pH 7.2. This solution was stabilized with mercaptoethanol and stored at –20°C. The activity of this preparation was measured in the ATP $\rightleftharpoons$ PP exchange reaction.

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[20] C. R. Lea and G. D. Novelli, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, p 171.

[21] G. D. Novelli, *Ann. Rev. Microbiol.* 14, 65 (1960).

Preparation of S-RNA (Amino Acid Acceptor RNA). – Dried cells of *E. coli* were suspended in 0.1 *M* cacodylate buffer (pH 7.0). Most of the nucleic acids, exclusive of S-RNA, were precipitated by treatment of the suspension with Duponol and NaCl. The S-RNA was then precipitated by ethanol at 0°C. After centrifugation, the precipitate was dissolved in cacodylate buffer (pH 7.0). This solution was incubated with pancreatic DNase to remove any DNA which might not have precipitated with the Duponol and NaCl. The nucleotides resulting from DNase action were removed by precipitation with 1.5 *M* NaCl. S-RNA was concentrated by ethanol fractionation and stored as a solution in cacodylate buffer (pH 7.0).

Studies on the Transfer of Activated Amino Acids to S-RNA. – Several experiments have been performed with the above materials. In the first series, the transfer of isotopically labeled C^{14} amino acids present in a yeast hydrolysate was examined. The incubation mixture contained buffer, ATP, Mg^{2+}, K^{+}, and GSH as stabilizer. After incubation at 30°C, the RNA with any labeled amino acids attached was precipitated with one of the following solutions: (1) 0.5 *M* NaCl in 67% C_2H_5OH, (2) 5% TCA, or (3) $HClO_4$ (PCA). Since the amino acid linkage to S-RNA is labile to mild alkali treatment, the precipitate was subjected to 0.01 *N* KOH for 30 min. The results indicated that not all of the counts were solubilized. It was our assumption that yeast hydrolysate may contain contaminating protein to which the charged S-RNA transfers the amino acid. In further experiments, isotopically labeled C^{14}-leucine was employed. The incubation mixture was supplemented with an energy generator of PEP and PEP kinase. After incubation, with subsequent precipitation of the S-RNA with PCA, attachment of the C^{14} label was found on the RNA. This transfer was maximum at 30 min and decreased with further incubation. The transfer of labeled amino acid was dependent upon the addition of both enzyme and S-RNA. Further studies are being done on this problem with C^{14}-leucine and C^{14}-valine.

Studies on the Synthesis of RNA

K. B. Jacobson

Since protein synthesis is intimately related to the presence of RNA, and, presumably, to specific forms of RNA, the study of the synthesis of RNA by cells will reveal information about mechanisms of protein synthesis, the mechanism of information transfer from the genetic to the enzymic levels, and other roles of nucleic acids.

Various approaches to understanding the means by which cells synthesize RNA will be needed, since the enzymological evidence accumulated by various workers suggests that there are at least two general mechanisms for the polymerization of ribonucleotides, and perhaps more. The ribonucleotides in the diphosphate form have been polymerized by bacterial enzymes; in the triphosphate form, ribonucleotides have been polymerized by enzymes derived from mammals, bacteria, and plant seedlings. In addition to these two types of mechanisms, other factors of regulation are indicated by the characteristics of the polymer produced. In some cases its base composition reflects the concentrations of the ribonucleotide precursor and in other cases an apparently inherent information code.

A system in which one could hope to observe the synthesis of a specific RNA is that in which the bacterial enzyme β-galactosidase is induced to appear by $\beta(D)$thiomethyl galactoside. Cultures of *Escherichia coli* were induced in the presence of P^{32}-orthophosphate, and the extent of P^{32} incorporation was compared with that for noninduced cultures. No appreciable increase of P^{32} could be detected in the total RNA of the induced culture. When the ribosomes were separated from the rest of the cell and a specific fraction of the ribosomes obtained by treatment with the antiserum to purified β-galactosidase, the RNA labeling was distinctly different in the induced culture. This observation will be studied further.

A second approach to understanding RNA synthesis was made by measuring the incorporation of α-labeled P^{32}-UTP into RNA by cell-free preparations. Such incorporation was readily demonstrable using extracts of *E. coli* and will be of use in pursuing the synthesis of a specific RNA such as the one associated with β-galactosidase induction. On the other hand, this type of incorporation was not observed when extracts of *Tetrahymena pyriformis* were tested. This failure may be related to the finding of a mononucleotide pool in *Tetrahymena* that appears to be either 2′ or 3′ ribonucleotide and not 5′(work in collaboration with D. M. Prescott, Cytology and Genetics Group).

Studies on Antibody Synthesis

C. J. Wust

Introduction. – Experiments designed to develop a cell-free system for antibody synthesis[22] have been continued.

Results and Discussion. – In the last report,[22] particles obtained from the homogenates of spleen and liver by centrifugation at 105,000 × *g* were said to agglutinate with specific antigen when the tissues had been excised from immunized animals. Particles similarly obtained from mice immunized to bovine serum albumin were treated with sodium deoxycholate at pH 7.6 and recentrifuged at 105,000 × *g*. The soluble fraction or supernatant was dialyzed to remove the bile salt and then concentrated by flash evaporation or by lyophilization. The concentrated material brought about hemagglutination with sheep red cells sensitized with bovine serum albumin. The material also reacted with rabbit anti-mouse globulin in the precipitin test. Material obtained in a similar manner from unimmunized mice reacted in the precipitin reaction with anti-mouse globulin but not with sensitized sheep cells. These serological reactions were confirmed with the Ouchterlony agar-diffusion technique.[23]

Emphasis was placed on the ability of particles, denuded of reactive material, to incorporate isotopically labeled amino acid (C^{14}-leucine) into protein or protein-like material which subsequently could be stripped off with deoxycholate in order to demonstrate its reactivity with antigen or antiglobulin. Since this new protein would be isotopically labeled, we hoped to show that any serological reactivity would be commensurate with *de novo* synthesis of antibody.

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[22] C. J. Wust, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, pp 183–84.

[23] M. W. Wilson and B. H. Pringle, *J. Immunol.* **73**, 232 (1954).

The evidence previously reported,[22] that liver particles react with antigen, suggested a scavenging or storing role for liver in contrast to this being a site of antibody synthesis.[24] Our attention turned to the spleen, which is known to be involved in antibody formation. Although incorporating systems have been developed in this and other laboratories on an elegant scale,[25,26] it has been found that spleen particles do not incorporate well. Initial experiments indicated that varying the extraction procedure or conditions of incorporation would not enhance a spleen system to more than 30 counts/min per milligram of particle protein. The combination of spleen particles and liver supernatant obtained after centrifugation of the homogenate at 105,000 × *g* has been successful, and incorporation of labeled amino acid up to 200 counts/min per milligram of particle protein were recorded.

In this series of experiments, both mice ($C_3H \times C_{57}BL$) and rats (Sprague-Dawley) have been used to study the incorporating system composed of liver supernatant and spleen particles. The rat system incorporates two to four times as much labeled amino acid as does the mouse system and has been chosen as the preferred animals. Studies with the mouse system are continuing in an effort to enhance the incorporation. Some studies were performed with immunized chickens and rabbits.

In the rat system, we have found that deoxycholate-treated particles do not incorporate any labeled amino acid in the absence of 105,000 × *g* supernatant (liver better than spleen, as indicated above), ATP, and an energy generator and Mg^{2+}. There is a reduction of up to 50% in the system when GTP is omitted from the incubation mixture. Potassium ion is required although Na^+ can be substituted with no apparent loss in activity. The pH optimum is between 7.8 and 8.0. It is interesting to note that an unbuffered homogenate of spleen was observed to have a pH 7.7–7.8. Optimum concentrations of Mg^{2+} and K^+ have been studied.

