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BIOLOGY DIVISION

SEMIANNUAL PROGRESS REPORT

For Period Ending August 15, 1958

Alexander Hollaender, Director

Stanley F. Carson, Assistant Director

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BIOLOGY DIVISION SEMIANNUAL PROGRESS REPORT

PUBLICATIONS AND LECTURES

Publications. – During the period covered by this report, 52 papers, 60 abstracts, and a Semiannual report have been published. Another symposium – "Genetic Approaches to Somatic Cell Variation" – was prepared and sent to the publisher. Also in press are 76 papers and a few abstracts.

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Baker, W. K.	Induced Loss of a Ring and a Telomeric Chromosome in *Drosophila melanogaster*	*Genetics* **42**, 735–748 (1957)
Benedict, W. H.	Progressive Lamellar Radiation Cataracts in the Newborn Mouse	*Am. J. Ophthalmol.* **45**, 822–826 (1958)
von Borstel, R. C.	"Biochemical Cytology" by Jean Brachet, Academic Press, Inc., New York, 1957, 516 pages	*Am. Scientist* **46**, 160A–162A (1958) (Book Review)
von Borstel, R. C., and M. L. P. Rekemeyer	Division of a Nucleus Lacking a Nucleolus	*Nature* **181**, 1597–1598 (1958)
von Borstel, R. C., and R. W. Rogers	Alpha-Particle Bombardment of the *Habrobracon* Egg. II. Response of the Cytoplasm	*Radiation Research* **8**, 248–253 (1958)
Bruce, A. K.	Response of Potassium Retentivity and Survival of Yeast to Far-Ultraviolet, Near-Ultraviolet and Visible, and X-Radiation	*J. Gen. Physiol.* **41**, 693–702 (1958)
Congdon, C. C.	Method of Acceleration of Regeneration of Hematopoietic Tissues	In *Proceedings of the Sixth International Congress of the International Society of Hematology* (ed. by W. Baker *et al.*), Grune & Stratton, New York, 1958, p 398–400
Congdon, C. C., T. Makinodan, N. Gengozian, I. C. Shekarchi, and I. S. Urso	Lymphatic Tissue Changes in Lethally Irradiated Mice Given Spleen Cells Intravenously	*J. Natl. Cancer Inst.* **21**, 193–211 (1958)
Davis, J. W., and G. D. Novelli	The Activation of Amino Acids in Extracts of Pea Seedlings	*Arch. Biochem. Biophys.* **75**, 299–308 (1958)
Ferrebee, J. W., D. Billen, I. S. Urso, W. C. Lu, E. D. Thomas, and C. C. Congdon	Preservation of Radiation Recovery Factor in Frozen Marrow	*Blood* **12**, 1096–1100 (1957)
Gailey, F. B., and N. E. Tolbert	Effect of Ionizing Radiation on the Development of Photosynthesis in Etiolated Wheat Leaves	*Arch. Biochem. Biophys.* **76**, 188–195 (1958)
Gaulden, M. E., and R. P. Perry	Influence of the Nucleolus on Mitosis as Revealed by Ultraviolet Microbeam Irradiation	*Proc. Natl. Acad. Sci. U.S.* **44**, 553–559 (1958)
Gengozian, N., and T. Makinodan	Relation of Primary Antigen Injection to Time of Irradiation on Antibody Production in Mice	*J. Immunol.* **80**, 189–197 (1958)
Gengozian, N., T. Makinodan, C. C. Congdon, and R. D. Owen	The Immune Status of Long-Term Survivors of Lethally X-Irradiated Mice Protected with Isologous, Homologous, or Heterologous Bone Marrow	*Proc. Natl. Acad. Sci. U.S.* **44**, 560–565 (1958)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Gude, W. D.	An Inexpensive Paraffin Knife	*Am. J. Clin. Pathol.* **29**, 601–602 (1958); also, *Tech. Bull.* **28**, 109–110 (1958)
Hollaender, A	Protection and Recovery from Radiation Damage	In 4^a *Rassegna Internazionale Elettronica E Nucleare, Atti del Congresso Scientifico*, vol I, Sezione Nucleare. Palazzo dei Congressi, Rome, 1957, p 413–427
Hollaender, A., and S. F. Carson	Biology Division Semiannual Progress Report for Period Ending February 15, 1958	ORNL-2481
Horn, E. C.	Ascites Tumor Development. III. Analysis of an Antiserum Prepared Against the Soluble Phase of the Cell	*Growth* **21**, 221–237 (1957)
Jagger, J.	Photoreactivation	*Bacteriol. Revs.* **22**, 99–142 (1958)
Khym, J. X., D. G. Doherty, and R. Shapira	Ion-Exchange Studies of Transguanylation Reactions. II. Rearrangement of 3-Aminopropylisothiourea and N-Substituted Aminoethyl- and Aminopropylisothioureas to Mercaptoalkylguanidines and 2-Aminothiazolines or Penthiazolines	*J. Am. Chem. Soc.* **80**, 3342–3349 (1958)
Kimball, R. F.	Experiments with *Stentor coeruleus* on the Nature of the Radiation-Induced Delay in Fission in the Ciliates	*J. Protozool.* **5**, 151–155 (1958)
