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For Period Ending August 15, 1957

Alexander Hollaender, Director

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PUBLICATIONS AND LECTURES

Publications. – The Symposium on Biocolloids (1956) was completed and published during the period and the Symposium on "Antibodies: Their Production and Mechanism of Action" was prepared and sent to the publisher. In addition, 63 papers, a semiannual report, and 24 abstracts have been published since the middle of February. Seventy-five papers and 14 abstracts are in press.

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Anderson, N. G.	Cell Division. Part II. A Theoretical Approach to Chromosomal Movements and the Division of the Cell	*Quart. Rev. Biol.* **31**, 243–269 (1957)
———	Labile Colloidal Complexes of the Cytoplasm	*J. Cellular Comp. Physiol.* **49**, Suppl. 1, 221–241 (1957)
Astrachan, L., and E. Volkin (with technical assistance of M. H. Jones)	Chromatographic Detection of Differences Between Bacteriophage-Related Deoxyribonucleic Acids	*J. Am. Chem. Soc.* **79**, 130–134 (1957)
Atwood, K. C., R. C. von Borstel, and A. R. Whiting	An Influence of Ploidy on the Time of Expression of Dominant Lethal Mutations in Habrobracon	*Genetics* **41**, 804–813 (1956)*
Baker, W. K.	Chromosome Segregation in *Drosophila virilis* Males with Multiple Sex Chromosomes	*Genetics* **41**, 907–914 (1956)
Billen, D.	Modification of the Release of Cellular Constituents by Irradiated *E. coli*	*Arch. Biochem. and Biophys.* **67**, 333–340 (1957)
———	Recovery of Lethally Irradiated Mice by Treatment with Bone Marrow Cells Maintained *in vitro*	*Nature* **179**, 574–575 (1957)
von Borstel, R. C.	Nucleocytoplasmic Relationships in Early Insect Development	*The Beginnings of Embryonic Development* (eds. A. Tyler, R. C. von Borstel, and C. B. Metz), Publ. No. 48 AAAS, Washington, D.C., 1957, p 175–199
Brosseau, G. E., Jr.	Fast Green as a Useful Counterstain for Neuroblast and Salivary Smears	*Drosophila Info. Serv.* **30**, 160 (1956)
———	The Recovery of Detachments from a Reversed Acrocentric Compound-X Chromosome	*Drosophila Info. Serv.* **30**, 106 (1956)
Cohn, W. E.	Nucleic Acid Derivatives	*Ion Exchangers in Organic and Biochemistry* (eds. C. Calmon and T. R. E. Kressman), p 345–359, Interscience Publishers, Inc., New York, 1957

*Publications bearing 1956 date did not appear until 1957.

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Cohn, W. E., and E. Volkin	Acid Degradation Products of Deoxyribonucleic Acid	*Biochim. et Biophys. Acta* **24**, 359–364 (1957)
———	The Nucleic Acids	*Ann. Rev. Biochem.* **26**, 491–522 (1957)
Congdon, C. C., T. Makinodan, and N. Gengozian	Effect of Injection of Rat Bone Marrow on Reticular Tissues of Mice Exposed to X Radiation in the Midlethal Dose Range	*J. Natl. Cancer Inst.* **18**, 603–613 (1957)
Congdon, C. C., and I. S. Urso	Homologous Bone Marrow in the Treatment of Radiation Injury in Mice	*Am. J. Pathol.* **33**, 749–767 (1957)
Conger, A. D.	Some Cytogenetic Aspects of the Effects of Plant Irradiation	Symposium on Radiation in Plant Breeding (Sponsored by University of Tennessee and Oak Ridge Institute of Nuclear Studies, Jan. 1957), p 59–62
de Serres, F. J.	Genetic Studies of Purple Adenine Mutants in *Neurospora crassa*	*Microbial Genetics Bull.* **14**, 8–9 (1956)
DiLuzio, N. R., and K. A. Simon	The Effect of X Irradiation on the Plasma Lipide Fractions of the Rabbit	*Radiation Research* **7**, 79–84 (1957)
Doherty, D. G., W. T. Burnett, Jr., and R. Shapira	Chemical Protection Against Ionizing Radiation. II. Mercaptoalkylamines and Related Compounds with Potential Protective Activity	*Radiation Research* **7**, 13–21 (1957)
Dolin, M. I.	The *Streptococcus faecalis* Oxidases for Reduced Diphosphopyridine Nucleotide. III. Isolation and Properties of a Flavin Peroxidase for Reduced Diphosphopyridine Nucleotide	*J. Biol. Chem.* **225**, 557–573 (1957)
Edington, C. W.	The Induction of Recessive Lethals in *Drosophila melanogaster* by Radiations of Different Ion Density	*Genetics* **41**, 814–821 (1956)
———	The Production of Dominant Lethals by Gamma Rays, 1-Mev and 14-Mev Neutrons	*Drosophila Info. Serv.* **30**, 111 (1956)
Gengozian, N., and T. Makinodan	Antibody Response of Lethally X-Irradiated Mice Treated with Rat Bone Marrow	*J. Immunol.* **77**, 430–436 (1956)
Green, J. A.	Ovarian Weight and Responsiveness to Gonadotrophin Throughout the Estrous Cycle of Mice	*Proc. Soc. Exptl. Biol. Med.* **95**, 504–506 (1957)
Gude, W. D	Controlled Humidity of Autoradiographic Stripping Film During Storage	*Stain Technol.* **32**, 197 (1957)
Haber, A. H., and N. E. Tolbert	Photosynthesis in Gibberellin-Treated Leaves	*Plant Physiol.* **32**, 152 (1957)
Hinton, C. W.	The Analysis of Rod Derivatives of an Unstable Ring Chromosome of *Drosophila melanogaster*	*Genetics* **42**, 56–65 (1957)
Hollaender, A. (Division Director)	Symposium on Biocolloids	*J. Cellular Comp. Physiol.* **49**, Suppl. 1 (1957)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Hollaender, A., and S. F. Carson	Biology Division Semiannual Progress Report for Period Ending Feb. 15, 1957	ORNL-2267
Hollcroft, J., E. Lorenz, E. Miller, C. C. Congdon, R. Schweisthal, and D. Uphoff	Delayed Effects in Mice Following Acute Total-Body X Irradiation: Modification by Experimental Treatment	*J. Natl. Cancer Inst.* **18**, 615–640 (1957)
Khym, J. X., L. P. Zill, and W. E. Cohn	Separation of Carbohydrates	*Ion Exchangers in Organic and Biochemistry* (eds. C. Calmon and T. R. E. Kressman), p 392–417, Interscience Publishers, Inc., New York, 1957
Kimball, A. W., W. T. Burnett, Jr., and D. G. Doherty	Chemical Protection Against Ionizing Radiation. I. Sampling Methods for Screening Compounds in Radiation Protection Studies with Mice	*Radiation Research* **7**, 1–12 (1957)
Kirby-Smith, J. S.	"Actions Chimiques et Biologiques des Radiations," Collection dirigée par M. Haissinsky	*Am. J. Roentgenol., Radium Therapy, and Nuclear Med.* **75**, 1012 (1956) (Book Review)
Kirby-Smith, J. S. and D. L. Craig	The Induction of Chromosome Aberrations in Tradescantia by Ultraviolet Radiation	*Genetics* **42**, 176–187 (1957)
Lindsley, D. L., and E. Novitski	Influence of the Proximal Regions of the Fourth Chromosomes on Their Meiotic Behavior	*Drosophila Info. Serv.* **30**, 130–131 (1956)
Lindsley, D. L., and L. Sandler	The Effect of a Free Heterochromatic X-Chromosome Duplication on the Disjunction of Normal Fours	*Drosophila Info. Serv.* **30**, 131–132 (1956)
Melville, G. S., Jr., F. P. Conte, M. Slater, and A. C. Upton	Acute Lethality of Mice as Influenced by the Periodicity of Paired Exposures to Fast Neutrons or X Rays	*Brit. J. Radiol.* **30**, 196–199 (1957)
Morris, D. M., F. F. Wolff, and A. C. Upton	The Influence of the Thyroid Gland on the Survival of Rats and Mice Bearing Transplanted Lymphoid Leukemia	*Cancer Research* **17**, 325–328 (1957)
Novelli, G. D.	Water-Soluble Vitamins. Part III. Pantothenic Acid, Thiamine, Lipoic Acid (Thioctic Acid), Riboflavin, and Inositol	*Ann. Rev. Biochem.* **26**, 243–274 (1957)
Novitski, E.	The Possibility of Another Cytoplasmically Inherited Factor in *D. melanogaster*	*Drosophila Info. Serv.* **30**, 143 (1956)
Novitski, E., and I. Sandler	Are All Products of Spermatogenesis Regularly Functional?	*Proc. Natl. Acad. Sci. U.S.* **43**, 318–323 (1957)
Oakberg, E. F.	A Description of Spermiogenesis in the Mouse and Its Use in Analysis of the Cycle of the Seminiferous Epithelium and Germ Cell Renewal	*Am. J. Anat.* **99**, 391–414 (1956)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
———	Duration of Spermatogenesis in the Mouse and Timing of Stages of the Cycle of the Seminiferous Epithelium	*Am. J. Anat.* **99**, 507–516 (1956)
———	Gamma-Ray Sensitivity of Spermatogonia of the Mouse	*J. Exptl. Zool.* **134**, 343–356 (1957)
Odell, T. T., Jr., R. D. Owen, F. G. Tausche, and D. L. Lindsley	The Homotransplantation of Functional Erythropoietic Elements in the Rat Following Total Body Irradiation	*Ann. N.Y. Acad. Sci.* **64**, 811–823 (1957)
Randolph, M. L.	Energy Deposition in Tissue and Similar Materials by 14.1-Mev Neutrons	*Radiation Research* **7**, 47–57 (1957)
Randolph, M. L., C. C. Congdon I. S. Urso, and D. L. Parrish	Effect of Bone Marrow Treatment on Mortality of Mice Irradiated with Fast Neutrons	*Science* **125**, 1083–1084 (1957)
Russell, L. B.	Effects of Low Doses of X Rays on Embryonic Development in the Mouse	*Proc. Soc. Exptl. Biol. Med.* **95**, 174–178 (1957)
Russell, L. B., and M. H. Major	Radiation-Induced Presumed Somatic Mutations in the House Mouse	*Genetics* **42**, 161–175 (1957)
Russell, W. L.	Shortening of Life in the Offspring of Male Mice Exposed to Neutron Radiation from an Atomic Bomb	*Proc. Natl. Acad. Sci. U.S.* **43**, 324–328 (1957)
Sandler, I.	Segregation in Females Heterozygous for $T(1;4)B^S$	*Drosophila Info. Serv.* **30**, 151 (1956)
Sandler, L.	Additional Evidence on the Role of the Centromere in Determining Disjunctional Patterns	*Drosophila Info. Serv.* **30**, 150 (1956)
Sandler, L., and E. Novitski	Meiotic Drive as an Evolutionary Force	*Am. Naturalist* **91**, 105–110 (1957)
Shapira, R., D. G. Doherty, and W. T. Burnett, Jr.	Chemical Protection Against Ionizing Radiation. III. Mercaptoalkylguanidines and Related Isothiuronium Compounds with Protective Activity	*Radiation Research* **7**, 22–34 (1957)
Sheppard, C. W., M. Slater, E. B. Darden, A. W. Kimball, G. J. Atta, C. W. Edington, and W. K. Baker	Biological Effects of Fast Neutrons from an Internal Target Cyclotron: Physical Methods and Dominant Lethals in Drosophila	*Radiation Research* **6**, 173–187 (1957)
Slater, J. V.	Radiocobalt Accumulation in *Tetrahymena*	*Biol Bull.* **112**, 390–399 (1957)
Smith, L. H., and C. C. Congdon	Leukemoid Blood and Leukocytes in the Treatment of Radiation Lethality	*Arch. Pathol.* **63**, 502–507 (1957)
Smith, L. H., T. Makinodan, and C. C. Congdon	Circulating Rat Platelets in Lethally X-Radiated Mice Given Rat Bone Marrow	*Cancer Research* **17**, 367–369 (1957)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Upton, A. C., and J. Furth	Host Factors in the Pathogenesis of Leukemia in Animals and in Man	*Proceedings of the Third National Cancer Conference*, p 312–324, J. B. Lippincott Company, Philadelphia, 1957
Urso, I. S., and C. C. Congdon	Short Term Preservation of Mouse Bone Marrow at Refrigeration and Room Temperature for Irradiation Experiments	*J. Appl. Physiol.* **10**, 314–316 (1957)
Urso, P., and C. C. Congdon	The Effect of the Amount of Isologous Bone Marrow Injected on the Recovery of Hematopoietic Organs, Survival, and Body Weight After Lethal Irradiation Injury in Mice	*Blood* **12**, 251–260 (1957)
Volkin, E., and L. Astrachan	RNA Metabolism in T2-Infected *Escherichia coli*	*The Chemical Basis of Heredity* (eds., W. D. McElroy and H. B. Glass), p 686–695, The Johns Hopkins Press, Baltimore, 1957
Ward, C. L., and M. L. Alexander	Cytological Analysis of X-Ray-Induced Mutations at Eight Specific Loci in the Third Chromosome of *Drosophila melanogaster*	*Genetics* **42**, 42–54 (1957)
Welshons, W. J.	N^{Co}:Notch-Confluens Welshons. 1-3.0	*Drosophila Info. Serv.* **30**, 79 (1956)
Welshons, W. J., and W. L. Russell	The Effect of X-Rays on the Drosophila Testis and a Method for Obtaining Spermatogonial Mutation Rates	*Proc. Natl. Acad. Sci. U.S.* **43**, 608–613 (1957)

Scientific Society Lectures and Traveling Seminars. – Exclusive of the lectures given at the Summer Institute of Radiobiology, mentioned in the section on **Education**, 102 lectures were given on Biology Division work during the period of this report.

AUTHOR(S)	TITLE	PLACE PRESENTED
Adler, H. I.	Gamma-Ray Resistance of a Hemin Dependent Bacterium [*Bacteriol. Proc.* (1957), p 50]	Soc. Am. Bacteriol., Detroit
Amy, R. L., and R. C. von Borstel	The Effects of Different Wave Lengths of Ultraviolet Light on the Habrobracon Egg	2nd Intern. Congr. Photobiol., Turin, Italy
Anderson, N. G.	Cellular Molecular Populations	AIBS Conf. on Basic Problems of Biological Aging, Gatlinburg
———	Techniques for Isolating Cell Components	Gordon Research Conf. on Cell Structure and Metabolism, Kimball Union Academy, Meriden, N.H.
Astrachan, L., and E. Volkin	RNA Turnover and DNA Synthesis in Bacteriophage-Infected Bacteria [*Federation Proc.* **16**, 147, (1957)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Chicago
Atwood, K. C.	Radiation and Tracer Applications in Biological Research	Conf. on Peaceful Uses of Atomic Energy, Tokyo

AUTHOR(S)	TITLE	PLACE PRESENTED
———	Somatic Mutation Theories of Aging	AIBS Conf. on Basic Problems of Biological Aging, Gatlinburg
Benedict, W. H., K. W. Christenberry, and A. C. Upton	Ocular Effects of Ionizing Radiation	Inter-American Symposium (sponsored by Brookhaven National Laboratory), Long Island
Billen, D.	The Use of Bone Marrow Cells Maintained *in vitro* to Modify X-Radiation Damage in Mice	Radiation Research Soc., Rochester
———	As above	Dept. of Bacteriol., University of Tennessee, Knoxville
———	The Use of the Tissue Culture Method for the Maintenance of Mammalian Cells Capable of Modifying X-Ray Damage in Mice	Dept. of Biology, M. D. Anderson Hospital and Research Institute, Houston
Bradford, R. H., R. Shapira, and D. G. Doherty	Selective Intracellular Binding of Radiation-Protective Agents by Mammalian Tissues [*Federation Proc.* 16, 157 (1957)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Chicago
Bruce, A. K.	Comparative Effects of Far-Ultraviolet, Near-Ultraviolet, and X Irradiation on Potassium Rententivity and Survival in Yeast	Radiation Research Soc., Rochester
———	Effects of Radiation on Potassium Exchange and Survival in Yeast	Dept. of Bacteriology, Pennsylvania State University, State College
———	As above	Dept. of Biology, University of Buffalo
Carson, S. F.	Mechanism of Carboxylation Reactions by Propionic Acid Bacteria	Biochemical Research group, Harvard University
———	Succinic Acid Decarboxylation Systems	Hopkins Marine Station, Stanford University
Cohn, W. E.	Minor Constituents of Ribonucleic Acids [*Federation Proc.* 16, 166 (1957)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Chicago
Congdon, C. C.	Bone Marrow Transplantation in Animals Exposed to Whole-Body Radiation	Tenth Ann. Biol. Symp. on "Antibodies: Their Production and Mechanism of Action," Gatlinburg
———	Pathologic Findings in the Delayed Heterologous Bone Marrow Reaction	Radiation Research Soc., Rochester
———	Radiation as a Factor in Experimental Lymphomas and Leukemias [*Proceedings on the Third National Cancer Conference*, p 330, J. B. Lippincott Company, Philadelphia (1957)]	3rd National Cancer Conf., Detroit
Congdon, C. C., and D. G. Doherty	Experimental Treatment of Radiation Injury	Brookhaven National Laboratory, Long Island (Exhibit for Inter-American Symposium on Peaceful Uses of Atomic Energy)

AUTHOR(S)	TITLE	PLACE PRESENTED
Congdon, C. C., T. Makinodan, and N. Gengozian	Causes of Death in Sublethally Irradiated Mice Given Rat Bone Marrow [*Federation Proc.* 16, 354 (1957)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Exptl. Pathol.), Chicago
Cormier, M. J., and J. R. Totter	Quantum Efficiency Determinations on Components of the Bacterial Luminescence System [*Federation Proc.* 16, 167 (1957)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Chicago
Davis, J. W., A. N. Best, and G. D. Novelli	The Activation of Amino Acids in Plant and Animal Tissues [*Federation Proc.* 16, 170 (1957)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Chicago
Dent, J. N., and C. W. Sheppard	Distribution of Lithium^6 in Gastrulae of *Amblystoma punctatum* [*Anat. Record* 127, 285 (1957)]	Am. Assoc. Anat., Baltimore
Doherty, D. G.	Chemical Protection of Biological Systems from Ionizing Radiation	Dept. of Electrical Engineering (Bioacoustics Laboratory), University of Illinois, Champaign
———	As above	Basic Science Seminar, University of Nebraska College of Medicine, Omaha
———	As above	School of Biological Sciences, University of Tennessee, Memphis
———	As above	Departments of Biological Sciences, University of Texas, Austin
———	Sulfhydryl Compounds and Chemical Protection Against Ionizing Radiation	Eastman Kodak Company, Rochester
Dolin, M. I.	DPNH Oxidation by *Clostridium perfringens* [*Bacteriol. Proc.* (1957), p 110]	Soc. Am. Bacteriol., Detroit
———	DPNH Oxidation Reactions of Facultative and Strict Anaerobes	Edsel Ford Institute of Medical Research, Detroit
Fairbanks, A. J., Jr.	Paramagnetic Resonance Determination of Free Radicals in Irradiated Cells With and Without a Protective Agent	Radiation Research Soc., Rochester
———	The Role of Free Radicals in Radiation Protection	National Biophysical Conf., Columbus, Ohio
Gaulden, M. E.	Discussion Parts: Work Conference on Mitogenesis, Argonne National Laboratory, September 1955	Work Conference on Mitogensis, Argonne National Laboratory
———	Effects of Low Doses of X Rays on Mitosis as Observed in Living Neuroblasts of Grasshopper	French Atomic Energy Installation, Saclay, France
———	Study of Deoxyribonucleic Acid Synthesis and X-Ray Effects in Single Mitotic Cells with Thymidine 2-C^{14}	1st Pan-American Cytology Congress, Miami
Gengozian, N., and T. Makinodan	Mortality of Mice, as Affected by Varying the X-Ray Dose and Number of Nucleated Rat Bone Marrow Cells Injected	Radiation Research Soc., Rochester

AUTHOR(S)	TITLE	PLACE PRESENTED
Gude, W. D., and A. C. Upton	Modification of Radiation-Induced Intestinal Injury in Rats by Chilling After Irradiation	Radiation Research Soc., Rochester
Hollaender, A.	Applications of Nuclear Energy to Medicine	Comitato Nazionale per le Ricerche Nucleari, Rome
———	Discussion of Radiation Protection and Recovery Investigations in the Biology Division	Dept. of Zoology, University of Milan, Milan, Italy
———	As above	Mount Vernon Hospital, Northwood, Middlesex, England
Jagger, J., and G. E. Stapleton	Compared Effects on Bacteria, with Special Reference to Protection and Restoration	2nd Intern. Photobiol. Congr., Turin, Italy
Kirby-Smith, J. S., and M. L. Randolph	The Relative Effects of Gamma Rays, Fast Neutrons and α Particles on Free Radical Production [*Bull. Am. Phys. Soc.* **2** (Ser. 11), 278 (1957)]	Am. Phys. Soc. (Southeastern Section), Lexington, Kentucky
Limbaugh, B., and M. E. Gaulden	The Lethal Effects of X Rays on the Neuroblasts and Embryos of the Grasshopper *Chortophaga viridifasciata*	Assoc. Southeastern Biologists, Athens, Georgia
Lindsley, D. L.	Transplantation of Tissues into Irradiated Mammals	Dept. of Biology, University of Colorado, Boulder
———	As above	Denver Medical Center, Denver
Makinodan, T.	Effect of Irradiation on Immune Response	Bacteriol. Soc., University of Kentucky, Lexington
———	Radiation Immunology	Society for Research on RES, Chicago
———	Fate of Injected Rat Bone Marrow Cells in Irradiated Mice	Tenth Ann. Biol. Symp. on "Antibodies: Their Production and Mechanism of Action," Gatlinburg
———	Immunology of Bone Marrow Transplantation	As above
Makinodan, T., and I. Shekarchi	Effect of X Irradiation on the Antigen-Host Genetic Relationship [*Federation Proc.* **16**, 423 (1957)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Immunol.), Chicago
Novelli, G. D.	Activation of Amino Acids	Dept. of Biochemistry, Duke University, Durham
———	The Activation of Amino Acids in the Biosynthesis of Proteins	Dept. of Biochemistry, Emory University, Georgia
———	As above	Am. Chem. Soc. (Pittsburgh Section), Pittsburgh
Novelli, G. D., and J. A. DeMoss	The Activation of Amino Acids and Concepts of the Mechanism of Protein Synthesis	Tenth Ann. Biol. Symp. on "Antibodies: Their Production and Mechanism of Action," Gatlinburg
Oakberg, E. F.	Survival of the Spermatogonia of the Mouse Following Low Radiation Exposure	Beta Beta Beta Chapter, Berea College, Kentucky

AUTHOR(S)	TITLE	PLACE PRESENTED
Odell, T. T., Jr.	Production, Life Span, and Fate of Blood Platelets: Studies with Radioisotope Labeling Techniques	Dept. of Biological Sciences, Florida State University, Tallahassee
———	As above	Dept. of Biology, University of Florida, Gainesville
———	As above	Dept. of Zoology, Louisiana State University, Baton Rouge
Odell, T. T., Jr., and B. C. Caldwell	Influence of Dose of Total-Body Irradiation on Successful Transplantation of Homologous Bone Marrow in Rats	Fed. Am. Soc. Exptl. Biol. (Am. Physiol. Soc.), Chicago
Perry, R. P.	Changes in the Ultraviolet Absorption Spectrum of Parts of Living Cells Following Irradiation with an Ultraviolet Microbeam	Natl. Biophysical Conf., Columbus, Ohio
Phares, E. F., R. J. Koeppe, and S. F. Carson	Intermediate Compounds in the Conversion of Succinate to Propionate and Carbon Dioxide by Bacterial Extracts [*Bacteriol. Proc.* (1957), p 128]	Soc. Am. Bacteriol., Detroit
Randolph, M. L.	Interactions of 14.1-Mev Neutrons with Biological Tissue [*Bull. Am. Phys. Soc.* **2** (Ser. II), 278 (1957)]	Am. Phys. Soc. (Southeastern Section), Lexington, Kentucky
———	Presentation of Radiobiological Effect Versus Physical Characteristics of Radiation	Radiation Research Soc., Rochester
Russell, L. B.	Genetic Considerations in the Practice of Ovarian Irradiation for the Treatment of Sterility	Am. Soc. for the Study of Sterility, New York City
———	Radiation Hazards to the Embryo and Fetus	The Medical Circle, New York Acad. Med., New York City
Russell, W. L.	Genetic Hazards of Radiation	The Medical Circle, New York Acad. Med., New York City
———	As above	Am. Nuclear Soc., Pittsburgh
———	Spontaneous and Induced Mutations and the Problems of Measuring Their Frequencies and Effects	Natl. Acad. Sci. Symp. on Genetics with Special Reference to Radiation Hazards, Washington, D.C.
Schwartz, D.	Cytogenetic Studies with Maize	Dept. of Botany, Duke University, Durham
———	As above	Genetics Seminar, University of Kentucky, Lexington
Sheppard, C. W.	Catheter Artifacts in Indicator Dilution Experiments [*Federation Proc.* **16**, 118 (1957)]	Fed. Am. Soc. Exptl. Biol. (Am. Physiol. Soc.), Chicago
———	An Electromathematical Theory of Circulatory Mixing Transients	Natl. Biophys. Conf., Columbus, Ohio
———	Realities of Biophysics	Am. Phys. Soc. (Southeastern Section), Lexington, Kentucky

AUTHOR(S)	TITLE	PLACE PRESENTED
———	Use of Isotopes in Experimental Pathology	East Tennesse Assoc. Pathologists, Oak Ridge
Smith, L. H.	Modification of Irradiation Damage by Hematopoietic Tissue	Dept. of Zoology, Syracuse University, Ithaca
———	The Recovery Action of Leukemoid Blood	Radiation Research Soc., Rochester
Smith, L. H., T. Makinodan, and C. C. Congdon	Rat Platelets in Irradiated, Rat Bone Marrow-Injected Mice [*Federation Proc.* 16, 120–121 (1957)]	Fed. Am. Soc. Exptl. Biol. (Radiobiol. Intersociety Meeting), Chicago
Sproul, J. A., Jr., and M. L. Randolph	Relative Biological Effectiveness of 14-Mev Neutrons for Acute Lethality in Mice	Radiation Research Soc., Rochester
Stapleton, G. E.	Basic Mechanisms in Radiation Damage to Living Cells	Dept. of Biology, Marquette University, Milwaukee
———	Basic Studies on Radiation Effects on Living Cells	Am. Nuclear Soc. (Special Session on Biological Effects of Radiation), Pittsburgh
Stapleton, G. E., and D. H. Woodbury	Protection of *Escherichia coli* B/r Against Gamma-Ray Inactivation by β-Mercaptoethylamine [*Bacteriol. Proc.* (1957), p 50]	Soc. Am. Bacteriol., Detroit
Tolbert, N. E.	Phosphorylcholine in Plants [*Federation Proc.* 16, 262 (1957)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Chicago
Tolbert, N. E., and F. R. Gailey	Greening of Normal and Gamma-Irradiated Etiolated Plants in the Light	2nd Intern. Photobiol. Congr., Turin, Italy
Tolbert, N. E., and R. Rabson	Light Control of Glycolate Metabolism and Excretion	2nd Intern. Photobiol. Congr., Turin, Italy
Upton, A. C.	Ionizing Radiation and the Aging Process	AIBS Conf. on Basic Problems of Biological Aging, Gatlinburg
———	Hazards to Man of Ionizing Radiations	Dept. of Biology, Spring Hill College, Mobile
Upton, A. C., R. F. Buffett, J. Furth, and D. G. Doherty	Radiation-Induced "Dental Death" in Mice [*Federation Proc.* 16, 274–375 (1957)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Exptl. Pathol.), Chicago
Upton, A. C., K. W. Christenberry, R. J. Elliott, and J. A. Sproul	Comparison of Acute and Chronic Gamma Irradiation in the Induction of Lens Opacities in Mice	Radiation Research Soc., Rochester
Upton, A. C., F. F. Wolff, and J. Furth	The Influence of Splenectomy Preirradiation on the Induction of Leukemia in RF Mice by X Radiation	Am. Assoc. Cancer Research, Chicago
Urso, I. S., N. Gengozian, and C. C. Congdon	Thymus Specificity of Lethally Irradiated Mice Treated with Rat Bone Marrow	Radiation Research Soc., Rochester

AUTHOR(S)	TITLE	PLACE PRESENTED
Urso, P.	The Effect of Chemical Protection and Bone Marrow Treatment on the Response of the Hematopoietic Organs and Body Weight in X-Irradiated Mice	Radiation Research Soc., Rochester
Volkin, E.	RNA Metabolism in Bacteriophage-Infected coli	Dept. of Biochemistry, University of Illinois, Urbana
Whiting, A. R., S. Caspari, M. Koukides, and P. Kao	Time of Death of X-Ray-Induced Embryo Lethals in Haploid and in Heterozygous Habrobracon	Radiation Research Soc., Rochester
Wolff, S.	Biochemical Aspects of Chromosome Break Rejoining	Depts. of Bacteriology, Botany, and Zoology, Indiana University, Bloomington
_____	Chromosome Breakage and Reunion	Dept. of Zoology, Louisiana State University, Baton Rouge
_____	As above	Dept. of Botany, Duke University, Durham
_____	Studies on the Chemical Nature of Radiation-Induced Chromosome Breaks	Radiation Research Soc., Rochester
Woodbury, D. H.	The Atomic Age and the College Student	Open Forum of the Lyceum Committee, Johnson C. Smith University, Charlotte
Zill, L. P	Ultraviolet Inhibition of Sucrose Synthesis in Plants [*Federation Proc.* 16 276 (1957)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Chicago

Visiting Lecturers. – Of the 40 well-known visitors who have lectured to Biology Division groups during this period, 14 have discussed research being done in ten foreign laboratories. This is in accord with the plan of world coordination of research.

LECTURER	AFFILIATION	TOPIC
L. Jacobson	Argonne Cancer Research Hospital, Chicago	The Dynamic Equilibrium of the Erythron
B. Nebel	Argonne National Laboratory, Chicago	1. Microfibrillae in the Chromosome of Iceryine Coccoid Sperm 2. An Entoptic Phenomenon Related to Posterior Vitreous Detachment in Man
J. Mayer	Department of Nutrition, Harvard University School of Public Health, Boston	The Regulation of Food Intake
W. F. Loomis	Loomis Laboratory, Greenwich, Connecticut	pCO_2 Control of Sexual Differentiation in Hydra
T. Shedlovsky	The Rockefeller Institute for Medical Research, New York	Electrical Conductance and pH in Water-Methanol Mixtures

LECTURER	AFFILIATION	TOPIC
J. S. Laughlin	Memorial Center, New York	1. Radiation Chemical Evidence of the Polarization Effect 2. The Gonadal Dose Received by the U.S. Public in Medical X-Ray Exposures
P. B. Medawar	University College, London	Recent Advances in the Immunology of Transplantations
C. S. Pittendrigh	Department of Biology, Princeton University	Biological Clocks
J. A. V. Butler	Chester Beatty Research Institute, Royal Cancer Hospital, London	Recent Works on DNA and the Action of Radiation
G. C. Webster	Department of Agricultural Biochemistry, Ohio State University, Columbus	Discussion of Amino Acid Incorporation into Protein
C. E. Ford	Radiobiological Research Unit, Harwell, England	1. Chromosomes as Cell Tags: Further Observations on Radiation Chimaeras 2. The Cytogenetic Individuality of Some Neoplasms in the Mouse
D. W. van Bekkum	Medical Biological Institute, Rijswijk, The Netherlands	Observations on Bone Marrow Therapy of Irradiated Mice
P. Alexander	Chester Beatty Institute, London	Nucleic Acids
O. G. Fahmy	Pollards Wood Research Station, Buckinghamshire, England	Evidence for Differential Mutagenesis in *Drosophila*
R. F. Ruth	Department of Embryology, Carnegie Institution of Washington, Baltimore	Cellular Changes During Antibody Formation
J. M. Sturtevant	Sterling Chemical Laboratory, Yale University, New Haven	Some Observations on Chymotrypsin and Trypsin Catalyses
P. W. Whiting	University of Pennsylvania, Philadelphia	Recent Developments in Genetics of *Mormoniella*
D. Marrian	Department of Radiotherapeutics, University of Cambridge, Cambridge, England	1. The Metabolic Stability of Deoxyribonucleic Acid 2. Radiosensitizing Agents *in vivo*
B. E. Holmes	Department of Radiotherapeutics, University of Cambridge, Cambridge, England	Attempts at Using the Regenerating Rat Liver as a Material for the Study of the Mitotic Cycle in Mammals
H. A. Itano	National Institutes of Health, Bethesda	Genetic Control of Human Hemoglobin Synthesis
F. Vester	Department of Pharmacology, Yale University School of Medicine, New Haven	A New Approach to the Role of Optical Activity in Living Systems
I. Tipton	Department of Physics, University of Tennessee, Knoxville	Spectrographic Analysis of Human Tissue

LECTURER	AFFILIATION	TOPIC
H. Krauch	Department of Chemistry, Yale University, New Haven	Relation of Cumulenes and Polyethylene to Eye Pigments
F. Vester H. Krauch T. L. V. Ulbricht	Yale University, New Haven	Beta-Ray-Induced Optical Activity
H. Laser	Molteno Institute, Cambridge, England	Stimulation of Induced Enzyme Formation and of Growth by *E. coli* B Following upon X-Irradiation
J. M. Mitchison	Department of Zoology, University of Edinburgh, Edinburgh, Scotland	Growth of Single Cells
D. R. Parker	Department of Biology, University of Mississippi, University	Chromosome Breakage and Rejoining in Oocytes of *Drosophila melanogaster*
E. E. Osgood	Division of Experimental Medicine, University of Oregon Medical School, Portland	Observations on the Growth of Human Hemic Cells *in vivo* and *in vitro*
B. Volcani	The Weizmann Institute of Science, Rehovot, Israel	1. A Unique Disruption of TMV with Bacteria 2. The Dead Sea – Bacteria, Salt, and Civilization
D. von Wettstein	Department of Genetics, Statens Skogsforsknings-Institut, Stockholm, Sweden	1. Mutations in the Experimental Evolution of a Plant Species 2. Genetics and Submicroscopic Cytology of Plastids
C. O. Doudney	M. D. Anderson Hospital and Tumor Institute, Houston	The Influence of Nutritional Supplements on the Mutation Response of *E. coli* Exposed to Ultraviolet Light
M. Warringa	Edsel B. Ford Institute for Medical Research, Henry Ford Hospital, Detroit	Succinate, Fumarate Conversion in *M. lactilyticus*
S. Brown	Department of Genetics, University of California, Berkeley	The Cytology and Sex Determination of the Scale Insects
R. Lewontin	Department of Genetics, North Carolina State College, Raleigh	1. The Adaptations of Populations to Varying Environments 2. The Maintenance of Polymorphism in Human Populations
R. Mason	Department of Chemistry, University College London, London, England	Chemical Carcinogenesis and Molecular Structure
C. W. Cotterman	Department of Genetics and Biometry, Wadley Research Institute and Blood Center, Dallas	Some New Approaches to the Study of Blood Groups

LECTURER	AFFILIATION	TOPIC
V. Massey	Edsel B. Ford Institute for Medical Research, Henry Ford Hospital, Detroit	The Role of Iron in Succinic Dehydrogenase
K. C. Bora	Biology Division, Atomic Energy Establishment, Govt. of India, Bombay, India	Studies on Radiation-Induced Chromosome Breakage

Speakers at Professional Meetings, Autumn 1957. – Seventeen papers are scheduled for presentation at professional society meetings before October 11. The societies and speakers are listed.

American Institute of Biological Sciences – R. C. von Borstel (coauthor, M. L. Pardue), A. C. Fabergé, D. F. Lindsley (coauthor, C. W. Edington), E. F. Oakberg (coauthor, R. L. DeMinno), L. B. Russell (by title: coauthor, L. Wickham), A. H. Haber (two papers: coauthor, N. E. Tolbert), R. Rabson (two papers: coauthor, N. E. Tolbert), L. M. Rohrbaugh (coauthor, N. E. Tolbert), E. Novitski (coauthor, E. R. Dempster), E. F. Oakberg, L. B. Russell (by title: coauthor, M. K. Freeman)

Gordon Research Conference on Cancer – A. C. Upton

American Physiological Society – J. F. Albright (coauthor, N. G. Anderson)

Society for Experimental Biology and Medicine (Southeastern Section) – B. Anderson (coauthor, T. T. Odell)

Conjoint Clinical Conference, University of Washington Medical School – C. C. Congdon

European Travel. – Alexander Hollaender attended the Second International Congress of Photobiology in Turin, Italy. As president of the Comité International de Photobiologie, which sponsored the Congress, Dr. Hollaender presided over the meetings. After the Turin congress, he attended the International Photoperiodism Conference in Parma, where he participated informally in discussions. He visited various research laboratories on the continent and in England, including the University of Milan, the Department of Genetics of the University of Naples, the Zoological Station at Naples, the French Atomic Energy Commission installation at Saclay, and the Mount Vernon Hospital in Northwood, England, and gave seminars before the Biology Department of the University of Milan and Radiology group at Mount Vernon Hospital. Dr. Hollaender's last European travel was to Rome, where he presented a paper at the Second Technical Meeting on Nuclear Energy of the Comitato Nazionale per le Ricerche Nucleari, the title of which was "Studies of the mechanism of irradiation of biological materials, with special emphasis on protection and recovery."

John Jagger attended the Second International Congress on Photobiology in Turin, Italy, and gave a paper entitled "Compared effects on bacteria, with special reference to protection and restoration" as a part of a symposium on "Comparison of the Biological Effects of Ionizing and Ultraviolet Radiation." He also visited the Citta Universitaria in Rome, Institut Pasteur in Paris, the French Atomic Energy Commission installation at Saclay, and the French Centre National de la Recherche Scientifique.

N. E. Tolbert also attended the Photobiology Congress, where he presented two papers, "Greening of normal and gamma-irradiated etiolated plants in the light" and "Light control of glycolate metabolism

and excretion." The first paper was coauthored by F. R. Gailey and the second by R. Rabson. Dr. Tolbert also served as an official discusser at the symposium on "Photoreceptors in Biology." The laboratories visited during the trip were as follows: Laboratorium voor Plantenphysiologisch, Onderzoek der Landbouwhogeschool, in Wageningen; Medisch-Biologisch Laboratorium, Rijswijk; the atomic energy laboratories of Holland in Delft; Carlsberg Laboratory in Copenhagen, Lund University in Sweden; Forest Research Institute and Karolinska Institutet in Stockholm, Kungl University in Uppsala; University College and King's College in London; Oxford, Cambridge, and Edinburgh Universities; and the Atomic Energy Research Establishment, at Harwell, England.