In the mouse system with deoxycholate-treated particles there is an absolute requirement for 105,000 × *g* supernatant (liver better than spleen), ATP and energy generator, GTP, Mg^{2+}, and K^+.

Employing these incorporating systems, experiments are in progress with mice immunized with triose phosphate dehydrogenase (TPD) and with rats immunized with bovine plasma albumin or TPD.

Primary Immune Response of Mice to an Enzyme – Triose Phosphate Dehydrogenase

C. J. Wust Sara L. Page

Introduction. – Subsequent to the injection of antigen before the appearance of circulating antibody, histological observations of lymphoid tissue have indicated that certain cellular types are present at specific time intervals.[27] With some organs, gross changes can be observed. It was the purpose of this study to evaluate changes in tissue weights and to measure the capacity of the spleen and liver to synthesize protein during the primary immune response.

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[24] J. S. Garvey and D. H. Campbell, *J. Exptl. Med.* 110, 355 (1959).

[25] C. R. Lea and G. D. Novelli, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, p 171.

[26] R. J. Mans and G. D. Novelli, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, p 172.

[27] C. C. Congdon *et al.*, *Rev. arg. cancerol.* (1960).

Results and Discussion. – Triose phosphate dehydrogenase was crystallized from rabbit muscle and injected in an aqueous vehicle into mice. In the animals that received an intramuscular or intravenous injection, no significant increase in weight was observed in the lungs, liver, spleen, and lymph nodes. Other mice that received the antigen intraperitoneally, emulsified with Freund's adjuvant, showed a spleen weight increase up to fourfold. No other tissue weight was increased significantly. The administration of adjuvant alone but not the oils of the adjuvant resulted in a spleen weight increase of two- to threefold. This increase was noted for both the wet and dry weight, indicating hypertrophy, as evidenced also in histological section. The increase in splenic weight attributable to antigen was not greater than fourfold with a tenfold increment in concentration (0.35 to 3.5 mg).

The spleen weight increase was noted from the 4th day after injection and reached a maximum on the 7th to 8th day. On the 9th day there was a decrease in both the wet and dry weights. The appearance of circulating antibody was evident early but only as a flocculating type. On the 9th day, correlated with the decrease in spleen weight, a marked increase in precipitating antibody was seen which neutralized enzymic activity. On the 11th day, the spleen weight increased to fourfold and then slowly decreased. The relative titer of antibody remained high through the 20th day.

In order to understand the hypertrophy of spleen which developed in response to antigen in adjuvant, a study of the incorporation of C^{14}-glycine into protein was made in vivo at time intervals after antigen injection. Although the liver weight did not increase significantly in any of the animals, the incorporation of the C^{14}-glycine into liver protein was examined. The labeled amino acid was administered intravenously. After 90 min the animals were bled out, the weights of spleen and liver recorded, and a portion prepared for the determination of radioactivity. Only the animals injected with antigen showed a significant increase in liver protein, reaching a maximum on the 7th day. On the 9th day, the level of labeled protein was in the normal range; however, it increased again on the 10th and 11th days. The results obtained with splenic protein indicated a significant increase in animals given antigen in adjuvant, adjuvant, or oils of the adjuvant. The maximum was twofold on the 5th day, which slowly decreased through the 9th day. On the 10th day, the maximum level of twofold was observed.

Serum proteins were labeled during the incorporation experiments. A higher specific activity was noted with sera from animals injected with antigen in adjuvant. This increase was shown to be due to a greater activity in the gamma globulins.

These results indicate that prior to the appearance of high-titering circulating antibody, the spleen and liver are more active in protein synthesis and nonspecific globulin formation is increased. The decrease in this activity and loss of spleen weight on the 9th day, correlated with a marked rise in antibody appearance, seems to indicate a prior production and release phenomenon.

Studies on the Biosynthesis and Properties of Dihydroorotic Dehydrogenase in *Escherichia coli*

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

We have been attempting to take advantage of the repression and release of repression of enzyme formation to develop a system which would permit a detailed analysis of specific protein synthesis

under conditions where initiation of synthesis is under control of the investigator. As shown by Yates and Pardee,[28] if *E. coli* is grown in the presence of uracil, washed, and placed in mineral salts medium *minus* uracil, there is a rapid synthesis of dihydroorotic dehydrogenase (DHO dh.) and aspartate transcarbamylase (ATC). We have reported[29] that although lysozyme protoplasts of *E. coli* synthesize ATC with the same efficiency as cells, DHO dh. is not formed by protoplasts. Since synthesis of ATC and DHO dh. is initiated simultaneously in whole cells by removal of uracil, the inability of protoplasts to form DHO dh. could provide an insight into the site and mechanism of synthesis of this enzyme. Accordingly, we are studying the localization and properties of the DHO dh. of *E. coli.*

Results and Discussion. – Studies on the localization of DHO dh. in protoplasts and in cells have indicated that the enzyme is associated with a cell membrane fraction. Protoplasts prepared with lysozyme and Versene from derepressed cells were subjected to lysis with water and then fractionated. All the DHO dh. in the protoplast lysate sedimented at 10,000 × *g* and was bound to the "ghost" or membrane fraction. Differential centrifugation of extracts prepared from cells with the French cell has demonstrated that 90% of the enzyme activity was sedimented at 10,000 × *g*. The remaining 10% of the activity sedimented at higher centrifugal forces. If the enzyme is synthesized on the membrane, failure of protoplasts to form the enzyme could be due to damage of the enzyme-forming site during preparation of protoplasts.

The DHO dh. of *E. coli* does not appear to be linked to pyridine nucleotides and is therefore different from the enzyme in other organisms. The membrane-bound DHO dh. uses oxygen as the electron acceptor. The aerobic conversion of dihydroorotic acid (DHO) to orotic acid is inhibited by 0.006 *M* KCN, suggesting involvement of cytochromes in the reaction; however, ferricyanide will act as electron acceptor for DHO oxidation, and this electron transfer is not inhibited by KCN. Thus it is probable that DHO dh. is bound to an electron transport system involving cytochromes and that ferricyanide reduction is a more direct assay of the enzyme than aerobic conversion of DHO to orotic acid. Experiments are in progress to solubilize and purify the enzyme by using the ferricyanide-reduction assay method. Knowledge of the properties and prosthetic groups of the enzyme may suggest an approach to developing a system for cell-free synthesis of DHO dh.

Evidence for the Reversibility of Adenosine-5′-Phosphosulfate Reductase

H. D. Peck, Jr. T. E. Deacon

Introduction. – Adenosine-5′-phosphosulfate (APS) is the form in which sulfate is reduced to sulfite by extracts of *Desulfovibrio desulfuricans.*[30] The enzyme, APS reductase, occurs in the "sulfate-reducing bacteria" and also in the thiobacilli that oxidize reduced compounds of sulfur to sulfate. In

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[28] R. A. Yates and A. B. Pardee, *J. Biol. Chem.* **209**, 677 (1957).

[29] W. H. Taylor, K. Tonomura, and G. D. Novelli, *Bacteriol. Proc.*, pp 148–49 (1960).

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

contrast, most other microorganisms reduce sulfate in the form of 3′-phosphoadenosine-5′-phosphosulfate. From a consideration of the O^{18} experiments of Santer[31] with whole cells of *Thiobacillus thioparus*, the requirement of phosphate or arsenate for the complete oxidation of thiosulfate, and the presence of certain enzymes concerned with the metabolism of inorganic sulfur compounds in extracts of these organisms, a scheme for the oxidation of thiosulfate to sulfate involving APS as an intermediate has been proposed.[32] The critical reaction in this scheme is the formation of APS from AMP and sulfite, that is, the reversibility of the enzyme, APS reductase. Direct evidence has now been obtained for the reversibility of APS reductase from *T. thioparus* and *D. desulfuricans*.

Results. – Extension of preliminary observations concerning the reversibility of APS reductase was limited by the fact that naturally occurring electron acceptors would not function in this system. Recently we have observed that extracts of *T. thioparus* and *D. desulfuricans* catalyze an AMP-dependent reduction of $Fe(CN)_6^{3-}$ in the presence of SO_3^{2-}. The rate of $Fe(CN)_6^{3-}$ reduction is directly proportional to enzyme concentration and constitutes a simple and rapid assay for this activity. Incubation of AMP, enzyme, $S^{35}O_3^{2-}$, and $Fe(CN)_6^{3-}$ in the presence of a large pool of unlabeled SO_4^{2-} yields a radioactive nucleotide that has the same mobility as APS upon electrophoresis and has been identified as APS. From the partial stoichiometry of the AMP-dependent reduction of $Fe(CN)_6^{3-}$ with SO_3^{2-}, it is postulated that the reaction proceeds as follows:

$$AMP + SO_3^{2-} + 2Fe(CN)_6^{3-} \longrightarrow APS + 2Fe(CN)_6^{4-} .$$

The reactivity of various nucleotides in this system was examined. IMP and deoxy-AMP are as active as AMP, while GMP shows only about 40% the activity of AMP. CMP, UMP, deoxy-GMP, deoxy-CMP, TMP, and 3′,5′-diphosphoadenosine are inactive. Studies on the stability, inactivation, and fraction of APS reductase activity with the reduced methyl viologen assay and the $Fe(CN)_6^{3-}$ assay indicate that both these activities are catalyzed by a single enzyme.

Discussion. – The formation of APS from AMP and sulfite by extracts of *T. thioparus* demonstrates that these organisms can form a high-energy sulfate during the oxidation of reduced sulfur compounds that can then be exchanged for phosphate to form ADP. The contribution of this reaction to the total energy production of this autotrophic organism is at present unknown; however, it is possible that this unique organism obtains a large portion of its energy from this substrate sulfurylation.

The Mechanism of Thiosulfate Oxidation by Thiobacilli

M. P. Stulberg H. D. Peck, Jr.

Introduction. – The requirement of inorganic phosphate (P_i) for the oxidation of thiosulfate to sulfate by whole cells and cell-free extracts of *Thiobacillus thioparus* has been partially elucidated by

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[31] M. Santer, *Biochem. Biophys. Research Communs.* **1**, 9 (1959).

[32] H. D. Peck, Jr., *Proc. Natl. Acad. Sci. U.S.* **46**, 1053 (1960).

Fisher and Peck[33] into the following scheme:

$$\text{thiosulfate} \xrightarrow{\text{thiosulfate reductase}} \text{sulfite} + H_2S\,, \tag{1}$$

$$\text{sulfite} + \text{AMP} \xrightarrow{\text{APS reductase}} \text{APS}\,, \tag{2}$$

$$\text{APS} + P_i \xrightarrow{\text{ADP sulfurylase}} \text{ADP} + \text{sulfate}\,, \tag{3}$$

$$2\text{ADP} \xrightarrow{\text{adenylic kinase}} \text{AMP} + \text{ATP}\,. \tag{4}$$

Santer[31] has demonstrated the transfer of oxygen from P_i to sulfate during the oxidation of thiosulfate by whole cells of *T. thioparus*, indicating a covalent bond formation between phosphate and sulfate or sulfite during the over-all reaction. The elucidation of this interaction has been attempted by use of appropriate O^{18}-labeled substrates incubated with various extract preparations, as detailed by Fisher and Peck.[33]

Methods. – AMP-O^{18} was prepared by isolation of AMP from an alkaline digestion of ATP in H_2O^{18} catalyzed by Ca^{2+} ions. P_i-O^{18} and sulfate-O^{18} were prepared by acid catalysis of an oxygen exchange reaction between H_2O^{18} and P_i or sulfate oxygens. Oxygen-18 analysis of $BaSO_4$ isolated from incubations was performed by a modification of the carbon-reduction method of Hoering.[34]

Results. – When AMP-O^{18} was incubated with extracts, treated to eliminate recycling of adenosine nucleotides (reaction 4), a quantitative transfer of one oxygen from AMP to sulfate produced was realized. This transfer confirmed reactions (2) and (3) as postulated by previous balance studies.[33] When P_i-O^{18} was incubated with extracts now treated to allow all four reactions to take place, the O^{18} found in the sulfate was much lower than that predicted. Partial explanation for this result was found by a reisolation of the P_i-O^{18} which had been diluted threefold from the initial O^{18} concentration. Labile phosphate (ADP and ATP) isolated from the experiment also reflected this dilution.

Discussion. – The dilution difficulties encountered may be a reflection of oxidative phosphorylation carried out by these extracts. Conclusive interpretations await results on the effect of dinitrophenol on this diluting reaction of P_i-O^{18}. Further confirmation can be expected by experiments now in progress whereby reaction (4) again has been inhibited in order to determine whether the bridge oxygen of the ADP formed (i.e., the oxygen which is donated to sulfate) is derived from P_i oxygen in reaction (3).

Experiments with whole cells are also in progress, using H_2O^{18}, P_i-O^{18}, and O_2^{18} as oxygenated substrates. Active cultures defined by oxygen uptake, sulfate production, and P_i pool size are needed for the success of these experiments. The results once obtained should further confirm and elucidate the mechanism of inorganic sulfur metabolism in microorganisms.

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[33] E. Fisher, Jr., and H. D. Peck, Jr., *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, p 176.

[34] T. C. Hoering, Ph.D. thesis, Washington University, 1952.

Analysis of a Bacterial Sulfated Polysaccharide

Mary-Lowell B. Taylor G. D. Novelli

Introduction. – A sulfated heteropolysaccharide-protein complex produced by a soil diphtheroid has previously been reported by us[35] to contain glucose, galactose, and fucose. The complex can be obtained as an extracellular component and concentrated by alcohol precipitation or obtained from whole cells by Waring Blendor agitation. Although sulfated polysaccharides have been known to occur in animals, there have been no previous reports of a sulfated bacterial polysaccharide. There have been many attempts to elucidate the mechanism of sulfation of polysaccharides. The presence of sulfate in the polysaccharide can act as an analytical marker in studies involving the specific synthesis of the polysaccharide.

Results and Discussion. – The polysaccharide-protein complex cannot be dissociated by Geon electrophoresis at pH 4.1, 7.8, or 9.2, by ultracentrifugation at pH 7.5, by precipitation with Cetavlon or cetylpyridinium chloride at pH 6.0 or 8.1, or by the Sevag procedure. These findings suggest that the polysaccharide and protein are linked by covalent bonds.

The constituent amino acids found after hydrolyzing 24 hr in 1 *N* HCl at 105°C accounted for 12% of the dry weight of the sample analyzed. Amino acid analysis of the polysaccharide-protein complex showed the presence of 14 amino acids. On a micromolar basis, alanine, serine, threonine, glycine, glutamic acid, and aspartic acid account for 73% of the amino acids present. No sulfur-containing amino acids could be detected. Glycine was the only *N*-terminal amino acid found.