Still another paper on work done in the Biology Division was presented by R. L. Amy at the Photobiology Congress. The paper, coauthored by R. C. von Borstel, was entitled "The effects of different wave lengths of ultraviolet light on the Habrobracon egg."

W. L. Russell attended a gathering of British geneticists at Harwell at the invitation of the British Medical Research Council. During his stay in England, Dr. Russell visited several laboratories, including University College, Chester Beatty Research Institute, and Guy's College in London; Mount Vernon Hospital in Northwood, Middlesex; University of Cambridge and Strangeway's Research Laboratories in Cambridge; and the University at Edgbaston, Birmingham.

After attending the Inter-American Symposium on the Peaceful Application of Nuclear Energy at Brookhaven National Laboratory, Mary Esther Gaulden traveled to Europe, where she visited laboratories in France, England, Scotland, and Belgium. She presented a seminar before the Biology Division of the French Atomic Energy Commission installation at Saclay, entitled "Effects of low doses of X rays on mitosis as observed in living neuroblasts of the grasshopper embryo."

K. C. Atwood served as a member of the Far East Atomic Energy Mission; he was one of four members from Oak Ridge. A period of about six weeks was spent in Japan, the Phillippines, and Formosa in various conferences with industrial, university, and government groups.

1957 Biology Symposium. Antibodies: Their Production and Mechanism of Action. – The manuscript for the Tenth Annual Biology Symposium held in Gatlinburg in April 1957 has gone to press for publication as another supplement to the *Journal of Cellular and Comparative Physiology*. This publication is scheduled for distribution about the middle of December 1957. The table of contents follows, as it will appear in the publication.

ALEXANDER HOLLAENDER. Introduction

WILLIAM H. TALIAFERRO. General introduction: Synthesis and degradation of antibody. Eight figures

FRANK J. DIXON. Characterization of the antibody response. Five figures

S. J. SINGER. Physical-chemical studies on the nature of antigen-antibody reactions. Five figures

ELVIN A. KABAT. Size and heterogeneity of the combining sites on an antibody molecule. Eight figures

Special session: Bone Marrow Transplantation

C. C. CONGDON. Bone marrow transplantation in animals exposed to whole-body radiation

C. E. FORD, P. L. T. ILBERY, AND J. F. LOUTIT. Further cytological observations on radiation chimeras. Five figures

D. W. H. BARNES, C. E. FORD, P. L. T. ILBERY, P. C. KOLLER, AND J. F. LOUTIT. Tissue transplantation in the radiation chimera

D. W. VAN BEKKUM AND O. VOS. Immunological aspects of homo- and heterologous bone marrow transplantation in irradiated animals. Seven figures

T. MAKINODAN. Fate of injected rat bone marrow cells in irradiated mice. Four figures

General discussion

G. DAVID NOVELLI AND J. A. DEMOSS. The activation of amino acids and concepts of the mechanism of protein synthesis. Four figures

R. S. SCHWEET AND R. D. OWEN. Concepts of protein synthesis in relation to antibody formation. Three figures

DAVID W. TALMAGE. Diversity of antibodies. Four figures

N. A. MITCHISON. Adoptive transfer of immune reactions by cells. Two figures

ROBERT W. WISSLER, FRANK W. FITCH, MARIANO F. LA VIA, AND CARL H. GUNDERSON. The cellular basis for antibody formation. Six figures

ELMER L. BECKER. Hypersensitivity. One figure

T. MAKINODAN. Immunology of bone marrow transplantation. Eight figures

Eleventh Annual Biology Research Conference. – The subject of papers and discussions to be presented at the 1958 Biology Research Conference will be "Genetic Approaches to Somatic Cell Variation." This conference, to be held in Gatlinburg on April 2–5, will again be sponsored by the Biology Division and supported by the Atomic Energy Commission. Those who have assisted in arranging the program are: D. L. Lindsley, K. C. Atwood, and A. D. Conger. The program follows.

WEDNESDAY, APRIL 2

Chairmen: Curt Stern, University of California, Berkeley
George D. Snell, Roscoe B. Jackson Memorial Laboratory, Bar Harbor

Introduction: Curt Stern, Department of Zoology, University of California, Berkeley

Cellular and Possibly Nuclear Changes upon Immunization: Albert H. Coons, Harvard University Medical School, Boston

Erythrocyte Antigen Mosaicism: Charles W. Cotterman, Wadley Research Institute and Blood Center, Dallas

THURSDAY, APRIL 3

Chairman: J. F. Danielli, Department of Zoology, University of London King's College, London

Histocompatibility Changes in Tumors: George Klein, Institute for Cell Research, Karolinska Institutet, Stockholm

Excursion – Great Smoky Mountains National Park: In case of inclement weather, the program will continue and Saturday morning will remain open for a tour of the Biology Division of Oak Ridge National Laboratory.

FRIDAY, APRIL 4

Chairmen: Hilary Koprowski, The Wistar Institute of Anatomy and Biology, Philadelphia
S. E. Luria, Department of Bacteriology, University of Illinois, Urbana

Correlation of Chromosomal with Physiological Changes in Tumors: Theodore S. Hauschka, Roswell Park Memorial Institute, Buffalo

Chromosome Rearrangement and the Formation and Alteration of Tumors: Charles E. Ford, Medical Research Council, Radiobiological Research Unit, Harwell

Genetic Changes in Cells in Tissue Culture: Marguerite Vogt, Kerckhoff Laboratory, California Institute of Technology, Pasadena

EVENING

Round-Table Discussion: *The Molecular Basis of the Cause and Expression of Somatic Cell Variation.* Chairman: E. L. Tatum, Rockefeller Institute for Medical Research, New York. Discussion leaders:

Rollin D. Hotchkiss, The Rockefeller Institute for Medical Research, New York
Sol Spiegelman, Department of Bacteriology, University of Illinois, Urbana
Daniel Mazia, Department of Zoology, University of California, Berkeley
Michael Heidelberger, Institute of Microbiology, Rutgers University, New Brunswick
A. Katchalsky, Weizmann Institute of Science, Rehovot, Israel

SATURDAY, APRIL 5

MORNING

Chairman: Herschel Roman, Department of Botany, University of Washington, Seattle

Cell Population Dynamics and Somatic Change: Werner Braun, Institute of Microbiology, Rutgers University, New Brunswick

Summary: Joshua Lederberg, Department of Genetics, University of Wisconsin, Madison.

Serving as official discussers at the Conference will be:

F. M. Burnet, Director, The Walter and Eliza Hall Institute of Medical Research, Melbourne
Peter Brian Medawar, University College, London
C. A. Clarke, David Lewis Northern Hospital, Liverpool
N. A. Mitchison, University of Edinburgh, Edinburgh

Education. – Mary Esther Gaulden had a major part in arranging the Inter-American Symposium on the Peaceful Application of Nuclear Energy, which was sponsored by the United States. The Symposium was held at Brookhaven National Laboratory on May 13–17, 1957, after which various interest groups of the participants were escorted on a two-week tour. The Biology and Agriculture Tour group stopped in Oak Ridge and visited the Biology Division for two days. The time was taken up with group lectures by members of the Biology Division; laboratory tours, exhibits, and informal discussions.

Two members of the Biology Division have been honored with awards from the John Simon Guggenheim Memorial Foundation. J. S. Kirby-Smith has a one-year Fellowship (from May 1, 1957) for study with Prof. Joseph Rotblat at the Medical College of St. Bartholomew's Hospital, London. R. F. Kimball, whose Fellowship is for 10 months, left September 1 for the Karolinska Institutet in Stockholm.

W. A. Arnold is completing his third consecutive summer of work at the Institute for Muscle Research, Wood Hole, Massachusetts.

Alexander Hollaender, A. C. Upton, Liane B. Russell, E. Novitski, and G. E. Stapleton served as lecturers in the Summer Institute of Radiation Biology held at the University of Tennessee at Knoxville. The Summer Institute was sponsored by the National Science Foundation and the Atomic Energy Commission. Members of the Biology Division listed as faculty lecturers for the past regular session of the Institute of Radiation Biology are: W. A. Arnold, A. D. Conger, Mary Esther Gaulden, R. F. Kimball, G. E. Stapleton, and N. E. Tolbert. C. W. Sheppard, who left the Laboratory in June, was also a faculty member.

Five members of the Division presented lectures to the ORSORT student body during February of this year. G. E. Stapleton and A. C. Upton each gave two lectures, and W. L. Russell, Liane B. Russell, and E. F. Oakberg were responsible for one lecture each.

A. C. Upton participated in the Fourth Chemistry Institute sponsored by the National Science Foundation and the Division of Chemical Education of the American Chemical Society and financed by the National Science Foundation. This institute, which was open to college chemistry teachers, was held at the Institute of Natural Sciences, University of North Carolina, Chapel Hill. Dr. Upton lectured for one-half day and led an open discussion and question session for another half day.

CYTOLOGY AND GENETICS

CYTOGENETIC EFFECTS OF RADIATION

R. F. Kimball
K. C. Atwood
A. D. Conger
R. R. Cowden[1]
W. E. Jefferson[2]
D. R. Krieg
M. Mota[3]
T. H. Pittenger
D. Schwartz
F. J. de Serres[4]
M. A. Sicard[5]
S. Wolff
N. Gaither
H. E. Luippold
H. J. Luippold
M. Patteson
I. L. Sandler
S. M. Wilson

Independence of the Postirradiation Modification of Mutation to the Kind of Radiation

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

Introduction. – It has been shown in previous reports[6] that x-ray-induced lethal and deleterious mutations in *Paramecium aurelia* can be decreased by various postirradiation treatments of which the most used has been exposure to streptomycin. It has now been possible to show that streptomycin posttreatment is just as effective after alpha-particle irradiation and after x irradiation in the absence of oxygen as it is after x irradiation in the presence of oxygen. Clearly, the reversible part of the mutational process is much the same under all three conditions and so cannot be very dependent on the nature of the original absorption events.

Results. – Alpha particles from a Pu^{239} disk[7] were used to irradiate paramecia, compressed to about 25 μ thickness in a hanging drop on a thin Mylar film. A number of exposures were made and half the animals from each exposure were put into culture medium with streptomycin and half into ordinary culture medium. The streptomycin-treated animals were transferred to ordinary culture medium after 4 hr. The data in Table 1 show that there were appreciably more normal clones after autogamy, that is, less mutation in the streptomycin group. A similar finding was made for animals x-irradiated in nitrogen instead of air. The magnitude of the streptomycin effect was approximately the same in all three treatments shown in the table.

Discussion. – The events during and immediately after the absorption of radiation should have been considerably different for the three kinds of irradiation used. The fact that there is approximately the same streptomycin effect in all three cases must mean that the reversible process is a later step in the mutational process, a step that is common to all the radiations studied. Indeed, there is evidence that a similar step occurs in mutation production by ultraviolet, since results

[1] USPHS Fellow.
[2] Research Participant.
[3] ICA-NRC Fellow.
[4] Research Associate.
[5] Visiting investigator from abroad.
[6] R. F. Kimball, N. Gaither, and S. M. Wilson, *Biol. Semiann. Prog. Rep. Aug. 15, 1956*, ORNL-2155, p 13–14; *Biol. Semiann. Prog. Rep. Feb. 15, 1957*, ORNL-2267, p 13–14.
[7] J. S. Kirby-Smith and M. L. Randolph of the Biophysics group made the alpha-particle exposures possible.

Table 1. Streptomycin Effect with Ionizing Radiation

Radiation	Gas	Streptomycin	Number of Treated Animals (25 autogamous animals from each)	Percentage Frequency of Normal Exautogamous Clones
X rays	20% O_2	–	60	20.9
		+	60	32.1
	N_2	–	60	49.9
		+	60	63.3
Alpha particles	Air	–	120	50.6
		+	120	62.9

obtained by Witkin[8] for the ultraviolet-induction of prototrophs in bacteria resemble in many ways our results with ionizing radiations.

AET Protection in Mice, Mouse Tumor, and Tumor-bearing Mice

A. D. Conger
D. G. Doherty[9]
H. J. Luippold
R. Shapira[9]

Introduction. – Extensive experience in this laboratory with the protective chemical AET (see Appendix for alphabetical list of abbreviations) has shown that AET-injected mice survive a gamma- or x-ray dose two times as great as unprotected mice. It was desirable to have some basic information on the protective effect of AET on mammalian cells, to compare with the protective effect on the whole body. This was done with cells of the Ehrlich mouse ascites tumor, a suspension of free cells which grows in the peritoneal cavity.

Results and Discussion. – Cytological damage (per cent of chromosomally abnormal cells at the time of division) and inhibition of division were the criteria of effect. When tumor cells were treated with or without AET and irradiated *in vitro*, treated and untreated cells showed the same amount of radiation damage, though the appearance of damage was delayed somewhat in the AET-treated cells. The result was surprising, since the chemical was in contact with the membrane of the free cells and the cells were metabolizing, though at a reduced rate. When cells were treated *in vivo* by injection of the AET into the peritoneal cavity, however, protection by a factor of about 1.7 was found. Apparently, active metabolism is required for the AET to enter these mammalian cells; when it does enter, the protection afforded is less, at least for this effect, than for survival of the whole animal.

An analysis of the distribution of orally administered S^{35}-labeled AET within an ascites-tumor-bearing mouse showed the AET appeared more rapidly and in higher concentration in the body tissues than in the tumor cells. This transitory difference in body and tumor concentration of AET suggested that improved radiotherapy of the tumor might be achieved by injecting AET into a tumor-bearing mouse shortly before irradiation, and then delivering about twice the normally tolerable x-ray dose. Ideally, with an AET-injected animal, the AET-unprotected tumor cells would receive effectively the full x-ray dose, the AET-protected body cells would receive effectively only half this dose. Results from a first experiment with tumor-bearing mice are given in Table 2.

Death by weight loss can be considered very crudely as "radiation" death, by weight gain as "tumor" death. The results indicate that both x-ray doses, with or without AET, prolong life by retarding tumor growth. At 450 r, AET apparently achieved the desired result – protection of the body, but not the tumor – since both unprotected and AET-protected mice showed about the same tumor growth until the unprotected animals began to die by radiation death; the AET-protected animals survived the period of radiation death, to die 2.3 days later by tumor death. Tumor growth was suppressed still more by the 900-r dose, but the AET did not prevent radiation death as it would have in normal animals; apparently the tumor mice, being sick already, are less able to survive radiation than a normal mouse. The experiment has

[8] E. M. Witkin, *Cold Spring Harbor Symposia Quant. Biol.* 21, 123–140 (1956).

[9] Biochemistry Section.

Table 2. Survival and Weight Change in Ascites-Tumor-Bearing Mice

Treatment		Mean 50% Survival Time (days)	Weight Change After Treatment	
X Rays (r)	AET (8 mg)		Initially	As Death Commences
0	+	6.3	Gain	Gain
450	0	9.2	Slight gain	Loss
450	+	11.5	Slight gain	Gain
900	+	7.6	None	Loss

been repeated, but on a larger scale and using smaller doses, and with an additional group of animals for cytological observation; this experiment is still in progress.

A Cytochemical Consideration of the Nucleoli of the Slug in Growing Oocytes

R. R. Cowden

Introduction. – Oogenesis in the slug was investigated with special reference to the nucleoli with the use of cytochemical procedures. An unusual nucleolar system has been described in the slug *Deroceras reticulatum* in which "secondary" nucleoli appear subsequent to the development of an original or "primary" nucleolus. Bridgeford and Pelluet[10] have described this process in detail, and have contended that the secondary nucleoli arise from the primary nucleoli during the course of development.

Results. – In this investigation, an attempt was made to characterize these two types of nucleoli as to their chemical constitution, and to detect differences between them. Several differences were found. The secondary nucleoli contained a lower concentration of RNA than did the primary nucleoli. While neither type of nucleoli contained DNA, the primary nucleoli did possess a circumnucleolar region of Feulgen-positive material, possibly indicating nucleolus-associated chromatin. The same region was positive for histone when stained by the fast-green reaction of Alfert and Geschwind.[11] No such reaction was observed in the secondary nucleoli. The primary nucleoli were found to possess a mucopolysaccharide-containing nucleolar membrane, which stained with the periodic acid–Schiff reaction, and with acid aqueous Alcian blue. This material was not removable by prior incubation in malt diastase or hyaluronidase. The secondary nucleoli showed no evidence of staining by these methods. Both classes of nucleoli were positive for dibasic amino acids as demonstrated by the naphthol yellow S method of Deitch.[12] Neither class of nucleoli was sudanophilic, indicating the absence of demonstrable lipids, and phospholipids were also absent.

Discussion. – The results of this investigation indicate that the secondary nucleoli do not play any major role in RNA synthesis; they may function as a reserve for protein. No direct morphological evidence for the origin of the secondary nucleoli from the primary nucleoli was observed, nor was there any simple relationship between the volumes of the two classes of nucleoli in a given cell. A cytochemical study of the behavior of the secondary nucleoli during the meiotic divisions and zygote formation, particularly with respect to dibasic amino acids, might tend to clarify the function of the latter.

[10] H. B. Bridgeford and D. Pelluet, *Can. J. Zool.* 30, 323–337 (1952).

The General Occurrence of the Effect of Postirradiation Inhibition of Cellular Respiration on Chromosome Aberration Yield

S. Wolff

Introduction. – The early studies of Giles and Riley[13] on the "oxygen effect" of increased chromosomal aberration yield after irradiation in the presence of oxygen led to the conclusions that in *Tradescantia* the oxygen had to be present at the time of irradiation to be effective, that the increased number of aberrations resulted from a higher frequency of initial breakage, and that the postirradiation presence or absence of oxygen had no effect on the recovery (namely, rejoining) process. Although the studies of Wolff and coworkers[14–16] on the seed of *Vicia faba* confirmed that the increased aberration yield was caused by increased breakage, they also demonstrated that the postirradiation presence or absence of oxygen *can* modify the numbers of aberrations. Since these extensive experiments have been thought to be exceptions,[17] it has been necessary to demonstrate that the same phenomena occur in *Tradescantia*, the classical cytological material for studies of this kind.

[11] M. Alfert and I. I. Geschwind, *Proc. Natl. Acad. Sci. U.S.* 39, 991–999 (1953).

[12] A. Deitch, *Lab. Invest.* 4, 324–351 (1955).

[13] N. H. Giles and H. P. Riley, *Proc. Natl. Acad. Sci. U. S.* 36, 337–344 (1950).

[14] S. Wolff and K. C. Atwood, *Proc. Natl. Acad. Sci. U.S.* 40, 187–192 (1954).

[15] S. Wolff and H. E. Luippold, *Science* 122, 231–232 (1955).

[16] S. Wolff and H. E. Luippold, p 217–221 in *Progress in Radiobiology* (eds. J. S. Mitchell, B. E. Holmes, and C. L. Smith), Oliver & Boyd, Edinburgh, 1956.

[17] L. H. Gray, *Indian J. Radiol.*, Souvenir No. 1–8 (1956).

If the radiation dose to inflorescences of *Tradescantia paludosa* (clone 3 Sax) is fractionated into two doses separated by 1 hr, then the number of 2-hit chromosome aberrations observed is simply the sum of those produced by each of the two doses. Previous experiments on *Vicia*[15,16] and on *Tradescantia*[18] have shown that postirradiation anoxia (after the first dose) inhibits the rejoining of the breaks. These breaks then can rejoin with those produced by the second dose and thus result in an aberration yield that is greater than the simple sum of that induced by the two doses. Experiments with *Vicia* have shown this particular postirradiation effect of anoxia to be the result of the inhibition of cellular respiration.

[18] S. Wolff, *Biol. Semiann. Prog. Rep. Feb. 15, 1957*, ORNL-2267, p 15–16.

Results. – In Table 3 may be seen the results of dose fractionation experiments performed on interphase cells of *Tradescantia* microspores. Between the two doses the inflorescences were placed in 95% CO + 5% O_2 in the dark or in the light.

Discussion. – In those inflorescences that were placed in carbon monoxide in the dark the breaks did not restitute until after the completion of the second dose. The aberration yield thus is equal to that obtained when the dose is not fractionated. If, however, the material in the carbon monoxide is exposed to the light from a sodium arc lamp, then the breaks restitute and only the additive amount of aberrations is obtained.

Since the light reversibility of the effect of carbon monoxide indicates that cytochrome oxidase activity is necessary for chromosome breaks to rejoin, it is apparent that in *Tradescantia*, as in

Table 3. The Effect of Postirradiation Inhibition of Cellular Respiration on Chromosome Break Rejoining in *Tradescantia*

Dose I in Air, 200 r/min (r)	Treatment for 1-hr Interval	Dose II in Air, 200 r/min (r)	Number of 2-Hit Aberrations / Number of Cells Scored	2-Hit Aberrations per 100 Cells	Expected Yield if Doses I and II Are Additive
150	95% CO + 5% O_2 in dark	0	38/200	19.0 ± 3.1	
0	95% CO + 5% O_2 in dark	150	52/300	17.3 ± 2.4	
150	95% CO + 5% O_2 in dark	150	155/300	50.7 ± 4.2*	36.3
150	95% CO + 5% O_2 in light	0	46/300	15.3 ± 2.3	
0	95% CO + 5% O_2 in light	150	48/250	19.2 ± 2.8	
150	95% CO + 5% O_2 in light	150	132/350	37.4 ± 3.3	34.5
150	Air in light	0	42/300	14.0 ± 2.2	
300	Air in light	0	135/244	55.2 ± 4.8*	
150	Air in light	150	105/300	35.0 ± 3.4	28
150	95% CO + 5% O_2 in light	150	106/300	35.3 ± 3.4	28
150	95% CO + 5% O_2 in dark	150	141/300	47.0 ± 4.0*	28

*If the breaks induced by dose I remain open to rejoin with those of dose II, the aberration yield should be the same as that observed after an unfractionated dose of 300 r.

Vicia, the aberration yield can be modified by postirradiation treatments that inhibit cellular respiration. Since this effect on radiation-induced genetic damage has now also been observed in *Drosophila*[19] and in *Allium*,[20] it may be assumed that it occurs generally and is not an exception. Note should be taken that the postirradiation effect of anoxia is the opposite of the effect preirradiation anoxia has on aberration yield.

Somatic Breakdown of Disomic Nuclei in *Neurospora*

T. H. Pittenger

Introduction. – In *Neurospora* it has been found that in the progeny of crosses between closely linked mutants some of the phenotypically wild-type progeny are not recombinants but are heterozygous disomics probably resulting from nondisjunction.[21,22] Such disomic nuclei are unstable and by some postmeiotic process give rise to genetically different haploid nuclei and such strains are then heterokaryotic and referred to as pseudowild types (PWT). In a recent report[23] it has been shown that the somatic reduction giving rise to haploid nuclei from the disomics takes place in the early nuclear divisions within the ascospore. The present experiments are an attempt to determine whether the disomic breaks down in the nuclear division which follows the delimitation of the spore and results in the binucleate condition of the mature ascospore.

It has previously been shown[24] that the two nuclei of the ascospore are inactivated independently by x rays, and calculations have shown that in a population of spores having 20% survival following x irradiation roughly 95% of the surviving spores will be uninucleate. If the progeny from a cross containing both crossover wild types and PWT's is irradiated with a dose sufficient to reduce survival to 20% or less, then in the surviving uninucleate population spores originally having either two wild type or two disomic nuclei will still be capable of producing a wild-type strain. If, however, an ascospore containing two different mutant nuclei, as the result of somatic breakdown of the original disomic nucleus, has one nucleus inactivated, then such a spore will no longer be phenotypically wild. Thus if an appreciable number of the original disomic nuclei have formed two different nuclei as the result of the division within the ascospore so that the spore is heterokaryotic then it would be expected that a difference would be found in the proportion of crossover wild types and PWT's following x-ray treatment.

Results. – In a cross of *A,lys*-3 × *a,al*-2,*nic*-2, the wild-type crossovers are usually albino in phenotype, whereas the PWT's, which carry *al*-2 in addition to its wild-type allele, have a wild or pink phenotype.[22] From the progeny of this cross in which the frequency of total wild types was about 1.8%, a sample of 375 was isolated; 57% had a pink phenotype (PWT) and 43% were albino (crossovers). In the same experiment, in which the survival following a dose of 50,000 r was only 0.04%, a total of 28 wild types recovered; 14% were pink and 86% were albino. In another experiment using this cross in which the survival following x irradiation was approximately 20%, the frequency of wild types was 1.4% and, in a sample of 219, 36% were pink and 64% were albino.

In another cross of two closely linked pantothenate-requiring mutants (kindly supplied by M. Case of Yale University) it has been found that the PWT's are temperature sensitive and show only limited growth at 35°C whereas the crossover wild types are not temperature sensitive. In several different experiments having an average frequency of 1.6% phenotypically wild-type progeny, the proportion of crossover wild types (minimal growers at 35°C) in the controls averaged 16% (range 12 to 19%). In populations irradiated to give less than 20% survival, the proportion of crossover wild types averaged 39% (range 35 to 47%).

Discussion. – In all the experiments the proportion of wild types recovered which are PWT's is significantly lower following x irradiation. The results are consistent with the hypothesis that an appreciable fraction of the mature PWT spores are heterokaryotic at the time of irradiation. Inactivation of one of the two nuclei by the radiation generally precludes the formation of a wild-type strain and as a result there is a decrease in the

[19] S. Abrahamson, *Genetics* 41, 631 (Abs) (1956).

[20] N. S. Cohn, *Genetics* 41, 639 (Abs) (1956).

[21] M. B. Mitchell, T. H. Pittenger, and H. K. Mitchell, *Proc. Natl. Acad. Sci. U.S.* 38, 569–580 (1952).

[22] T. H. Pittenger, *Genetics* 39, 326–342 (1954).

[23] T. H. Pittenger, *Biol. Semiann. Prog. Rep. Feb. 15, 1957*, ORNL-2267, p 19–20.

[24] K. C. Atwood and T. H. Pittenger, *Genetics* 40, 563–564 (1955).

proportion of PWT's recovered after x irradiation. Thus it would appear that the abnormal division of the disomic nucleus resulting in a heterokaryotic spore very often takes place as early as the first nuclear division in the ascospore, but the presence of PWT's in irradiated populations whose survival is only a few per cent suggests that certainly this is not always the case.

The Mating Type Alleles and Heterokaryon Formation in *Neurospora crassa*

T. H. Pittenger

Introduction. – The recent work of Garnjobst and Wilson[25] has shown that the failure of heterokaryon formation as controlled by the C and D genes and their alleles in *Neurospora* is the result of protoplasmic incompatability. Similarly, they have shown somewhat unexpectedly that the same type of protoplasmic incompatability occurs in heterokaryons between strains of the opposite mating types. Since there is a good deal of unpublished evidence showing that genes in addition to C and D control heterokaryon formation, the observation that strains of the opposite mating type show the same pattern of protoplasmic incompatability as C and D suggested that one of these still unidentified genes controlling heterokaryon formation may be linked to mating type and that it is such a gene and not mating type that is responsible for the incompatability reaction so often observed in bisexual heterokaryons. Although it is well established that strains of the opposite mating type usually do not form compatible heterokaryons, there is still no critical evidence to show that the incompatibility is caused by mating type rather than a closely linked gene. In fact, the rare cases where it has been shown that bisexual heterokaryons are compatible[26] could most easily be explained as involving recombinants between mating type and a closely linked gene similar to C and D in its effect on heterokaryon formation.

Pseudowild types,[27] believed to originate as nondisjunctional disomics that break down somatically to give identical haploid nuclei, except for the linkage group originally present in duplicate, have been found involving the mating type chromosome, but unlike those previously described[28] involving this linkage group, some have now been found that are bisexual. When the two strains recovered from homokaryotic conidia of a bisexual pseudowild type are crossed, any segregation noted in the progeny involves only genes in the mating type chromosome, since the other six linkage groups are identical. Such strains provide especially favorable material in which to test whether the incompatability of bisexual heterokaryons is the result of heterozygosis at the mating type locus or at a closely linked locus. From the cross of the linked mutants *a,al*-2,*nic*-2 × *A,lys*-3, phenotypically wild-type bisexual strains were recovered from single normal-sized ascospores, but subsequent growth was erratic and characterized by definite albino and wild-type sectors on minimal agar slants. From homokaryotic conidia of such a strain, it was possible to isolate two strains, *a,al*-2,*nic*-2 and *A,lys*-3, believed to be identical except for genes in the mating type chromosome. When these two strains were crossed it was expected that among the progeny all combinations of *nic*-2 and *lys*-3 strains of the same mating type would form normal heterokaryons. However, if two strains of the same mating type were incompatible as a heterokaryon it would mean that some gene other than mating type was involved in the failure to form a normal heterokaryon.

Results. – A cross was made between *a,al*-2,*nic*-2 × *A,lys*-3 (both strains were recovered from homokaryotic conidia of the same bisexual pseudowild type) and 179 progeny were recovered of the following genotypes: 38 *A,lys*-3; 41 *a,al*-2,*nic*-2; 29 *a,lys*-3; 33 *A,al*-2,*nic*-2; 10 *a,al*-2,*lys*-3; 11 *A,nic*-2; 7 *a,nic*-2; and 10 *A,al*-2,*lys*-3. Heterokaryon tests were then made by first combining each of the 44 *A,nic*-2 strains (the albino marker will be disregarded in what follows) with the parental *A,lys*-3. The 39 *a,lys*-3 strains were tested with the parental *a,al*-2,*nic*-2 strain. All such combinations formed normal heterokaryons. Then the 48 *a,nic*-2 strains were tested with *a,lys*-3 strains that in the previous tests were shown to be heterokaryon positive with the parental *a,al*-2,*nic*-2. Likewise, the 48 *A,lys*-3 progeny

[25] L. Garnjobst and J. F. Wilson, *Proc. Natl. Acad. Sci. U.S.* 42, 613–618 (1956).

[26] C. C. Lindegren, *J. Genet.* **28**, 425–435 (1934).

[27] M. B. Mitchell *et al.*, *Proc. Natl. Acad. Sci. U.S.* 38, 569–580 (1952).

[28] T. H. Pittenger, *Genetics* 39, 326–342 (1954).

were tested with *A,nic*-2 strains previously shown to be heterokaryon-positive with the parental *A,lys*-3. Every A/A and every a/a combination of *lys*-3 and *nic*-2 formed a compatible heterokaryon.

Discussion. – These experiments give no evidence of segregation in this linkage group of any gene affecting heterokaryon formation other than the mating type alleles. According to Stevens'[29] formulation and the 5% confidence interval, recombination between a gene controlling heterokaryon formation and mating type would have been detected unless the gene were less than 1.98 crossover units from the mating type locus.

Complementation Between *ad-3A* Alleles In *Neurospora crassa*

F. J. de Serres

Introduction. – Recent studies[30–33] on series of allelic mutants at complex loci in *Neurospora* have given evidence for partial or complete complementation in certain pairwise combinations of alleles when present in the same cytoplasm (heterokaryons) and/or in the same nucleus (PWT's).[34] Previous heterokaryon tests[35] with a series of 3 *ad-3A* and 21 *ad-3B* alleles, derived from the same wild-type strain, gave no evidence for complementation between all possible pairwise combinations within each series. However, since unmarked strains were used, the argument might be advanced that the negative tests are inconclusive, since proof of hyphal fusion and nuclear mixing in the *ad-3A* + *ad-3A'* combinations can be obtained only by indirect methods. The present studies were designed to overcome this difficulty by using the forced heterokaryon test on genetically marked strains to determine whether the phenomenon of allelic complementation is of more general occurrence in *Neurospora*. In addition, adenine-independent progeny from intercrosses of *ad-3A* mutants were tested to determine whether any such progeny were actually PWT's resulting from complementation between different *ad-3A* alleles present in the same disomic nucleus.

Results. – Thirteen *ad-3A* mutants (of independent origin), 1 *ad-3B* mutant, and wild-type strain 74A (all of similar or identical genetic background) were crossed to an *a inos pan* double mutant strain; and *A ad-3 inos*, *A ad-3 pan*, or *A inos* and *A pan* derivatives were obtained for each of the 15 strains. All possible pairwise combinations of conidia from each of the 15 *pan* strains were made with each of the 15 *inos* strains on minimal medium supplemented with adenine. Conidia from such forced heterokaryons were then tested on minimal (and supplemented minimal) medium to test for complementation. Whereas conidia from all *ad-3A* + *ad-3A'* heterokaryons grew on adenine-supplemented medium, no growth was obtained on minimal medium. However, all *ad-3A* + *ad-3B* or *ad-3A* + *ad-3A*$^+$ combinations grew on minimal medium, and at wild-type rate, as determined in separate experiments using the growth tube method. In addition, all pairwise combinations were tested on growth-rate-limiting levels of adenine in growth tubes. No evidence for complementation was obtained for different pairwise combinations of *ad-3A* mutants in these tests. All heterokaryons grew at the same submaximal growth rates as each of the homokaryotic components (i.e., A1 *pan* + A3 *inos* as compared with A1 *pan* + A1 *inos* and A3 *pan* + A3 *inos*) on the levels of adenine tested.

Adenine-independent progeny from *ad-3A* × *ad-3A*(2) crosses were tested by crossing each isolate to the tester strain *hist-2 ad-3A ad-3B nic-2*. All crosses were then examined to determine whether the expected ratio of 1:1 of adenine-dependent to adenine-independent progeny was obtained. In extensive tests of the progeny from such intercrosses, no PWT progeny of the type *ad-3A* + *ad-3A*(2) were recovered. The fact that PWT's do occur in such crosses, however, was ascertained by the recovery of PWT's of the type *ad-3A* + *ad-3A*$^+$ (parental type + adenine-independent recombinant).

Conclusions. – The present analyses have provided unequivocal evidence for the lack of complementation between *ad-3A* alleles when present in the same cytoplasm or the same nucleus. They have also shown that the negative tests obtained previously, using unmarked strains of similar or identical genetic background, cannot be attributed to a failure to obtain hyphal fusion

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[29] W. L. Stevens, *J. Genet.* 43, 301–307 (1942).

[30] M. B. Mitchell and H. K. Mitchell, *Genetics* 41, 319–326 (1956).

[31] M. B. Mitchell, *Compt. rend. trav. lab. Carlsberg, Sér. physiol.* 26, 285–298 (1956).

[32] M. E. Case, *Ph.D. thesis*, Yale University, 1957.

[33] N. H. Giles *et al.*, *Proc. Natl. Acad. Sci. U.S.* 43, 305–317 (1957).

[34] T. H. Pittenger, *Genetics* 39, 326–342 (1954).

[35] F. J. de Serres, *Genetics* 41, 668–676 (1956).

and nuclear mixing. It would appear that complementation between members of allelic series is not of general occurrence in *Neurospora*, but that the phenomenon may be restricted to allelic series of a particular (biochemical) type.

Recombination and Interference in the *ad-3* Region of *Neurospora crassa*

F. J. de Serres

Introduction. – Experiments reported previously[36] have shown marked differences in the linkage relations of 11 *ad-3A* mutants in the *ad-3A* – *ad-3B* region in crosses with *ad-3B* mutant 35203. On the basis of these linkage relations, the mutants can be divided into two groups: the first, consisting of 8 mutants (derived from wild-type strain 74A), gives an average value of 0.67 unit for this region, whereas the second group, consisting of mutants 38701, 38709, and 68306 (ref 37) (related in origin to each other, but different from 74A), gives an average value of 0.18 unit. It has also been shown[35] that these differences in linkage in the *ad-3A* – *ad-3B* region are not correlated with proportionate differences in linkage with the closely linked, adjacent markers *hist-2* and *nic-2*; all 11 mutants have the same linkage relations in the *hist-2* – *ad-3A* and *ad-3A* – *nic-2* regions. In addition, the proportion of the total adenine-independent progeny of "exceptional" (parental or reciprocal crossover) genotype obtained in each group of crosses provided evidence for no or slight interference in the *hist-2* – *ad-3A* – *ad-3B* – *nic-2* regions in the first group, but negative interference for the second group. The present studies were designed to determine whether the same type of differences in linkage and patterns of interference would be obtained from crosses of the *ad-3A* mutants to marked strains of *ad-3B* mutants B10 and B10-R2, which previous studies[38] have shown to be more closely linked to *ad-3A* mutant A2 than is *ad-3B* mutant 35203.

Results. – The data from analyses of the crosses of the 8 mutants in the first group gave an average value of 0.19 unit for the *ad-3A* – *ad-3B* region. In addition, many more adenine-independent progeny of "exceptional" genotype were recovered than expected in these crosses, indicating marked negative interference in the *hist-2* – *ad-3A* – *ad-3B* – *nic-2* regions. However, the 3 mutants in the second group were not more closely linked to mutant B10 or B10-R2 than the 8 mutants in the first group, as expected on the basis of previous crosses with mutant 35203. Instead, the data from analyses of these crosses gave an average value of 0.73 unit for the *ad-3A* – *ad-3B* region. In addition, fewer adenine-independent progeny of "exceptional" genotype were recovered than expected, indicating marked positive interference in these crosses.

Discussion. – A hypothesis that might be advanced to explain these results takes into account the origin of the mutants under study. The 8 mutants in the first group, B10 and B10-R2, were all derived from the same wild-type strain, and the 3 mutants in the second group are closely related in origin to mutant 35203. From the crossing analyses we can see that when crosses are made between mutants with identical or closely related genetic background, recombination in the *ad-3A* – *ad-3B* region is low, accompanied by negative interference. However, when crosses are made between mutants of different genetic background, recombination in the *ad-3A* – *ad-3B* region is higher, accompanied by positive interference. Unfortunately, the *hist-2 nic-2* strain used to determine the linkage relations of the *ad-3A* mutants in adjacent regions[36] is not related in origin to either group of mutants. Thus, the fact that differences in linkage in the *ad-3A* – *ad-3B* region are not correlated with differences in linkage in adjacent regions, using markers 2 and 3 units away, is probably of dubious significance. More pertinent, perhaps, are the results of crosses using another closely linked marker, induced in 74A, with *ad-3A* mutants from both groups. Preliminary data from such crosses provide additional evidence that recombination in regions adjacent to the *ad-3A* locus is lower in crosses between strains of identical genetic background than in crosses between strains of different genetic background. Thus, it would appear that the higher estimates of recombination in the *ad-3A* – *ad-3B* region obtained in the present experiments can be attributed to the action of unknown genetic modifiers, and that the best estimates of the map distance for this region are about 0.1 to 0.2 unit.

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

[37] G. W. Beadle and E. L. Tatum, *Am. J. Botany* **32**, 678–686 (1945).

[38] F. J. De Serres, *Genetics* (in press).

The pattern of interference can be explained if it is assumed that adenine-independent progeny arise through two different processes: "gene conversion"[39] and "conventional" genetic recombination, with the latter subject to the action of genetic modifiers in these crosses. Under this assumption, the progeny of "exceptional" genotype would result from "gene conversion" rather than "conventional" genetic recombination. Thus, in one type of cross, the proportion of the total progeny of "exceptional" genotype is high, giving the impression of negative interference, and in the other type of cross, the proportion is low, suggesting positive interference.