Although no sulfur amino acids were detected, the $S^{35}O_4$-polysaccharide-protein complex could be prepared by growing cells on a mineral salts medium containing $S^{35}O_4$. The $S^{35}O_4$-polysaccharide-protein complex was isolated and shown to be isotopically homogeneous by paper electrophoresis. The S^{35}-sulfate could not be freed from the complex during paper electrophoresis.

After a crude extract of *Limax* digestive gland was incubated with $S^{35}O_4$-polysaccharide-protein complex at 37°C, free $S^{35}O_4$ could be detected by paper electrophoresis. Free $S^{35}O_4$ was also liberated from the $S^{35}O_4$-polysaccharide-protein complex by hydrolysis at 100°C in 1 *N* HCl for 30 min. Experiments are now in progress to isolate intermediates containing $S^{35}O_4$.

In the presence of ATP, $S^{35}O_4$, polysaccharide-protein complex, and diphtheroid extract, a small amount of $S^{35}O_4$ appears to be transferred to the polysaccharide. The transfer of $S^{35}O_4$ into the polysaccharide-protein complex can be correlated with the ability of the diphtheroid extract to form 3′-phosphoadenosine-5′-phosphosulfate (PAPS).

A bacterium has been isolated by the enrichment technique from the soil that is capable of using the polysaccharide-protein complex as a sole source of carbon. It is hoped that extracts of this organism will offer a method for obtaining components of the complex in an unaltered state.

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[35] M. L. Taylor and G. D. Novelli, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, p 184.

Enzymes of Starch Biosynthesis in Maize: UDP-D-Glucose–D-Fructose Transglucosylase (Saccharese)

Audrey N. Best G. D. Novelli

Introduction. – This is a continuing and cooperative project with the Maize Genetics Group that is concerned with the mechanism of gene action at the molecular level.[36]

Results and Discussion. – We have concentrated our efforts on the enzyme saccharese that catalyzes the following reaction:

$$\text{UDP-glucose} + \text{fructose} \rightleftharpoons \text{sucrose} + \text{UDP} .$$

Since this is a reversible reaction, it is possible that sucrose can be converted to UDP-glucose and thence into starch by a reaction similar to glycogen synthesis in mammals. If this were the case, saccharese would be the first enzyme in starch biosynthesis. A recent report by Fekete *et al.*[37] that a fraction from germinating string beans can incorporate UDP-glucose-C^{14} into starch suggests that the above assumption may be correct.

We have purified saccharese from waxy maize endosperm twelve- to fifteenfold and have an enzyme 20 times more active than any previously described.[38] Nevertheless, electrophoretic analysis indicates that our preparation has at least four protein peaks, and we must therefore continue with the purification.

We have made attempts to determine whether a maize extract can incorporate C^{14} from sucrose into starch. In carrying out these experiments, crude extracts of maize endosperm were incubated with sucrose-C^{14}, UDP, amylase or amylopectin and fortified with our purified saccharese. Under these conditions, the starch that was precipitated at the end of the reaction contained considerable radioactivity. Much of the C^{14} in the starch could be washed or dialyzed away; so it is difficult to know whether the C^{14} in the starch pellet was contaminating unreacted sucrose or was present as small oligosaccharides that may be involved in starch synthesis. In running control reactions we observed that saccharese itself could transfer C^{14} from glucose to a fraction precipitating with starch. Here again much of the radioactivity could be removed by washing the starch pellet; so the results were again equivocal. We then discovered that our saccharese preparation is contaminated with amylases; so it is possible that saccharese is indeed transferring glucose residues to a primer but that the amylases are reducing the primers to short-chain polysaccharides. The investigation is continuing along two lines: (1) further purification of saccharese to eliminate the amylases, and (2) the preparation of UDP-glucose-C^{14}, in order to test whether the transfer activity is catalyzed by saccharese or by a different enzyme contaminating our preparation.

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[36] A. N. Best and G. D. Novelli, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, p 182.

[37] M. R. de Fekete, L. F. Leloir, and C. E. Cardini, *Nature* 189, 918 (1960).

[38] C. E. Cardini, L. F. Leloir, and J. Chiriboga, *J. Biol. Chem.* 214, 149 (1955).

MICROBIOLOGY

Enzyme and Tracer Studies on Bacterial Metabolism

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Mechanism of Methylmalonyl Isomerase

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Introduction and Experimental. – Eggerer *et al.*[1] have shown that methylmalonyl isomerase acts by causing a migration of the thioester group of methylmalonyl Co-A to its beta methyl carbon to form succinyl Co-A and proposed that this is a free-radical reaction involving the cobamide coenzyme as a reduced intermediate. We have tested this theory by use of a variety of compounds that might react with the free radicals. Methylmalonyl isomerase and the cobamide coenzyme were isolated from *Propionibacterium pentosaceum*. A methylmalonyl isomerase involving the separation of succinate-C^{14} from methylmalonate-C^{14} by ion exchange chromatography was developed to avoid interference by the test compounds. The compounds tested included radiation-protection compounds known to be "free-radical scavengers," some common one-electron oxidation-reduction reagents, and other compounds with a wide range of molecular orbital energies. Most compounds had little effect on the system, but one group of related compounds, with interesting molecular orbital constants, were effective inhibitors. This group included methylene blue and Nile blue, which have high "lowest-empty" orbital constants (good electron acceptors), and chlorpromazine, with an extremely low "highest-filled" orbital constant (good electron donor). However, strongest inhibition was obtained by preincubation of the test compound with the apoenzyme; in these cases the presence of the cobamide coenzyme afforded some protection.

Discussion. – A free-radical trap would be expected to be a competitive inhibitor, functioning only during the reaction itself, whereas an inhibiting complex with the apoenzyme would be enhanced by prolonged contact of the inhibitor with the enzyme. Thus, the inhibitors in question are probably of the latter type and could mask any effect on free radicals. Since charge-transfer processes depend on the fit between reacting molecules as well as on the molecular orbital constants, the magnitude of inhibition of free-radical reactions might well be small, especially where competition with a large coenzyme having a very small K_m is involved. Therefore, a closer examination of the less effective inhibitors may be required. Further evidence concerning the nature of the isomerase mechanism also is being sought by the use of electron-spin resonance.

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[1] H. Eggerer *et al.*, *J. Am. Chem. Soc.* 82, 2643 (1960).

Polynucleotide Phosphorylase of *Clostridium perfringens*

M. I. Dolin

Introduction. – Previous work[2] has shown that the activity of polynucleotide phosphorylase isolated from *Clostridium perfringens* is markedly dependent upon the presence of basic proteins such as histone and protamine or the polypeptide polylysine. With the last three compounds, the rate of polyadenylic acid synthesis may be stimulated some twentyfold. This dependency is observed only after partial purification of the enzyme, which suggests that the basic proteins may replace a naturally occurring activator. The mechanism and physiological significance of this polymerase system are being further investigated.

Results. – The specificity of the requirement for basic proteins is being investigated together with the effect of these compounds on the stoichiometry and kinetics of polyadenylic acid synthesis. In general, the phenomenon seems to be rather specific for basic polypeptides. Polyornithine has been added to the list of activators and is almost as effective as polylysine. Natural and acidic polyamino acids are inactive, as is the anionic polyelectrolyte, heparin. Streptomycin is inhibitory.

Protamine and polylysine do not alter the stoichiometry of the reaction. In the presence or absence of activator, 1 mole of inorganic phosphate is released for every mole of AMP incorporated into polyadenylic acid.

In addition to stimulating the rate of polyadenylic acid synthesis, it has been found that protamine and polylysine markedly increase the affinity of the enzyme for ADP. In the presence of either protamine or polylysine, the K_s for ADP decreases from 1.6×10^{-2} *M* to 5×10^{-4} *M*. The K_s for polylysine (mol. wt 16,500) is 3.4×10^{-6} *M*.