Biochemical Studies on Cytoplasmically Inherited Mutants in *Neurospora crassa*

W. E. Jefferson T. H. Pittenger

Introduction. – A series of cytoplasmically inherited mutations in *Neurospora* are characterized by slow growth, insensitivity to cyanide, the absence or deficiency of cytochrome oxidase, and the accumulation of abnormal amounts of cytochrome pigments other than cytochrome oxidase.[40] It would appear that the defective cytochrome systems could be the basis for the other characteristics, yet two situations are known in which certain of these mutants attain normal growth rates without a normal complement of cytochrome pigments. Mitchell and Mitchell[41] found a gene which permits normal growth in Mi-1 (poky) and Pittenger[42] found that heterokaryons of Mi-1 and Mi-4 will grow at a normal rate. Neither of these conditions results in a normal cytochrome content.

Atwood and Pittenger[43] observed abnormally high (500 × wild type) accumulations of ATP when extracts of either Mi-1 or Mi-4 were incubated with ADP and DPNH. Heterokaryons of Mi-1 and Mi-4 were intermediate between wild type and the mutants, thus raising the possibility that an abnormal phosphorus or nucleotide metabolism occurs in the mutants and that it could be related to the increased growth of the heterokaryons. Although the accumulation of ATP had proved variable in the hands of its discoverers, an investigation of the phosphorus and nucleotide metabolism of the mutants appeared desirable.

Results. – Experiments similar to those reported by Atwood and Pittenger[43] were carried out with crude cell-free extracts of Mi-1, Mi-4, and wild type, and with various fractions obtained by differential centrifugation of these extracts. Orthophosphate and ATP were determined in these preparations in a variety of conditions. In no case was more ATP accumulated in mutant than in wild-type extracts. Two consistent differences between wild-type and mutant extracts were found, however. The mutant extracts contained more (usually 2 to 3 ×) orthophosphate and destroyed ATP at a rate at least twice that of similar wild-type extracts. In general, the mutant extracts showed more hydrolytic activity (conversion of ATP, ADP, and DPN to orthophosphate) and less synthetic activity (uptake of orthophosphate in the presence of substrate amounts of DPNH, succinate, or glucose) although, except for the effect on ATP, these varied considerably.

The response to DPN and DPNH was quite variable in both mutant and wild-type extracts. Some attacked these materials rapidly with a transient accumulation of ATP while others showed only a slow release of inorganic phosphate. When present, the rapid breakdown of DPN was associated with particles which could be removed by mild centrifugation (7000 × *g* for 15 min).

Discussion. – The failure to find the previously reported accumulation of ATP in the mutant extracts could be due to a variety of causes. In the crude extracts ATP can be formed by at least three separate series of reactions: from ADP by myokinase, from ADP by oxidative phosphorylation, and from DPN probably by its conversion to ADP-ribose and subsequent decomposition of this compound. These extracts also rapidly destroy ATP with the liberation of orthophosphate. A change in the activity of any of these enzymes could lead to a change in the accumulation of ATP. On the basis of the experiments described here, a decrease in the rate of destruction of ATP or an increase in the breakdown of DPN is the most likely source of the discrepancy, since these processes are far more rapid than the other reactions.

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[39] M. B. Mitchell, *Compt. rend. trav. lab. Carlsberg, Sér. physiol.* 26, 285–298 (1956).

[40] A. Tissieres and H. K. Mitchell, *J. Biol. Chem.* 208, 241–249 (1954).

[41] M. B. Mitchell and H. K. Mitchell, *J. Gen. Microbiol.* 14, 84–89 (1956).

[42] T. H. Pittenger, *Proc. Natl. Acad. Sci. U.S.* 42, 747–752 (1956).

[43] K. C. Atwood and T. H. Pittenger, *Biol. Semiann. Prog. Rep. Aug. 15, 1956*, ORNL-2155, p 18–19.

The elevated ATPase and inorganic phosphate content of the mutants suggests that phosphorus metabolism may play some role in their abnormality.

More recent results indicate that the major formation of ATP is brought about by cell fragments. These were removed with the grinding material in the experiments described above, but were not removed in the experiments described by Atwood and Pittenger.[43] The explanation for the discrepancy may lie in this difference.

Oxidative Phosphorylation in Cytoplasmic Particles from *Neurospora crassa* Without the Formation of ATP

W. E. Jefferson

Introduction. – Cytoplasmic particles from a wide variety of organisms couple the formation of ATP from orthophosphate and ADP with the oxidation of DPNH and other substrates.[44] Particles obtained by high-speed centrifugation of extracts of *Neurospora crassa* 73a appear to take up orthophosphate and ADP without forming ATP.

Results. – Particles obtained by centrifuging extracts of *Neurospora* 1 hr at 40,000 rpm in a Spinco ultracentrifuge after first removing heavier particles at 25,000 × *g* for 15 min were taken up in 0.15 *M* KCl. One-milliliter aliquots were incubated with 1 μmole of ADP, 1 μmole of orthophosphate, and either 1 μmole of DPNH or 10 μmoles of sodium succinate. Each milliliter also contained 10 μmoles of NaF, 2 μmoles of $MgSO_4$, and 100 μmoles of tris buffer at pH 7. The reactions were stopped after 5 and 30 min. Phosphate was determined by the method of Fiske and Subbarow,[45] and ATP by the use of an extract of firefly tails.[46] There was a disappearance of inorganic phosphorus as expected but a reduction rather than an increase in "ATP." The results from one of several similar experiments are shown in Table 4. When similar experiments were conducted with ATP substituted for ADP the additional substrates had no effect.

Discussion. – The occurrence of myokinase in the firefly extracts, and probably in the *Neurospora* preparation, make quantitation of the "ATP" uncertain since both ATP and ADP will cause the production of light by the firefly extract. Under the conditions used either ATP or ADP gave satisfactory standard curves but combinations of the two did not, since the production of light from ATP decreased with time while that from ADP increased. For that reason, the data are reported as counts per minute rather than micromoles of ATP or ADP. The reduction in "ATP" observed when succinate is added appears to indicate the complete disappearance of both ADP and ATP. A possible alternative explanation would be the formation of some inhibitor of the firefly luciferin reaction. Preliminary attempts to demonstrate such an inhibitor failed, however.

[44] F. E. Hunter, p 610–666 in *Methods in Enzymology*, Vol. II (eds. S. P. Colowick and N. O. Kaplan), Academic Press, Inc., New York, 1955.

[45] O. Lindberg and L. Ernster, p 1–22 in *Methods in Biochemical Analysis*, Vol. III (ed. D. Glick), Interscience Publishers, Inc., New York, 1956.

[46] B. L. Strehler and J. R. Totter, p 341–356 in *Methods in Biochemical Analysis*, Vol. I (ed. D. Glick), Interscience Publishers, Inc., New York, 1954.

Table 4. The Influence of Succinate and DPNH on the Metabolism of ADP by Cytoplasmic Particles from *Neurospora crassa*

Substrate	Orthophosphate-Blank*		"ATP" counts/min × 512	
	5 min (μmoles)	30 min (μmoles)	5 min	30 min
ADP	0.41	0.48	860	520
ADP and DPNH	0.34	0.47	480	380
ADP and succinate	0.04	0.64	0	0

*Amount of orthophosphate contained in particle suspension.

Further Studies on the Effect of High-Dose Gamma Radiation on Seedling Growth

M. A. Sicard

Dry wheat and barley (var. Himalaya) seeds were irradiated with gamma rays from the cobalt source, with doses up to 700,000 r. They were planted in sand, in the greenhouse, immediately after radiation. Ten days after germination the seedlings were harvested and the height of coleoptile and first leaf was measured. At this time growth had ceased for seedlings irradiated with more than 60,000 r. The measurements are plotted against dose (Figs. 1 and 2).

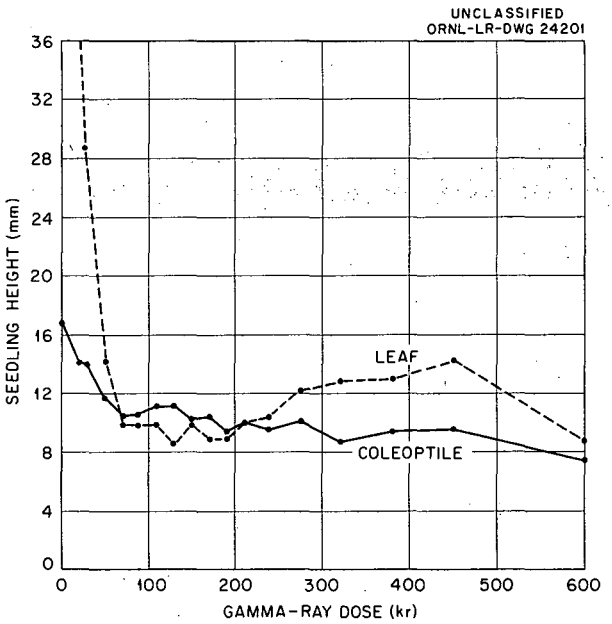

Fig. 1. Wheat Planted in the Greenhouse Immediately After Irradiation; Sun Every Day.

The growth of the leaves for both barley and wheat shows the same response to radiation dosage as was previously observed with corn. With increasing dosage, growth decreases until a point is reached, around 110 kr for wheat and 200 kr for barley, where the relationship is reversed and growth increases.

However, the growth of coleoptile in wheat does not show this reversal. Figure 1 strikingly shows the difference in the response of the leaf and coleoptile tissue. Between 140 kr and 450 kr, the former is increasing in growth while the latter is

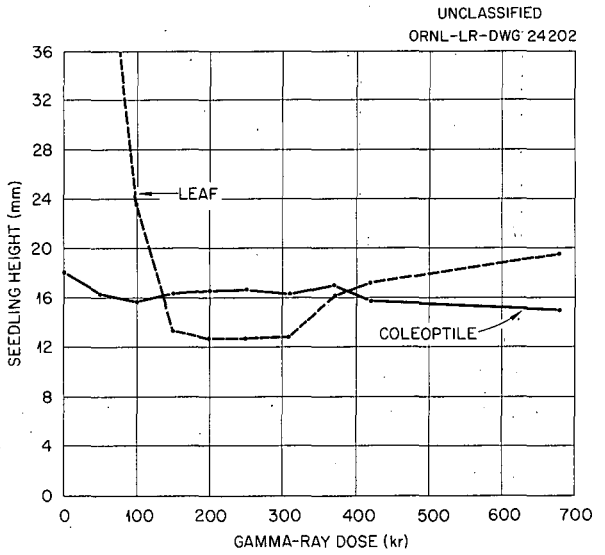

Fig. 2. Barley Planted in the Greenhouse Immediately After Irradiation; Sun Every Day.

decreasing. Since there are only a few dividing cells in the coleoptile as compared with the leaf, this result supports the hypothesis that the reversal in the dose curve, where seedling height is plotted against dose, is dependent on the interaction of chromosome damage and rate of mitosis.[47]

It is difficult to compare number of mitoses for these two different tissues. The coleoptile growth is very short. The number reaches a maximum 24 hr after germination at 16°C. After 64 hr there are only very few cell divisions.

In barley (Fig. 2) the coleoptile growth shows a slight reversal like the leaves, and, in fact, we found about 10 times more cell divisions in barley coleoptile than in wheat coleoptile.

Oat and sorghum were used in the same kind of studies. These plants showed the effect only when grown in darkness. It is possible that under these conditions the effect is simply amplified due to increased elongation and thus easier to detect. However, this may result from increased cell division in darkness. Thompson[48] reported an inhibition of cell division in oat mesocotyl by light.

In similar studies with the barley coleoptile, Moutchen[49] reported that the reversal in the dose

[47] D. Schwartz, *Am. Naturalist* 40, 323–327 (1956).

[48] B. Thompson, *Am. J. Botany* 41, 326–332 (1954).

[49] J. Moutchen *et al.*, *Experientia* 12, 314–315 (1956).

curve is eliminated by storage of the seeds for 20 days between radiation and planting.

This work was repeated with wheat and corn stored for 0, 3, 6, 13, and 21 days, and we failed to find a significant change in the shape of dose response curve for both leaf and coleoptile.

With barley the results of Moutchen[49] have been confirmed for the coleoptile in strong light. However, in the case of leaf, where the reversal is most striking, this storage effect was not observed, even after two months.

When grown in darkness, the reversal in both coleoptile and leaf persists even after two months of storage. In fact, under these conditions the most striking effect on the coleoptile growth was observed (Fig. 3).

With corn, the reversal in the dose response curve was found to persist even after 4 years of storage.

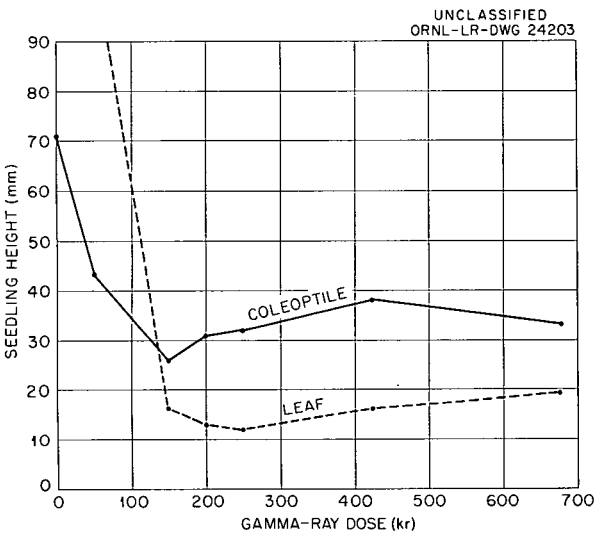

Fig. 3. Barley Planted Two Months After Irradiation; Growing in Darkness.

Behavior of a Highly Mutable Gene in Maize

D. Schwartz M. Patteson

The behavior of an unstable recessive gene at the *c* locus in maize is under investigation. This gene, designated c^m, mutates with a high frequency to a stable dominant allele, *C* (colored aleurone), and also to a stable recessive, *c* (colorless aleurone). The mutations occur at all stages in the life cycle of the plant. Late c^m – *C* mutations, occurring after fertilization, appear as colored spots on a colorless background. The size of such spots varies from only a few cells to half of the endosperm, depending on the time of the mutation. Where only very late mutations occur, more than 100 spots can be counted on a single kernel. Early mutations to *C*, occurring before fertilization, give rise to fully colored kernels. Very early mutations appear as clusters of colored kernels on the ear when the unstable gene is introduced in the cross by the female parent. These mutations are germinal. When colored kernels arising from the cross of $c^m/c \times c/c$ are backcrossed to homozygous recessive *c/c* plants, the progeny show 1:1 segregation for fully colored and colorless seeds, no mosaics.

It is difficult to recognize late mutations to the stable *c*. Many early mutations, which appear as clusters of completely colorless kernels, have been found. The progeny of such kernels are completely colorless.

It has been possible to select for early and late occurring mutations. Plants heterozygous for the unstable gene and a normal recessive allele were used as female parents in backcrosses to recessive tester plants, and the progeny were scored for the ratio of fully colored, mosaic, and colorless kernels. Ears from late mutating plants give half colorless kernels; the other half being mainly mosaic with a few germinals. With the early mutating plants this cross yields well over 50% colorless kernels. There is a good correlation between the per cent germinal kernels in the colored class and the per cent colorless kernels (Table 5). The excess of colorless kernels over 50% represents the early mutations to the stable *c*.

There is no detectable chromosome breakage involved in these mutations. Pachytene configurations show no aberrations. Chromosome breakage in maize endosperm tissue gives rise to the breakage-fusion-bridge cycle which results in variegation for markers located proximal to the point of breakage. No such variegation was observed within the mutation sectors either for *C* itself or for *Wx*. These mutations appear to involve intragenic changes. An explanation of these data on the basis of the *Ac-Ds* system of McClintock would require that the c^m gene is compound, composed of *C* and a suppressor of color, *S*. Mutations to *C* would result from deletions of the suppressor alone, while changes to the stable *c* would occur as a result of

Table 5. Results of Cross $c^m/c \times c/c^{♂}$

	Number of Ears	Mosaic	Fully Colored (germinals)	Colorless	Per Cent Germinal in the Colored Class	Per Cent Colorless
Late mutating plants	105	13,874	390	15,898	2.7	52.7
Early mutating plants	17	292	456	4,034	61.0	84.4

the deletion of the entire complex, *CS*. These events would have to occur either without breakage of the chromosome or with complete restitution. It would be very difficult to distinguish such a process from true gene mutation.

On the Production of Wild-Type Recombinants from Mixed Infection of K12(λ) Bacteria by T4rII Phage

D. R. Krieg

Introduction. – This work concerns bacteriophage T4 mutants of the type designated rII, which do not reproduce normally in K12(λ) strains of *Escherichia coli*. If such bacteria are mixedly infected with two rII mutants, either phage reproduction is essentially normal and the mutants are said to be complementary, or there is very little phage reproduction and the mutants are noncomplementary. Even in the case of infection by a single rII mutant, however, a small fraction of the infected bacteria lyse and produce a small burst of progeny phage; this fraction is called the "transmission coefficient." In the case of mixed infection by two pseudoalleles (noncomplementary mutants capable of recombining), a fraction of the bacteria produce wild-type recombinant phage. The original investigation[50] of these phenomena did not ascertain whether recombinants were detected because of the transmission coefficient, or were attributable in some way to the action of their wild-type genes.

This latter alternative implies the following two assumptions: phage in an rII-infected K12(λ) bacterium reach the vegetative state but cannot mature. In a suitable mixed infection, genetic recombination occurs, producing wild-type recombinants competent to express their phenotype, leading to the production of mature phage. This alternative has now been ruled out by experiments demonstrating that mixedly infected bacteria do not produce any T4 phage in excess of the number attributable to transmission coefficient.

Results. – Three experiments were performed, each using a different pair of rII pseudoalleles in a set of two experimental crosses and two controls to evaluate the transmission coefficient. In addition, four similar experiments were performed by using ultraviolet-irradiated phage as parents. This treatment increases the frequency of genetic recombination in crosses, so that an increased percentage of recombinant phage are found even among the first mature phage formed.[51] Since strain 112-12(λh) was used as the host bacteria, the transmission coefficient was low,[52] and, even if only one potentially competent vegetative phage were formed per mixedly infected bacterium, a manyfold increase in the production of progeny phage would have been detected. Phage production was determined both by assaying infected bacteria before lysis and by assaying progeny released after lysis. No appreciably increased titers were found in the experimental crosses in excess of that found in the controls.

Discussion. – It is concluded that there is no interaction between T4rII pseudoalleles in mixedly infected K12(λ) bacteria to produce a wild-type phenotype; transmission coefficient can account

[50] S. Benzer, *Proc. Natl. Acad. Sci. U.S.* **41**, 344–354 (1955).

[51] R. H. Epstein, personal communication.

[52] D. R. Krieg, UR-482 (May 7, 1957).

for all the phage produced. If vegetative phage are formed in such complexes, any rII^+ genes formed by recombination are not competent to express their phenotype; mature recombinants are released only in the bursts attributable to transmission coefficient. This indicates that the rII^+ gene of bacteriophage T4 in K12(λ) bacteria must initiate its phenotypic action prior to the occurrence of genetic recombination, possibly while the parental phage is in a "prevegetative" state.

INSECT CYTOLOGY AND GENETICS

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Radiation-induced Inherited Partial Sterility in *Habrobracon*

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Introduction. – Previously there has been no study of chromosome translocations in *Habrobracon*. The lack of such a study may be attributed to the large number of linkage groups (10) that should be marked for a complete genetic analysis, and the small size of the chromosomes which would make a cytological analysis tedious at best.

In mice, inherited partial sterility has been shown to be a reliable method for locating lines that are heterozygous for translocations.[55] The normal sequence of chromosomal events during meiosis results in a large number ($\frac{1}{2}$ to $\frac{2}{3}$) of the offspring of translocation heterozygotes deficient for one chromosome segment and having another segment duplicated. Gene balance is upset and death of the individual usually occurs during embryogenesis.

Techniques for analysis of radiation-induced dominant and recessive lethality are well worked out in *Habrobracon*. These methods appear to be the most suitable way to locate and analyze translocations in *Habrobracon* chromosomes.

Methods. – It is well known that translocations are more easily induced by x rays in sperm chromosomes than in egg chromosomes. This has been shown in *Sciara*, *Drosophila*, and mice. Consequently, *Habrobracon* males were used in this experiment; males were irradiated with 6000 r of x rays and were crossed with unirradiated females.

In *Habrobracon* fertilized eggs become females; unfertilized eggs become males. In order to perform the test for translocations, the regular procedure for finding recessive lethal mutations is used. In this test all female offspring (F_1) of the irradiated male and the normal female are collected as virgins and bred unmated. If a female is heterozygous for one or more recessive lethal mutations or translocations, then half or more of the F_2 embryos will die. If the deaths are to be attributed to aneuploidy resulting from segregation of a translocation heterozygote, one-half of the surviving F_2 males must carry the translocation. Several F_2 males from each F_1 female whose eggs showed reduced hatchability are mated to normal females and the eggs of F_3 daughters are tested for reduced hatchability. If the F_4 embryos show lowered hatchability, the F_1 female was a translocation heterozygote instead of having been heterozygous for a recessive lethal mutation.

Results. – At 6000 r there were 8 surviving female offspring from 320 fertilized eggs. The females were set as virgins and hatchability of the F_2 embryos recorded. All 8 females showed 50% hatchability or less. When the test for translocations through inherited partial sterility was employed, three of the original eight were shown to be heterozygous for one translocation, and the drastically reduced hatchability of one other indicated that she was heterozygous for two translocations. Since testing of the other four females did not indicate a pattern of inherited partial sterility, they were regarded as being heterozygous for recessive lethal mutations.

The hatchabilities of unfertilized eggs of the translocation heterozygotes are shown in Table 6. Hatchabilities were based on counts of at least 400 eggs for each translocation. As can be seen, hatchabilities vary from 35% to 50% for T1-T4. A survival of 33% is theoretically possible from known behavior of translocation heterozygote segregants in other organisms, but a value this low is rare. Fifty per cent survival is much more common, and T3 and T4 are of this type. The expected hatchability for a combination of two translocations, each of which would give 50% survival,

[53]Consultant.

[54]Research Associate.

[55]G. D. Snell, *Genetics* 31, 157–180 (1946).

Table 6. Hatchability of Fertilized and Unfertilized Eggs from *Habrobracon* Translocation Heterozygotes

Translocation	Egg Hatchability (%)	
	Unfertilized	Fertilized
T1	35.6	78.7
T2	38.3	81.0
T3	49.4	89.8
T4	47.4	86.1
T4,5	26.7	90.8

is 25%. The value obtained for T4,5, 26.7%, conforms closely to the expected hatchability. Translocation T4 was derived from stock T4,5.

The hatchability of fertilized eggs was considerably higher than that of the unfertilized eggs (Table 6) but adult survival was the same. A considerable number of aneuploid zygotes that were fertilized with normal sperm reached larval or pupal stages before dying.

By backcrossing, lines homozygous for all the translocations except T1 have been made. When homozygous, T1 appears to cause female sterility.

Discussion. – The behavior of these cases of inherited partial sterility in *Habrobracon* is in every way consistent with known behavior of chromosome translocations in other animals. There is no possibility that the effect can be that of a semilethal mutation, since hatchability of a semilethal heterozygote virgin would be above 50%, and for fertilized eggs adult survival would be 100%.

It is of utmost interest that the fertilized aneuploid eggs of translocation heterozygotes are delayed in time of death, most of them dying in larval or pupal stage, whereas the unfertilized aneuploid eggs die before hatching. This phenomenon has been observed in dominant lethality induced by radiation in eggs in the first meiotic metaphase.[56] This state is called conditionally delayed dominant lethality and has been attributed to chromosome breakage with consequent gene imbalance. The translocations provide a known source of gene imbalance, and the action of aneuploid segments in the haploid and diploid zygote is like that of radiation-induced conditionally delayed dominant lethality. Comparative evidence is thus provided that gene imbalance is the end result of this type of radiation-induced dominant lethality.

DROSOPHILA GENETICS

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An Analysis of the Fertility Factors on the Y Chromosome of *Drosophila melanogaster*

G. E. Brosseau, Jr.

Introduction. – In spite of its many extraordinary properties, the Y chromosome of *Drosophila* has been very poorly analyzed and little is known about this chromosome. The Y chromosome is certainly much more than simply a homologue for the X chromosome in the male; however, outside of the + allele of *bb* and a few male fertility factors, the Y is particularly poor in gene content as measured by more usual methods. When unusual genetic setups are used, however, a number of other properties are uncovered. An analysis of the fertility factors is one more or less direct approach for further investigation of the Y chromosome. While the presence of the factors is well established, nothing is known about the number or genetic nature of these factors. The purpose of this investigation is to obtain further information on these latter points.

Results. – By means of a special technique a total of 63 sterile Y chromosomes was recovered from 1650 irradiated Y chromosomes. By further genetic tests it was possible to determine that 13 of the sterile Y's were involved in translocations with the autosomes (these were discarded); of the remainder, 12 carried deficiencies for the fertility factors located on the short arm of the Y (Y^S) and 36 had deficiencies for factors on the long arm (Y^L). To obtain an estimate of the number of fertility factors on each arm of the Y,

[56] R. C. von Borstel, K. C. Atwood, and A. R. Whiting, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 36–39.

[57] American Cancer Society Fellow.

[58] Temporary Employee.

[59] Research Associate.

the sterile Y's were combined in all possible combinations (yielding $X/Y^{St}/Y^{St}$ males) to determine which ones are able to complement each other. The tests have been completed for 8 Y^{S}'s and 12 Y^{L}'s. The results of these tests are as follows: (1) Y-short deficiencies 3, 4, 8, 10, 12, and 13 are sterile *inter se* but fertile with number 5; therefore, there must be at least 2 fertility loci on this arm of the Y. Deficiencies numbers 11 and 14 are sterile in all combinations and hence must be at least double deficiencies. (2) Y-long deficiencies numbers 29 and 33 are fertile in any combination but sterile with each other, thus revealing one distinct locus. The remaining 10 deficiencies fall into 9 unique groups according to their behavior in the test crosses. Deficiency number 3 is fertile with itself and is being investigated further.

Discussion. – While the situation for Y^{S} is a straightforward one, the situation with regard to Y^{L} is much less clear. The tests reveal one distinct locus and an additional region that is characterized by nine unique deficiencies. On the basis of the information now available it is not possible to derive a linear sequence of units for this region if it is assumed that multiple deficiencies must lack adjacent loci. It is possible that this analysis has uncovered a new type of genetic region that is not composed of discrete genes in the classical sense, but it is also possible that the genes for male fertility show a curious type of interaction in their function. It is hoped that the results of crosses with the deficiencies not yet tested will help to clarify this point.

The Effect of AET Pre- and Posttreatment on the Induction of Dominant Lethals in *Drosophila*

C. W. Edington

Introduction. – Considerable attention in the last few years has been focused on the protection against radiation-induced death in animals. Some of the most effective chemicals thus far discovered which offer protection against radiation death are AET and its related compounds.[60,61] The present experiments were initiated to determine whether AET protected against radiation-induced genetic effects.

Results. – Oregon-R males were injected intra-abdominally with 0.15 μl saline, 0.15 μg AET per 0.15 μl saline, or 0.30 μg AET per 0.15 μl saline immediately before or after exposure to 4000 r of x rays. Appropriate controls were carried out simultaneously. The males were pair-mated to two successive sets of Oregon-R, virgin females for 24 hr each (sperm samples 1 and 2) and the frequency of dominant lethals (eggs unhatched/total eggs laid) was determined. The results of these experiments are given in Table 7. No difference can be demonstrated between the control series and any individual AET pretreatment series. However, if the controls plus the posttreatment series are pooled to increase the sample size and these are compared with the pooled AET pretreatment series, a significant increase in the percentage of dominant lethals in the AET pretreatment series can be demonstrated ($\chi^2 = 6.45$, $P = 0.01$ to 0.02). In sperm sample 2 there is a significant increase in the dominant lethal frequency in the AET injected series as compared to the saline controls when individual or pooled χ^2 tests are carried out. This effect is not due to either mutagenicity of AET or to metabolic poisoning (posttreatment series). Furthermore, replacement of the Br·HBr, that was thought to be possibly deleterious, in the AET compound with Cl·HCl did not alter the effect.

Discussion. – These results show that the induced genetic damage, as measured by dominant lethals, is not decreased but is increased, at least in the second sperm sample, when AET is injected prior to radiation.

The Analysis of Irradiated *Drosophila* Populations for Abnormally Segregating Chromosomes

G. D. Hanks E. Novitski
R. D. Wilkerson

Introduction. – The chromosomes analyzed in these experiments for abnormal behavior come from population cages of *Drosophila melanogaster* maintained at the Cold Spring Harbor Biological Laboratory by B. Wallace. These populations have been maintained for about 200 generations under constant irradiation; samples have been tested from a total of seven such populations. The genetic tests consist of mating both males and females heterozygous for chromosomes derived from the populations and for standard marked chromosomes to homozygous mutant stocks.

[60] D. G. Doherty and W. T. Burnett, Jr., *Proc. Soc. Exptl. Biol. Med.* 89, 312–314 (1955).

[61] D. G. Doherty *et al.*, *Biol. Semiann. Prog. Rep., Feb. 15, 1956*, ORNL-2060, p 45–46.

Table 7. The Percentage of Dominant Lethals Induced in *Drosophila* After Pre- and Postirradiation Injection of AET

Injected Material	Amount per Injection	Radiation Dose (r)	Sperm Samples 1: Unhatched Eggs / Eggs Laid	Sperm Samples 1: Per Cent Dominant Lethals	Sperm Samples 2: Unhatched Eggs / Eggs Laid	Sperm Samples 2: Per Cent Dominant Lethals
			Pretreatment			
		4000	177/244	79.02	370/524	70.61
AET·Br·HBr	0.15 μg		7/171	4.09	7/393	1.78
Saline	0.15 μl	4000	617/744	82.93	727/1010	71.98
AET·Br·HBr	0.15 μg	4000	271/310	87.42	604/754	80.11
AET·Br·HBr	0.30 μg	4000	342/400	85.50	707/858	82.40
AET·Cl·HCl	0.204 μg	4000	161/186	86.56	250/325	76.92
			Posttreatment			
Saline	0.15 μl	4000	249/295	84.41	253/387	65.37
AET·Br·HBr	0.30 μg	4000	275/335	82.09	215/333	64.56

A system of sequential analysis devised by A. W. Kimball of the Mathematics Panel permitted the early discard of those tests in which, with a probability of 1 in 20, an abnormally segregating chromosome would be mistakenly discarded, and which requires the retesting of chromosomes which show a deviation in the anticipated direction, an excess recovery of the population chromosome, expected by chance in only 1 out of every 50 trials.

Results. – Out of a total of 1654 initial tests, 33 were retested because there was an excess of progeny carrying the population chromosome. Of the latter group, two were singled out for special attention because they consistently behaved in this fashion, both in different kinds of crosses and over a number of generations. One of these involves the X chromosome and the other the second. In each case there are recovered in the progeny of male heterozygotes about twice as many of the "abnormal" chromosome as of the normal homologue, instead of the equality ordinarily expected.

Discussion. – The recovery of abnormal chromosomes of this type from irradiated populations is of some interest, since it suggests that irradiation may induce new chromosome types which have the property of appearing more frequently in the progeny of heterozygotes carrying that chromosome than would be expected. It can be shown that such a curious behavior would under certain conditions lead to an increase in the frequency of that chromosome in the population, even if it happened to carry moderately deleterious genes along with it. This is quite unlike the situation for usual deleterious radiation effects, which tend eventually to eliminate themselves from the population.

The Use of the Oracle[62] in Analyzing Data from Laboratory Populations of *Drosophila melanogaster*

E. Novitski E. Dempster[63]

Introduction. – Heterosis, or hybrid vigor, is a well-established genetic phenomenon. In some cases, however, heterosis is not directly observable as such but is inferred because a population appears to contain more heterozygotes than can be accounted for by ordinary genetic theory, that is, by the Hardy-Weinberg Law.

A precise mathematical model based on a maximum likelihood formulation has been set up which takes into account the expected frequencies of

[62] This automatic computer is operated by the Mathematics Panel of the Oak Ridge National Laboratory.

[63] Department of Genetics, University of California, Berkeley, California.

the different zygotic types if they have different viabilities, thus allowing for a numerical estimation of the degree of heterosis compatible with a set of observed zygotic types. The analysis of data using such a model is not practicable by ordinary methods, and for this reason the Oracle was programed to estimate the extent to which heterosis might be responsible for the apparent excess of heterozygotes in a population. The specific data analyzed were those presented by Hexter[64] as evidence for heterosis in laboratory populations of *Drosophila melanogaster*.

Results. – The output of the Oracle showed that the earlier[64] conclusion that heterosis was responsible for the increased frequency of heterozygotes in the populations under consideration was unwarranted and that the deviations from expectations based on the Hardy-Weinberg Law might be as readily explained by a reduced viability of individuals homozygous for mutant genes as by heterosis. The computer further indicated the algebraic nature of the above ambiguity, which was simply that any arithmetic values of viabilities would equally well fit the data, provided only that the square of the viability of the heterozygote divided by the viability of the homozygote equals a constant determined by the data.

Discussion. – This analysis is of some importance to the field of population genetics because it shows that one of the most commonly applied tests for heterosis, a statistical test for deviations from the Hardy-Weinberg Law in the direction of an excess of heterozygotes in a population, is in fact quite ambiguous. The analysis shows also the great advantages in the use of automatic computers in treating genetic data, where the mathematical formulation is so complex that there may exist ambiguities or a multiplicity of solutions not obvious without making a detailed study of the properties of those equations in the systematic way provided by a computer program.

[64] W. H. Hexter, *Genetics* 40, 444–459 (1955).

[65] Mathematics Panel.

A Note on the Frequency Distribution of Tetrads by Rank in *Drosophila melanogaster*

L. M. Sandler M. A. Kastenbaum[65]

Introduction. – Typical frequencies of no-exchange (E_0), one-exchange (E_1), two-exchange (E_2) and three-exchange tetrads (E_3) for free X chromosomes marked from *sc* to *f* have been computed by Weinstein.[66] These frequencies have been reported by Sandler[67] for reversed acrocentric compound X chromosomes. For free X chromosomes $E_0 = 0.06$, $E_1 = 0.64$, $E_2 = 0.28$, and $E_3 = 0.02$; for reversed acrocentrics $E_0 = 0.44$, $E_1 = 0.12$, $E_2 = 0.44$, and E_3 is assumed to equal 0. A model is presented here which attempts to rationalize these two distributions.

Results. – The proposed model is as follows: Consider first the case of free X chromosomes. Suppose that the physical basis of exchange (say chromosome replication) starts at one end of the chromosome and proceeds towards the other end and that in every genetic unit of length the probability of an exchange is α. After an exchange, however, the per-unit probability of an additional exchange is different, say β. If a second exchange takes place, the probability reverts to α, a third exchange changes it back to β, and so forth. Now if this is so, it seems likely that $\alpha > \beta$, in which case the distribution of exchange values from reversed acrocentrics (i.e., a high frequency of E_0 and E_2, with a low frequency of E_1) might be accounted for simply by assuming that this same model applies except that the first probability is β instead of α.

Chiasma interference is taken into account by supposing that, after every exchange, there are i genetic units in which an exchange cannot occur, after which interference is not operative. This is equivalent to replacing the true interference function by a step function whose value is zero for i units after every exchange and one thereafter.

[66] A. Weinstein, *Genetics* 21, 155–199 (1936).

[67] L. Sandler, *Genetics* 39, 923–942 (1954).

The probabilities of exchanges of ranks 0, 1, 2, and greater than 2 (P_0, P_1, P_2, and P_3) have been derived for a chromosome N genetic units long:

$$P_0 = (1-\alpha)^N ,$$

$$P_1 = \frac{\alpha(1-\beta)^{N-i} - \beta(1-\alpha)^{N-i}}{(\alpha-\beta)} - P_0 ,$$

$$P_2 = (1-\alpha)^{N-2i-1}\left[1 + \frac{(N-2i-1)\alpha\beta}{\beta-\alpha}\right] + \frac{\alpha^2(1-\beta)}{(\beta-\alpha)^2}\left[(1-\beta)^{N-2i-1} - (1-\alpha)^{N-2i-1}\right] - (P_0 + P_1) ,$$

$$P_3 = 1 - P_0 - P_1 - P_2 .$$

The solution of these equations (by iteration), for various sets of data, gives the results in Table 8.

Discussion. – From the foregoing, it can be seen that, with reasonable approximations for interference, for $0.04 < \alpha < 0.05$ and $0.02 < \beta < 0.01$, the data from free X and reversed acrocentric compound X chromosomes are approximately satisfied.

Table 8. Comparison of Observed Distribution of Tetrads by Rank with That Calculated from the Equations in the Text, Using the Parameters Shown

Free X's and reversed acrocentric X's are represented by two sets of data each

Genetic Situation	Tetrad Data			Estimates of Parameters			
	Rank	Observed	Calculated	N	α	β	i
Free X's	0	0.09	0.09	57	0.041	0.018	17
		0.06	0.06	57	0.047	0.017	17
	1	0.62	0.63				
		0.64	0.64				
	2	0.28	0.25				
		0.28	0.26				
	3	0.01	0.03				
		0.02	0.04				
Reversed acrocentrics	0	0.39	0.42	67	0.050	0.013	11
		0.44	0.48	67	0.048	0.011	11
	1	0.17	0.21				
		0.12	0.19				
	2	0.44	0.37				
		0.44	0.33				

GENETIC AND DEVELOPMENTAL EFFECTS OF RADIATION IN MICE

W. L. Russell, Section Chief

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The Nature of Spontaneous and Radiation-induced Mutations in the Mouse

W. L. Russell

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It is often suggested that most radiation-induced mutations may turn out to be deficiencies and that, on the other hand, most spontaneous mutations may be point mutations. The question is of considerable importance. Among the loci used in our studies on mutations at specific loci, there are two that were chosen in the expectation that they would provide information on this subject. These are the *D* and *Se* loci. Their value lies in their extremely close linkage (0.16 average crossover percentage). This is closer than that in maize between the *A* locus and an adjacent locus which is now known to have been involved in the two smallest deficiencies at the *A* locus studied by Stadler and Roman.[3] It was, therefore, reasonable to expect that if deficiencies can be induced at the *D* or *Se* locus in the mouse, then they might sometimes, perhaps often, involve loss at the associated locus, thereby giving the phenotypic appearance of simultaneous mutation to the *d se* genotype. It has already been reported[4] that no such double losses were found among a small sample of induced mutations at the two loci. The evidence on this subject is now much more extensive and is presented briefly here.