Charge effects are probably important in the activation phenomenon, since at high ionic strength (0.2 *M* KCl) there is also a stimulation of polyadenylate synthesis (approx fourfold) and a decrease in K_s for ADP (to 1.3×10^{-3} *M*). However, on a weight basis, it takes more than 1000 times as much KCl as polylysine to produce this effect. The effects produced by the basic proteins are greater in magnitude and take place at physiological concentrations.

Conclusions. – The effect of basic proteins in lowering the K_s of the enzyme for ADP may be physiologically significant. One of the difficulties involved in considering polynucleotide phosphorylase as a synthetic (rather than a phosphorylyzing) enzyme is that for all of the enzymes isolated so far, the concentration of ADP required to saturate the system is very high (K_s approx 2×10^{-2} *M*). The presence of basic proteins may make nucleoside diphosphates more reasonable substrates for polymer synthesis.

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[2] M. Dolin, E. Godiniaux, and M. Grunberg-Manago, "Polynucleotide Phosphorylase of *Clostridium perfringens*," *Bacteriol. Proc.* (*Soc. Am. Bacteriologists*) (to be published).

High-Catalase Mutant of *Rhodopseudomonas spheroides*

R. K. Clayton Carolmarie Smith

In a high-catalase mutant of *Rhodopseudomonas spheroides*, obtained in this laboratory,[3] as much as one-fourth of the total protein can be catalase. This remarkable capacity for synthesis of a single enzyme was studied, first, by characterizing the enzyme. The constitutive catalase of the mutant and the inducible[4] catalase of the parent wild type were purified and characterized. It was found that the catalase of the mutant is homogeneous and identical to that of the wild type by the following criteria: measurement of enzyme activity, spectrophotometry, ultracentrifugal analysis, chromatography on DEAE-cellulose, electrophoresis on Geon resin and on starch gel, and immunochemical analysis. In both the mutant and the wild type, less than 1% of the catalase is bound to subcellular particles.

Earlier Syntheses and Viability in X-Irradiated *Rhodopseudomonas spheroides*

R. K. Clayton H. I. Adler

Earlier literature[5-7] on the effects of x rays on protein synthesis and viability in bacteria have indicated that the syntheses of proteins are much more resistant than viability; the loss of protein-forming ability has been regarded as a post-mortem phenomenon. We have found that the ability of *Rhodopseudomonas spheroides* to perform an induced,[4] *de novo*[8] synthesis of catalase is inhibited by x rays to the same degree as is viability. The inhibition is manifested immediately after irradiation; it does not become progressively more severe or less severe. The effects of environmental factors and the interpretation of kinetic experiments indicate that x rays act in an all-or-none manner at the level of the genetic apparatus. The syntheses of constitutive and inducible catalase, of chromatophore proteins, and of general cellular proteins are all inhibited by x rays to about the same degree in *R. spheroides*.

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[3] R. K. Clayton and C. Smith, *Biochem. Biophys. Research Communs.* 3, 143 (1960).

[4] R. K. Clayton, *Biochim. et Biophys. Acta* 37, 503 (1960).

[5] D. Billen and H. C. Lichstein, *J. Bacteriol.* 63, 533 (1952).

[6] L. S. Baron, S. Spiegelman, and H. Quastler, *J. Gen. Physiol.* 36, 631 (1953).

[7] H. Pauly, *Nature* 184, 1570 (1959).

[8] R. K. Clayton, *J. Biol. Chem.* 235, 405 (1960).

PLANT PHYSIOLOGY AND PHOTOSYNTHESIS

Plant Physiology

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Photosynthesis

W. A. Arnold
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Metabolic Studies of Gamma-Irradiated Wheat Growing Without Cell Division

A. H. Haber W. L. Carrier

Introduction. – Corn grains given certain high doses (~500,000 r) of ionizing radiation were found by Schwartz and Bay[2] to germinate and produce small seedlings without undergoing cell division. Subsequent studies have suggested this may occur with some other cereal grains.[3–5] Such growth without cell division offers promise for both physiological and anatomical studies.

To the physiologist, "gamma-plantlets" (i.e., seedlings growing without cell division from 800-kr gamma-irradiated grain) offer a system for studying the actions of chemicals on growth by cell expansion uncomplicated by the occurrence of cell division.[5,6] Such studies presuppose that the growth of the heavily irradiated grain, although obviously not typical of normal growth in all respects, is nonetheless typical of normal growth in some respects (e.g., increase in protein content). Our investigations have established that gamma-plantlets carry on certain anabolic processes and that their growth cannot be attributed to a swelling of the embryo that is unrelated to metabolism.

To the anatomist, studies of gamma-plantlets make it possible to follow the fate during germination of the cells originally present in the dormant embryo. It is important to know whether there is any replication of genetic material, that is, DNA synthesis, because of the surprisingly high degree of cellular differentiation. Although cell division and DNA synthesis are usually associated processes, there is abundant evidence that mitosis does not always follow replication of chromonema[7,8] or DNA synthesis.[9–11] That no mitosis necessarily implies no DNA synthesis is especially questionable in systems given large doses of ionizing radiation. Thus Kelly[12] found net

[1]Consultant.

[2]D. Schwartz and C. Bay, *Am. Naturalist* 90, 323 (1956).

[3]J. Moutschen, Z. M. Bacq, and A. Herve, *Experientia* 12, 314 (1956).

[4]M. A. Sicard and D. Schwartz, *Radiation Research* 10, 1 (1959).

[5]A. H. Haber and H. J. Luippold, *Am. J. Botany* 47, 140 (1960).

[6]A. H. Haber and J. D. White, *Plant Physiol.* 35, 495 (1960).

[7]F. D'Amato, *Caryologia* 4, 311 (1952).

[8]A. Lorz, *Botan. Rev.* 13, 597 (1947).

[9]N. K. Das, K. Patau, and F. Skoog, *Chromosoma* 9, 606 (1958).

[10]A. Ficq, C. Pavan, and J. Brachet, *Exptl. Cell Research* (suppl 6), 105 (1958).

[11]R. Vendrely, pp 155–80 in *The Nucleic Acids*, vol II (ed. by E. Chargaff and J. N. Davidson), Academic Press, New York, 1955.

[12]L. S. Kelly, *Proc. U.N. Intern. Conf. Peaceful Uses Atomic Energy, 2nd, Geneva, 1958* 22, 521–23 (1958).

increases in DNA in nondividing ascites tumor cells in mice given 800 r, and concluded that radiation-induced mitotic inhibition is not caused by any possible effect on DNA synthesis. Similarly, McGrath[13] found that the nuclei of certain nondividing crypt cells in the small intestine of mice given 5000 r incorporated H^3-thymidine into insoluble material (presumably DNA) to an extent comparable with that of unirradiated controls. Similar results with irradiated fibroblasts have been reported recently by Harrington.[14] Consequently, another purpose of our investigation was to demonstrate that there is no DNA synthesis in growing gamma-plantlets.

Summary. – Wheat grains given 800 kr of Co^{60} gamma radiation before moistening can germinate and grow into small seedlings without cell division or deoxyribonucleic acid (DNA) synthesis. This growth is sustained by metabolism and is not merely water uptake. The growing seedlings increase in dry matter, protein, and ribonucleic acid (RNA). They can perform photosynthesis by fixing carbon from CO_2 into sugar phosphates, sucrose, amino acids, and organic acids. These results suggest that such irradiated cereals may be used for certain physiological and anatomical studies of germination and development in an intact growing organism in the absence of DNA synthesis, mitosis, and cell division.

Photosynthesis

W. A. Arnold E. B. Gassner

This work is an attempt to extend the reversible spectral changes found in purple bacteria by Arnold and Clayton[15] to higher plants. A species survey indicated that similar changes do occur and that these changes are nearly identical for all higher plants examined. They were not easily interpreted in the light of the original theory, which is based on bacterial chromatophores. Higher plant material gives no light signal in dried films. A surprisingly high concentration of water (>80% saturation) is required to promote signal response. Various poisons useful on bacteria gave very different results in chloroplasts. In spite of these differences several similarities have been found. The signals from chloroplasts suspended in glycerol are temperature independent (300 to 70°K). Further, both materials show similar signal titration curves, in the presence of the ferri-ferro redox couple.

Dichromate was found to be a reversible oxidant which is easily washed out while leaving the material oxidized. This could then be reduced with buffered ascorbate, thus regenerating the signal. Using this technique (not possible with highly colored iron salts), the two signal maxima (approximately 700 and 430 mμ) could be examined together. This, along with other kinetic evidence, indicates that these two maxima are the result of changes caused by the same process.

Spectral examination of a sonicated cell suspension of *Synechococcus cedrorum* showed an unexpected absorption maximum at 750 mμ (a wavelength known to inhibit photosynthesis; see Govindjee *et al.*[16]). The nature of the material with this absorption band is now being investigated.

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

[14] H. Harrington, *Biochim. et Biophys. Acta* 41, 461 (1960).

[15] William Arnold and R. F. Clayton, *Proc. Natl. Acad. Sci. U.S.* 46, 6 (1960).

[16] Govindjee *et al.*, *Biophys. J.* 1, 91 (1960).

BIOPHYSICS

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Postirradiation of Electron Spin Resonance Signals

J. S. Kirby-Smith

Introduction. – Studies of the changes in radiation-induced spin resonance patterns produced by postirradiation heating, exposure to moisture, and ultraviolet radiation offer a means of elucidating the nature of the primary resonances. In some materials, such as the crystalline amino acids, such investigation may help to distinguish whether the observed patterns arise from true free radicals or from some other phenomena such as lattice defects. Postirradiation exposure to heat, high humidity, and ultraviolet radiation have been carried out in amino acids, proteins, and *Tradescantia* plant pollen.

Results and Discussion. – In crystalline solids, in proteins, and in *Tradescantia* pollen, exposure to ultraviolet radiation (2537 A as well as mixed radiation in the 2200 to 3000 A band) results in a drastic decrease in the magnitude of the primary ionizing-radiation-induced signal. The magnitude of the signals (number of resonant centers) decreases in an apparent exponential manner with the applied dose of ultraviolet radiation. This fact is in agreement with one-hit kinetics in which a single ultraviolet quantum is presumed to interact with a primary resonant site, either by releasing a trapped electron or by the formation of a free radical by the ultraviolet radiation. The ultraviolet-induced radical might then interact with a primary (ionizing-radiation-induced) radical. This latter possibility may be more likely in *Tradescantia* pollen, for in this system ultraviolet radiation alone in the dose ranges used produces an appreciable free radical signal.

As for heat treatments, in all systems studied the signals decay to a constant level, after which further exposure to temperature in the 80 to 100°C range has no effect. In single glycine crystals the changes in spin resonance correlate with changes in the ultraviolet absorption spectra. Studies of combined ultraviolet radiation and heat treatment are in progress to determine the maximum decrease in signal which can be obtained.

Kinetics of Electron Spin Resonances Produced by Irradiation

M. L. Randolph

Introduction. – An approach to an ultimate understanding of free radical processes is to study the kinetics of electron spin resonances produced by irradiation. We are attempting to describe the

[1]Loanee.

measured numbers of resonances present at times t following irradiations of durations τ in mathematical forms based on classical kinetic models.

Results. – Our data on crystalline amino acids and seed materials indicate qualitatively that: (1) at high doses (greater than 10 megarads) saturation effects are appreciable; (2) as measured starting about 30 min after irradiation, decay of resonances is not exponential like radioactive decay, but rather more rapid at first; and (3) at any time t after irradiation, the probability of decay of a resonance in a given time is somewhat greater for highly irradiated samples than for slightly irradiated samples.

Discussion. – Because the environment of the resonances – in terms of concentrations of free electrons, ions, and excited states – is markedly different during irradiation and postirradiation decay, we may expect some quite different constants in the differential equations describing the kinetics during and after irradiation. From our measurements during the decay period we may hope to determine directly the kinetics of decay, and from extrapolations back to the end of irradiation, the kinetics during irradiation. Making the usual assumption of kinetic theories that $i = 1, 2, 3$, etc., resonances are involved in the recombination or cancellation of a center, the differential equations for the variation of the numbers of resonances N present during irradiation times τ and decay times t are:

$$\frac{dN}{d\tau} = \alpha I - \sum_{i=1}^{k} \beta_i N^i , \tag{1}$$

$$\frac{dN}{dt} = - \sum_{i=1}^{k} \gamma_i N^i , \tag{2}$$

where the Greek letters are coefficients, with the β_i expected to equal or exceed γ_i, and I the dose rate. The qualitative results suggest that during decay first-order kinetics will be involved but inadequate to describe all the data. Reasonably good separate fits can be made to our sets of glycine data taken 4 and 47 days postirradiation, using either first- or second-order kinetics, but the constants which serve at one time fail at the other. Since the decay curves on a logarithmic scale are almost independent of τ, it would seem that decay should be mostly first-order kinetics, despite the rapid initial decay. An explanation now being tested is that the coefficient γ_1 depends on the concentration of some excited state or other abnormality which itself decays. All preliminary calculations indicate that recombination rates must be greater during irradiation than during decay. A logical extension of these studies would be a detailed study of the kinetics in lettuce seed, for which we have variations in decay rate with AET concentrations and saturation effects, all in the radiation and AET dose ranges of biological significance.[2]

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[2]M. L. Randolph and A. H. Haber, "Production and Decay of Free Radicals Induced by X-Irradiation of Dry Lettuce Seeds," presented at the *Symposium on the Effects of Ionizing Radiation on Seeds and Their Significance for Crop Improvement, Karlsruhe, Aug. 8–12, 1960* (in press).

Electron Spin Resonance Signals from Oxygen-Exposed Lyophilized Bacterial Cells

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

Introduction. – When *E. coli* cells are lyophilized in distilled water and then exposed to small amounts of air or oxygen they rapidly lose viability, while no such effect is produced when the cells are kept under vacuum.[3] Dried *E. coli* cells may also be protected against this lethal effect of oxygen by thiourea or more effectively by sodium iodide, but cysteine is completely ineffective in this respect.[4] In a typical experiment the viability of dried cells after exposure to oxygen was found to be: control, 0.05%; in presence of cysteine, 0.001%; in presence of thiourea, 42%; in presence of NaI, 80%.[4] Certain aspects of these observations suggested that free radicals might be involved in this reaction. An electron spin resonance (ESR) study was therefore undertaken.

Results. – Lyophilized bacteria kept in vacuum showed no perceptible spin resonance signal. In the presence of oxygen a relatively strong signal was apparent 30 min after exposure and reached its final value after several hours. This signal remained stable for at least six weeks. Contrary to experiments on lyophilized tissues by Miyagawa and Gordy,[5] the signal also remained stable even after re-evacuation and removal of oxygen. In experiments where the additives, cysteine, NaI, or thiourea, were used, a definite correlation between the ability of these substances to suppress free radical signals and viability was observed. Dried bacteria irradiated with 750 kr of x rays showed a broad, weak, and double-peaked signal which became higher and sharper after heat treatment and rapidly decayed on exposure to oxygen. This change in pattern after heating in the irradiated material suggests a heat-induced transition from one radical species to another, and a fundamental difference in radiation-induced radicals and those occurring after lyophilization alone.