The number of mutations occurring separately at either the *D* or *Se* locus that have now been recovered from irradiated spermatogonia, and that have been completely tested for their allelism and effect in homozygous condition, is now 17 (14 at the *D* locus and 3 at the *Se* locus). Several additional mutations have been found which, although they have not been tested, are almost certainly correctly classified as mutations at these loci. Not one of those mutations from irradiated spermatogonia has involved the associated locus.

In surprising contrast to this is the fact that, in the control (nonirradiated) groups, where only two *D*-locus and no *Se*-locus mutations have been observed, there have, nevertheless, been two cases of simultaneous loss of the phenotypic effects of *D* and *Se*, that is, apparent deficiencies involving both *D* and *Se* loci. This, incidentally, indicates that the *D* and *Se* loci are in close proximity on the chromosome, a fact which was suggested, but not proved, by their low crossover percentage. The surprise, of course, is the fact that deficiencies, or deficiencies of this size, may constitute a sizable proportion of the spontaneous mutations, but have yet to be found among the induced mutations recovered from irradiated spermatogonia.

One explanation for the lack of irradiation-induced deficiencies that seemed plausible to us was the possibility that such deficiencies are induced in spermatogonia, but fail to go through gametogenesis. This hypothesis seemed to gain support when the two spontaneous *d se* mutants were tested. Details of these tests are too extensive to give here, but the results indicated that both of these mutations failed to go through gametogenesis to the next generation. It then seemed likely that deficiencies of this kind would be recovered in experiments in which sperm, or postspermatogonial cells, were irradiated, rather than spermatogonia.

For this reason, and others, such experiments have now been performed, and already three apparent *D Se* deficiencies have been recovered in a much smaller total number of animals than that obtained from irradiated spermatogonia. Two of these have been tested and have furnished another

[1]Consultant.

[2]Temporary Employee.

[3]L. J. Stadler and H. Roman, *Genetics* 33, 273–303 (1948).

[4]W. L. Russell, *Cold Spring Harbor Symposia Quant. Biol.* 16, 327–336 (1951).

surprise: both are transmitted through gametogenesis.

It is clear that further explanation is needed for the absence of deficiencies, or, at least, deficiencies of this size, in experiments involving irradiated spermatogonia. Whatever the explanation may be, it appears that the commonly held view that induced mutations differ from spontaneous mutations in being mostly deficiencies does not apply to mutations recovered from irradiated spermatogonia. If anything, the reverse appears to be true.

It may seem surprising that such basic findings as those reported here have not been clearly demonstrated in *Drosophila*. This is explained by the fact that so little work has been done on the spermatogonial stage in *Drosophila*. Since the spermatogonial stage is the important stage in spermatogenesis so far as genetic effects of radiation in man are concerned, the mouse results are of considerable practical importance. In this connection it should be emphasized that, although the mutations recovered from irradiated spermatogonia have so far shown no deficiencies of a size that apparently occurs in high proportion among spontaneous mutations and mutations induced in postspermatogonial stages, nevertheless the spermatogonial mutations are markedly deleterious, most of them being lethal in homozygous condition.

Comparison of the Effects of Acute and Fractionated Irradiation on Fertility of the Female Mouse

L. B. Russell M. K. Freeman

The fact that female mice become permanently sterile soon after even relatively low doses of acute radiation has made it difficult to study genetic effects of radiation in females. The present experiment was undertaken to determine whether this difficulty could be circumvented by dose fractionation.

Female (101 × C3H)F_1 mice, in groups of 12, were exposed to 0, 50, 100, 200, or 300 r of x rays (delivered at about 80 r/min) (a) in a single exposure, (b) in 25-r fractions given twice weekly, or (c) in 10-r fractions given five times weekly. The irradiations were arranged such that the last exposures in all groups fell on the same day, following which the females (3 months old at the time) were mated singly for the rest of their lifetime. The total number of young obtained was 5810.

The average number of litters per control female was 14.4. Single exposures markedly decreased this number even at the lowest dose used. Dividing the dose into 10-r fractions in all cases allowed more litters per female than single exposures: 12.2 vs 4.0 for total doses of 50 r, given in 10-r fractions and as a single dose, respectively; 9.5 vs 2.0 for 100 r; 4.2 vs 1.7 for 200 r; 2.0 vs 1.4 for 300 r. Division of the total dose into 25-r fractions was less effective in delaying sterility than division into 10-r fractions, although in each case 50 r was accumulated per week. Parallel results obtain with respect to litter size; its decline in the last litter or last few litters becomes less steep with increasing fractionation. Table 9 shows the average number of young per female obtained in the various groups.

Table 9. Average Number of Young per Female* Produced During the Reproductive Life Span Following Various Doses of X Rays Administered in Various Fractions

Total Dose (r)	Average Number of Young per Female Exposed in the Following Way		
	Single Exposure	25 r, Twice Weekly	10 r, 5 Times a Week
0	91.2		91.7**
50	23.2	52.3	80.0
100	14.1	17.8	61.8
200	10.0		21.0
300	7.6		13.7

*Twelve females in each of the 12 groups.

**Females taken through all the motions of irradiation 30 times, which was the maximum number of exposures for any of the irradiated groups.

Since more young are produced when a given dose is divided into 10-r fractions than when it is divided into 25-r fractions, it seems highly probable that chronic irradiation of females will be even more compatible with the retention of fertility. Experiments now under way indicate that this is indeed the case.

The Incidence of Disturbed Fertility Among Male Mice Conceived at Various Intervals After Irradiation of the Mother

L. B. Russell L. Wickham

Strain (101 × C3H)F_1 females were mated at various intervals after receiving 400-r x rays and their sons were tested for fertility. Groups A to E consisted of 320 males conceived 1½ to 34½ days after irradiation; group O, of 80 contemporary sons of nonirradiated mothers. Results are based on about 7000 matings, made with 2454 females.

(1) Complete sterility occurred only among males conceived 13½ days or longer after irradiation. Its incidence in group C (13½ to 17½ days) is 6.2%; summed over groups A to E, 1.9%. (2) Males giving infrequent litters due to many infertile matings were found only among sons of irradiated females (0.9%). (3) The incidence of males giving infrequent litters due to infrequent mating is equal among sons of irradiated and control females (1.3% in each); among the former, however, there are another 2.8% with questionably reduced libido. (4) Slightly but consistently reduced litter sizes characterized 1.3% of group O, 1.3% of groups A to E; while another 1.9% of groups A to E gave questionably reduced litter sizes. Only one male, conceived 25½ days after irradiation, was clearly "semisterile," gave semisterile sons, and thus presumably carries a translocation. This result confirms that in mice, as in *Drosophila*, translocation induction in females is much less frequent than in males.

Summing all categories of disturbed fertility, the incidence among sons of irradiated mothers (5.4%; or 8.4%, if questionable cases are included) is higher, but not significantly so, than among sons of control mothers (2.5%). Group C has the highest incidence of disturbed fertility.

X-Ray Sensitivity of Primary Spermatocytes of the Mouse

E. F. Oakberg R. L. DiMinno

Spermatozoa utilized in successive matings after an acute radiation exposure initially represent cells irradiated as mature sperm, followed in turn by gametes derived from spermatids and spermatocytes. It is well known that doses even above those required for a 100% incidence of dominant lethals in the mouse have no effect on development of cells irradiated as spermatids, or on fertilizing capacity of mature sperm. Irradiated primary spermatocytes, however, may undergo selection during meiotic division. While this possibility is generally recognized, the extent of this loss of cells has not been adequately evaluated.

Hybrid 101 × C3H male mice were exposed to 0, 100, 200, 300, 400, 600, 800, and 1000 r of x rays. Doses in this range have almost no effect on meiotic prophase, but do result in increased degeneration of primary spermatocytes in metaphase I of the meiotic divisions. Quantitative evaluation of total cell loss is most accurately made by allowing degeneration to occur, then scoring the number of surviving spermatids in stage II tubules. On the basis of the timing of spermatocyte development given in Table 10, mice were killed at times

Table 10. Timing of Primary Spermatocyte Development in the Mouse

Cell Stage	Duration (hr)		Interval to Meiotic Divisions (days)	Interval to Stage 2 Spermatids (days)
Preleptotene	31.0		12.3	14.1
Leptotene	31.2		10.8	12.5
Zygotene	37.5		9.5	11.3
Pachytene	175.3	early	7.7	9.5
		mid	5.1	6.9
		late	2.1	3.8
Diplotene	21.4		0.88	2.6
Diakinesis-metaphase I	10.4			

appropriate for scoring sensitivity of spermatocytes irradiated during preleptotene, leptotene, zygotene, early pachytene, mid-pachytene, late pachytene, diplotene, and diakinesis to metaphase I. Spermatids were counted in 10 randomly selected stage II tubule cross sections for each mouse.

Anisocytosis of spermatids arising from irradiated spermatocytes obfuscates the relationship between spermatid count and production of functional gametes, since the most extreme size variants probably do not show continued normal development. Accordingly, data of Table 11 are based only on those spermatids of approximately normal size. The LD_{50} for preleptotene is significantly lower than for any other stage. The LD_{50}'s for leptotene through diakinesis and metaphase I range from 314 to 411 r, with no significant differences. Additional data at properly selected doses possibly would indicate stage differences within the leptotene to diakinesis group, but it is unlikely that such differences would be large. These results do indicate that gamete contribution of primary spermatocytes in presterile period matings is markedly reduced by doses of 200 to 400 r. With doses of 800 r or more, only very few gametes are derived from cells irradiated as primary spermatocytes.

These results indicate that the relationships of fertility and genetic effects observed in successive weeks after exposure to irradiation probably are dose dependent. The latter part of the presterile period, when sperm available for fertilization have been derived from irradiated spermatocytes, would be primarily affected.

Table 11. LD_{50}'s for Various Primary Spermatocyte Stages

Based on number of approximately normal stage 2 spermatids derived from irradiation of specified meiotic prophase stages

Stage Irradiated	LD_{50}	95% Limits	
		Lower	Upper
Preleptotene	112.9	10.9	177.3
Leptotene	321.1	227.9	395.4
Zygotene	364.2	308.9	413.4
Early pachytene	313.8	290.1	336.1
Mid-pachytene	350.5	320.3	379.3
Late pachytene	387.6	340.5	430.5
Diplotene	350.5	281.7	412.3
Diakinesis and metaphase I	411.3	328.1	478.7

The Linear Arrangement of Loci Which Constitute a Probable Pseudoallelic Series in *Drosophila melanogaster*

W. J. Welshons

Three recessive mutants are available which appear to constitute a pseudoallelic series at the Notch locus. It was reported earlier[5] that the linear arrangement of these three mutants from left to right on the X chromosome appeared to be *facet* (fa), *facet-notchoid* (fa^{no}), and *split* (spl). Recombination in heterozygotes of *facet* and *facet-notchoid* yielded the double mutant chromosome carrying both *facet* and *facet-notchoid* and the reciprocal wild-type recombinant. Heterozygotes of *facet-notchoid* and *split* also produced the expected double mutant and the wild-type recombinants. However, the heterozygotes of *facet* and *split* produced only the wild-type product, and it was assumed that the double mutant could not be detected because the expression of the mutant *facet* could not be observed when *split* was present. The following experiments indicate the validity of this assumption.

Females of the genotype $++fa^{no}spl+/w^{a}fa++rb$ were mated to $fa^{no}spl$ males. If crossing over occurs between fa^{no} and spl, it would be expected that $++fa^{no}+rb$ and $w^{a}fa+spl+$males would be recovered in the next generation. If the expression of *facet* cannot be detected when *split* is present, the latter product of crossing over would be phenotypically *apricot* and *split*, but this chromosome should also carry the mutant *facet*.

Approximately 28,200 males were obtained from the above cross, of which five were $fa^{no}rb$ and seven were presumably $w^{a}fa\,spl$. These latter seven were mated to homozygous *facet* females, and the heterozygotes, $+fa+/w^{a}fa\,spl$, were found to resemble *facet* phenotypically. Thus it seemed likely that the mutant *facet* was present on the same chromosome which carried *split*.

[5] W. J. Welshons, *Biol. Semiann. Prog. Rep. Feb. 15, 1956*, ORNL-2060, p 29–30.

In an earlier cross, a recombinant chromosome had been obtained which was expected to carry both *facet* and *split* although males carrying such a chromosome were *split* in phenotype. This chromosome was used to construct heterozygous females of the genotype w^a++rb+/+fa spl+ sn. If *facet* is present, a crossover between fa and spl should yield hemizygous males which are phenotypically *facet* and *ruby*. Eleven such males were observed among the 28,300 male progeny.

These experiments indicate that both reciprocal products of an exchange between *facet* and *split* do occur among the progeny of heterozygous females, but only the wild-type recombinant is normally recovered since the *facet* phenotype cannot be detected in flies which are also phenotypically split. Therefore, the linear array of these loci, which constitute a probable pseudoallelic series, was properly indicated in the earlier report.[5]

RADIATION PROTECTION AND RECOVERY IN BACTERIA

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X-Ray Sensitivity of Synchronous Cultures of *Escherichia coli*

G. E. Stapleton N. A. Sicard

Introduction. – Stapleton[4] demonstrated the phasic changes in x- and gamma-ray sensitivity of *Escherichia coli* B/r at the various stages of the culture cycle. Similar changes have been shown for ultraviolet sensitivity. Bruce and Maaloe[5] have attempted unsuccessfully to demonstrate such changes in "synchronous" cultures of *Salmonella typhimurium*. Synchronous cultures should be ideal for such studies in that essentially the cells should be homogeneous with respect to biochemical activity as well as cell division. A number of systems have been successfully used to obtain bacterial cultures synchronous in cell division including temperature shift[4] and recently by use of auxotrophic mutants with periodic withholding and supplying the requirement.[6] It was decided that the technique of Barner and Cohen[6] might be most appropriate, since the synchrony with these cells (strain 15_{T-}) apparently depends on synthesis of DNA (see Appendix for alphabetical list of abbreviations) and is precluded if thymine is withheld from the culture medium. The techniques used were similar to those of Barner and Cohen with minor changes.

Results. – Several preliminary experiments were carried out, by using 250-kvp x rays at 30 ma, in which stationary phase cells of *E. coli* (15_{T-}) were irradiated for 3 min at 8600 r/min. In several experiments, in each of which 5 samples were irradiated, there was less than 5% variation in survival among the various samples, indicating that it would be possible to demonstrate changes in sensitivity based on differences in survival greater than this amount. It was planned to irradiate samples taken from synchronous and nonsynchronous cultures as frequently as practical. Aliquots were removed simultaneously from the culture for viable cell count, for chemical determination of nucleic acids, and for irradiation. Samples were irradiated under the normal conditions of growth, that is, in growth medium with aeration at 37°C, and then diluted and plated on nutrient agar.

The results to be reported are preliminary, and since synchrony was not obtained routinely, the data are selected according to whether or not synchronous division was obtained. Figure 4 shows the comparison between the relative viable cell count of a synchronous and a normal logarithmic culture during 100-min incubation time, the first 30 min of which is in the absence of thymine, the latter 70 min following thymine

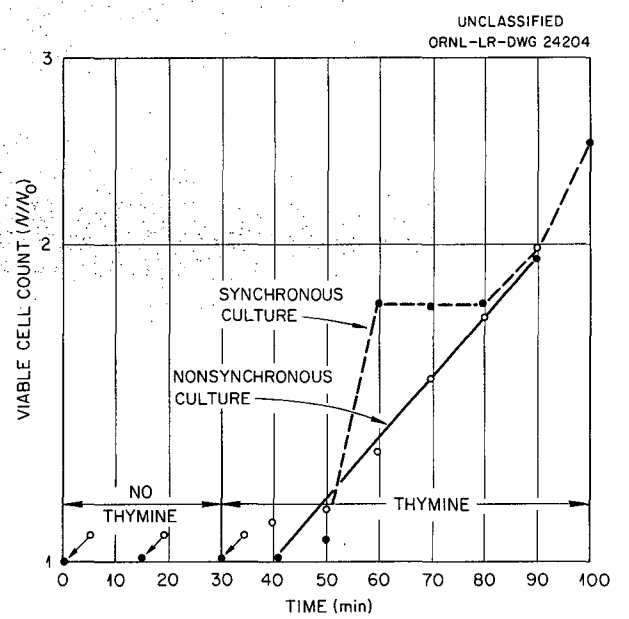

Fig. 4. Comparison of Synchronous and Nonsynchronous Division in *Escherichia coli* (15_{T-}).

[1]Research Associate.

[2]Visiting investigator from abroad.

[3]Research Participant.

[4]G. E. Stapleton, *J. Bacteriol.* 70, 357–362 (1955).

[5]V. G. Bruce and O. Maaloe, *Biochim. et Biophys. Acta* 21, 227–233 (1956).

[6]H. Barner and S. Cohen, *J. Bacteriol.* 72, 115–123 (1956).

addition (2 μg/ml). It is clear that when synchrony is obtained a large increase in viable cell count occurs within a short time period (10 min) preceded and followed by a lag phase. On the other hand, when synchrony does not occur the cells divide logarithmically with a mean generative time of 45 to 50 min. Figure 5 compares the change in sensitivity as a function of incubation time for selected experiments with synchronous and nonsynchronous cultures. It appears that drastic changes in sensitivity occur in synchronous cultures as compared with logarithmically dividing cultures. Since these are preliminary results and since synchrony is not obtained as yet in every experiment, it is too early to attempt the correlation with biochemical activity or with the processes preceding or following cell division.

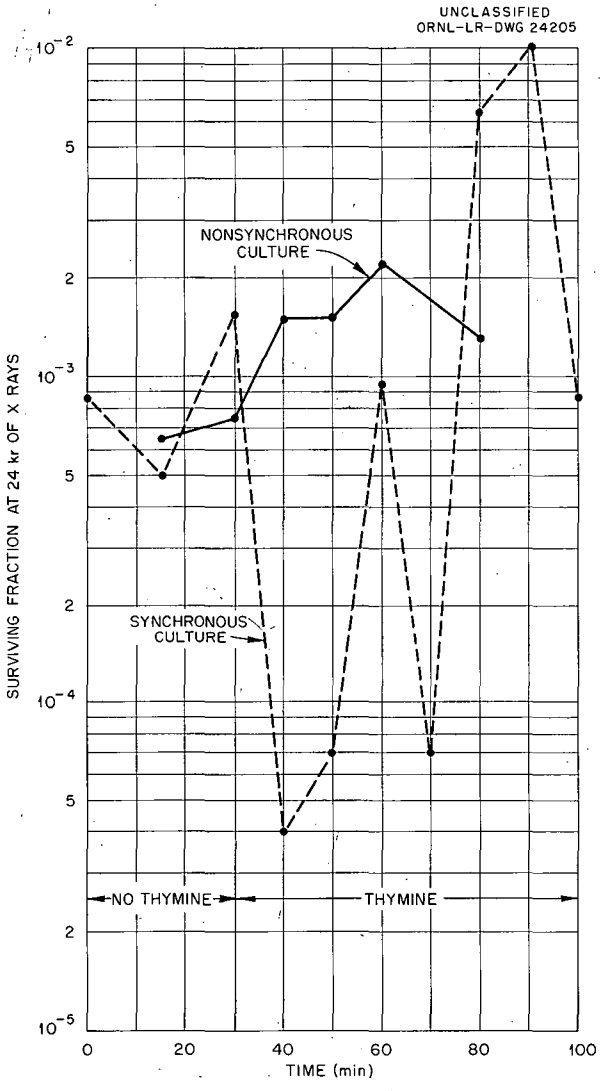

Fig. 5. Variations in X-Ray Sensitivity During the First Division Cycle for Synchronous and Nonsynchronous Cultures.

An After-Effect of Ionizing Radiation on a Mutant of *Escherichia coli*

H. I. Adler

Introduction. – The bacterium under study is a mutant of *Escherichia coli* Monod, which must have hemin supplied in its growth medium in order to synthesize the iron-containing enzymes, notably catalase and the cytochromes.[7] Since the role of these enzymes in the radiation behavior of biological systems is of considerable interest, a study of the response of this bacterium to ionizing radiation was undertaken.

Results. – If this bacterium (H_7) is grown with hemin (H_{7+}) and then exposed to an x-ray dose sufficient to inactivate approximately 99% of the cells (26 kr in 3 min), little if any inactivation of the survivors is observed as a function of post-irradiation holding time at 25°C. If, however, the bacterium has been grown without hemin (H_{7-}) and exposed to the same dose, inactivation of the survivors continues after the end of irradiation. This after-effect may be prevented by washing or diluting the irradiated cells in unirradiated buffer. However, if irradiated H_{7-} cells are diluted in irradiated phosphate buffer or irradiated water (26 kr), the inactivation proceeds. If nitrogen is bubbled through the buffer before and during irradiation, less inactivating material is produced than in oxygen. Since the inactivating material is present in oxygen-containing irradiated buffer or water and has been shown to be stable over several hours, hydrogen peroxide is implicated.

Figure 6 shows that reagent hydrogen peroxide (9.3 μg of H_2O_2 per milliliter) and buffer exposed to 26 kr of x rays produce the same after-effect. Figure 7 shows that the after-effect can be prevented by adding catalase to the irradiated buffer before adding irradiated H_{7-} cells. This is a specific function of the enzymatic action of the

[7] M. Beljanski, *Compt. rend.* 240, 374–377 (1955).

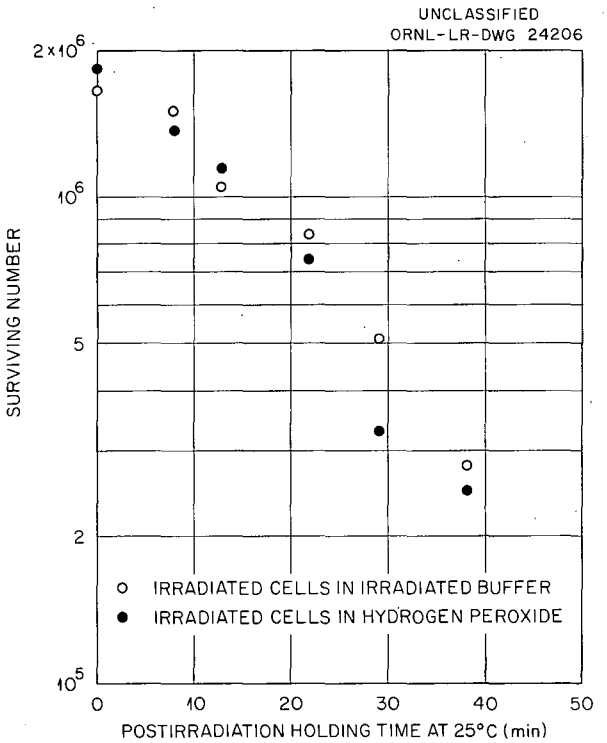

Fig. 6. Comparison of Inactivation of *Escherichia coli* (H_{7-}) Produced by Buffer X-irradiated with 26 kr and Reagent Hydrogen Peroxide.

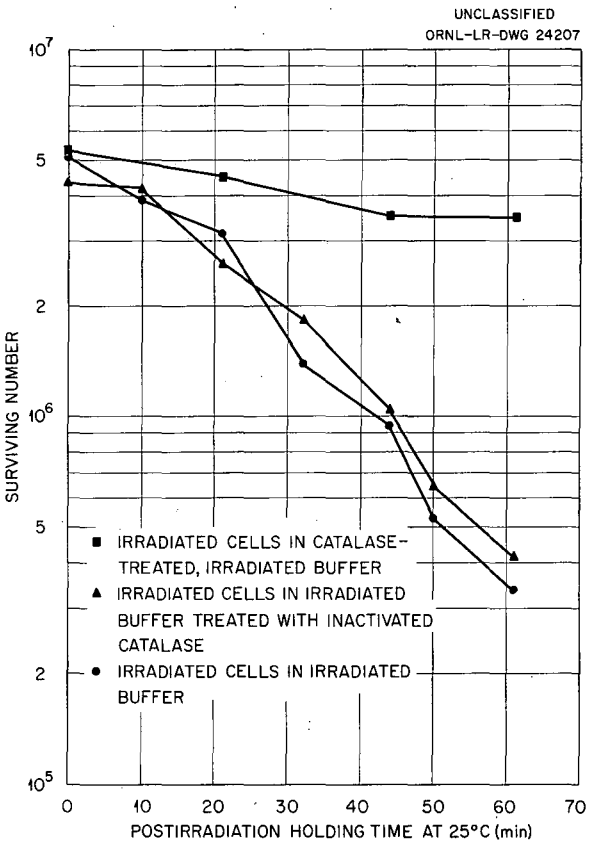

Fig. 7. Catalase Prevention of the After-Effect.

catalase, since catalase inactivated by heat or acid treatment cannot prevent the after-effect on H_{7-} cells.

Discussion. – From the above evidence it has been concluded that irradiated H_{7-} cells can be inactivated by hydrogen peroxide produced by irradiation of the suspending medium. This is not true for unirradiated H_{7-} cells, since it can be demonstrated that buffer irradiated with 26 kr has no toxic effect on such cells. Catalase negativity *per se* is not sufficient to make cells susceptible to the levels of hydrogen peroxide studied here. Apparently, radiation causes a "sensitization" to hydrogen peroxide, similar to the "part inactivation" of bacteriophage described by Alper.[8] The nature of the "sensitization" process will be investigated.

[8]T. Alper, *Brit. J. Radiol.* **27**, 50–54 (1954).

Response of Potassium Retentivity and Survival in Yeast to X Irradiation in Oxygen and Nitrogen Atmospheres

A. K. Bruce

Introduction. – Studies on the effect of near-ultraviolet and far-ultraviolet radiation on potassium retentivity and survival in yeast[9] have indicated that responses of these two criteria of radiation damage result from independent processes. This investigation extends these studies to x irradiation under both aerobic and anaerobic conditions.

Experimental. – Suspensions of fresh, starved, baker's yeast have been exposed to x radiation under oxygen and nitrogen. Viability has been determined by plate count after irradiation at 25°C and potassium retentivity after irradiation

[9]A. K. Bruce, *Biol. Semiann. Prog. Rep. Feb. 15, 1957*, ORNL-2267, p 37.

at both 0° and 25°C by methods previously described.[9] Irradiation was carried out by the use of a G-E Maxitron x-ray unit operated at 250 kvp and 30 ma, filtered with 1 mm of aluminum. Yeast suspensions of 20 mg/ml (wet weight) were exposed while being bubbled vigorously with either oxygen or nitrogen in a Petroff-type pyrex culture flask, placed in a controlled temperature water bath, constructed of Lucite 7 mm thick, attached to the x-ray machine. The distance from the target to the center of the suspension was 11.3 cm. The dose rate was determined as 4100 rep/min by chemical dosimetry by the use of the ferrous sulfate system[10] with the oxidation of ferrous ion assayed by titration with potassium dichromate to the end-point of diphenylamine.

Results and Discussion. – The dose-responses to 250-kvp x radiation at 25°C under oxygen and nitrogen upon survival of this strain of yeast are exponential and are shown in Fig. 8. A dose reduction factor of 2.5 is found which is similar to that demonstrated for a number of organisms.[11,12] When potassium retentivity is taken as the criteria of radiation damage, as is shown in Fig. 9, a much more dramatic difference is seen. For irradiation in oxygen this response is exponential, being only about one-fifth as sensitive as is survival. This is intermediate between the responses from far and near ultraviolet radiations.[9] If, however, irradiation is carried out under anaerobic conditions, not only is the response decreased in magnitude, but its shape departs from exponential, showing an initial gradual decrease in potassium retentivity followed by a sharp decrease at high doses. Lowering the temperature to 0°C during irradiation moves the response under nitrogen to considerably higher doses than when carried out at 25°C. Little, if any, difference in response is observable between irradiation at 0° or 25°C if carried out in the presence of oxygen.

These results further substantiate the conclusion that radiation effects on the potassium retentivity mechanism are not directly related to those responsible for the loss of viability as determined

[10] C. J. Hochanadel and J. A. Ghormley, *J. Chem. Phys.* 21, 880–885 (1953).

[11] A. Hollaender, G. E. Stapleton, and F. L. Martin, *Nature* 167, 103–106 (1951).

[12] W. T. Burnett, Jr., *et al.*, *Proc. Soc. Exptl. Biol. Med.* 77, 636–638 (1951).

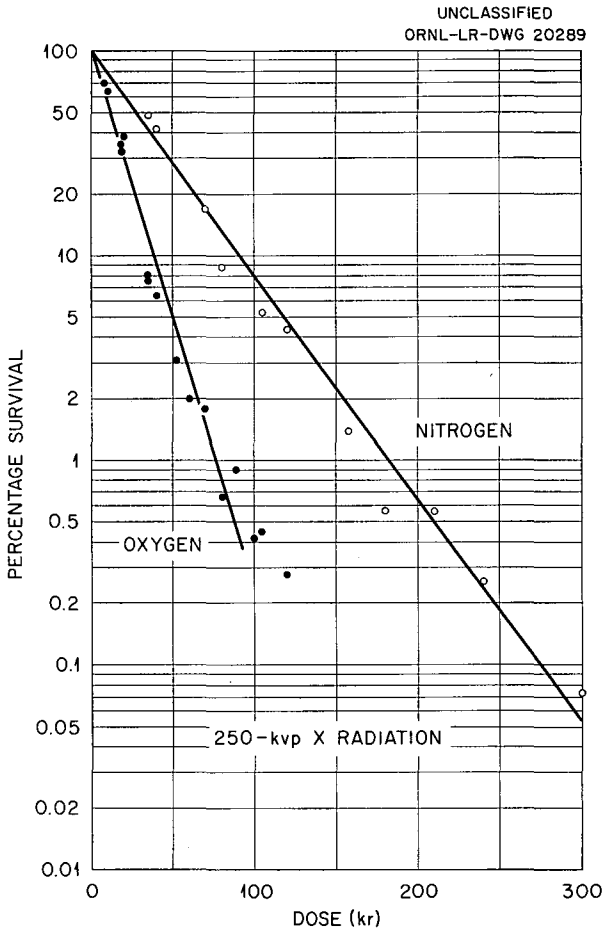

Fig. 8. Dose Response of Survival in Yeast to 250-kvp X Rays in Nitrogen and in Oxygen.

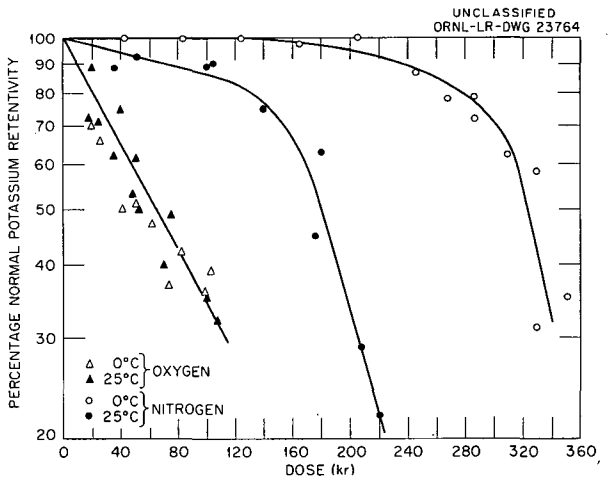

Fig. 9. Dose Response of Potassium Retentivity in Yeast to 250-kvp X Rays in Nitrogen and Oxygen at 0°C and at 25°C.

by plate counts.[9] The response of the potassium retentivity mechanism would appear to be due to damage at a site which is separate from that which controls the response of viability of the cell. Loss of potassium by irradiated cells does have an effect on their behavior when, as in the assay of viability, they were studied on plates. Studies by Bair and Stannard[13] on the influence of removal of potassium from normal and irradiated yeast by treatment with the cation exchange resin Dowex-50 found a lower metabolic rate, as measured by gas exchange, in the irradiated cells than the controls. If potassium was added back to the cells, then the metabolic rate of the irradiated cells approached that of the controls. This would be expected to result in a lengthened lag time for the formation of visible colonies, and under some conditions to prevent visible colony formation. In these experiments, as with those using ultraviolet radiation,[9] irradiated yeast was indeed found to result in delayed colony formation, requiring 5 to 6 days for visible colonies rather than 2 days as in the controls. The loss of potassium may or may not be the explanation for this behavior, since the addition of potassium to the medium did not result in a higher colony count; in fact, somewhat fewer colonies were observed.

The hyperprotection afforded the system responsible for potassium retentivity by irradiation in nitrogen as compared with oxygen, in contrast to survival, requires an explanation. Several possibilities exist in this respect: (1) The curves as shown could possibly represent a multiple-hit type of phenomenon in nitrogen. An extrapolation according to the Atwood and Norman analysis[14] yields hit numbers from about 10 to 100 depending on the fitting of the experimental points and the temperature at which irradiation was carried out. Such values do not correspond to any known biological entity, and the variation with temperature renders this type of analysis invalid. (2) During anaerobic metabolism compounds possessing considerable reducing potential, as alcohol, are accumulated. Such compounds could be expected to act as "protective" substances such as has been demonstrated for ethanol and cysteamine.[12,15] The shapes of the curves are indeed what would be expected for a limiting quantity of such a compound, that is, protection until the quantity of material present is exhausted by reaction with oxidizing products of the irradiation followed by an inactivation at the rate of the unprotected system. The temperature data, however, do not support such a conclusion, since increased temperature would be expected to yield a greater quantity of this "protective" material and result in a displacement of the sharply dropping portion of the curve to the right or higher doses. Instead, at the higher temperature the curve is displaced to the left. (3) Since metabolic reactions occurring at or near the cell surface, the area where potassium exchange is presumed to be controlled, are progressing at a rapid rate, the removal of oxygen from the proximity of these sensitive "sites" may be considerably more efficient than from the nucleus by enzymatic processes.[16] From studies of the effect of external pH upon potassium exchange,[17] it seems probable that effects upon this system are more dependent upon the conditions of external environment than upon those inside the cell. From the combined processes of nitrogen bubbling and enzymatic oxygen removal the external oxygen tension is probably rapidly reduced to a very low level, resulting in little contribution to radiation damage from indirect effects dependent on oxygen. The change in shape and temperature displacement of the curves, however, are not explained by this postulate. (4) A change in slope could result if the curve found for irradiation in nitrogen is not due to the effect upon a single system, but rather upon two processes contributing to the retention of potassium. At the lower dosage levels the effect could presumably be upon the system primarily responsible for monovalent cation retention, while at the higher doses a more general damage to the membrane could result, releasing a number of intracellular constitutents, including potassium. Microscopic examination of the cells at high doses

[13] W. J. Bair and J. N. Stannard, *J. Gen. Physiol.* 38, 493–504 (1955).

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

[15] P. Alexander *et al.*, *Radiation Research* 2, 392–413 (1955).

[16] G. E. Stapleton, D. Billen, and A. Hollaender, *J. Bacteriol.* 63, 805–811 (1952).

[17] A. Rothstein and C. Demis, *Arch. Biochem. and Biophys.* 44, 18–29 (1953).

delivered in nitrogen and preliminary studies on the dose-response of the loss of 260-mμ absorbing material and amino acids[18] do not substantiate this explanation. Further studies are in progress and projected which, it is hoped, will test the above hypotheses and elucidate the behavior of the system responsible for potassium retention in the yeast cell in response to irradiation under anaerobic conditions.

Effect of X Rays on the Efflux of Potassium from *Escherichia coli*, Strain B

A. P. Harrison, Jr.

Introduction. – The increase in potassium efflux that occurs upon x irradiation of baker's yeast has been studied in some detail;[19] the purpose of the present study has been to determine whether irradiation has a similar effect upon potassium efflux in bacteria. Although a few experiments have been undertaken with other species, this report will deal exclusively with *Escherichia coli*, strain B.

Methods. – Cells harvested from trypticase broth were washed three times with distilled water, then suspended in distilled water at a concentration of 20 mg (wet weight) per milliliter. Ninety-milliliter aliquots of this suspension were placed into each of two Petroff flasks. One flask was irradiated with 250-kvp x rays for 15 min (60 kr). The other flask served as the control. Both flasks were oxygenated at a rate of 1 liter per minute for 15 min prior to irradiation, and also during irradiation. After irradiation, an aliquot from each flask was centrifuged at 4000 rpm (Serval Angle-Head centrifuge) for 10 min, and the clear supernatants were decanted directly into test tubes or decanted through millipore filters into the test tubes. The cell pellets were resuspended in fresh water and the manipulations were repeated. Each such cycle of events required 15 min. In some experiments the resuspended cells were held for a brief period prior to the next centrifugation; this extended the cycle to 20 min. Variations in the above efflux technique were sometimes employed; for example, compounds such as glucose or dilute HCl were added to the water, or the suspensions were bubbled with different gases between centrifugations. Each supernatant was analyzed for its potassium content by flame photometry. A summation of the individual analyses was made, and the quantity of potassium effluxed per kilogram (wet weight) of cells was calculated. The quantity of potassium (millimoles) effluxed per kilogram of bacteria was plotted vs time.

Results and Discussion. – Typical efflux-time curves are presented in Fig. 10 (I, II, III). The efflux rate of the irradiated cells is greater than that of the control. Moreover, it appears that leakage of potassium commences sooner in the case of irradiated cells (Fig. 10-III). Lowering the pH of the wash water by means of HCl increases the efflux rate of both irradiated and nonirradiated cells. However, the effect on nonirradiated cells is proportionally greater, so with lowering pH and the concomitant increase in efflux, the difference between irradiated and nonirradiated efflux rates becomes less pronounced. Variations in the pH of different distilled waters and differences in the buffering capacity of different cell crops have been considered to be responsible for the lack of quantitative reproducibility from experiment to experiment. Accordingly, the use of buffers has been contemplated, although it is admitted that addition of foreign ions to the test system is not desirable. The presence of an energy source such as glucose (also possibly citrate) depresses the efflux rate of both irradiated and nonirradiated cells and, like low pH, lessens the difference between the two rates. The efflux rates are not affected by bubbling with air, oxygen, or nitrogen. Most important, potassium efflux and the conditions which govern it appear to be influenced by the manner of cultivation of the test organism (*E. coli* B).

[18] D. Billen, *Arch. Biochem. and Biophys.* **67**, 333–340 (1957).

[19] A. K. Bruce and J. N. Stannard, *J. Cellular. Comp. Physiol.* (in press).

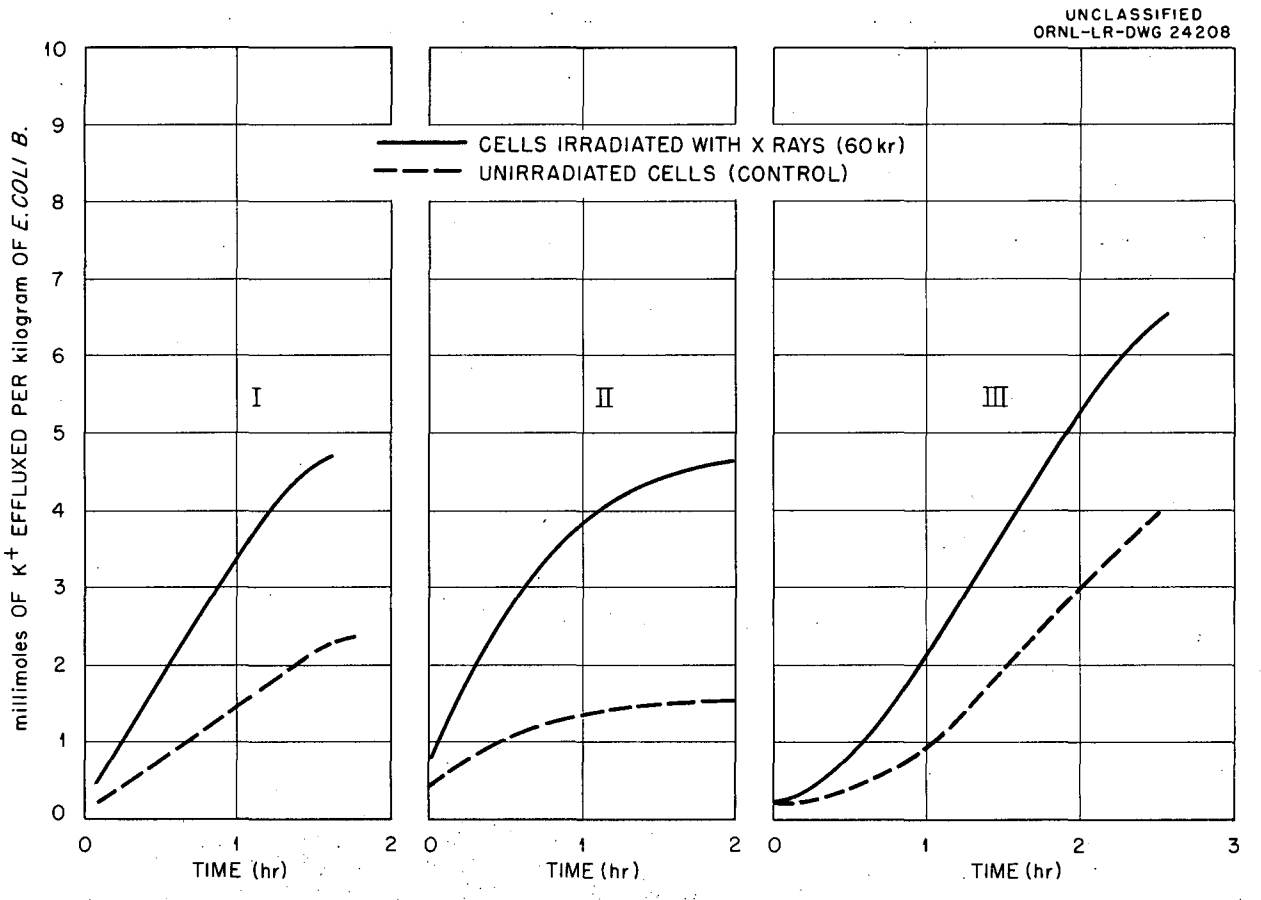

Fig. 10. Potassium Efflux of X-irradiated and Nonirradiated Cells of *Escherichia coli*, Strain B.

MAMMALIAN RECOVERY

C. C. Congdon

J. W. Goodman[1] L. H. Smith
D. Billen I. S. Urso
J. Toha[2] G. W. Royal[3]
A. P. Henley

Bone Marrow Cell Separation[4]

J. W. Goodman

Introduction. – The technique developed by Anderson, Makinodan, and Shekarchi[5] for separation of rat bone marrow cells was tried on mouse bone marrow cell suspensions. Isologous bone marrow suspensions were obtained from the femurs of normal (C57BL × 101)F_1 mice. The bone marrow cell suspension was layered onto a density gradient made up of BSA (see Appendix for alphabetical list of abbreviations) and Tyrode's solution. On centrifugation, essentially the same layers were obtained as those reported earlier.[5] The layers of densely packed cells were then taken off and kept separate from the intervening clear areas. Cell counts were made on each layer and the intervening clear areas. Appropriate numbers of cells from each cut were then injected into lethally irradiated mice.

Results. – The survival of lethally irradiated mice given different amounts of the separated cells in comparison with fresh whole bone marrow suspensions is shown in Fig. 11. Table 12 shows the approximate contents of each layer for a typical experiment. Layer A, which contained relatively few intact cells, did not promote survival of lethally irradiated isologous mice. In separate experiments not illustrated, the separated layers were reconstituted and tested in irradiated mice. The results did not differ from whole, fresh bone marrow cell suspensions.

Discussion. – None of the layers or intervening clean areas was better than whole, fresh bone marrow when compared on a cell dose basis. The clear area in the lower half of the gradient and D contained cells as effective as whole, fresh bone marrow. Layers B and C, although not so effective as whole, fresh marrow on a total-count basis, did show the greatest evidence of true morphological cell separation. Further investigation of these layers is being carried out. The toxicity of various materials used, or potentially useful, in the cell separation procedures was tested on fresh bone marrow, with subsequent injection of the bone marrow into lethally irradiated mice. BSA, PVP, Kel-F_1, dextran, hyaluronidase, heparin, and DNAse did not show toxicity under the conditions tested. Chymotrypsin showed some toxicity by this test.

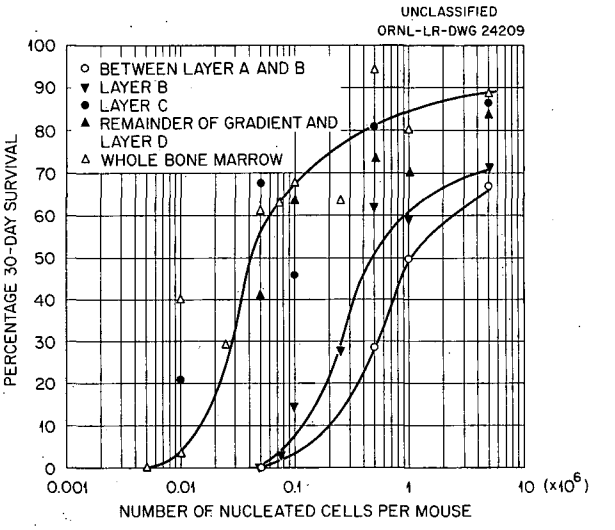

Fig. 11. Effect of Separated Cell Dosage on 30-Day Survival of Lethally Irradiated Mice. All experiments were done at 900 r. Whole-bone-marrow curve represents survival data on 466 animals; other data represent tests on 711 animals.

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[1]Consultant.

[2]Visiting investigator from abroad.

[3]Research Participant.

[4]This project is being carried out in collaboration with the Cell Physiology and Radiation Immunology Groups.

[5]N. G. Anderson, T. Makinodan, and I. C. Shekarchi, *Biol. Semiann. Prog. Rep. Feb. 15, 1957*, ORNL-2267, p 59–61.

Table 12. Isologous Bone Marrow Cell Layers

Layer	Cell Content	Volume (ml)	Total Nucleated Cell Count	Percentage of Total Nucleated Cell Count
			($\times 10^6$)	
A	Platelets, debris, rare mononuclear cells	2.9	0.28	0.18
Between A and B	Many mononuclear cells, few RBC	5.0	1.31	0.83
B	Very many mononuclear cells, many RBC	6.3	18.80	11.83
C	Rare RBC, blast cells, and maturing cells	8.9	48.75	30.65
Remainder of gradient and D	Megakaryocytes, blast cells, and maturing cells	38.5	89.55	56.51
	Total	61.6	158.69	100.00

Pathologic Findings in the Delayed Heterologous Bone Marrow Reaction

C. C. Congdon

Introduction. – The delayed foreign bone marrow reaction in lethally irradiated mice that received rat bone marrow resembled that seen with homologous bone marrow. The appearance, behavior, and time of death were nearly identical. An autopsy study was performed to compare the two situations from a pathologic standpoint.

Results. – Eighty-one male and female (101 × C3H)F_1 mice were exposed to 950 r of x radiation and given an intravenous injection of rat bone marrow. The cumulative mortality at 15 days after irradiation was 6%; at 30 days, 36%; at 60 days, 67%; at 90 days, 74%; and at 120 days, 75%. Gross autopsy examination of 50 mice dying between 8 and 104 days after exposure and treatment with rat bone marrow showed marked weight loss, with reduction of adipose tissues. Graying of hair was delayed. Occasional animals showed edema of the body wall and sometimes anasarca. Atrophy of lymph nodes, thymus, and Peyer's patches was almost invariably present. The spleen was sometimes enlarged but also often extremely atrophic. Focal necrosis throughout the liver and ulceration of the gastrointestinal tract were sometimes seen. Marked congestion of the lung was a common autopsy finding. Massive accumulations of parasites in the intestine were observed in a number of mice. These could sometimes be seen invading the lymphatics of the mesentery. Histologic examination of the autopsy material showed essentially the same changes reported for the delayed homologous bone marrow reaction. Reactive changes in the lymph nodes progressed from an early granulomatous reaction to complete atrophy and fibrosis of the lymph nodes. Partial reconstitution of lymph nodes with lymphocytes was infrequently seen, but none could be considered normal. Simple atrophy of the thymus was always present. The white pulp of the spleen was never normal. It was atrophic in all animals. Fibrinoid necrosis of the white pulp of the spleen was not seen in this autopsy series but has been observed in other irradiated mice dying after treatment with rat bone marrow. The bone marrow was intact in most of the mice at autopsy; only rarely was it depressed enough to help account for the death of the animal.

Discussion. – These pathologic changes do not differ appreciably from those reported for the delayed homologous bone marrow reaction.[6]

[6] C. C. Congdon and I. S. Urso, *Am. J. Pathol.* 33, 749–767 (1957).

The Potentialities of Tissue Cultured Cells of Hemic Origin in X-irradiated Mice

D. Billen

Introduction. – The use of isologous bone marrow cells maintained in tissue culture for short periods in the treatment of irradiation injury has been reported.[7] McCulloch and Parker described two rapidly proliferating, mouse-bone-marrow-derived, "altered" cell lines.[8] They could be grown continuously in tissue culture and were reported to have some effect in the treatment of irradiation injury.[8] The two cell lines, MC32 (derived from the bone marrow of a C57BL mouse) and CF_1C9 (derived from the bone marrow of a CF_1 mouse), were obtained for testing on lethal irradiation injury.

Results. – *Protective activity:* The MC32 cells (1.0 to 10.0 × 10^6 cells) were injected both intravenously and intraperitoneally into (C57BL × 101)F_1 (900 r) and C57BL (700 r) irradiated recipients. Of a total of 21 recipients so treated, none survived 30 days.

For testing of the CF_1C9 cell line, (C3H × 101)F_1 (900 r) mice were used, since the isologous strain CF_1 was not available. Again, the results were negative in that all 38 animals injected (0.6 to 2.0 × 10^6 cells) intravenously died. There was no significant increase in mean survival time for recipients receiving either line, except in one experiment involving the MC32 line in which several animals did survive a number of days past the control death times.

Tumor formation: In experiments originally designed to test for possible immunologic potential of these cell lines, it was found that when fresh homologous bone marrow cells were injected, together with the MC32 cells, a number of the surviving recipients develop tumors on the greater omentum with the accumulation of a bloody ascites fluid. In this experiment, C57BL mice (750 r) received (C3H × 101)F_1 cells intravenously and MC32 cells intraperitoneally. Five animals remaining alive at 25 days died within the next three days with tumor formation. In a similar experiment, using isologous fresh bone marrow cells in place of homologous cells, two of three animals dying past day 15 showed tumors at autopsy.

Discussion. – The tissue cultured, bone-marrow-derived cell lines as used here were unable to bring about the hematopoietic repopulation required for survival. Two possible explanations can be considered. The cell line may have originated from a nonhemic cell present in the primary explant. The other possibility is a loss of hematopoietic potential *in vitro*. Dedifferentiation or loss of normal cell function *in vitro* could well be involved in the lack of protection by the cell lines tested. This same phenomenon may be operative in cultures of fresh bone marrow cells in which the effectiveness of such cells was lost at two weeks of *in vitro* life.[7] The fact that the MC32 cell line was derived from supposedly normal bone marrow and is now capable of uncontrolled growth in irradiated recipients illustrates the profound alterations that occur *in vitro*. From the viewpoint of maintaining *in vitro* effective bone marrow cells for extended lengths of time, it would seem that the maintenance of hemic cell differentiation is essential.

Long-Term Survival of Irradiated Mice After Bone Marrow Therapy

G. E. Cosgrove[9] C. C. Congdon
A. C. Upton[9]

Introduction. – In a previous report, survival of lethally irradiated (L × A)F_1 mice treated with isologous and homologous bone marrow was shown for a 12-month period.[10] Some of these animals have now been observed for an additional 6 months. The survival of irradiated mice given a delayed injection of homologous bone marrow on day 1, 2, or 3 after exposure is also shown.

Results. – The data are presented in Fig. 12. Between 7 and 12 months after exposure and bone marrow treatment, all groups showed an increase in mortality. The mice given a delayed injection of homologous bone marrow showed no increase in mortality between 4 to 11 months after treatment. In unirradiated controls taken from a separate experiment, the mortality associated with aging did not begin until 21 months of age.

Discussion. – The survival after isologous bone marrow treatment is essentially the same as that

[7] D. Billen, *Nature* 179, 574–575 (1957).

[8] E. A. McCulloch and R. C. Parker, *Proc. Second Canad. Cancer Res. Conf.* 2, 152–167 (1957).

[9] Pathology and Physiology Section.

[10] C. C. Congdon, A. C. Upton, and I. S. Urso, *Biol. Semiann. Prog. Rep. Feb. 15, 1957*, ORNL-2267, p 39–40.

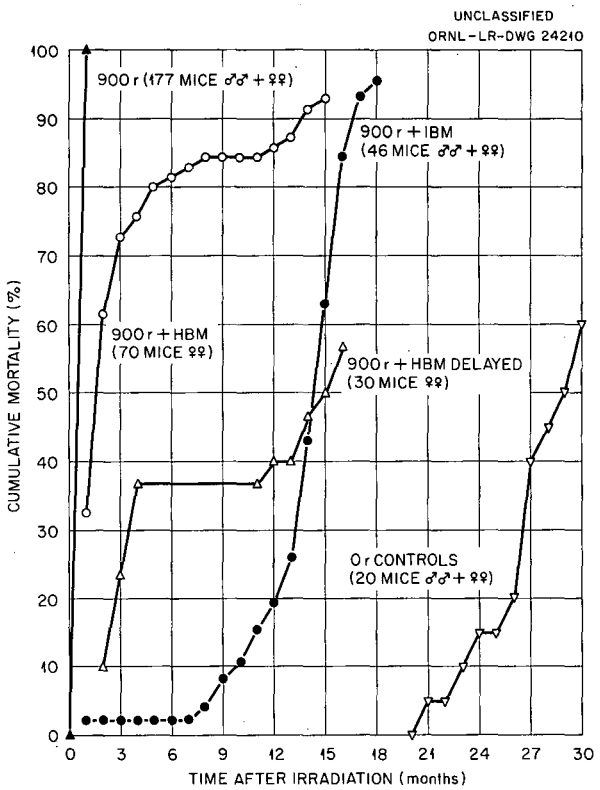

Fig. 12. Cumulative Mortality in Lethally Irradiated Mice With and Without Bone Marrow Treatment in Comparison to a Group of Unirradiated Controls.

reported by Barnes and Loutit[11] and by Cole, Nowell, and Ellis[12] for treatment with isologous spleen cell suspensions. Spleen shielding during lethal irradiation also gave a similar long-term survival pattern.[13] The irradiation effect, which shortens the life span by 14 months at the 50% death point in the comparison made between unirradiated controls and irradiated animals treated with isologous bone marrow, constitutes one of the major problems in prevention and treatment of irradiation injury.

Survival Time of Erythrocytes Taken from Irradiated Mice Treated with Rat Bone Marrow

J. Toha L. H. Smith

Introduction. – With immunohematological techniques, it was shown that the circulating erythrocytes of lethally irradiated mice given rat bone marrow are of rat origin.[14] Experiments were started, using Cr^{51}, to study the *in vivo* survival time of these mouse-grown rat erythrocytes in normal rats. The erythrocytes were labeled *in vitro* with the isotope. Three groups of normal rats were used. Group R-R and group M-R received labeled rat erythrocytes and labeled mouse erythrocytes, respectively; and group MR-R received tagged erythrocytes from x-irradiated, rat-bone-marrow-injected mice that showed 100% rat erythrocytes by agglutination tests. The rats were transfused intravenously with the labeled erythrocytes and the activity of tail blood samples determined at intervals. The initial 24-hr blood sample was taken as the 100% level for each rat, and the activity of the succeeding samples was plotted as the per cent of the initial activity.

Results. – The distribution of activity values plotted against time resembled an exponential curve. The slopes for each of the groups, therefore, were determined and plotted on semilogarithmic paper. The slope for groups R-R, MR-R, and M-R was -0.0178 ± 0.001, -0.0304 ± 0.004, and -1.46 ± 0.52, respectively (Fig. 13). The slopes for groups R-R and MR-R were significantly different ($P < 0.001$) from the slope of group M-R. The slope of group R-R was also significantly different ($P < 0.001$) from that of group MR-R.

Discussion. – The slope for group M-R indicates that the disappearance rate of normal mouse erythrocytes transfused in the rat is very rapid (less than four days). The erythrocytes from an irradiated rat-bone-marrow-treated mouse (MR-R), however, circulate much longer. To date, the data indicate that the slope for normal rat erythrocytes is significantly less than that of erythrocytes from irradiated rat bone marrow-treated mice. Inspection of the nonlogarithmic curve, however, indicates that the greater slope of group MR-R results primarily from a more rapid disappearance rate of the labeled erythrocytes during the first 15 to 20 days.

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[11] D. W. H. Barnes and J. F. Loutit, *Nucleonics* 12(5), 68–71 (1954).

[12] L. J. Cole, P. C. Nowell, and M. E. Ellis, *J. Natl. Cancer Inst.* 17, 435–445 (1956).

[13] J. Hollcroft *et al.*, *J. Natl. Cancer Inst.* 18, 615–640 (1957).

[14] T. Makinodan, *Proc. Soc. Exptl. Biol. Med.* 92, 174–179 (1956).

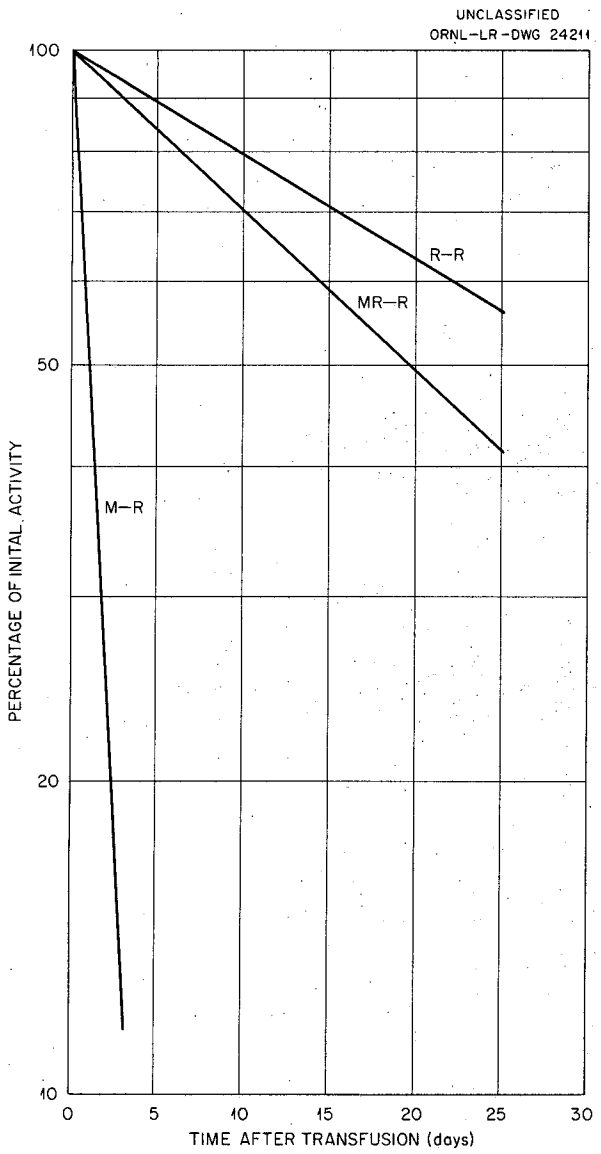

Fig. 13. Survival of Normal Rat, Normal Mouse, and Mouse-grown Rat Erythrocytes.

Effect of Fetal Liver and Newborn Spleen from Homologous and Heterologous Donors on Long-Term Survival of Lethally Irradiated Mice

I. S. Urso

Introduction. – Isologous blood-forming tissues, whether from fetal liver, spleens of newborn mice, or adult bone marrow, do not cause the delayed foreign bone marrow reaction. Adult homologous and heterologous bone marrow, although preventing the acute radiation death, causes a foreign bone marrow reaction that kills most of the mice 2 to 3 months after irradiation. In preliminary experiments, homologous fetal liver treatment was associated with less of these delayed deaths than would be expected with homologous or heterologous bone marrow.[15] Additional experiments, using homologous and heterologous fetal liver and newborn spleen, are reported here. The recipients of homologous blood-forming tissues were irradiated $(L \times A)F_1$ mice, and irradiated $(101 \times C3H)F_1$ mice or the reciprocal hybrid were used as recipients of the heterologous blood-forming tissue. Homologous fetal liver and newborn spleen came from $(101 \times C3H)F_2$ mice. The liver was taken at 15 to 21 days gestation. One homologous fetal liver was used for each recipient. Fifty to 75% of a fetal heterologous liver was used per irradiated recipient. Smaller amounts gave very little 30-day survival.

Results. – Figure 14 shows the results. Homologous fetal liver showed less delayed mortality than adult homologous bone marrow. The early data on heterologous fetal liver also suggest less delayed mortality.

[15] C. C. Congdon and I. S. Urso, *Am. J. Pathol.* **33**, 749–767 (1957).

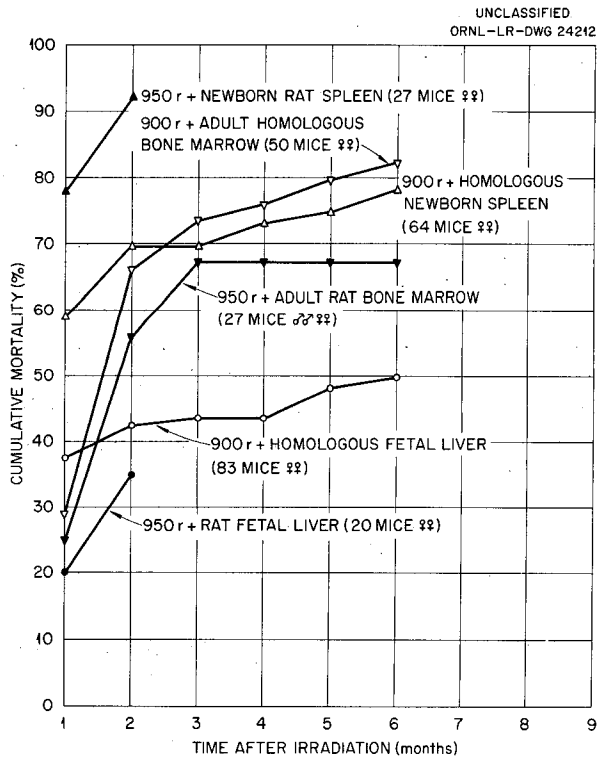

Fig. 14. Delayed Mortality in Lethally Irradiated Mice Given Foreign Blood-forming Tissues.

Discussion. – The data on newborn heterologous spleen may be of limited significance, since the highest dose of cells given (approximately 24×10^6) is much less than the standard rat bone marrow cell dose. The killing effect of foreign spleen (homologous newborn spleen varying from 1 to 10 days of age and heterologous from 1 to 24 days of age) may also influence the 30-day mortality. The reason for less delayed mortality with foreign fetal liver is not apparent. Sufficient fetal bone marrow cannot be obtained to make a comparison with fetal liver.

RADIATION IMMUNOLOGY

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Relative Effects of Splenic and Bone Marrow Cells on Lethally Irradiated Mice

T. Makinodan N. Gengozian
I. C. Shekarchi

Introduction. – Based on our previous studies,[3–5] the following hypothesis was formulated to explain the delayed foreign bone marrow reaction. Lethal irradiation has caused a temporary curtailment of the immune mechanism of the host, and this permitted the transplantation of closely related foreign bone marrow. In time, however, there is a gradual recovery of the host antibody-producing cells which will react against the proliferating foreign transplant. Subsequent validity tests substantiated this hypothesis, in part.[6–8] Barnes *et al.*[9] submitted an alternative hypothesis; that is, the antibody-producing cells present in the transplanted bone marrow tissue are reacting against the host tissues and thereby causing the delayed foreign bone marrow reaction. In view of this expressed sentiment, it became necessary to further investigate the problem of the fate of lethally irradiated mice treated with foreign bone marrow. Since spleen is one of the few organs, among the many implanted, agreed by all investigators as being a site of antibody production, it was felt that information obtained from studies dealing with the relative effects of spleen and bone marrow cells on the immune mechanism, as well as survival patterns, of lethally irradiated mice should contribute to the existing problem. The following reports such findings.

Results. – The 950-r mice that received rat RBC (see Appendix for alphabetical list of abbreviations) simultaneously with IBM (isologous bone marrow) did not produce any detectable circulating antibody during the 30-day period of observation. In contrast, the 950-r mice that received rat RBC simultaneously with ISp (isologous spleen) responded. Circulating antibodies were first detected 5 days after treatment, and the peak titer was attained 12 days after treatment. The results are shown in Fig. 15.

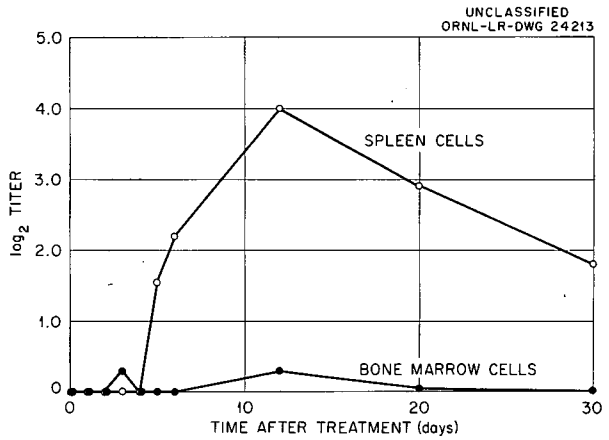

Fig. 15. Antibody Production of 950-r Mice Treated with 12 × 10^6 Spleen Cells or Bone Marrow Cells and Injected with RBC Antigen.

Of the 93 (C3H × 101)F_1 mice exposed to 950 r, all died within 13 days after irradiation. Excellent protection, however, was afforded to 950-r mice treated with IBM or ISp. During the 60-day period of observation, none of the 22 950 r-IBM mice died, and only 1 out of 24 950 r-ISp mice died. Of the 51 950 r-HBM (homologous bone marrow) mice, 20 died in 30 days, and 14 died during the ensuing 30- to 60-day period. In contrast, all the 119 950 r-HSp (homologous spleen) mice died in 36 days, of which 116 died within the first 16 days.

Discussion. – Two types of data are presented. The first demonstrates circulating antibodies in lethally irradiated mice treated with ISp and the

[1]USPHS Fellow.

[2]Temporary Employee.

[3]T. Makinodan, *Proc. Soc. Exptl. Biol. Med.* **92**, 174–179 (1956).

[4]N. Gengozian and T. Makinodan, *J. Immunol.* **77**, 430–436 (1956).

[5]I. C. Shekarchi and T. Makinodan, *Radiation Res.*, in press.

[6]C. C. Congdon, T. Makinodan, and N. Gengozian, *J. Natl. Cancer Inst.* **18**, 603–612 (1957).

[7]N. Gengozian and T. Makinodan, *Cancer Research*, in press.

[8]T. Makinodan, *J. Cellular Comp. Physiol.* **50**, Suppl. (1957), in press.

[9]D. W. H. Barnes *et al.*, *J. Cellular Comp. Physiol.* **50**, Suppl. (1957), in press.

antigen. On the other hand, lethally irradiated mice treated with IBM and the antigen did not synthesize detectable amounts of circulating antibodies during the 30-day period of observation. These results substantiate the assumption that the spleen of a normal mouse contains more antibody-producing cells than the bone marrow.

In accepting this conclusion, it would be expected, then, that injection of HSp into a lethally irradiated mouse would further accentuate the deleterious effects of radiation, while the bone marrow would operate in favor of recovery. Our second series of experiments confirmed this expectation. Only 10% of the 950 r-HBM mice died by the 10th day, 16% by the 15th day, 39% by the 30th day, and 67% by the 60th day. In contrast, 87% of the 950 r-HSp mice died by the 10th day, 96% by the 15th day, and 100% by the 36th day.

These results further substantiate our hypothesis discussed earlier concerning the delayed death patterns of lethally irradiated mice treated with foreign bone marrow.

Relation of Primary Antigen Injection to Time of Irradiation on Antibody Production in Mice

N. Gengozian T. Makinodan

Introduction. – The depressing and often inhibitory effect of total-body x irradiation on antibody production, shown initially by Benjamin and Sluka[10] to be dependent upon the time of antigen injection relative to x-ray exposure, has been repeatedly corroborated in the literature.[11] This report is a study of the effects of the temporal relation of x irradiation and antigen injection on agglutinin production, using the following criteria for immunologic efficiency: (a) induction period, (b) rate of appearance of circulating antibody, (c) mean peak titer, (d) time of peak titer, and (e) mean total titer.

Results. – Intraperitoneal injection of antigen (1 ml of 1% sheep RBC) was performed at intervals of 10, 5, 3, 1, and ½ days before 710-r x irradiation and 0, ½, 1, 5, 15, 30, and 45 days after a similar exposure; antigen injection on day 0 was given 5 min after x ray. For each group, agglutinin production was followed for 50 days after antigen injection; the data are shown in Table 13. (a) Induction period: at antigen injection intervals from 1 day before to 5 days after x irradiation, the induction period was lengthened from 5 to 9 times the normal, the greatest suppression of the immune mechanism under this criterion occurring when antigen was given on day 0. (b) Rate of appearance of circulating antibody: in the groups receiving antigen from 1 day before to 45 days after x ray, a considerable slowing in the appearance rate was observed, the $\log_2$ titer per day ranging from 0.07 to 0.77, as compared to a normal value of 2.25. (c) Mean peak titer: peak titers significantly lower than the normal were obtained with the nine groups injected with antigen 3 days before to 30 days after irradiation. The greatest suppression of antibody production under this criterion occurred among the groups receiving antigen on day 0, ½, or 1 after irradiation. Antigen injection 5 days before x ray produced a marked increase in peak titer, significantly greater than the normal control ($\log_2$ titer 10.3 vs normal 8.7). (d) Time of peak titer: in general, the greatest delay in attaining peak titer (40 or 50 days) occurred among the groups receiving antigen injection from 3 days before to 30 days after x irradiation. The absence of any definite relation of time of peak titer with the varying sequences of irradiation and antigen injection makes this criterion of immunologic efficiency a variable and inconsistent one. (e) Mean total titer: with the exception of the group receiving antigen injection 5 days before x ray, all experimental groups showed a mean total titer significantly lower than the normal, the $\log_2$ titer ranging from 2.33 to 7.46 as compared to the control value of 8.01. Maximum inhibition occurred with antigen injection on day 0, ½, or 1 after irradiation. In the group receiving antigen 5 days before x ray, the mean total titer was significantly greater, not less, than that of the normal control.

A general appraisal of the results shows that when all five criteria are considered as a whole, the greatest suppression of antibody formation occurred when antigen was injected on day 0, ½, or 1 after irradiation. Analysis of the data in Table 13 in terms of the degree of immunologic suppression relative to the normal (i.e., ratio of experimental/normal) shows that irradiation did not affect the different indices of antibody response to the same degree. Thus, the degree of inhibition

[10] E. Benjamin and E. Sluka, *Wien. klin. Wochnschr.* 21, 311–313 (1908).

[11] W. H. Taliaferro and L. G. Taliaferro, *J. Immunol.* 66, 181–212 (1951).

Table 13. Effect of 710-r Radiation on the Immunologic Efficiency of Mice Receiving Antigen Injection at Varying Intervals Before (–) or After (+) X-Ray Exposure

Time of Antigen Injection in Relation to Time of 710 r (days)	Criterion of Immunologic Efficiency				
	Induction Period (days)	Primary Rate of Appearance of Circulating Antibodies ($\log_2$ titer day^{-1})	Mean Peak $\log_2$ Titer (L.S.D.* at 5% level = 1.11)	Time of Peak Titer (days after antigen injection)	Mean Total $\log_2$ Titer (L.S.D.* at 5% level = 0.40)
Normal	2	2.25	8.70	18	8.01
−10	2	2.25	8.20	30	7.46
−5	2	2.25	10.30	24	8.61
−3	2	2.00	7.20	40	5.16
−1	10	0.21	6.40	50	3.83
−0.5	14	0.28	5.50	40	3.23
+0.003	18	0.29	4.40	50	2.34
+0.5	13	0.32	3.80	50	2.39
+1	12	0.07	3.60	40	2.33
+5	10	0.08	5.20	50	2.90
+15	5	0.30	6.80	30	4.96
+30	3	0.34	6.70	50	5.40
+45	3	0.77	7.90	12	5.44

*L.S.D. = least significant difference.

observed with x irradiation is, in ascending order, (1) rate of appearance of antibody $\geq$ (2) induction period > (3) mean total titer > (4) mean peak titer.

Discussion. – Suppression of the immune mechanism was obtained in both pre- and postirradiation antigen injection groups. The degree of this inhibition varied for the different criteria of antibody response and was also dependent upon the temporal relation of x irradiation and antigen injection. The immune mechanism of irradiated mice never returned to a completely normal status, although mice receiving 710 r of x rays 5 days after antigen showed above normal mean peak and mean total agglutinin titers.

Thymus Specificity in Lethally Irradiated Mice Treated with Rat Bone Marrow

N. Gengozian

I. S. Urso[12] C. C. Congdon[12]
A. D. Conger[13] T. Makinodan

Introduction. – The transplantation and repopulation of erythrocytic, granulocytic, and platelet precursors in lethally irradiated mice injected with heterologous (rat) bone marrow has been demonstrated by immunohematological,

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cytochemical, and chromosome marker techniques.[14–18] A preliminary report concerning the specificity of thymus cells of lethally irradiated mice treated with rat bone marrow (RBM) has been made.[19] This study, involving the identification of the thymocytes by a specific *in vitro* agglutination technique and also by cytological analysis of dividing cells by the technique of Ford and Hamerton,[20] has now been completed. Data concerning the recovery of the thymus in terms of weight and histologic appearance were also obtained.

Results. – The results of the agglutination tests on the thymuses of 950 r-RBM mice are shown in Table 14. On days 12 and 15, tests with antimouse thymus serum showed strong agglutination, while only slight reactivity was obtained with antirat thymus serum. From days 18 through 30, there was a gradual increase in the degree of agglutination

[14] T. Makinodan, *Proc. Soc. Exptl. Biol. Med.* **92**, 174–179 (1956).

[15] O. Vos *et al.*, *Acta physiol. pharmacol. neerl.* **4**, 482–486 (1956).

[16] P. C. Nowell *et al.*, *Cancer Research* **16**, 258–261 (1956).

[17] C. E. Ford *et al.*, *Nature* **177**, 452–454 (1956).

[18] L. H. Smith, T. Makinodan, and C. C. Congdon, *Cancer Research* **17**, 367–369 (1957).

[19] I. S. Urso, N. Gengozian, and C. C. Congdon, *Biol. Semiann. Prog. Rep. Feb. 15, 1957*, ORNL-2267, p 40.

[20] C. E. Ford and J. L. Hamerton, *Stain Technol.* **31**, 247–251 (1956).

Table 14. *In vitro* Agglutination Tests and Cytological Analyses of the Thymuses of Lethally Irradiated Mice Treated with Rat Bone Marrow

Days After Treatment	Agglutination Tests (degree of agglutination*)		Cytological Analysis (%)			
			Rat Cells		Mouse Cells	
	Antirat Thymus Serum	Antimouse Thymus Serum	Spleen and Bone Marrow	Thymus	Spleen and Bone Marrow	Thymus
7	–	3+	100	0	0	100
12	±	3+	96	7	4	93
14	–	4+	100	22	0	78
15	±	3+				
18	1+	3+				
19			100	58	0	42
20	1+	2+	100	88	0	12
21	2±	2+	100	95	0	5
22	2+	2+	100	97	0	3
23	3+	1+	100	100	0	0
25	3+	–				
28	4+	±				
30	4+	±				
40	4+	±				
45	4+	±				
56	4+	–				
83	4+	–				

*Degree of agglutination graded from – to 4 +.

when the thymuses were tested with antirat thymus serum and a corresponding decrease in reactivity with antimouse thymus serum. The 50% level of rat and mouse thymus cells present in the test organ occurred on approximately the 21st day after treatment; and by 30 days, all the thymus cells appeared to be of the rat type, as indicated by maximum agglutination.

The cytological analysis of the proportion of dividing mouse and rat cells in the bone marrow, spleen, and thymus of the 950 r-RBM mice is shown in Table 14. Replacement of mouse cells by rat cells occurs much sooner in the bone marrow and spleen than in the thymus; both the bone marrow and spleen were pure rat, or almost so, by at least the seventh day after irradiation, whereas the thymus does not achieve this until about the twenty-first to twenty-second day.

The thymic weight pattern of lethally irradiated mice treated with rat or isologous mouse bone marrow (IBM) is shown in Fig. 16. As recorded by the mean average ratio of milligram of thymus weight per gram of body weight (T/B), a sharp reduction in size occurred within a few days after irradiation; and among the 950 r-IBM mice, this was followed by almost complete recovery within 15 days after treatment. In contrast, thymic recovery of the 950 r-RBM mice was only partial, reaching a peak value (~40% of normal) on the fifteenth to eighteenth day, followed by a second period of relative weight loss from the nineteenth through the twenty-fifth days. There was extreme variation in the thymus weight response of the 950 r-RBM mice, and several animals showed thymic recovery comparable to that of the IBM-treated mice.