Discussion. – The experiments of Miyagawa and Gordy[5] were done on ground up and obviously unviable animal tissues, and, therefore, their results are not directly comparable with ours. They show that molecular species of biological origin which in the dry state are able to react quickly with oxygen are not restricted to bacteria. They differ in that the reaction between oxygen and the sensitive site of bacteria seems to be irreversible. This last fact would exclude a normal metabolic role for this reaction, for otherwise one might expect an ESR signal in dried bacteria kept in vacuum, which are intact and viable cells. One might rather imagine the oxygen-sensitive site to be protected in a wet cell system by the normal metabolic machinery removing excess of oxygen. Once this normal machinery is stopped in the dry cell, the site becomes easily accessible to oxygen, unless other protecting materials are present, with consequent inactivation and loss of the capability of the cell to recover, when reintroduced into its normal environment. This last capability makes the bacterial cell an interesting model in ESR studies of biological phenomena.

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[3] M. B. Lion and E. D. Bergman, *J. Gen. Microbiol.* (1960), in press.

[4] M. B. Lion and E. D. Bergman, *J. Gen. Microbiol.* (1960), in review for publication.

[5] I. Miyagawa and W. Gordy, *Proc. Natl. Acad. Sci. U.S.* **44**, 613 (1958).

Modification of Ultraviolet-Induced Aberration Frequencies in *Tradescantia* Pollen

H. L. Cromroy

Introduction. – We have investigated the effects of a variety of pre- and posttreatments on ultraviolet-induced chromosomal aberrational frequencies in *Tradescantia* pollen. These studies were done to explain the variability of the magnitude of the synergistic effect previously reported by Kirby-Smith, Nicoletti, and Gwyn.[6] The investigated parameters included humidity, temperature, and visible radiation. All the ultraviolet treatments were made at 50% relative humidity and with an exposure of 1×10^6 ergs/cm^2 of 2537-A radiation. Visible radiation pretreatments were made with a reflector spot lamp filtered with either copper sulfate (10 g per liter of distilled water at a depth of 5 cm) or a Corning No. 9863 glass filter and the copper sulfate. The two filters together limited light within 3100 to 4100 A to pass, whereas the copper sulfate alone permitted light within region of 3000 to 6000 A to pass. The exposure with visible radiation was 6500 ergs cm^{-2} sec^{-1}.

Results. – Pretreatments of pollen in a water-saturated chamber decreased frequency of both chromatid and isochromatid aberrations, whereas posttreatment in a similar type of chamber caused a slight decline in chromatid breaks alone. Heat treatment of pollen (40°C for 30 min) prior to ultraviolet radiation exposure resulted in an increased aberration frequency; a similar postultraviolet heat treatment decreased aberration frequencies. Pretreatment with light in the 3000 to 6000 A range led to a reduction in the ultraviolet aberration frequencies, whereas pretreatment with radiation in the 3000 to 4000 A band was ineffective. An unusual observation was that dehydrated pollen which received the radiation pretreatment in the 3000 to 6000 A region and no ultraviolet exposure had a high aberration frequency, whereas wet pollen under similar conditions (i.e., exposed to 50% humid atmosphere) did not.

Discussion. – The effects of humidity and heat correlate with factors affecting the magnitude of the synergistic effect previously reported.[6] In contrast to the direct dependence of this synergism on pretreatment with visible light in the wavelengths 4000 to 6000 A, the present experiments show a reduction in the ultraviolet aberration frequencies. Although the experiments with visible radiation show a variation in the frequency of isochromatid aberrations, the percentage of incomplete isochromatids remains constant. This factor would tend to rule out a simple explanation based on interference with a chromosome rejoining mechanism. The interpretation of the results with visible protection appears to be compatible with a mechanism similar to that proposed for photoprotection in certain bacteria. Further studies are being carried out on the elucidation of the action spectrum of this effect.

Biological and Physical Ranges of Photoprotection in Bacteria

John Jagger R. S. Stafford

Introduction. – Photoprotection (PP) is a phenomenon in which irradiation with near-ultraviolet light (near uv) diminishes the amount of biological damage incurred by *subsequent* irradiation with

[6] J. S. Kirby-Smith, B. Nicoletti, and M. L. Gwyn, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, pp 197–98.

far-ultraviolet light (uv). The present work is an investigation of the occurrence of PP among the bacteria and of the wavelengths of light involved.

Results. – Table 26 lists bacteria in which PP has been sought. In attempts to photoprotect *P. fischeri*, which is very sensitive to killing by near uv, many different light bands throughout the range 3000–6000 A were tried, but none produced PP. The protection found with *P. aeruginosa* seems more satisfactory than that found with *E. coli* B, for the former occurs with plating on plain agar and the uv survival curve is exponential and highly reproducible, whereas the latter requires salt agar and the survival curves are complex and highly dependent on environment both before and after uv irradiation.

Table 26. Photoprotection in Bacteria

Order	Family	Genus, Species, Strain	Photoreactivation	Photoprotection
Pseudomonadales	Pseudomonadaceae	*Pseudomonas aeruginosa*	+ (*)	+ (*)
		Photobacterium fischeri	+ (*)	– (*)
Eubacteriales	Enterobacteriaceae	*Escherichia coli* B	+	+
		B/r	+	–
		K12(λ)	+	+
		K12S		–
	Micrococcaceae	*Staphylococcus aureus* (induction)	+	+
Actinomycetales	Streptomycetaceae	*Streptomyces griseus* sp.	+	– (*)

*Determination made in this laboratory.

The action spectrum of photoprotection from killing in *E. coli* B was determined. Eleven light bands, of which seven were effective, were used, and in the latter case, the bands were all 100 A wide, with no overlap between adjacent bands. The bacteria were handled as reported by Jagger,[7] the plating being done on salt agar. The average uv survival was about 4%, from which level PP curves rose, at least for the most effective wavelengths, with almost linear initial slope on a plot of logarithm of survival vs dose. Since the uv survival level could not be reproduced with accuracy from one experiment to the next, the criterion chosen was the inverse of the number of incident quanta required to produce an effective dose-reduction factor (DRF) of 0.6, which is proportional to the efficiency per incident quantum. Usually, a complete spectrum was done in each experiment in order to minimize biological variation. These complete spectra were plotted logarithmically and then displaced vertically to obtain the best fit. In only one case did this displacement amount to more than 15%. Figure 23 shows the action spectrum. It is based upon six experiments, which yielded five points for each effective wavelength. The curve is normalized to 100% at 3341 A, which wavelength was included in all experiments.

[7] J. Jagger, *Radiation Research* 13, 521–39 (1960).

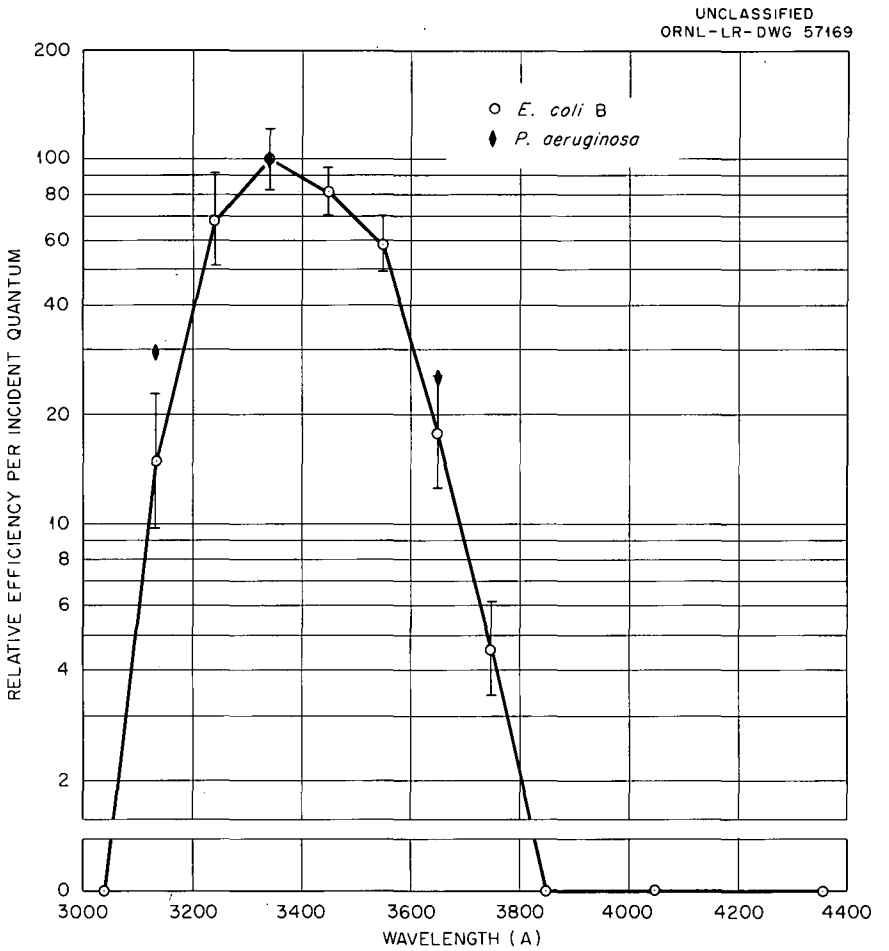

Fig. 23. Action Spectra for Photoprotection from Killing in *Escherichia coli* B and *Pseudomonas aeruginosa*. Both spectra normalized to 100% at 3341 A. Errors shown are standard deviation from the mean.

Using exactly the same procedure, except that a DRF of 0.75 was used, and only four wavelengths were tested, a rough action spectrum for PP from killing in *P. aeruginosa* was obtained. The four points are shown in Fig. 23, each point representing at least three determinations.

Discussion. – Table 26 shows that PP is not as universal an effect among the bacteria as is photoreactivation; on the other hand, it is not an isolated phenomenon.

Figure 23 shows that the action spectrum for PP is very narrow and of simple form, having a single bell-shaped peak. The curve is much narrower than for photoreactivation in *E. coli* B/r (note difference in strain), for which 4047-A light is very effective. As with the photoreactivation spectrum, the sharp drop at low wavelength is due to superimposed killing. If one makes a correction for killing, the PP efficiency remains high in this region. The similarity of the spectra for PP in two organisms from different orders of bacteria suggests that the chromophore may be the same in all bacteria.

Our present knowledge of PP supports but does not prove the hypothesis that the chromophore is nucleic acid.

The Intracellular State of the Virus ϕX174

R. B. Setlow

Introduction. – The results of experiments on the irradiation of nucleic acids by ultraviolet light of wavelength 2537 A indicate that the pyrimidines, cytosine and thymine, are destroyed with much higher quantum efficiencies than are the purines, adenine and guanine.[8] A detailed investigation of the action spectrum for inactivation of the single-stranded DNA-containing virus, ϕX174, indicated that the major inactivating events arose from quanta absorbed in cytosine and thymine.[9] These results led to the hypothesis that the action spectra for affecting single-stranded polynucleotide chains should be different from those for affecting double-stranded chains.[10] The hypothesis has been confirmed by experiments on the action spectrum for inactivation of transforming principle DNA in double- and single-stranded states[11] and has been used to identify a single-stranded stage in the development of intracellular T2 phage (a double-stranded virus).[12] In an attempt to determine the strandedness of developing ϕX174 in its host, *E. coli* C, the virus-host complex has been irradiated by monochromatic ultraviolet light at various times during the latent period.

Results and Discussion. – The normal minimum latent period is 15.0 min. Action spectra for inactivation of virus-host complexes have been obtained on complexes of ages 0.7, 3.0, 4.0, and 6.0 min. At later times, the complexes making up the irradiated suspension are not all at the same stage of development because of a spread in latent periods among the complexes. As development proceeds, not only does the sensitivity to ultraviolet light decrease, but the *shape* of the action spectrum changes. Extracellular phage and 0.7-min complexes have spectra similar to those for single-stranded polynucleotides, but, from 3 min on, the spectra indicate that the sensitive component is a double-stranded polynucleotide. Thus this initially single-stranded virus goes through a double-stranded state during its multiplication, but there is no indication as to whether the double strand represents two single strands of DNA, one of DNA and one of RNA, or one strand of DNA folded back on itself. Sinsheimer[13] has obtained density-gradient centrifugation data which indicate that at after 7 min of development the viral DNA is in a double-strand structure.

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[8] D. Shugar, in *The Nucleic Acids*, vol III (ed. by E. Chargaff and J. N. Davidson), Academic Press, New York, 1960.

[9] R. Setlow and R. Boyce, *Biophys. J.* 1, 29 (1960).

[10] R. Setlow, *Biochim. et Biophys. Acta* 39, 180 (1960).

[11] J. K. Setlow and R. B. Setlow, this report.

[12] J. K. Setlow and R. B. Setlow, *Proc. Natl. Acad. Sci. U.S.* 46, 791 (1960).

[13] R. L. Sinsheimer, Abstracts, Biophysical Society, St. Louis, Mo., Feb. 16–18, 1961.

The Destruction of Thymine Dimers by Ultraviolet Light

R. B. Setlow

Introduction. – If thymine in frozen solution is irradiated by 2537-A radiation, the major photoproduct formed is a thymine dimer which has a very low absorption coefficient for wavelengths above 2700 A.[14] If the dimer in liquid solution is irradiated by ultraviolet light, it is split, and two thymine molecules are produced.[15] In an attempt to determine the relations between this reversible photochemical reaction and the biological process of photoreactivation, the action spectrum for splitting the thymine dimer has been obtained.

Results and Discussion. – The absorption coefficient of the purified dimer increases monotonically as the wavelength decreases. There is no absorption maximum at 2600 A. The action spectrum is very similar to the absorption spectrum. The quantum yield for splitting the dimer varies slightly, but not beyond systematic experimental errors, with wavelength. Table 27 shows a summary of the results of these experiments. It is apparent that the quantum yield for the splitting

Table 27. Parameters of Thymine Dimer at Different Wavelengths

Wavelength (A)	Absorption Coefficient (10^{-18} cm^2)	Sensitivity to Splitting (10^{-18} cm^2)	Quantum Yield
2300	7.9	7	0.89
2375	4.3	2.4	0.56
2482	1.75	0.95	0.54
2537	1.1	0.70	0.64
2652	0.22	0.055	0.25
2805		0.0024	
3131		<0.0003	

is very high and that longer wavelengths are ineffective because they are not absorbed. This process does not have the same wavelength sensitivity as photoreactivation, for which the most effective wavelengths are above 3000 A. Nevertheless, the formation of thymine dimers by ultraviolet light may be important in biological inactivation, because such dimers have been isolated from irradiated DNA.[16] It is pertinent that it has not been possible to form such dimers with 5-bromouracil.

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