Among the 950 r-IBM mice, histologic examination of the thymus showed marked atrophy on the seventh day after treatment, while the thymus of mice sacrificed on days 12, 18, and 21 after treatment showed a normal histologic architecture. The thymus of 950 r-RBM mice on the seventh day after treatment was similar to that of the 950 r-IBM mice. On days 12 and 15, the architecture was essentially normal, although mild, irregular atrophy of the cortex was seen in one thymus. From days 18 through 22, there was a progressive increase in the atrophy of the cortex. On days 28, 40, and 56, nearly complete regeneration of the cortex was observed, and the thymuses appeared normal.

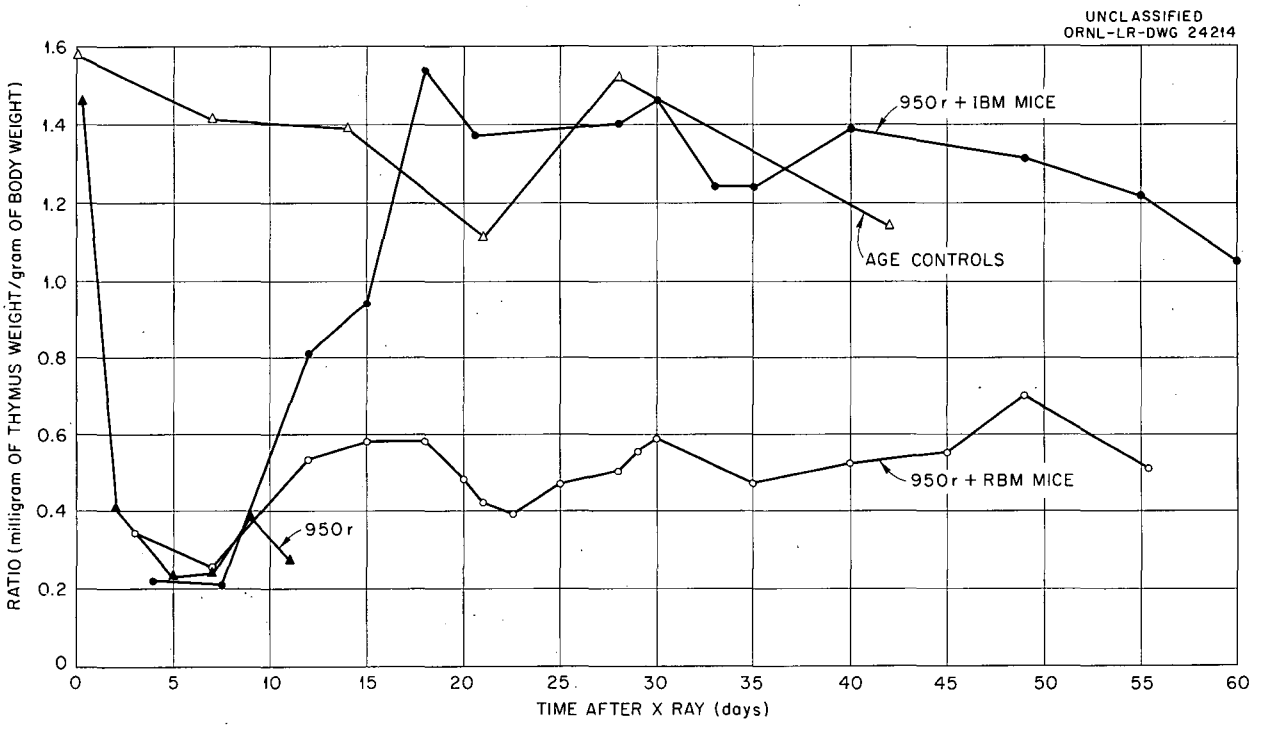

Fig. 16. Thymic Recovery of Lethally Irradiated Mice Treated with Rat Bone Marrow (RBM) or Isologous Bone Marrow (IBM).

Discussion. – The specific *in vitro* agglutination tests and the cytological analysis indicate a repopulation of the thymus by rat-type cells in lethally irradiated mice treated with rat bone marrow. Although the histologic architecture of these thymuses was often normal, recovery of the thymuses in terms of weight was incomplete.

Survival of Lethally X-irradiated Mice Injected with Bone Marrow from X-irradiated Mice Protected with Isologous, Homologous, or Heterologous Bone Marrow

P. Urso

Introduction. – Donor cells have been shown to transplant and remain functional in the majority of lethally x-irradiated mice protected with homologous or heterologous (rat) bone marrow for several months after treatment.[16–18,21] Transfer of the bone marrow of HBM-protected mice 14 days after treatment into lethally x-irradiated mice resulted in only 5% 30-day survival of the host strain but resulted in 90 to 100% 30-day survival of the donor strain.[15] Serial transfer of the bone marrow of lethally x-irradiated, rat-protected mice on days 10 to 42 into lethally x-irradiated mice of the host strain did not provide protection for these animals.[22] This study was undertaken to determine the activity of the bone marrow of lethally x-irradiated (900 r), IBM-, HBM-, and HeBM-protected mice in affording protection to lethally x-irradiated (900 r) mice of the host strain when it is transferred at various time intervals after initial treatment.

Results. – Transfer of the bone marrow of irradiated (C × DBA)F_1 mice protected with IBM on day 15 after initial treatment did not provide protection for irradiated mice of the host strain. When the bone marrow was transferred on days 21 to 97 after initial treatment, survival of host-strain mice was similar to that of mice receiving normal IBM. Cole, Habermeyer, and Nowell[22] obtained similar results in LAF_1 mice.

Irradiated host-strain mice injected with 15- and 30-day bone marrow from one femur of (101 × C3H)F_1 HBM-protected CAF_1 mice showed the typical delayed foreign bone marrow deaths which occur with normal HBM. When the bone marrow was transferred on days 60 and 64, excellent 30- and 60-day survival (100 and 80%) was obtained which was comparable to that from an IBM experiment. Transfer of the marrow from HBM-protected mice into irradiated mice of the donor strain on the same time intervals specified above gave consistently high 30- and 60-day IBM survival.

Thirty- to 36-day transfer of the bone marrow from irradiated CAF_1 mice protected with HeBM into irradiated host-strain mice resulted in no 30-day survivors. Fifty- to 64-day transfer of this marrow gave 25% 30-day survival and 19% 60-day survival, as compared to no 30-day survival of irradiated host-strain mice receiving normal RBM in one-mouse-femur equivalents. The bone marrow of the survivors receiving 50- to 64-day transfer was tested for rat cells by the alkaline phosphatase staining method and found to be negative for such cells.

Discussion. – Early transfer of bone marrow from HBM- and HeBM-protected mice into lethally irradiated mice of the host strain demonstrated that the transferred bone marrow acts similar to the bone marrow of the donor. The implication is that at this time, the bone marrow of HBM- and HeBM-protected mice is that of the donor. This conclusion was first reached by Vos *et al.*[15] for the HBM experiment and later by Cole, Habermeyer, and Nowell[22] for the HeBM experiment. The results obtained in this study also show that transferred bone marrow of lethally irradiated mice protected with IBM, HBM, or HeBM is functionally active in its ability to modify the effects of lethal x irradiation in the host strain, provided that it is transferred at the proper time interval after initial treatment. Thus, it is apparent that a change takes place in the bone marrow of bone-marrow-protected mice which increases the protective activity of the transferred bone marrow for the host strain as the time interval between initial treatment and transfer increases.

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[21] T. Makinodan, *Proc. Soc. Exptl. Biol. Med.* 92, 174–179 (1956).

[22] L. J. Cole, J. G. Habermeyer, and P. C. Nowell, *Am. J. Physiol.* 188, 555–558 (1957).

PATHOLOGY AND PHYSIOLOGY

PATHOLOGIC AND PHYSIOLOGIC EFFECTS OF RADIATION

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A Fatal Reaction Induced in Irradiated F_1 Hybrid Mice by Implantation of Parental Strain Spleen Cells

E. E. Schwartz C. C. Congdon[6] F. F. Wolff

Introduction. – In exploring the usefulness of the implantation of antitumor antibody-forming cells in the experimental radiotherapy of mouse leukemia, it was observed that irradiated F_1 hybrids inoculated with parental strain spleen cells died more rapidly than nontreated controls.

Results. – Young adult F_1 hybrid mice exposed to lethal (800 to 1000 r) or sublethal (400 r) x radiation and subsequently inoculated with viable spleen cells from a donor of one of the parental strains exhibited a heavy mortality within 2 to 4 weeks after treatment, regardless of whether isologous or parental strain bone marrow were administered concomitantly (Table 15). This reaction was not observed if small numbers of spleen cells were injected, nor was it seen in nonirradiated recipients or in those injected with parental marrow alone or isologous F_1 spleen. Autopsy findings disclosed atrophy and necrosis of hemopoietic elements except in those mice inoculated with parental strain marrow in addition to parental spleen.

Discussion. – Although the mechanism of death is not yet clear, this reaction is ascribed to the production by the grafted spleen cells of antibodies against antigens in the recipient that are inherited from the homologous parent. Despite the fact that the foreign spleen reaction occurs sooner after treatment than the foreign bone marrow reaction, the two diseases have certain features in

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Table 15. Survival of Irradiated F_1 Hybrid Mice Injected with Spleen Cells Obtained from Mice of a Parental Strain

Recipients		Transplanted Spleen		Recipients Surviving 30 Days	
Dose (r)	Strain	Donor Strain	Number of Cells in Inoculation	Number	Per Cent
			$\times 10^6$		
800–1000	(C3H × 101)F_1	101	100	2/24	8
800–900	(C3H × 101)F_1	101	50	42/49	86
0	(C3H × 101)F_1	101	100	9/9	100
800–1000	(C3H × 101)F_1	(C3H × 101)F_1	100	16/16	100
400	(RF × AKR)F_1	RF*	20	0/9	0
900	(C57BL × 101)F_1	C57BL	100	0/20	0
900	(C57BL × 101)F_1	C57BL	50	2/7	29
900	(C57BL × 101)F_1	(C57BL × 101)F_1	100	10/10	100

*RF donor mice were preimmunized against a transplanted AKR lymphoma.

common. The foreign spleen system appears to be of great potential value in the study of the mechanism of tissue immune reactions as well as in the experimental treatment of cancer.

The Inhibitory Effect of Splenectomy on the Induction of Myeloid Leukemia in RF Mice

F. F. Wolff E. S. Ledford W. D. Gude

Introduction. – In an effort to explore the physiological factors influencing susceptibility to the induction of myeloid leukemia by ionizing radiation, the effects of removal of the spleen were investigated.

Results. – Splenectomy one week before whole-body x irradiation greatly inhibited the induction of myeloid leukemia, whereas the production of lymphomas was not affected (Table 16). Preliminary results of more recent experiments indicate that splenectomy as late as one month after exposure also greatly inhibited the induction of myeloid leukemia. A lesser inhibition was also produced by sham splenectomy before but not after irradiation.

Discussion. – These results demonstrate a hitherto unsuspected importance of the spleen in the induction of myeloid leukemia by irradiation. The precise role of this organ and the mechanism of the smaller effect caused by sham splenectomy remain to be elucidated.

The Relative Effectiveness of Chronic Neutron and Gamma Irradiation for the Production of Cataracts in Mice

K. W. Christenberry J. A. Sproul, Jr.
R. J. Elliott

Introduction. – Although it has been known that neutrons have a high RBE (see Appendix for alphabetical list of abbreviations) for the production of lens opacities, precise data on the relative cataractogenic effectiveness of chronic neutron irradiation are lacking. Accordingly, this study was undertaken to compare the RBE of neutrons and gamma rays for lens damage in RF mice exposed at varying intensities to neutrons from a Po-Be source and to Co^{60} gamma rays.

Results. – Preliminary observations (Fig. 17) indicate that exposure to gamma rays at relatively low intensities (0.055 r/min) is several times less damaging to the lens than exposure at higher intensities (7 r/min); whereas, the dose rate of neutron irradiation less markedly affects the dose response (Fig. 18).

Discussion. – Because of the pronounced dependence of the effects of gamma radiation on the dose rate and the less marked intensity-dependence of the neutron response, the RBE of neutrons varies inversely with the dose rate. If these results may be extrapolated to man, they help to explain the occurrence of radiation cataracts in

Table 16. The Effect of Splenectomy on the Induction of Myeloid Leukemia by X Rays

Dose (r)	Treatment	Total Number of Mice	Percentage Dying with Leukemia		Mice Still Surviving (%)
			Myeloid	Lymphoid, Thymic	
0	Control	197	4	5	0
150*	Intact	104	34	9	0
300*	Intact	104	38	16	0
300*	Splenectomy preirradiation	92	13	15	0
300**	Splenectomy preirradiation	66	5	5	30
300**	Splenectomy postirradiation	68	15	7	20
300**	Sham preirradiation	61	26	16	23
300**	Sham postirradiation	60	43	7	17

*Whole-body 250-kvp x radiation at 5 to 6 weeks of age.
**Whole-body 250-kvp x radiation at 10 weeks of age.

those exposed chronically to neutrons and the absence of cataracts in those occupationally exposed to x rays or gamma rays.

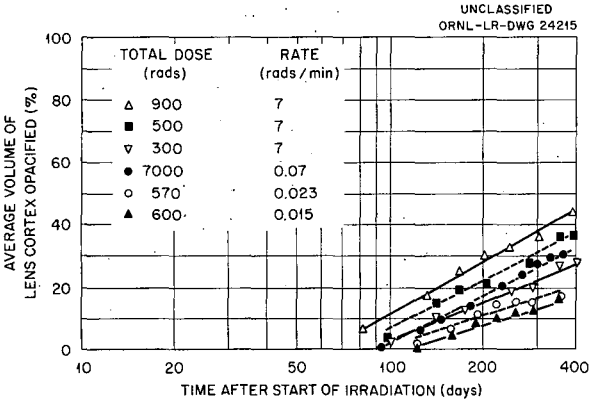

Fig. 17. Lens Opacities in RF Female Mice Exposed to Co^{60} Gamma Rays. The mice exposed at an intensity of 7 rad/min were irradiated in a single sitting. The others were exposed daily for 23 hr/day.

Radiation-induced "Dental Death" in Mice

R. J. Elliott T. Mack

Introduction. – Adult mice exposed to localized x radiation administered to the head developed deformities of the incisor teeth leading to death from starvation during the third or subsequent months after irradiation.

Results. – In RF mice exposed at ten weeks of age, dental defects became evident clinically within 60 days after exposure of the head to 1250 to 1800 r of x rays [half-value level (hvl) 0.44 mm Cu, 100 r/min]. These defects consisted of varying degrees of loss, erosion, or tipping of one or more of the incisors. Loss of teeth was attended by ulceration of the gums. Mice with severe defects were unable to feed adequately on food pellets, became progressively malnourished, and eventually starved to death unless provided with a soft diet (powdered food pellets). The time of onset of dental deformities and the resulting effects on survival varied with

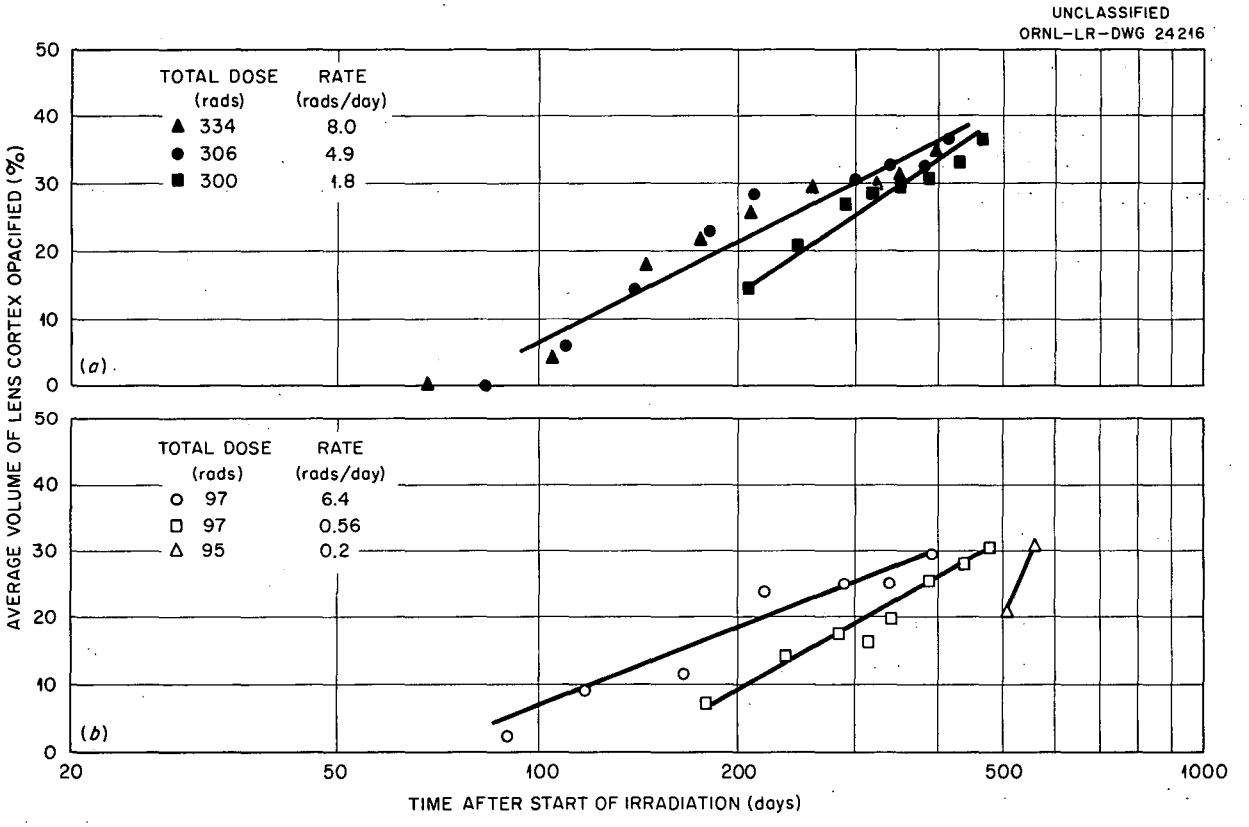

Fig. 18. Lens Opacities in RF Female Mice Exposed to Po-Be Neutrons. The mice were irradiated daily, 23 hr/day.

the dose of radiation; the administration of AET before irradiation lessening the effectiveness of a given dose (Fig. 19). Dental defects were not noted in mice exposed to doses below 1000 r or in the nonirradiated controls.

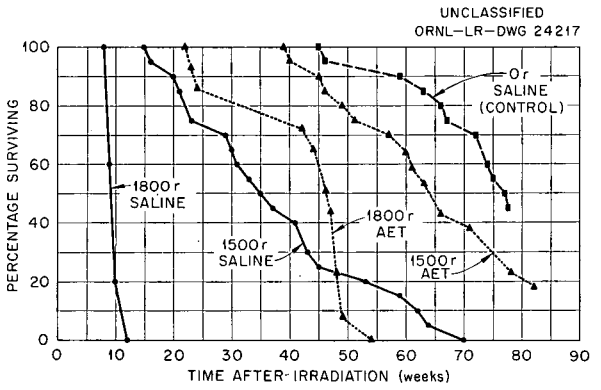

Fig. 19. Survival of RF Male Mice Exposed to Localized Head X Radiation. All mice, except the controls, died with dental defects and emaciation.

Discussion. – Since the radiosensitivity of tooth-forming tissues is well known and since in mice the incisor teeth continue to grow throughout life, it is not surprising that the observed dental defects were induced by irradiation even in adulthood. Similar effects on the incisors of the rat have been reported by English, Schlack, and Ellinger.[7]

Chemical Studies of Mucopolysaccharides in Rat Blood Platelets

B. Anderson T. T. Odell, Jr.

Introduction. – Following the observation that S^{35}-labeled sulfate is incorporated into a mucopolysaccharide in rat platelets,[8] attempts have been made to characterize the mucopolysaccharide chemically.

Results. – Two mucopolysaccharides isolated from rat blood platelets treated with alkali or with trypsin have been separated by paper electrophoresis. The electrophoretic mobility of one of them (MPS 1) is the same as that of chondroitin sulfuric acid (CSA), while the other (MPS 2) moves more slowly. Other electrophoretic data indicate that MPS 2 is not heparin, and probably not hyaluronic acid. An autoradiogram of a paper strip on which S^{35}-sulfate-labeled material was run indicated that only MPS 1 is sulfated, but that MPS 2 is contaminated with MPS 1 on the paper strip.

Each mucopolysaccharide was eluted and tested for uronic acids by the Dische procedure; MPS 1 was positive, MPS 2 was negative.

Each eluate was hydrolyzed in the presence of Dowex 50 (H^-), and the hydrolysis products were studied by paper chromatography. Since the hexosamines were absorbed in the resin, they were eluted after the other hydrolysis products had been removed and were chromatographed separately. Water-saturated collidine was used to develop the hexosamine chromatograms. Butyl alcohol–pyridine–water (5:3:2) was the solvent for the chromatograms on which the other hydrolysis products were run. Each chromatogram was developed three times in the latter solvent. Some of the products have been tentatively identified as follows: MPS 1 – galactosamine, glucuronic acid; MPS 2 – galactosamine, glucosamine, glucuronic acid. In addition, four monosaccharides were found on the chromatograms in the glucose, galactose, mannose, and arabinose areas and the ribose and lyxose areas. Blanks indicated that the compound tentatively identified as glucose is present as a contaminant. The compound in the galactose area may be a component of MPS 2, and the compound in the mannose-arabinose area may also come from MPS 2 but is present to a very small extent. Hydrolysis of a known sample of CSA which had been electrophoresed and eluted in the same manner as the samples yielded some of the sugar which goes to the ribose-lyxose area, thus indicating that it is an artifact. The fact that this sugar occurs to the same extent in hydrolyzates of both unknowns also indicates that it is an artifact.

Discussion. – If all the monosaccharide in the ribose-lyxose area is an artifact, MPS 1 may be CSA. Not enough is known about MPS 2 to indicate its identity. The possible importance of these mucopolysaccharides in the maintenance and repair of the interendothelial cement of the capillary wall or in hemostasis remains to be investigated.

[7] J. A. English, C. A. Schlack, and F. Ellinger, *J. Dent. Research* **38**, 377 (1954).

[8] T. T. Odell, Jr., and B. Anderson, *Proc. Soc. Exptl. Biol. Med.* **94**, 151–156 (1957).

Influence of AET on Leukemia Induction in RF Mice by X Radiation

R. J. Elliott W. D. Gude D. G. Doherty[9]

Introduction. – Since administration of AET before irradiation increases the $LD_{50}/30$ days in mice by a factor of nearly two,[10] this experiment was undertaken to study the effects of AET on the rate of induction of lymphoma and myeloid leukemia.

Results. – Preliminary data (Table 17) indicate that the incidence of both myeloid leukemia and thymic lymphoma is lower in the mice that received AET than in the saline-injected controls.

Discussion. – These results are consistent with the thesis that the protective effect of AET represents a dose-reducing action of the compound. Although it is too early to estimate the degree of protection afforded against the induction of leukemia, it is noteworthy that the incidence of both hematologic types was reduced, in keeping with the observed effects of AET on the reduction of longevity by irradiation.[11]

The Influence of the Intensity of Irradiation on the Induction of Leukemia by Ionizing Radiation

J. A. Sproul, Jr. R. J. Elliott

Introduction. – These observations are preliminary results of a large-scale investigation, mentioned in earlier reports,[12] on the relative effectiveness of neutron and gamma radiation as influenced by radiation intensity.

Results. – In RF female mice exposed to whole-body Co^{60} gamma radiation at ten weeks of age, the leukemia incidence varied directly, and the survival time varied inversely, in relation to the dose (Table 18). However, reduction of the dose rate or fractionation of the radiation greatly reduced the effectiveness of the radiation at the one dose level (300 r) tested so far.

Discussion. – These results indicate clearly that the relative effectiveness of gamma radiation for leukemia induction and for shortening of the life span depends markedly on the dose rate at which the radiation is administered, 300 r being less than one third as leukemogenic when protracted over a 3-day period as when administered within an interval of 40 min.

Effect of Bone Marrow Injections on Myleran Toxicity in Rats

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

Introduction. – It was reported by Weston[13] that rats injected with a lethal dose of Myleran, a marrow depressant, were induced to recover by the implantation of bone marrow. The present experiment was undertaken to investigate whether this

[9] Biochemistry Group.

[10] D. G. Doherty and W. T. Burnett, Jr., *Proc. Soc. Exptl. Biol. Med.* 89, 312–314 (1955).

[11] C. C. Congdon *et al.*, *Biol. Semiann. Prog. Rep., Feb. 15, 1957*, ORNL-2267, p 52–53.

[12] J. A. Sproul, Jr., *et al.*, *Biol. Semiann. Prog. Rep., Feb. 15, 1957*, ORNL-2267, p 49.

[13] J. K. Weston, *Federation Proc.* 16, 377 (1957).

Table 17. Effects of AET on the Induction of Leukemia by X Rays in RF Mice

Dose (r)	Treatment*	Number of Mice	Percentage Dying with Leukemia		
			Myeloid	Lymphoid, Thymic	Combined
0	Saline	68	4.4	2.9	7.3
	AET	43	7.0	0	7.0
150	Saline	49	26.6	4.1	30.7
	AET	70	17.4	5.7	23.1
300	Saline	59	42.4	15.3	57.7
	AET	61	34.4	4.9	39.3

*Male mice of the RF strain were injected intraperitoneally with 9.0 mg of AET in a 0.1% solution or with an equal volume of physiological saline 10 min before whole-body 250-kvp x irradiation (hvl 0.44 mm Cu, 100 r/min).

Table 18. Survival and Leukemia Incidence in RF Female Mice Exposed to Co^{60} Gamma Rays

Dose (r)	Total Number of Mice	Percentage Dying with Leukemia			Mice Still Alive (%)	Median Survival Time (months)
		Myeloid	Thymic	Combined		
0	109	3	6	9	11	20
100[a]	96	19	8	27	24	19
300[a]	94	22	21	43	19	16
300[b]	98	11	11	22	36	19
300[c]	95	14	16	30	37	18
500[a]	90	26	22	48	11	13

[a]Single exposure, 7 r/min.
[b]Single exposure, 0.07 r/min (~72 hr duration).
[c]Two exposures of 150 r each, 7 r/min, separated by 72 hr.

recovery-promoting action was associated with repopulation of the recipient's marrow, as is the case in irradiated animals.[14]

Results. – Of 10 Sprague-Dawley rats injected intravenously with 20 mg of Myleran (dissolved in 50% *N,N*-dimethylacetamide) per kilogram of body weight, the 5 controls that received no bone marrow died within 18 days. The other 5, which were injected intravenously with Sprague-Dawley bone marrow 4 days after administration of the Myleran, survived.

In contrast, of 10 rats of our D strain injected 4 days after Myleran treatment with bone marrow obtained from rats of our C strain, only 2 survived for 30 days. One of the latter exhibited increasing numbers of C-type erythrocytes in the circulating blood, eventually all of the erythrocytes becoming of the donor (C) type and remaining so until now (134 days), whereas in the other survivor donor-type erythrocytes have never been detected.

Discussion. – These results suggest that in Myleran-intoxicated rats implantation of antigenically foreign bone marrow occurs less readily and is less effective in promoting recovery than in rats exposed to whole-body x radiation.[14] Since the implantation of nonisologous bone marrow necessitates suppression of the immune response of the recipient, the observed differences between the action of foreign bone marrow in Myleran-treated and in irradiated rats may result from the fact that Myleran causes relatively less injury of lymphoid tissues for the same degree of marrow damage than do x rays. The difference in survival between the Sprague-Dawley rats and those of our D strain may be attributable to a lesser degree of histoincompatibility between Sprague-Dawley donors and recipients than between recipients of the D strain and donors of the C strain.

[14]T. T. Odell, Jr., *et al.*, *Ann. N. Y. Acad. Sci.* **64**, 811 (1957).

CELL PHYSIOLOGY

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Characterization of Rat Blood Proteins

N. G. Anderson M. L. Anderson
R. E. Canning

Introduction. – A major activity of the cell physiology group is the isolation and characterization of rat tissue proteins. Since blood proteins may be present in tissue protein extracts, either as contaminants or as products of the tissue studied, it is essential that blood proteins be isolated and characterized first.

Electrophoretic fractionations have been carried out by using three continuous curtain electrophoresis devices. To isolate serum albumin, serum was fractionated first by two runs at pH 5.5 followed by one run at pH 7.5. Gamma-globulin was isolated by repeated runs at pH 7.5.

Results. – Both proteins were homogeneous ultracentrifugally and showed less than 1% contamination electrophoretically when examined at pH 7.5 and pH 5.5. The ultracentrifuge pattern obtained with rat serum albumin is shown in Fig. 20.

[1]Consultant.

[2]Research Associate.

[3]Research Participant.

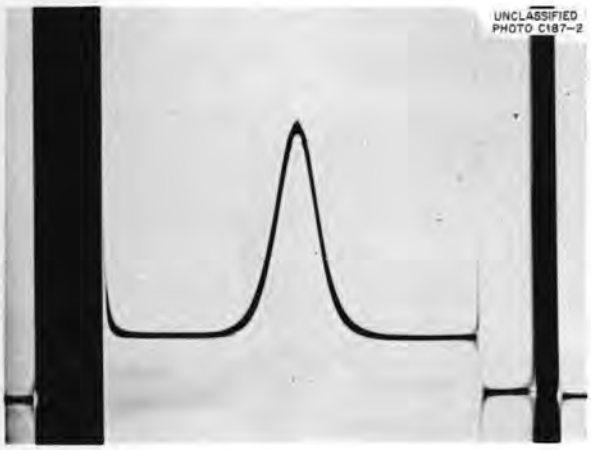

Fig. 20. Rat Serum Albumin; 112 min at 56,100 rpm.

The sedimentation constants extrapolated to zero concentration in water at 20°C were 6.94 for γ-globulin and 4.75 for serum albumin.

Discussion. – Precise data on the physical properties of serum components will be of value in determining which components vary after irradiation of the intact animal or in other pathological conditions and will facilitate the identification of blood proteins in tissue extracts.

Electrophoretic Mobility of Enzymes in Rat Liver Soluble Phase Preparations

W. D. Fisher N. G. Anderson
M. L. Anderson

Introduction. – Rat liver soluble proteins prepared by methods devised in this laboratory have been found to give a reproducible series of peaks when studied electrophoretically at pH 7.5 in a 0.1 ionic strength buffer. The distribution of enzymatic activity among these peaks is necessary for further electrophoretic fractionation of these mixtures and to determine whether a specific enzymatic activity resides in one or more peaks.

Methods. – To determine the distribution of various enzymes among these peaks, a sampling device has been constructed which allows the contents of each limb to be drawn off in approximately 35 consecutive aliquots. The sampling needle is lowered $\frac{1}{10}$ in. for each cut and 2 ml of fluid is withdrawn over a period of 4 min. The protein is removed in the first few drops withdrawn, the remainder of the 2-ml volume being buffer overlying the protein, which serves to wash out the needle, polyethylene tubing, and syringe. Sectioning of both limbs of an 11-ml cell requires approximately 8 hr. Photographs were taken of the electrophoretic pattern after every fourth cut so that the position of the samples withdrawn could be accurately determined.

Results. – Results obtained with β-glucuronidase and catalase are shown in Fig. 21. These two enzymes are found in very nearly the same peaks in the electrophoresis pattern in three separate

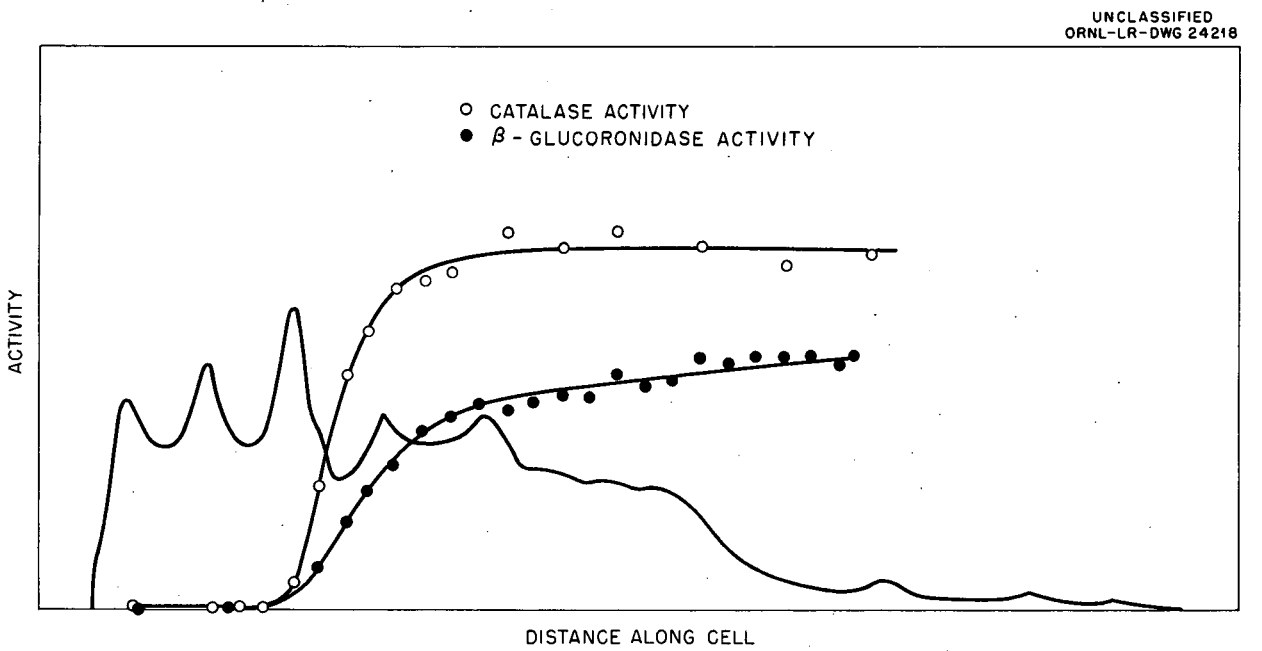

Fig. 21. Plot of Enzymatic Activity Along Descending Limb of Electrophoretic Pattern of Rat Liver Soluble Proteins.

experiments. Additional studies with other enzymes are in progress.

Discussion. – These data provide a ready starting point for isolation of these enzymes, using either zone or paper curtain electrophoresis.

Effect of X Rays on DNP from Rat Thymus

W. D. Fisher

Introduction. – It was reported previously[4] that the viscosity of crude nucleoprotein extracts from rat thymus was lowered by x-ray exposures of a few roentgens. To determine whether DNP (deoxyribonucleoprotein) within the nucleus was equally sensitive, the effect of irradiation on the thymus *in situ* was investigated.

Methods. – Male Sprague-Dawley rats were exposed without anesthesia and sacrificed immediately after irradiation. The x-ray source was a G-E Maxitron (250 kvp, 30 ma, 3 mm Al) and the dose rate 2238 r/min. Thymus extracts were made with 1 *M* NaCl. Viscosity measurements were made in modified Ostwald-Cannon viscometers at 25°C.

Results. – The data are presented in Table 19. Four animals were used at each dose level.

Table 19. Viscosity of DNP Extracts Prepared from Irradiated Tissue

Exposure (kr)	Specific Viscosity
0	3.53 ± 0.22
20	3.41 ± 0.15
40	2.00 ± 0.39

Discussion. – Although dilute DNP extracts are very sensitive to x rays *in vitro*, exposures of many kiloroentgens were necessary to produce any detectable change in the viscosity of extracts prepared from tissue irradiated *in situ*. The difference in sensitivity is probably due to the high concentration of DNP in the nucleus and to the protective action of materials such as proteins.

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

Activity of Injected Thymus in Newborn Mice

R. H. Shellhamer

Introduction. – Metcalf[5,6] reported the presence of a lymphocytosis-stimulating factor in chronic lymphatic leukemia which was not present in normal plasmas. It was shown also by Metcalf[7] that a similarly acting factor could be demonstrated in thymus. It is of significance that such a factor may be obtainable for use in animal radiation studies.

As a preliminary step toward isolating the lymphocytosis-stimulating substance from thymus, a method of assay was necessary. It was decided to essentially duplicate the techniques of Metcalf,[7] employing mice up to 36 hr of age to assay the thymus substance for lymphocytosis-stimulating activity.

Results. – Mice [(C57BL × 101)F_1] up to 36 hr of age were inoculated intracerebrally with 0.02 to 0.03 ml of thymus emulsion from rat, mouse, and calf obtained by homogenizing whole thymus glands in 0.14 *M* saline and centrifuging at 2000 rpm to remove the debris of cellular and intercellular fragmentation. The supernatant fluid so obtained was used for inoculations. Control mice were inoculated with saline. After inoculation, the mice were returned to their mothers for 6 days, at which time blood studies were performed to establish total WBC and differential WBC counts. Blood was obtained by cutting off a segment of the tail, the first flow of blood being used in all cases. Each animal was used for either total or differential WBC counts but not for both. Total counts were made by using the Levy hemacytometer, ordinary white blood cell pipettes, and 1% acetic acid diluent. Differential counts, routinely counting 100 cells, were made on cover-slip smears stained with Wright's stain. Human lymphocytic leukemia plasma was used for inoculation in one experiment.

Activity of inoculations was expressed in terms of (1) an increase or decrease in total white cells and (2) the lymphocyte-polymorph or L/P ratio. Normal L/P ratios, according to Metcalf, run as high as 2.5:1 in mice 6 days after inoculation.

A summary of the data obtained from these initial experiments on the effect of inoculations of mice with thymus emulsions can be found in Table 20. It was noted that a slight leukocytosis over the normal occurred in each experiment, but this is not appreciable, as can be seen by comparing mean total WBC readings in experimental and control groups and these in turn with the extremes of total WBC counts. The L/P ratios (Table 20) observed in day-old mice inoculated with rat, mouse (AK strain), and calf thymus in varying dilutions, as well as in those inoculated with chronic lymphocytic leukemia plasma, show no consistent lymphocytotic effect when compared with the L/P ratio in control mice inoculated with saline.

[5] D. Metcalf, *Brit. J. Cancer* 10, 169–178 (1956).

[6] D. Metcalf, *Brit. J. Cancer* 10, 431–441 (1956).

[7] D. Metcalf, *Brit. J. Cancer* 10, 442–457 (1956).

Table 20. White Blood Cells in Peripheral Blood in 6-Day-Old (C57BL × 101)F_1 Mice Inoculated When About 36 hr Old with Thymus, Human Leukemic Plasma, and with Saline

Donor	Inoculum		WBC on the 6th Day After Inoculation					
			Total			L/P Ratio		
	Milliliter	Dilution	Number of Mice	Mean	Extremes	Number of Mice	Mean	Extremes
Rat thymus	0.02	× 5	13	6255	4560–7640	18	2.2	0.62–5.3
Control			7	5965	4200–8920	11	1.7	0.49–3.5
Rat thymus	0.02	× 5	8	6440	3240–9160	6	2.2	0.59–3.2
Control			6	5173	3040–7360	6	2.3	1.1–3.5
Rat thymus	0.03	× 20	2	5920	5480–6360	4	1.6	1.1–2.3
Control			4	5340	4040–7120	4	1.5	1.4–1.6
Rat thymus	0.02	× 10	3	5213	4240–6200	6	4.0	2.2–7.9
Control			2	4560	4040–5080	7	2.1	1.2–3.5
AK mouse thymus	0.03	× 20	4	4800	4040–5720	8	1.8	1.2–2.2
Calf thymus	0.03	× 20	4	4800	4200–5440	7	3.4	2.2–6.6
Human plasma (chronic lymphatic leukemia)	0.03		4	5370	4640–5720	9	2.0	1.0–4.7
Control			3	4373	3480–5040	10	2.4	1.4–4.6

BIOCHEMISTRY

NUCLEIC ACIDS, ENZYMES, AND RADIATION-PROTECTIVE COMPOUNDS

W. E. Cohn

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Minor Constituents of Ribonucleic Acids

W. E. Cohn A. J. Bandy

Several hundred milligrams of the new nucleotide of RNA (see Appendix for alphabetical list of abbreviations) have been prepared for the investigation of its structure. The preparation involves the hydrolysis of 50- to 100-g batches of yeast RNA; precipitation of most of the cytidylic, adenylic, and guanylic acids with alcohol; and repeated ion-exchange fractionation of the soluble portion, which contains principally uridylic acid. The new nucleotide, 0.3% of the total, occupies a position between adenylic and uridylic acids in the conventional acid elution scheme and must be repeatedly recycled to remove these major contaminants.

Spectral and chemical properties and elemental composition indicate that the new nucleotide is a uridylic acid modified by not more than the substitution of a $-CH_2OH$ residue, probably in position 5. Periodate oxidation, 2′,3′ isomerism, and borate complex formation indicate that ribose is probably the sugar, although no conclusive orcinol reaction has yet been obtained. Separation of ribose from the base has not been accomplished although several methods have been tried (ultraviolet irradiation, bromination, hydrogenation, and prolonged acid hydrolysis).

Degradation to the free base has been tried in three ways: perchloric acid oxidation, formic acid hydrolysis, and periodate oxidation followed by alkaline hydrolysis. While the latter two methods yield some ultraviolet-absorbing products (in contrast to perchloric acid, which yields essentially nothing), multiple products of as yet unidentified character are the rule. It is the resemblance of one of these to 5-hydroxymethyl uracil in spectral and ion-exchange properties that leads to the tentative assumption that this is the base. Such a nucleotide would be analogous to the 5-hydroxymethyl cytosine of bacteriophage DNA and therefore not entirely unprecedented.

The possibility that this substance rearranges under relatively mild conditions, or that it is but one of a family of related minor nucleotides, is indicated by various peaks that separate from it in the course of isolation and purification. Most of these show the characteristic bathochromic shift in alkali although not to the same extent as the major substance.

RNA Turnover in Phage-Infected Bacteria

L. Astrachan E. Volkin
M. H. Jones

Introduction. – Previous reports have dealt with the turnover of RNA in bacteriophage-infected *Escherichia coli*. It was shown that when P^{32}-labeled inorganic phosphate was present in the medium for short time intervals, P^{32} was incorporated and subsequently released by RNA. In these experiments, several lines of evidence vaguely indicated that RNA could be a direct precursor of DNA. These indications were (1) P^{32} was incorporated at early times more rapidly into RNA than into DNA, (2) when the radioactive RNA was degraded to individual mononucleotides, the proportions of total counts associated with each mononucleotide suggested that a new kind of RNA was being synthesized and that its composition was related not to normal bacterial RNA but to phage DNA, and (3) in experiments where phage-infected bacteria were washed to remove a considerable proportion of the acid soluble radioactivity, the amount of radioactivity subsequently incorporated into DNA was so large that it could only have come, by however indirect the path, from RNA.

This report describes experiments in which RNA turnover and DNA synthesis were measured at various times after infection. The rationale of these experiments lies in the fact that immediately after infection of *E. coli* with bacteriophage T2, there is a 10-min period during which no net synthesis of DNA occurs. After this latent period,

[1]ORINS Fellow.

[2]Research Participant.

[3]Temporary Employee, summer 1957.

DNA is synthesized at a linear rate. If RNA turnover were directly and solely related to DNA synthesis, the rates of RNA turnover at different times should be proportional to the rates of DNA synthesis at those times.

Results. – Inorganic phosphate labeled with P^{32} was added to parallel cultures at successive 5-min intervals from 1 to 26 min after infection. Five to 10 min after isotope addition, excess inorganic phosphate was added to each of the cultures in order to dilute out the isotope. The greatest rate of P^{32} incorporation and turnover by RNA was observed when isotope was added 1 min after infection. This rate was 2 to 3 times the rate observed when P^{32} was added at 6 or 11 min after infection. A further gradual twofold fall in rates was observed over-all through the remaining time periods. While the rates of RNA turnover fell, the rates of DNA synthesis increased.

Discussion. – The theory that RNA is a direct precursor of DNA is apparently denied by these results. It is possible, however, that the observed rates of RNA turnover are not the true rates, but that, in the course of time after infection, the ratio of rates of incorporation to release becomes smaller. The following report indicates that even if this possibility were true, RNA could still not be a direct precursor of DNA.

RNA Turnover in Phage-Infected Bacteria: Effects of Chloramphenicol

L. Astrachan E. Volkin
M. H. Jones

Introduction. – It is known that if the protein synthesis inhibitor, chloramphenicol, is added to phage-infected bacteria around the time of infection, the synthesis of phage DNA is inhibited. If chloramphenicol is added 8 or more minutes after infection, no inhibition of DNA synthesis occurs. The present report compares the effects of chloramphenicol on RNA turnover and DNA synthesis.

Results. – Chloramphenicol was added 3 min after infection of *E. coli* with bacteriophage T2. Three minutes later, P^{32}-labeled inorganic phosphate was added. After 6 min of isotope incorporation, the cultures were centrifuged free of P^{32} and chloramphenicol and then resuspended in the presence or absence of chloramphenicol. During the period when isotope and chloramphenicol were both present, P^{32} was incorporated into RNA somewhat faster than it was in controls incubated in the absence of chloramphenicol. After removal of isotope, the continued presence of chloramphenicol prevented RNA turnover and DNA synthesis. Upon removal of the chloramphenicol, RNA exhibited turnover and DNA was synthesized. The proportion of total counts associated with each mononucleotide entering or leaving RNA suggested the synthesis and turnover of a new kind of RNA different from normal bacterial RNA.

When the same experiment was run except that chloramphenicol was added 3 min before infection, the results were similar except for one significant modification. In this case the proportion of total RNA counts associated with each mononucleotide during the period of isotope accumulation suggested the synthesis of normal bacterial RNA. Immediately following removal of isotope and chloramphenicol, the proportions of total counts coming out of RNA that were associated with each mononucleotide suggested the breakdown of normal bacterial RNA. At the same time very little DNA synthesis occurred. At later times the counts coming out of RNA were so distributed as to suggest the turnover of the new kind of RNA. At these times DNA synthesis occurred.

In another experiment, the effect of adding chloramphenicol 9 min after infection was studied. Two parallel cultures of *E. coli* were infected with bacteriophage at the same time. Nine minutes after infection chloramphenicol was added to one culture and then 3 min later P^{32}-labeled inorganic phosphate was added to both cultures. After another 5 min excess inorganic phosphate was added to both to dilute out the isotope. Whereas the control exhibited the expected turnover of RNA, chloramphenicol caused a three- to fivefold increase in isotope incorporation by RNA and inhibited turnover of RNA. The rate of DNA synthesis, however, was not affected by chloramphenicol.

Discussion. – The protein synthesis inhibitor, chloramphenicol, has apparently two effects on RNA metabolism of phage-infected bacteria. If chloramphenicol is added shortly after infection, a new kind of RNA, similar to that found in control cultures, is formed but is not degraded. If chloramphenicol is added before infection the new kind of RNA is not formed. In both cases when chloramphenicol is removed, RNA turnover and DNA synthesis occur. However, RNA turnover is not directly related to DNA synthesis, since the addition of chloramphenicol at later times after

infection allows DNA synthesis but still prevents RNA turnover.

A theory that fits the data of this and the preceding report is that RNA turnover is intimately connected to protein synthesis. Thus, as suggested by other investigators, a new protein must be formed shortly after infection before new phage DNA can be synthesized. We suggest that once this protein is formed, chloramphenicol would have no effect on DNA synthesis but would inhibit the RNA turnover associated with the synthesis of nonmetabolic phage protein.

The Cytoplasmic DNA of Frog Eggs

F. J. Finamore E. Volkin

Introduction. – These experiments were designed to demonstrate unequivocally the presence of DNA in the cytoplasm of the frog egg and to compare the composition and metabolic activity of the nuclear and cytoplasmic DNA's using P^{32}.

Female frogs were injected daily with P^{32} as orthophosphate for a period of one week and the nuclei were manually removed from the ovarian eggs. The cytoplasmic and nuclear fractions of these eggs were mixed with a "cold" ovary from a noninjected female and the nucleic acids were isolated and separated. The mononucleotides from DNA were obtained by column chromatography.

Results. – The results demonstrate conclusively that the mononucleotides from DNA contained P^{32} and that the per cent composition of the cytoplasmic DNA, based on counts, closely paralleled that obtained from the total ovary and both followed the base equalities postulated for the DNA molecule. Isolation of the DNA mononucleotides from the nuclear fraction of the eggs indicated that the per cent composition, based on counts, of the deoxyguanylic acid was considerably higher than expected. Isolation of the liver DNA mononucleotides from the same frog yielded a similarly high proportion of deoxyguanylic acid based on total counts. These results are shown in Table 21.

Determination of the per cent composition of liver DNA and total ovarian DNA (nuclear plus cytoplasmic) based on actual phosphorus content indicated that the liver DNA showed the expected base ratios and that deoxyguanylic acid was not disproportionately high.

Discussion. – These results indicate that the nuclear deoxyguanylic acid of the liver DNA and the nuclear deoxyguanylic acid of the oocyte DNA behave similarly and that it is not merely a peculiarity of the oocyte nuclear material. Also, within the egg, it appears that the deoxyguanylic acid precursor pool for nuclear DNA may be smaller than, and distinct from, the deoxyguanylic acid precursor pool for cytoplasmic DNA. Another interesting facet of this problem is that the total DNA counts of the nucleus of the egg are about one-third the total DNA counts of the cytoplasm and yet estimations of their respective DNA concentrations indicate that the cytoplasm contains at least 50 times the DNA of the nucleus.

Structure and Activity in Radiation Protection Sulfhydryl Compounds

D. G. Doherty T. W. McKinley, Jr.
R. Shapira D. E. Emmer

The oral administration of MEG and MPG has been shown to provide effective protection to irradiated mice.[4] Since both compounds must be

[4] D. G. Doherty *et al.*, *Biol. Semiann. Prog. Rep. Feb. 15, 1957*, ORNL-2267, p 63–64.

Table 21. Composition of Various DNA's Based on P^{32} Content

Acids	Oocyte Cytoplasmic DNA		Total Ovarian DNA		Oocyte Nuclear DNA		Liver Nuclear DNA	
	Total Counts	Per Cent	Total Counts	Per Cent	Total Counts	Per Cent	Total Counts	Per Cent
Deoxycytidylic	97.2	15.7	10,000	20.3	40.0	14.2	4350	16.8
Deoxyadenylic	191.5	31.0	15,120	30.7	78.0	28.8	6450	24.8
Thymidylic	195.0	31.6	13,200	26.8	76.6	27.2	6710	25.9
Deoxyguanylic	133.0	21.7	11,000	22.2	86.5	30.8	8460	32.5

prepared from their respective thiuronium salts – AET, APT – by neutralization and since they are susceptible to oxidation to the less-active disulfides, it would be obviously advantageous to administer them as the more stable thiuronium salts either as a solid or in solution. The pH of the animal is such that it could be expected that both AET and APT would be rearranged to MEG and MPG upon absorption into the body fluids. Indeed, both compounds were found to be just as effective when given in this manner, and, in addition, it was found that they were effective over a longer period of time, since they were probably absorbed more slowly. Since there is a relatively wide margin of activity for both AET and APT, a series of radiation LD_{50} experiments at different drug doses were performed. In Fig. 22 it may be seen that the elevation of the radiation LD_{50} is proportional to the log of the dose of drug and that APT (curve II) is more effective at lower dose levels than AET (curve I), the slope of the two lines being quite different. A compound – 3-aminopropyl-*N*′-methylisothiuronium·Br·HBr (APMT) – that had been found to be very effective at 900 r in screening tests, and to have a very desirable therapeutic ratio at this level, was subjected to the same test. It was found that APMT very rapidly saturated whatever sites it was protecting (Fig. 22, curve III)

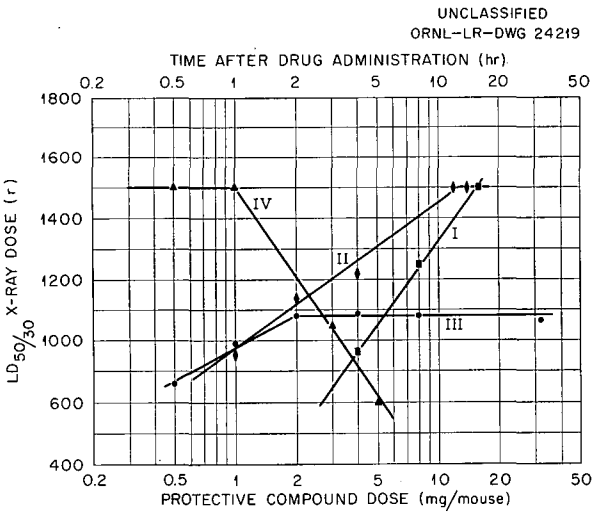

Fig. 22. Elevation of LD_{50}/30 Days of X-irradiated (101 × C3H) F_1 Mice Treated Orally with Varying Doses of AET (Curve I), APT (Curve II), APMT (Curve III); Elevation of LD_{50}/30 Days of X-irradiated (101 × C3H) F_1 Mice vs Time After Administration of AET or APT Orally (Curve IV).

and that even increasing the drug dose 16 times did not bring about any further increase in the radiation LD_{50}. These curves illustrate how dependent the protective activity is on the structure of the compound. The simplest one, AET, apparently is widely distributed, and small changes in structure are sufficient to alter the selectivity and distribution of the compounds markedly.

The period for which a single, maximum, oral dose of AET or APT remained effective was found by the determination of the radiation LD_{50}/30 days at several time intervals after administration of the drug. The protective activity remained at its maximum for an hour (Fig. 22, curve IV) and fell off until at 5 hr the LD_{50}/30 days was 900 r. An estimate of the rate of excretion of these compounds indicates that at 5 hr about 20% of the AET is still there, while the APT was more rapidly excreted with only about 10% remaining in the animal.

Chemical Protection Against Neutron Irradiation

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The finding that orally administered AET and APT provided 30-day survival to mice given 900-r x ray 5 hr after the administration of the drug and that the LD_{50}/30 days 3 hr after drug administration was 1050 r made it feasible to examine the protective action of these compounds against 14-Mev neutrons produced in a Cockcroft-Walton accelerator. At the maximum dose rate available, a lethal dose to mice of 900 rad could be obtained in 2½ to 3 hr. Fourteen groups of five 101 × C3H mice each were used, six being given orally 640 mg of AET per kilogram of body weight, six being given orally 480 mg of APT per kilogram of body weight, and two untreated control groups. The irradiation was started 10 min after drug administration and continued until 905 rad had been given. Sequential analysis of the time of death revealed a significant increase in the survival time of all the treated groups. The AET was more effective than APT, one-third of the animals surviving more than 30 days compared to only one-sixth of the APT animals. The survivors appear to be quite well and are grossly comparable to chemically protected x-rayed animals. Since neutron death is

[5]Biophysics Group.

presumably caused by intestinal damage, these compounds seem to provide at least marginal intestinal protection. The relative activities of the two compounds are also in agreement with the x-ray evidence that AET is more effective than APT at higher dose levels where intestinal damage becomes important.

Intracellular Distribution and Binding of Labeled Protective Compounds

R. H. Bradford R. Shapira
D. G. Doherty

The ability of the cellular fractions from mouse liver to bind protective mercaptoalkylguanidines selectively has been demonstrated previously by using MEG, D-MBG-2-S^{35}, and L-MBG-2-S^{35} (or C^{14}).[6] Similar results have now been obtained by using rat liver and spleen, indicating that this property of mouse liver, in which the majority of this study was carried out, was not unique for this tissue. Equilibrium dialysis experiments[6] had indicated that the labeled material, bound after the administration of either of the three tagged compounds, was apparently held by three types of binding. To discriminate between these, dialysis experiments in several solvent systems were performed. The results in Table 22 indicate that 60 to 85% of the activity, depending on the particular compound, was loosely bound and could be removed by dialysis against 2 *M* guanidine hydrochloride. An additional 10 to 30% of the original activity was removable after treatment with any one of several oxidizing or reducing agents. Approximately 5 to 10% of the original activity, however, was not dialyzable against any of the solvents used. An investigation was made as to the nature of the material to which was bound this nondialyzable activity. When a portion of the homogenate from the liver of a mouse treated with MEG-S^{35} was separated by the Schneider procedure[7] and the resulting fractions were studied by equilibrium dialysis, the nondialyzable activity was found in the Schneider protein fraction. A homogenate prepared from the liver of two rats given MEG-S^{35} was then separated into six fractions on the basis of solubility characteristics, following essentially the procedure of Mirsky and Pollister.[8] Subsequent separation of these six fractions by the Schneider procedure yielded six protein fractions,

[6] R. H. Bradford *et al.*, *Biol. Semiann. Prog. Rep. Feb. 15, 1957*, ORNL-2267, p 64.

[7] W. C. Schneider, *J. Biol. Chem.* **161**, 293 (1945).

[8] A. E. Mirsky and A. W. Pollister, *J. Gen. Physiol.* **30**, 117 (1946).

Table 22. Dialysis of Liver Homogenates from Mice Receiving MEG, D-MBG-2-S^{35}, or L-MBG-2-S^{35}

Compound[a] Administered	Maximum Nondialyzable in Solvent (percentage of original activity)					
	H_2O	Guanidine[b]	LTG[b]	LTG + Guanidine[b]	$NaBH_4$[c]	PFA[c]
MEG	50	40	15	14	12	11
D-MBG-2	35	25	15	10	7	6
L-MBG-2	25	15	14	8	7	6

[a] Each compound was administered at a dose of 0.55 mg.

[b] Guanidine was 2 *M* guanidine hydrochloride; LTG was 1% lithium thioglycolate; LTG + guanidine was a mixture containing 2 *M* guanidine hydrochloride and 1% lithium thioglycolate. An aliquot of the homogenate was allowed to dialyze to completion against the respective solvent, then this solvent was removed by dialysis against water.

[c] $NaBH_4$ was 5% sodium borohydride solution; PFA (performic acid) was prepared by mixing 5 ml of formic acid with 0.6 ml of 30% hydrogen peroxide immediately before use. The trichloroacetic acid–insoluble material of the homogenate, prepared by adding 2.5 ml of cold, 10% trichloroacetic acid to 1.0 ml of the homogenate and separating the resulting precipitate by centrifuging, was treated with either the $NaBH_4$ or PFA. The resulting material was then dialyzed against water. The activity present in the trichloroacetic acid–soluble fraction from this separation was found to be completely dialyzable against water.

five of which contained nondialyzable activity. The small total amount of nondialyzable activity was thus found to be well distributed among protein-type material of considerably different solubility characteristics.

The effect of radiation on the ability of the cellular fractions to bind D-MBG-2-S^{35} was investigated. When mice protected with this compound were subjected to irradiation, the intracellular distribution of activity was very similar to that obtained in nonirradiated, D-MBG-2 treated animals (controls). When, however, unprotected mice were subjected to irradiation and 5 min later treated with D-MBG-2, a marked alteration of the activity distribution was observed. The specific activity obtained in the mitochondrial fraction of the unprotected, irradiated mice was approximately twice that obtained in the controls. An immediate effect of radiation had apparently been to modify the capacity of the cellular constituents to bind this protective agent.

A consideration of the distribution results obtained previously[6] indicates the importance of the intracellular distribution in the protective activity of the mercaptoalkylguanidines. Taking into account the hypothesis of Doherty and Shapira[9] that the chemical protection realized from these compounds results from their ability to act as radical quenching agents, their *in vivo* mechanism of action may be explained as resulting from a localization of these compounds within the cell, preferentially covering any radiation-sensitive sites. Thus, it may be that radiation-produced free radicals are effectively removed, and the sensitive biological material is protected.

Further experiments are now being performed to characterize more completely the activity-containing material which was loosely bound to the cellular constituents.

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[9]D. G. Doherty and R. Shapira, to be published in *Radiation Research*.

ENZYMOLOGY AND PHOTOSYNTHESIS

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THE MECHANISM OF PROTEIN BIOSYNTHESIS

The Activation of Amino Acids in Extracts of Mammalian Tissues

G. D. Novelli A. N. Best

Introduction. – In a continuation[3] of the effort to understand why only 9 or 10 amino acids catalyze the amino-acid-dependent exchange of inorganic pyrophosphate (PP) with ATP (see Appendix for alphabetical list of abbreviations), a study of the exchange reaction has been extended to several other extracts of mammalian organs. In addition the formation of amino acid hydroxamates has been studied in these extracts, since hydroxamic acid formation has been used by other workers[4–6] as a measure of the activation of amino acids and these workers have reported the activation of some amino acids which have been found[7] to be inactive in the catalysis of the PP-ATP exchange in microorganisms.

Results. – Extracts of rat liver were found to catalyze the PP-ATP exchange with only the 10 amino acids previously found to be active in microbial extracts and rabbit liver.[3] Rat kidney extracts catalyzed this exchange with somewhat fewer amino acids (7 instead of 10), and, of these, all except alanine were members of the previously active group. Guinea pig pancreas and guinea pig liver, on the other hand, appeared to catalyze a small but probably significant PP-ATP exchange with most of the amino acids. In guinea pig pancreas the best exchange rates were seen with serine, threonine, histidine, tyrosine, and leucine, whereas in guinea pig liver, serine, valine, aspartic acid, lysine, and glycine were most active. The formation of amino acid hydroxamates in the presence of ATP, the individual amino acids, and high concentrations of hydroxylamine was studied with extracts of guinea pig liver. All the amino acids gave rise to small amounts of hydroxamates with phenylalanine, threonine and proline giving the largest amounts. In confirmation of the results with microbial extracts, there was no apparent correlation between the rate of the PP-ATP exchange and the rate of hydroxamate formation.

Discussion. – These studies with mammalian systems confirm and extend the conclusions that had been derived previously from microbial studies, that the amino acids can be divided into two large classes with respect to catalysis of the PP-ATP exchange.[7] In view of the present findings with guinea pig tissues, it might be better to have three classes of amino acids, (1) active, (2) borderline, and (3) inactive. When the rate of PP-ATP exchange by individual amino acids is compared with the formation of hydroxamates, no apparent correlation between these activities is evident. This observation suggests that the two methods are measuring different things, perhaps different stages in the over-all process of amino acid activation. The data also indicate that the findings with either method should be interpreted with caution as regards the mechanism of amino acid activation.

[1] Research Participant.

[2] ORINS Fellow.

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

[4] M. B. Hoagland, E. B. Keller, and P. C. Zamecnik, *J. Biol. Chem.* 218, 345–358 (1956).

[5] E. W. Davie, V. V. Koningsberger, and F. Lipmann, *Arch. Biochem. and Biophys.* 65, 21–38 (1956).

[6] R. Schweet, *Federation Proc.* 16, 244 (1957).

[7] J. A. DeMoss and G. D. Novelli, *Biochim. et Biophys. Acta* 22, 49–61 (1956).

Activation of Amino Acids in Extracts of Pea Seedlings

J. W. Davis G. D. Novelli

Introduction. – This report is a continuation of the study of the activation of amino acids in plant tissues.[8] In the present study some of the characteristics of the amino-acid-dependent exchange of PP with ATP have been examined.

[8] J. W. Davis and G. D. Novelli, *Biol. Semiann. Prog. Rep. Feb. 15, 1957*, ORNL-2267, p 70.

Results. – It had been previously observed that undialyzed extracts of the epicotyl portion of pea seedlings exhibited a PP-ATP exchange of 5.4% in the absence of added amino acids.[8] This exchange was increased to about 8% upon the addition of a complete mixture of amino acids. Since it was desired to test the catalysis of the PP-ATP exchange by individual amino acids, an attempt was made to reduce the endogenous exchange to as low a level as possible in order that reliable calculations of exchange rate with individual amino acids could be made. It was found that prolonged dialysis did not reduce the endogenous exchange rate, but simply lowered the total activity. However, centrifugation of the extracts at 100,000 × *g* followed by precipitation with 80% saturated ammonium sulfate and treatment of the extracts with hydroxylamine did reduce the endogenous exchange rate by about 50%. With such preparations the PP-ATP exchange was promoted by the following amino acids: leucine, tyrosine, valine, tryptophan, isoleucine, cysteine, and methionine. These are seven of the ten amino acids which had been found to be active in bacteria.[7] However, the rates of exchange with these amino acids in pea seedling extracts were only about one-tenth the rate in bacteria and about two times the rate in rat liver. It was found, surprisingly enough, that the presence of potassium fluoride, which had been added to suppress any ATPases or inorganic pyrophosphatases which might be present, inhibited the exchange reaction about 80%. The reason for this inhibition is not yet known, and the plant differs from the animal or bacterial systems in this repect. It was also observed that the exchange rate was somewhat higher when the experiment was done in the tris buffer instead of in the phosphate buffer. This led to the speculation that an orthophosphate exchange might be simultaneously operating. When this was tested, it was found that the extracts catalyze a slight orthophosphate exchange with ATP which was not stimulated by amino acids and hence did not interfere with calculations on the amino-acid-dependent PP-ATP exchange. The latter exchange was also found to be present in extracts of carrot roots and was somewhat higher than that in the pea seedling extracts. Carrot root mitochondria, in contrast to mitochondria from animal tissues, has an amino-acid-dependent exchange that is about one-third as active as the exchange in the soluble portion of the preparation.

Discussion. – All the results from studies of amino acid activation, as measured by PP-ATP exchange, in pea seedling preparations confirm the earlier observations in microbial extracts.[7] The fact that most of the same amino acids are active in plant tissue as in microbial preparations emphasizes that the catalysis of PP exchange by only these amino acids is a real phenomenon and not an artifact in the preparation of the extracts. It now becomes necessary to try to understand the manner in which the amino acids that do not catalyze the PP-ATP exchange reaction are activated. The technique for reducing endogenous activity by ammonium sulfate precipitation and treatment of the preparations with hydroxylamine should facilitate such studies.

Formation of ATP from Inorganic Pyrophosphate and Various Synthetic Aminoacyl-AMP Compounds by the Tryptophan-activating Enzyme from Beef Pancreas

R. A. McRorie G. D. Novelli

Introduction. – DeMoss, Genuth, and Novelli[9] have presented evidence suggesting that aminoacyl-AMP compounds are intermediates in the amino-acid-dependent exchange of inorganic pyrophosphate with ATP according to the following equation:

$$\text{ATP} + \text{R}-\underset{\underset{\text{NH}_2}{|}}{\text{CH}}-\text{COOH} \rightleftharpoons \text{AMP}-\text{O}-\overset{\overset{\text{O}}{\|}}{\text{C}}-\underset{\underset{\text{NH}_2}{|}}{\text{CH}}-\text{R} + \text{PP}$$

The evidence for this suggestion is based upon two facts, namely, (1) that chemically synthesized leucyl-AMP is enzymically converted to ATP in the presence of PP, and (2) the enzyme, which had been purified some twentyfold with respect to leucine-dependent exchange, exhibited the same specificity for ATP formation as had been observed for the PP-ATP exchange reaction. Berg,[10] however, working with an enzyme from yeast which

[9] J. A. DeMoss, S. M. Genuth, and G. D. Novelli, *Proc. Natl. Acad. Sci. U.S.* **42**, 325–332 (1956).

[10] P. Berg, *Federation Proc.* **16**, 152 (1957).

is specific for methionine in the PP-ATP exchange, reported that his enzyme catalyzes ATP formation from adenyl derivatives of several amino acids, including D-amino acids, which do not promote the PP-ATP exchange. This observation casts some doubt on the validity of the argument used previously[9] in suggesting these compounds as intermediates in the amino-acid-dependent PP-ATP exchange.

Since Davie, Koningsberger, and Lipmann[11] have reported the extensive purification of an enzyme from beef pancreas which is specific for the activation of tryptophan, it was felt that a study of ATP formation from a series of aminoacyl-AMP compounds by this purified enzyme might help to resolve the apparently contradictory observations reported above.

Results. – The tryptophan-activating enzyme was prepared from beef pancreas according to the directions of Davie, Koningsberger, and Lipmann.[11] The aminoacyl-AMP compounds were prepared by a modified Kohorana synthesis[12] by directions furnished by Berg.[13] The adenyl derivatives of the following amino acids have been prepared: D- and L-alanine, D- and L-valine, D- and L-methionine, D- and L-phenylalanine, L-tryptophan, L-*S*-methylcysteine, and L-leucine. These compounds have been tested for ATP formation at various stages of purification of the enzyme. When the enzyme had been purified tenfold all the above compounds led to a significant formation of ATP with D- and L-methionine, D- and L-phenylalanine, and L-tryptophan giving the best yields. With enzyme which had been purified 25-fold the same compounds were still active. Significantly, at all stages of purification so far tested the enzyme gives a good PP-ATP exchange, but this exchange rate cannot be influenced by added tryptophan. Similar tests with still more highly purified enzyme are now in progress.

Discussion. – The formation of ATP from D compounds as well as from an unnatural compound such as *S*-methylcysteine-AMP is indeed disturbing to the postulate that L-aminoacyl-AMP compounds are specific intermediaries in the PP-ATP exchange reaction. However, the fact that tryptophan cannot stimulate the PP-ATP exchange with even the 25-fold purified enzyme suggests that the enzyme contains bound tryptophan which may be involved in the catalysis of ATP formation from these various amino acid derivatives. It is clear that similar studies with more highly purified enzyme preparations will be necessary to understand the mechanism of ATP formation from adenylate derivatives that do not catalyze the PP-ATP exchange.

[11] E. W. Davie, V. V. Koningsberger, and F. Lipmann, *Arch. Biochem. and Biophys.* 65, 21–38 (1956).

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

[13] P. Berg, personal communication.

Synthesis of Proteins by Cell-Free Extracts of Bacteria

J. A. DeMoss G. D. Novelli

Introduction. – It would be most desirable for an understanding of the mechanism of protein synthesis to be able to study it in cell-free systems. For this purpose a study of the synthesis of certain constitutive enzymes in cell-free extracts of bacteria has been undertaken. Unfortunately cell-free extracts contain relatively large amounts of preformed enzyme, which makes measurements of a small net synthesis of the enzyme highly inaccurate. Therefore the present study was concerned with attempts to reduce the endogenous level of the enzymes whose net synthesis was to be measured in the cell-free extracts. Two different bacterial systems have suggested themselves as being amenable to the desired manipulations. The first of these studies was the temperature-sensitive pantothenate mutant of *Escherichia coli* described by Maas and Davis.[14] This organism grows like the wild type at temperature below 25°C, but above this temperature it requires an exogenous source of pantothenate because the pantothenate-synthesizing enzyme, which couples pantoic acid with β-alanine, is inactivated at the higher temperature. From these facts, it seemed that organisms grown at 15° would have the enzyme-forming system and the endogenous enzyme could be destroyed by a short incubation at 37°C. After this treatment, the net synthesis of enzyme at 15°C could be studied with little or no interference from endogenous enzyme.

The other system which appeared promising is the suppression of the synthesis of ornithine

[14] W. K. Maas and B. D. Davis, *Proc. Natl. Acad. Sci. U.S.* 38, 785–797 (1952).

transcarbamylase by arginine in *E. coli* B as described by Gorini and Maas.[15] They observed that high concentrations of arginine in the growth medium suppressed almost completely the formation of ornithine transcarbamylase. When such suppressed cells are washed free of arginine and grown in the absence of arginine, the full complement per cell of ornithine transcarbamylase is synthesized before one division has been completed. This appeared, also, to be a system in which the endogenous level of an enzyme could be greatly reduced before the preparation of the cell-free extract.

Results: *(a) Pantothenate-synthesizing enzyme.* – In order to measure the amount of this enzyme it is necessary to measure the pantothenate formed from β-alanine and pantoic acid; pantothenate is usually measured with a microbiological assay which requires 24 or more hours for results. A new procedure for the determination of pantothenate has been developed which is quite rapid and sensitive. The method takes advantage of the distinct color produced by β-alanine in the usual ninhydrin assay for amino acids. The reaction mixture which contains β-alanine, pantoic acid, and pantothenic acid is first freed of β-alanine by passage through a column of Dowex-50 which holds back the β-alanine and allows the pantothenate to pass through. The column effluent is then hydrolyzed with alkali, thereby liberating β-alanine from the pantothenate. The β-alanine is determined colorimetrically and expressed as pantothenate.

With the use of this assay, attempts to demonstrate the formation of the pantothenate-synthesizing enzyme in cell-free extracts of the mutant have not been successful. This has perhaps been due to the fact that the mutant is rather unstable and easily reverts to the wild type.

(b) Ornithine transcarbamylase in E. coli. – The suppression of this enzyme by arginine requires rather carefully controlled conditions, and we have not yet worked out the details for the complete suppression of this enzyme. Preliminary results with incompletely suppressed cells have indicated that incubation of such cells in the absence of arginine, but with glucose as an energy source, leads to a net increase in ornithine transcarbamylase.

[15] L. Gorini and W. K. Maas, *Biochim. et Biophys. Acta*, 25, 208–209 (1957).

Discussion. – The preliminary results reported here suggest that the suppression of ornithine transcarbamylase by arginine is a promising system for subsequent efforts to study cell-free protein synthesis.

Exchange of Inorganic Pyrophosphate with Ribonucleoside Triphosphates Catalyzed by Rat Liver Microsomes

G. D. Novelli A. N. Best

Introduction. – In a study of the amino-acid-dependent exchange of PP with ATP in rat liver extracts Hoagland, Keller, and Zamecnik[16] briefly noted that microsomes prepared from rat liver catalyzed a PP-ATP exchange which did not appear to be stimulated by amino acids. Since microsomes are a necessary component of the reconstructed system which incorporates C^{14}-labeled amino acids into protein (Zamecnik, Keller, and Loftfield[17]), it was decided to investigate the microsomal PP-ATP exchange to see if such studies might cast some light on the mechanism of protein synthesis.

Results. – Rat liver microsomes have been found to have a strong amino-acid-independent PP-ATP exchange. The reaction leads to about 20 to 30% exchange in 30 min at 37°C. With intact microsomes, the exchange reaction is unaffected by freezing and thawing. Indeed, microsomes which have been stored at –20° for as long as one year have retained 80 to 90% of their original activity. The exchange reaction is unaffected by treatment of the microsomes with RNAse, by dialysis, or by extraction with 1.0 *M* NaCl. The exchange reaction is 50% inhibited by the presence of 2.0 *M* hydroxylamine, which suggests that a hydroxylamine reactive intermediate may be formed during the exchange reaction. However, all attempts to detect an intermediate hydroxylamine derivative with the usual colorimetric procedures have been unsuccessful. The reaction is almost completely inhibited by pretreatment of the microsomes with deoxycholate.

One of the most interesting findings has been that certain lots of microsomes catalyze a PP exchange with GTP, CTP, ITP, and UTP as well

[16] M. B. Hoagland, E. B. Keller, and P. C. Zamecnik, *J. Biol. Chem.* 218, 345–358 (1956).

[17] P. C. Zamecnik, E. B. Keller, and R. B. Loftfield, *J. Histochem. Cytochem.* 2, 378–386 (1954).

as with ATP. With one preparation, for example, the precentage exchanges were as follows: ATP, 46%; UTP, 36%; CTP, 7%; GTP, 7%; and ITP, 5.5%. Unfortunately, the exchanges with the nucleoside triphosphates other than ATP are not reproducible in different preparations of microsomes, and it is impossible at the present time to predict when a given preparation of microsomes will exhibit all these activities. When these exchanges are present in a given preparation of microsomes, they are very reproducible in tests conducted at widely separated intervals.

Discussion. – The finding that microsomes will exchange PP with the pyrophosphoryl moiety of all four ribonucleoside triphosphates represents the first time that such an observation has been recorded. It suggests that the exchange reaction may be an indicator of some stage of the synthesis of nucleic acid. That different enzymes may be involved with each nucleoside triphosphate is suggested by the fact that some preparations of microsomes exhibit the PP-ATP exchange without having any activity with the other nucleoside triphosphates. It appears that the resolution of this problem will lie in the purification and separation of the enzymes involved in this reaction. The PP-ATP exchange reaction can be solubilized by sonication of the microsomes, and the soluble activity can be fractionated with ammonium sulfate. Further purification studies are now in progress.

Luminescence of *Achromobacter fischeri* as a Function of Salt Concentration

M. J. Cormier G. D. Novelli

Introduction. – It has been reported[18] that cultures of *Achromobacter fischeri* grown in a medium low in salt (1% NaCl) are practically nonluminescent, when compared to the relatively bright cultures obtained by growing the organism in 3% NaCl. In addition, it was reported that if cells which had been grown in 1% NaCl were suspended in a medium containing 3% NaCl then the cells would begin to luminesce after a short time. On the surface, such a system seemed a good one for the study of luciferase biosynthesis. In addition, it was hoped that an explanation of the "salt effect" might come about during the course of such an investigation.

Results. – In using a system such as the one described above, it was important to know whether differences in the light intensity of cells grown in 3% vs 1% NaCl were actually due to differences in luciferase content. Cells were grown in media containing both levels of salt, and the luciferase content was followed as a function of growth. On the basis of total units per cell, the cells grown in 3% salt contained 20 to 30 times as much luciferase as those grown in 1% salt. Although the difference in luciferase content seemed appreciable, a good bit (and perhaps all) of this difference could be explained in terms of a masking effect. For example, with cells grown in 1% salt, the apparent luciferase content per cell was increased two- to threefold depending upon whether the extracts were prepared by lysing with water or by extracting acetone-dried powders with water. The latter procedure gave the higher luciferase content. The luciferase content, per cell, of cells grown in 3% salt was the same regardless of which of the two methods was used in preparing the extracts. It was also found that cells grown in 1% NaCl were deficient with respect to long-chain aldehydes, since the addition of dodecylaldehyde to such a culture increased the luminescence intensity about fortyfold and, as a result, the luminescence of the culture became relatively bright to the eye. The luminescence of cultures which have been developed in 3% NaCl does not increase upon the addition of dodecylaldehyde indicating that such cells are saturated with respect to the aldehyde.

Discussion. – The differences in luciferase content between cells grown in 3% vs 1% NaCl are not so great as would be expected by observing the luminescence of such cultures. The effect of low salt concentration is at least twofold: (1) there is a masking of the enzyme which results in loss of activity, and (2) aldehyde production apparently becomes limiting. The effect of salt concentration on the production of aldehyde is being investigated further.

ONE-CARBON METABOLISM IN MICROBIAL EXTRACTS

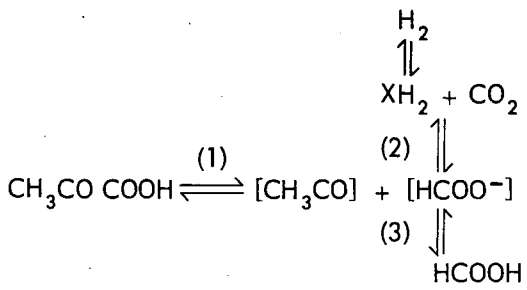
The Exchange Between Pyruvate Carboxyl and Formate or Bicarbonate

G. D. Novelli H. K. Sherwood

Introduction. – This is a continuing investigation into the problem of the mechanism by which

[18] A. H. Farghaly, *J. Cellular Comp. Physiol.* 36, 165–184 (1950).

certain bacteria exchange the carboxyl group of pyruvate with either formate or CO_2 (ref 19). Previous studies have indicated that the exchange reactions could be formulated as[20]



where the compounds in brackets represent hypothetical intermediates. This reaction scheme suggests that a one-carbon fragment at the level of oxidation of formate is derived from the carboxyl group of pyruvate and could be an intermediate in the conversion of formate to the carboxyl group of pyruvate or to hydrogen gas and CO_2. Similarly, such a compound might be an intermediate in the hydrogenlyase reaction in *E. coli*.

During the past several months an attempt has been made to set up an assay system in which various synthetic formate derivates could be tested as possible intermediates in catalyzing reactions (2) and (3) in the absence of pyruvate.

Results. – It has been found that extracts of *Micrococcus lactilyticus* catalyze a rapid CO_2-pyruvate exchange [reactions (1) and (2)] at *pH 6.8* but not at *pH 8.0*. On the other hand, the same extracts catalyze a rapid formate-pyruvate exchange [reactions (1) and (3)] at pH 8.0, but a very poor reaction at pH 6.5. Dihydrofolic acid inhibited the formate-pyruvate exchange in these extracts by about 90%, but stimulated the CO_2-pyruvate exchange by about 35%.

If cell-free extracts are incubated with $C^{14}O_2$ and formate, and at the end of the experiment formate is separated on a Celite column,[21] converted to $BaCO_3$, and counted, a measure of the reversibility of reactions (2) and (3) would be the extent of appearance of isotope in the recovered formate. This method permits the testing of synthetic compounds for catalysis of this exchange reaction in the absence of pyruvate. At pH 7.0 the extracts catalyze a 10% exchange between $C^{14}O_2$ and formate in the presence of a small amount of pyruvate but not in its absence. Dihydrofolic acid could not substitute for pyruvate in this exchange. Wood's[22] report of the reversibility of the hydrogenlyase reaction in *E. coli*, namely, $H_2 + CO_2 \rightleftharpoons HCOOH$, has been confirmed by showing that whole cells of anaerobically grown organisms bring about the complete exchange of $C^{14}O_2$ and formate. However, cell-free extracts prepared from such cells by alumina grinding, although still capable of decomposing formate into H_2 and CO_2, are unable to exchange $C^{14}O_2$ and formate.

Discussion. – A technique has been developed which will permit the testing of various formate derivatives as metabolically active intermediates in the above described exchanges. The synthesis of formyl phosphate, AMP-formate, and formyl CoA is in progress. These compounds will be tested for participation in these exchange reactions. The finding that hydrogenlyase, although freely reversible in whole cells, apparently operates only in one direction in extracts suggests that the mechanism of this reaction may have some properties in common with reactions (2) and (3) above, and a study of the hydrogenlyase reaction will be conducted together with the present studies.

Methionine Biosynthesis in *Neurospora crassa*

R. A. McRorie G. D. Novelli

Introduction. – A leaky, methionineless mutant of *N. crassa* accumulates a compound during growth on minimal medium which possesses methionine activity for other methionine-requiring mutants. On the basis of nutritional responses in experiments in which various mutants were used, the compound appears to be intermediate between homocysteine and methionine. The accumulated intermediate contains a thioether linkage which can be oxidized with H_2O_2 to an active sulfoxide and a free amino group. It is very labile and, on decomposition of solutions, the apparent

[19] G. D. Novelli and H. K. Sherwood, *Biol. Semiann. Prog. Rep. Aug. 15, 1956*, ORNL-2155, p 54.

[20] G. D. Novelli, *Biochim. et Biophys. Acta* 18, 594 (1955).

[21] E. F. Phares, E. H. Mosbach, F. W. Denison, and S. F. Carson, *Anal. Chem.* 24, 660–662 (1952).

[22] D. D. Woods, *Biochem. J.* 30, 515–527 (1936).

methionine content increases, indicating that the intermediate is a labile derivative of methionine.

Attempts have been made to compare the properties of the accumulated intermediate with those of methionine-AMP and to investigate the possibility of an ATP-activated pathway for methionine synthesis.

Results. – Synthetic methionine-AMP prepared by the use of dicyclohexylcarbodiimide[23] is not identical with the intermediate. Although enzyme extracts of wild-type and mutant mycelia of *Neurospora* and *E. coli* show PP^{32} exchange with cysteine and methionine, no evidence for cystathionine-AMP and homocysteine-AMP formation could be obtained.

S-Methylcysteine, previously reported to be inactive as a sole source of sulfur for some mutants, was found to be active for these mutants in the presence of sulfate. The corresponding sulfoxide is also active, but the properties of these compounds do not correspond to the accumulated intermediate.

Discussion. – The results obtained suggest that the intermediate is involved in an alternate pathway for the biosynthesis of methionine in which *S*-methylcysteine or a derivative serves as methyl donor. Preliminary experiments with enzyme extracts support this assumption. The chemical nature and role of the accumulated intermediate in this conversion remain to be determined.

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

TRACER STUDIES ON INTERMEDIARY METABOLISM

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

Role of H_2O_2 in Metabolism of *Clostridium perfringens*

M. I. Dolin

Introduction. – Electron transport enzymes from the strict anaerobe *Clostridium perfringens* are being used to study the role of peroxide in the metabolism of anaerobic bacteria, with special reference to the mechanism of oxygen toxicity.

Results. – The DPNH oxidase[1] (see Appendix for alphabetical list of abbreviations) of the organism has been further characterized with respect to (1) variety of H acceptors utilized, (2) inhibitors, (3) ultraviolet inactivation, (4) kinetics of inactivation by substrate (DPNH), and (5) role of peroxide in the DPNH inhibition reaction.

Conclusions. – The results demonstrate that even extremely low peroxide concentrations may be toxic to certain anaerobes and that these peroxide concentrations can be the result of DPNH oxidation reactions. The behavior of DPNH oxidase indicates that some enzymes may show enhanced sensitivity to peroxide when they are combined with substrate, reduced, or "turning over."

Mechanism of Succinate Decarboxylation by Bacterial Extracts

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

Introduction. – Earlier experiments[2] on the nature of the one-carbon intermediate compound (or complex), arising from succinate decarboxylation by *Propionibacterium pentosaceum* and *Micrococcus lactilyticus*,[3] concerned its possible similarity to certain intermediate compounds proposed for an analogous mammalian system. No evidence could be found for the participation of the ATP-activated carbon dioxide complexes ["AMP-CO_2" (ref 4) and "carbonyl phosphate" (ref 5); Eq. 1].

No substrate-dependent P^{32}-labeled ortho- or pyrophosphate exchange into ATP was detected. Although an exchange of labeled phosphorus *is* obligatory for formation of the proposed intermediate phosphate compounds, the possibility certainly remains that nucleotides other than ATP could be involved in the formation of the carbon dioxide complex. Hence substrate-dependent exchange of labeled ortho- and pyrophosphate into ITP, GTP, and UTP (in addition to ATP) was examined.

Results and Discussion. – Only in the case of ITP was there a substrate-dependent exchange of labeled phosphorus between P_i^{32} and PP^{32} (a mixture) and the nucleotide; in addition, a significant stimulation of CO_2 production was noted. Subsequently, P_i^{32} and PP^{32} were tested separately; PP^{32} was inhibitory, whereas with P_i^{32} the increase over control experiments was sevenfold for phosphorus exchange and eightfold for CO_2 production. These results demonstrate that the inosine nucleotide may well be concerned with CO_2 production from succinate in the bacterial system. The effect of ITP was also tested upon the reverse reaction, namely, the incorporation (or exchange) of CO_2 into succinate. A slight inhibition occurred with ITP for the reverse reaction, and hence there is as yet no direct evidence for a nucleotide-activated CO_2 complex. The latter result suggests that the relatively crude enzyme preparations used here may well contain interfering substances for the reverse reaction only; otherwise the interesting and quite rapid succinate-dependent exchange of P_i into ITP is equivocal.

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[1] M. I. Dolin, *Bacteriol. Proc.*, p 110 (1957).

[2] E. F. Phares, R. E. Koeppe, and S. F. Carson, *Bacteriol. Proc.*, p 128 (1957).

[3] E. F. Phares, E. A. Delwiche, and S. F. Carson, *J. Bacteriol.* 71, 604–610 (1956).

[4] M. J. Coon and B. K. Bachawat, *J. Am. Chem. Soc.* 78, 1505 (1957).

[5] M. Flavin, H. Castro-Mendoza, and S. Ochoa, *Nature* 176, 823–826 (1955).

$$(1)\quad \text{succinyl-CoA} \xrightarrow{AMP} \text{propionyl-CoA} + AMP - CO_2 \underset{}{\overset{P\sim P}{\rightleftharpoons}} CO_2 + ATP$$

$$\text{succinyl-CoA} \xrightarrow{P_i} \text{propionyl-CoA} + CO_2 - P \underset{ADP}{\rightleftharpoons}$$

Tracer experiments with cell extracts have not furnished satisfactory evidence concerning the nature of the one-carbon compound (or complex); hence intensive efforts have been initiated to purify the enzymes concerned. Until now the enzyme purification has not been promising as the enzymes concerned appear to be quite unstable despite the variety of treatments used. The handicap of enzyme instability has now been largely circumvented by the development of a far more sensitive assay procedure and by preparation of very large amounts of the bacterial extract.

PLANT BIOCHEMISTRY

N. E. Tolbert
R. Rabson[1]
A. H. Haber[2]
P. C. Kerr
D. A. Mondon
A. W. Naylor[3]

Conversion of Aspartic Acid to Homoserine

R. Rabson
N. E. Tolbert
A. W. Naylor
P. C. Kerr

Introduction. – This and the three following reports are concerned with amino acid metabolism *in vivo*, which is a subject of continuing investigation. In earlier reports[4] the conversion of aspartic-U-C^{14} acid to citric acid cycle constituents by a wide variety of plant tissues was found to be qualitatively essentially the same. In this report a conversion of aspartic acid to homoserine has been detected in the pea plant.[5]

Results. – Pea plants, when fed aspartic-U-C^{14} acid, convert considerable amounts of the C^{14} label into homoserine in short periods of time. The identity of the homoserine-C^{14} was established by cochromatography. The amount of conversion of aspartic acid into homoserine varied according to which organ of the plant was being studied. Roots showed the greatest capacity by converting about 15% of the aspartic acid in $2\frac{1}{2}$ hr to homoserine, while stems showed the least conversion. To date we have not been able to demonstrate this conversion in cell-free extracts, though the apparent formation of aspartyl phosphate can be demonstrated by the hydroxamic test.

Discussion. – Although homoserine has been known to occur in various higher plants, its biosynthesis has never been described for plants. In yeast, the pathway of biosynthesis from aspartic acid has been fully demonstrated.[6] It is probable that this same pathway exists in higher plants. The results may also explain how label from aspartic-U-C^{14} acid is detectable in threonine in most plants, in that homoserine is a precursor of threonine in yeast metabolism.[7]

A Ninhydrin Spray Test for α-Ketoglutaric Acid

R. Rabson
N. E. Tolbert

Introduction. – A number of methods exist for the detection of α-ketoglutaric acid on paper chromatograms. The ninhydrin spray test has now been adapted as a sensitive test for α-ketoglutaric acid and certain other α-keto acids such as pyruvic and α-ketobutyric acid. This allows the use of ninhydrin for detection of both amino acids and these keto acids simultaneously.

Method. – Ninhydrin (0.1% in ethanol) with about two drops of 2,6-lutidine per 100 ml of reagent is sprayed on paper chromatograms and heated 3 to 5 min at 90 to 95°C. The amino acid areas on the paper are examined. The paper is then placed in the dark for one or more days and afterwards observed in ultraviolet light (3660 Å). The keto acids fluoresce intensely yellow. As little as 1 μg of α-ketoglutaric acid, when added to a plant extract and chromatographed, markedly increased the intensity of the fluorescence in the position for α-ketoglutaric acid.

Glycine and Serine Biosynthesis During Photosynthesis

N. E. Tolbert
R. Rabson
P. C. Kerr

Introduction. – Serine and glycine are products of photosynthesis which often obtain a major portion of the C^{14} from short-time $C^{14}O_2$ fixation experiments. The synthesis of these two amino acids during photosynthesis is being investigated.[8]

Results. – In *Coleus* and *Sedum* leaves a diurnal variation was observed in the amount of C^{14} incorporated into serine by leaves excised at hourly

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[1]Temporary Employee.

[2]Research Associate.

[3]Consultant.

[4]R. Rabson, A. W. Naylor, N. E. Tolbert, and P. C. Kerr, *Biol. Semiann. Prog. Rep. Feb. 15, 1957*, ORNL-2267, p 76. See also *Plant Physiol.* 31, (Suppl.), x (1956).

[5]R. Rabson and N. E. Tolbert, *Plant Physiol.* 32, (Suppl.), xxxv (1957).

[6]S. Black and N. G. Wright, *J. Biol. Chem.* 213, 27–60 (1955).

[7]S. Black and N. G. Wright, *Amino Acid Metabolism* (ed. by W. P. McElroy and B. Glass), p 591–600, Johns Hopkins Press, 1955.

[8]R. Rabson and N. E. Tolbert, *Plant Physiol.* 32, (Suppl.), xxxv (1957).

intervals and allowed to photosynthesize for 10 min in $C^{14}O_2$ (Table 23). Almost half of the total $C^{14}O_2$ fixed into soluble constituents of *Coleus* leaves in 10 min was present in serine plus glycine, and, of this, the C^{14} in serine represented as much as 30% of the total soluble radioactivity during the middle of a 15-hr day. The ratio of radioactivity in glycine/serine varied from 2.0 at night to 0.5 during midday.

In an effort to determine which amino acid was synthesized first during photosynthesis, corn leaves were exposed to $C^{14}O_2$ for decreasing lengths of time, and then the amount of radioactivity in glycine and serine was determined (Table 24). The glycine/serine ratio of radioactivity increased at the shorter periods of time for this plant, which suggests that glycine was synthesized before serine.

Discussion. – The above data on C^{14} labeling does not offer direct evidence on the pathway of glycine and serine synthesis by plants during steady-state photosynthesis. It is suggestive that glycine synthesis precedes serine. A high glycine/serine ratio of C^{14} incorporation during 10 min of photosynthesis inserted in the middle of the night indicated that the glycine reservoir

Table 24. Change in Ratio* of C^{14} in Glycine and Serine During Photosynthesis by Corn Leaves

Experiment No.	Minutes of Photosynthesis			
	3	6	15	30
1	6.2	4.7	2.6	1.9
2	3.1	3.1	2.0	1.3

*Each figure represents

$$\frac{\text{counts in glycine}}{\text{counts in serine}}$$

in an aliquot of the sample.

Table 23. Diurnal Variation in C^{14} in Glycine and Serine of Golden Bedder Coleus Leaves After 10 min of $C^{14}O_2$ Photosynthesis

Hours of Light	Per Cent of C^{14} in Glycine + Serine	Ratio, $\frac{\text{Glycine-}C^{14}}{\text{Serine-}C^{14}}$	Hours of Dark	Per Cent of C^{14} in Glycine + Serine	Ratio, $\frac{\text{Glycine-}C^{14}}{\text{Serine-}C^{14}}$
0	33	2.0	1	48	0.6
1	37	1.5	2	36	0.4
2	31	1.5	3	36	1.3
3	32	0.7	4	41	1.3
4	29	1.0	5	39	1.9
5	41	0.6	6	37	1.6
6	37	0.7	7	41	2.1
7	37	0.4	8	39	1.4
8	47	0.6	9	44	1.7
9	39	0.4			
10	48	0.7			
11	34	0.7			
12	47	0.6			
13	40	0.9			
14	31	1.3			
15	44	1.0			

was replenished first upon exposure to light. Also, when the time for $C^{14}O_2$ fixation was decreased, a larger amount of C^{14} in glycine was found than in serine. There are several possible pathways for glycine and serine biosynthesis either from C_2 compounds to glycine or from C_3 compounds to serine. In order to explore these possibilities the C^{14} distribution in glycine, serine, and alanine from very short-term photosynthesis experiments with $C^{14}O_2$ is being determined.

Effect of Indoleacetic Acid on Metabolism in Tomato and Oat Plants

L. M. Rohrbaugh[9] N. E. Tolbert

Introduction. – Indoleacetic acid (IAA) has long been known to be a most important regulator of plant growth, but the mechanism of its action is unknown. The action of IAA on metabolic processes has been surveyed by chromatography of the soluble constituents from C^{14}-labeled plants which had been treated with this auxin. The results showed numerous changes in the C^{14} labeling of the amino acids, sugars, and organic acids of the plants.[10]

Results. – In short-time experiments the basal ends of leaflets of tomato and leaves of oat plants were placed in solutions containing 0 to 10,000 ppm (acid equivalent) of the potassium salt of indoleacetic acid for 1 or 2 hr. They were then exposed to $C^{14}O_2$ in the light for 20 or 30 min, after which they were extracted with hot ethanol and water. Soluble C^{14}-labeled products were analyzed by paper chromatography. The amount of labeling in each compound was determined by counting the radioactivity on the chromatograms.

In long-time experiments whole plants were labeled for two days. Different groups of these plants were then sprayed once with solutions containing 0, 100, 1000, and 10,000 ppm of the auxin and then returned to the $C^{14}O_2$ photosynthesis chamber for one to six days before analyzing as above.

In the short-time experiments total C^{14} fixation was sharply reduced at 1000 and 10,000 ppm of IAA. Also, increasing concentrations of IAA resulted in the following changes: (1) an increase in the percentage of the total C^{14} fixed which was in alanine, glutamine, maltose, and phosphorylcholine; (2) a decrease in the percentage of label in glycine, serine, aspartic acid, and malic acid.

In the long-time labeling experiments the results were less definite, but labeling of maltose and alanine as well as several other compounds (probably polysaccharides) increased with higher IAA concentrations.

Discussion. – The short-term experiments were designed to observe changes in synthesis patterns of the soluble constituents as affected by IAA. The long-term experiments were indicative of the reservoir size of these compounds. Changes in both the synthesis pattern and the reservoir size after IAA treatment cannot be interpreted as indicative of specific sites of action of IAA. One interpretation might be that these changes are only indicative of the metabolic changes induced by high concentrations of IAA but are not related to the site of IAA action. In any case further experimentation will be valuable to better understand plant growth as affected by IAA.

Evidence for Distinct Effects of Gibberellic Acid and Kinetin on the Germination of Lettuce Seeds

A. H. Haber N. E. Tolbert

Introduction. – Recently, two groups of substances have been shown to have profound effects on the growth and development of higher plants. These are gibberellins[11] and kinins.[12] Acceleration of germination rates of certain lettuce seeds by exposure to red light can be imitated either by treatment with gibberellin or kinin. In order to see whether these two groups of substances accelerate germination by affecting the same process(es) or different processes, we have compared the effects of a gibberellin (gibberellic acid) and a kinin (kinetin) on the germination of Grand Rapids lettuce seed under various conditions.

Results and Discussion. – At 35°C kinetin stimulates germination but gibberellic acid can no longer promote germination over controls. At 17°C and 7°C the reverse is true. Effects of

[9] Summer Research Participant in 1956.

[10] L. M. Rohrbaugh and N. E. Tolbert, *Plant Physiol.* 32, (Suppl.), xviii (1957).

[11] B. Stowe and T. Yamaki, *Ann. Rev. Plant Physiol.* 8, 181 (1957).

[12] F. Skoog and C. Miller, *Symposia Soc. Exptl. Biol.* 11, 118 (1957).

combinations of kinetin and gibberellic acid suggested synergism at 35°C, but the effects were less than additive at 22 or 17°C. These results clearly show that the effects of gibberellic acid and kinetin on seed germination can be separated, insofar as they must have different temperature optima for activity. This could be explained in two ways: (1) via different mechanisms gibberellic acid and kinetin affect the same basic process(es) controlling germination, or (2) gibberellic acid and kinetin affect different processes which share in the regulation of germination. Since the increases in germination caused by gibberellic acid plus kinetin appear to be more than additive at high temperature and less than additive at low temperature, we favor the second alternative.

Interactions of Kinetin with Adenine or 2,6-Diaminopurine in the Germination of Lettuce Seeds

A. H. Haber N. E. Tolbert

Introduction. – Although we have no knowledge of the mechanism(s) of action of kinins on stimulating cell division and growth, two considerations direct our attention to adenine.[12] First, active kinins thus far synthesized are derivatives of adenine. Second, biological effects of kinins are similar to adenine effects in several instances. These considerations suggest a hypothesis that kinins act by affecting the utilization or activity of adenine. This hypothesis has been tested by studying germination of two varieties of lettuce seed (Grand Rapids and Big Boston White) in the presence of kinetin plus either adenine or 2,6-diaminopurine, a growth inhibitor and antimetabolite of adenine.

Results and Discussion. – Effects of combinations of kinetin with either adenine or diaminopurine showed that the stimulation of germination by kinetin was neither enhanced by adenine nor depressed by inhibitory concentrations of diaminopurine. Germination of Big Boston White seed was slightly stimulated by adenine, but adenine and kinetin at low concentrations exhibited competition. The results did not support the hypothesis that kinetin acts by enhancing the activity or availability of free adenine to the cell.[13]

Greening in Particles in Etiolated Wheat Leaves Exposed to Light

A. H. Haber N. E. Tolbert

Introduction. – When etiolated wheat plants are exposed to light, they undergo many changes, including the development of chlorophyll and the photosynthetic process and the synthesis of protein. The development of the photosynthetic process[14] and the effect of ionizing radiation on these changes[15] have been studied. In the period covered by this report, we have attempted to gain information concerning the roles played by subcellular particles in these phenomena.[16] The greening process in particles of etiolated wheat leaves exposed to light was explored by differential centrifugation, chlorophyll analyses, and incorporation of C^{14}-leucine into protein.

Results. – The appearance of chlorophyll in "plastids," which sedimented at 1150 × *g*, occurred a few minutes after exposure to light. Particles not sedimenting at 4600 × *g* but sedimenting at 105,000 × *g* contained about 58% of the chlorophyll a. In 2 to 10 hr of continuous light, chlorophyll content increased in all fractions. The greatest rate of increase was in the chlorophyll fraction, whereas the least rate of increase occurred in the 105,000 × *g* pellet.

When C^{14}-leucine was fed to detached leaves, the protein of the fraction sedimenting at 1650 × *g* increased in specific activity upon exposure to light; specific activity of protein in the fraction sedimenting at 6260 × *g* decreased. These data on synthesis of chlorophyll a and incorporation of C^{14}-leucine into protein indicate that part of the initial greening process occurred in large particles (plastids).

[13] A. Haber and N. E. Tolbert, *Plant Physiol.* 32, (Suppl.), xlix (1957).

[14] N. E. Tolbert and F. B. Gailey, *Plant Physiol.* 30, 491–499 (1955).

[15] F. B. Gailey and N. E. Tolbert, *Biol. Semiann. Prog. Rep. Aug. 15, 1955*, ORNL-1953, p 103–106.

[16] A. Haber and N. E. Tolbert, *Plant Physiol.* 32, (Suppl.), ix (1957).

BIOPHYSICS

MOLECULAR BIOPHYSICS AND RADIOLOGICAL PHYSICS

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M. L. Randolph
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D. L. Parrish

Description of Radiobiological Effects in Terms of Dose and Linear Energy Transfer

M. L. Randolph

Introduction. – Attempts to correlate radiobiological effects observed with different ionizing radiations have led us to examine the concept that radiobiological effects – at least at a fundamental level such as the production of chromosome aberrations – may be described as continuous multidimensional surfaces whose axes are the biological effect and the physical parameters. Our present studies are limited to three-dimensional surfaces of effect vs dose and LET (see Appendix for alphabetical list of abbreviations). Since few irradiations are performed with discrete LET spectra, a major problem is the proper choice of an LET value to describe the wide LET spectra occurring in irradiated tissue.

Results. – Using previously described methods,[5] calculations have been completed of the energy average and track average LET values for several fast neutron spectra (see Table 25). The great difference between the two 14-Mev averages is caused by the hundredfold range of this LET spectrum. LET spectra, and therefore LET averages, depend both on the ionizing particles (electrons, protons, alpha particles, recoil nuclei, etc.) passing through the volume of biological significance and on the composition, particularly the number of electrons per unit volume, of that volume. Usually the pertinent calculation is for a small volume of interest surrounded by a larger volume in which the ionizing particles originate. The values given in Table 25 are for tissue surrounded by tissue. For typical protein surrounded by water or tissue the LET values are about 30% higher.

Three-dimensional models of the effect-dose-LET surfaces for chromosome exchange frequencies in irradiated *Tradescantia* inflorescences[6,7] were made based on both the energy average and the track average LET. Sections perpendicular to the axes of these models yield three families of curves – the dose response curves at various LET values, the dose required to produce a given effect vs LET, and the effect vs LET curves at various dose levels.

Discussion. – The last family of curves suggests what may be a useful index for description of radiobiological effects. The relative biological sensitivity (RBS) of a biological system to a radiation may be defined as the ratio of the biological effect produced by a given dose of that radiation to the effect produced by the same dose of a reference radiation. The RBS is numerically equal to the

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[5]M. L. Randolph, *Biol. Semiann. Prog. Rep. Feb. 15, 1957*, ORNL-2267, p 77–78.

[6]A. D. Conger, M. L. Randolph, and A. H. Johnston, *Genetics* 41, 639 (1956).

[7]M. L. Randolph, (abstr) Radiation Research Society, May 1957.

Table 25. Average LET Values in Tissue Surrounded by Tissue and Irradiated with Fast Neutrons

Neutron Spectrum	Track Average LET (kev/μ)	Energy Average LET (kev/μ)
Monoenergetic, Mev		
0.5	66.8	73.9
1.0	57.3	64.9
1.5	45.0	54.8
2.0	36.9	49.3
2.5	32.9	45.0
3.0	29.5	43.3
4.0	~24.2	~43.5
5.0	~22.9	~48.7
14.1	11.6	74.4
86-in. cyclotron, old facility*	45.8	66.8
86-in. cyclotron, new facility*	43.4	61.8

*Spectral analysis by C. W. Sheppard, personal communication.

RBE if the dose response curves are linear but describes the biological system rather than the physical radiation.

Primarily because of the very wide discrepancy between the two 14-Mev LET averages two different interpretations may be placed on the models. Based on the track average, biological sensitivity of *Tradescantia* inflorescences for the production of chromosome exchanges increases continuously with LET over the range studied. Based on the energy average, biological sensitivity passes through a broad maximum at roughly 150 kev/μ. Boag's suggestion that the proper choice of LET average may depend on the phenomenon studied[8] has been formalized and extended for cases with linear dose response by seeking LET values, $\bar{L}$, which satisfy the equation that the observed biological effect,

$$E = R(\bar{L}) \int_0^\infty f[\phi(L)]dL \ ,$$

where $R(\bar{L})$ is the RBE for the dose delivered at spatial rate $\bar{L}$, $\phi(L)$ is the dose delivered at rate L, and $f[\phi(L)]$ defines the dose response curve. Under these conditions, the energy average LET is the proper value if the RBE is a linear function of LET, and the track average LET is the proper value if RBE equals $A \pm (B/L)$ where A and B are constants. The second mathematical model better approximates the genetic data obtained in this laboratory on chromosome aberrations in *Tradescantia*[6] and recessive lethality in *Drosophila melanogaster*.[9] Other more general mathematical formulations are being studied.

Paramagnetic Resonance Studies of Free Radicals

A. J. Fairbanks, Jr.

Introduction. – Many of the effects of radiation on living cells are thought to be caused by radiation-induced free radicals. The development of paramagnetic resonance methods has made possible the direct detection of free radicals. Until this development very little direct information was available on the role of free radicals in the cell. The present investigations are a continuation of experiments which were set up to get a better understanding of the characteristics of the free radicals involved, and to elucidate their role in affecting survival of bacteria irradiated with and without treatment with AET·Cl.

Results. – Paramagnetic resonance curves were obtained from *Escherichia coli* B/r cells aerated during growth both in nutrient broth and in basal medium. Cells were grown in a 10-liter volume, centrifuged, and resuspended in approximately 40 ml of phosphate buffer, pH 6.8, to a final concentration of the order of 10^{12} cells per milliliter. An aliquot of 2.5 ml was used for each sample. Treatments consisted in incubation with 0.05 *M* AET·Cl, or irradiation with 170,000 r of gamma rays, or AET·Cl followed by irradiation. All treatments were performed in closed vessels. After treatment, survival was determined and the sample was lyophilized. The paramagnetic resonance spectrometer used was not sufficiently sensitive to detect free radicals in more than about 0.02 ml of water.

Samples treated only with AET·Cl show a lower free radical content than untreated control samples. Samples irradiated in the presence of AET·Cl show a lower free radical content than samples irradiated only. Irradiation generally increased the amount of free radicals compared with a sample treated in the same manner except for the irradiation. The presence of AET·Cl appears to alter the shape of the resonance curve so that an asymmetrical pattern is obtained. Comparison of irradiated and AET·Cl-treated, irradiated cells reveals a probable reciprocal correlation between per cent survival and free radical content.

All the halide salts of AET were prepared and x-irradiated. At room temperature and in the presence of air the resulting paramagnetic resonance patterns show increasing free radical content and increasingly resolvable fine structure in the order iodide, bromide, chloride, and fluoride. This is also the order of increasing electronegativity. The crystalline structures for the halide salts do not appear to be isomorphous.

Discussion. – Alterations in the paramagnetic resonance patterns obtained from *Escherichia coli* appear to reflect underlying changes in free radical components upon treatment with AET·Cl or gamma rays or both. The basic free radical changes are as yet obscure. The AET·Cl may act as a quenching or trapping agent for cellular free radicals

[8] J. W. Boag, *Radiation Research* 1, 323–341 (1954).

[9] C. W. Edington and M. L. Randolph, *Biol. Semiann. Prog. Rep. Feb. 15, 1957*, ORNL-2267, p 22–23.

whether induced by radiation or occurring naturally.[10] It appears also that AET·Cl perturbs the magnetic environment of the unpaired electron of some radical or radicals in the cell, so that resonance does not occur at exactly the same value of applied magnetic field strength for all the free radicals present.

The interesting inverse correlation between per cent survival and free radical content may indicate a cause and effect relationship. Further experiments are needed to clarify this point.

At present explanation for the resonance patterns obtained from the halide salts of AET is unknown.

An important phase of the paramagnetic resonance program is the improvement of the spectrometer with respect to sensitivity and general operating efficiency. This work is still in progress.

Behavior of Several Breakage Fusion Bridge Cycles in One Cell Lineage

A. C. Faberge'

The examination of maize endosperm has been completed. It seems to be the invariable rule that when two chromatid-type breakage-fusion-bridge cycles are generated in the same nucleus, they continue in subsequent cell divisions as separate, independent chromatid-type cycles, and never fuse to yield a chromosome-type cycle. This is probably an expression of the fact that free chromosome ends have to be in close physical proximity in a cell for an exchange to occur, which has been indirectly deduced from various radiation experiments. The present work is essentially of an all-or-none qualitative nature, and is evidenced by a good photographic record.

Radiation-induced Edema

E. B. Darden, Jr. C. W. Sheppard[11]

Introduction. – In sufficiently irradiated mammalian tissue an early sign of injury is the development of acute edema. In the rat forelimb a few hours after a localized exposure of 50 to 75 kr of x radiation, striking morphological changes are seen in the dermis: it becomes greatly swollen, wet, and gelatinous in consistency,[12] and the condition progressively spreads beyond the irradiated region. The underlying muscle tissue also becomes edematous. Previously it was reported[13] that postirradiation injection of large doses of diphenhydramine (Benadryl) reduced significantly the edema measured[12] in the forelimb muscle mass, possibly by interfering with radiation-induced liberation of histamine. Additional studies have provided a clearer understanding of the origin of the edema.

Results. – Within 6 hr following an exposure of 75 kr, plasma space in the irradiated left limb, as estimated by tail-vein injection of radioiodinated serum albumin (RISA) and subsequent determination of the activity per unit dry weight of forelimb tissue, had increased about 50% and by 22 hr had averaged a fourfold increase over that of the right or control limb. Concurrent wet weight gain is shown plotted in semilog coordinates in Fig. 23.

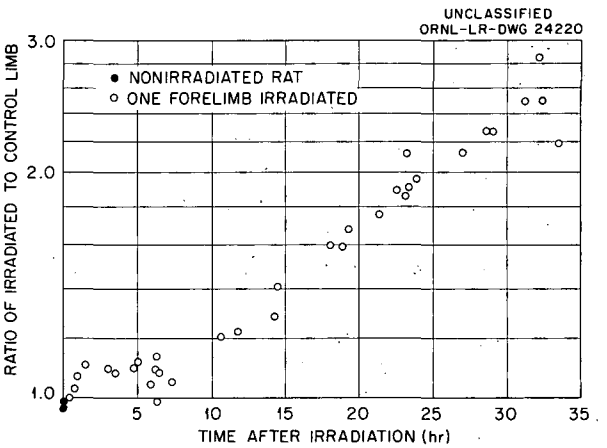

Fig. 23. Water Gain per Unit Dry Weight of Rat Forelimb After Exposure to 75 kr of X Radiation.

Weight changes in the controls were negligible. For nonpenetrating radiation effect the depilated forelimbs were exposed singly to a cross fire from two G-E H-4 ultraviolet lamps with envelopes removed (with precautions to prevent hyperthermia). Qualitatively similar but less pronounced changes were produced in skin and muscle (Table 26).

[10] D. G. Doherty *et al.*, *Biol. Semiann. Prog. Rep. Aug. 15, 1956*, ORNL-2155, p 48–50.

[11] Formerly of the Cell Physiology Group, now at the University of Tennessee Medical School, Memphis, Tennessee.

[12] W. S. Wilde and C. W. Sheppard, *Proc. Soc. Exptl. Biol. Med.* 88, 249–253 (1955).

[13] E. B. Darden, Jr., and C. W. Sheppard, *Biol. Semiann. Prog. Rep. Feb. 15, 1957*, ORNL-2267, p 61.

Table 26. Water Content in Muscle Tissue of Ultraviolet-irradiated Rat Forelimb

Hours of Exposure	Postirradiation Interval (hr)	Ratio of Irradiated to Control (Water content per gram dry weight)
1	69	1.003
2	22	1.064
		1.030
		1.038
		1.071
		1.052
		1.036
2.25	24	1.080

Histological examination[14] of the x-irradiated skin showed marked edema of the dermis, with a few scattered multinucleate polymorphs. Dermal elements as stained with hematoxylin and eosin generally appeared normal, although a larger amount of periodic acid–Schiff positive granular material increasing with time after irradiation was noted as compared to the control tissue. The ground substance exhibited variable metachromasia with toluidine blue but no consistent difference attributable to irradiation. Irradiated muscle tissue showed infiltration of fluid and expansion of interfascicular spaces but no fiber changes, except a questionably larger number of fibers shrunken in cross section as compared to the control. Lymph nodes on both sides generally showed depletion of lymphoid elements and tended to be glutted with fluid on the irradiated side. At about 40 hr after irradiation both skin and muscle tissue showed severe damage such as multiple petechiae, Zenker degeneration of many muscle fibers, and heavy leukocyte invasion. The entire upper left quadrant of body skin was stained by intravenous injection of Evans blue.

Discussion. – The results suggest that radiation injury in the heavily exposed forelimb is reflected in early development of an acute inflammatory condition characterized by progressive spreading dermal changes and by the accelerated loss of plasma from the irradiated blood vessels, with infiltration of the edema fluid from the dermis into the muscle tissue. Later the latter exhibits severe injury and necrosis presumably as a result of the vascular failure. The inhibitory effect of Benadryl is characteristic of the antiphlogistic properties exhibited by a number of the antihistamines whether they act by suppression of histamine liberation or not.[15]

[14]With the aid of A. C. Upton and W. D. Gude of the Pathology and Physiology Section.

[15]B. N. Halpern, *Rev. Can. Biol.* 12, 316–324 (1953).

APPENDIX

The standard abbreviations and corresponding explanations for terms used in this report are given alphabetically in the following list:

ABT – *S*,4-aminobutylisothiourea
ADP – adenosine diphosphate
AET – *S*,2-aminoethylisothiuronium bromide hydrobromide
AET·Cl – *S*,2-aminoethylisothiuronium chloride hydrochloride
AMP – adenosine monophosphate
APT – *S*,3-aminopropylisothiuronium bromide hydrobromide
ATP – adenosine triphosphate
BSA – bovine serum albumin
CoA – coenzyme A
CO_2-P – carbonyl phosphate
CTP – cytosine triphosphate
D-MBG-2-S^{35} – S^{35}-labeled D-mercaptobutyl-2-guanidine hydrobromide
DNA – deoxyribonucleic acid
DNAse – deoxyribonuclease
DNP – deoxyribonucleoprotein
DPN – diphosphopyridine nucleotide
DPNH – reduced diphosphopyridine nucleotide
GTP – guanosine triphosphate
ITP – inosine triphosphate
L-MBG-2-S^{35} – S^{35}-labeled L-mercaptobutyl-2-guanidine hydrobromide
LET – linear energy transfer
MEA – 2-mercaptoethylamine
MEG – 2-mercaptoethylguanidine
P^{32} – P^{32}-labeled inorganic phosphate
PP^{32} – P^{32}-labeled pyrophosphate
PVP – polyvinylpyrrolidone
RBC – red blood cells
RBE – relative biological effectiveness
RNA – ribonucleic acid
RNAse – ribonuclease
UTP – uridine triphosphate
WBC – white blood cells