Kirby-Smith, J. S., and G. W. Dolphin	Chromosome Breakage at High Dose Rates	*Nature* **182**, 270–271 (1958)
Lindsley, D. L.	Spermatogonial Exchange Involving the X but Not the Y Chromosome in *Drosophila melanogaster*	*Z. indukt. Abstammungs- u. Vererbungslehre* **89**, 103–122 (1958)
Lindsley, D. L., and C. W. Edington	Failure To Close the $Y^SX{\cdot}Y^L$ Chromosome	*Drosophila Inform. Serv.* **31**, 132–133 (1957)
———	A Screening Method for Recovering Sex-Linked Recessive Lethals Whose Expression Is Influenced by the Y Chromosome	*Drosophila Inform. Serv.* **31**, 131–132 (1957)
Makinodan, T., N. Gengozian, and I. C. Shekarchi	Relative Effects of Splenic and Bone Marrow Cells on Lethally Irradiated Mice	*J. Natl. Cancer Inst.* **20**, 591–600 (1958)
Odell, T. T., Jr., and B. C. Caldwell	Transplantation of Homologous Erythropoietic Elements in Rats After Sublethal Doses of X Radiation	*J. Natl. Cancer Inst.* **20**, 851–858 (1958)
Odell, T. T., Jr., and L. H. Smith	Localization of Injected Bone Marrow Cells by Autoradiography	*Acta Haematol.* **19**, 114–120 (1958)
Pandey, K. K.	On the Time of the S Allele Action (Communication to Editor)	*Nature* **181**, 1220–1221 (1958)
Patetta-Queirolo, M. A., M. L. Randolph, and J. A. Sproul, Jr.	Intestinal Weight Loss in Mice Irradiated by Roentgen Rays, Gamma Rays, and Fast Neutrons	*Acta Radiol.* **49**, 394–400 (1958)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Pittenger, T. H.	The Mating Type Alleles and Heterokaryon Formation in *Neurospora crassa*	*Microbial Genet. Bull.* 15, 21–22 (1957)
Repplinger, E., I. R. Schwartz, C. C. Congdon, and L. M. Tocantins	Species Characteristics of Platelets of X-Irradiated Mice Treated with Rat Bone Marrow	*J. Appl. Physiol.* 13, 105–108 (1958)
Sandler, L.	The Meiotic Behavior of Reversed Compound Ring X Chromosomes in *Drosophila melanogaster*	*Genetics* 42, 764–782 (1957)
Sandler, L., and M. Kastenbaum	A Note on the Frequency Distribution of Tetrads by Rank in *Drosophila melanogaster*	*Genetics* 43, 215–222 (1958)
Schwartz, D.	Deoxyribonucleic Acid Side-Chain Model of the Chromosome (Communication to Editor)	*Nature* 181, 1149–1150 (1958)
———	On the Coding Problem in Proteins	*Nature* 181, 769 (1958)
———	On the Stabilization of a Ring Chromosome in Maize	*Genetics* 43, 86–91 (1958)
———	Report for The News Letter	*Maize Genet. Coop. News Letter* 32, 113–115 (1958)
de Serres, F. J., Jr.	Studies with Purple Adenine Mutants in *Neurospora crassa.* III. Reversion of X-Ray-Induced Mutants	*Genetics* 43, 187–206 (1958)
Slater, M., G. B. Bunyard, and M. L. Randolph	Combination Ion Chamber–Proportional Counter Dosimeter for Measuring Gamma-Ray Contamination of Neutron Fields	*Rev. Sci. Instr.* 29, 601–605 (1958)
Slater, M., and G. S. Melville, Jr.	A Method for Improving the Precision of Inverse Square Measurements	*Brit. J. Radiol.* 31, 392–394 (1958)
Smith, L. H., and J. C. Tohá	Survival of Mouse-Grown Rat Erythrocytes	*Proc. Soc. Exptl. Biol. Med.* 98, 125–128 (1958)
Upton, A. C.	"Ciba Foundation Colloquia on Ageing, Vol 3, Methodology of the Study of Ageing," G. E. W. Wolstenholme and C. M. O'Connor, Editors, Little, Brown & Company, Boston, 1957, 202 pages	*J. Gerontol.* 13, 183 (1958) (Book Review)
———	The Radiobiology of the Cancer Cell	*Federation Proc.* 17, 698–713 (1958)
Upton, A. C., R. F. Buffett, J. Furth, and D. G. Doherty	Radiation-Induced "Dental Death" in Mice	*Radiation Research* 8, 475–479 (1958)
Upton, A. C., and J. Furth	Factors Influencing Induction of Myeloid Leukemia in RF Mice by Irradiation	In *Etiology and Treatment of Leukemia* (ed. by W. J. Burdette), The C. V. Mosby Co., St. Louis, 1958, p 59–70
Urso, P., C. C. Congdon, D. G. Doherty, and R. Shapira	Effect of Chemical Protection and Bone Marrow Treatment on Radiation Injury in Mice	*Blood* 13, 665–676 (1958)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Urso, P., T. W. McKinley, Jr., and C. C. Congdon	Survival of Irradiated Mice After Treatment with Repopulated Bone Marrow	*Transplant. Bull.* **5**, 60–63 (1958)
Welshons, W. J.	A Preliminary Investigation of Pseudoallelism at the *Notch* Locus of *Drosophila melanogaster*	*Proc. Natl. Acad. Sci. U.S.* **44**, 254–258 (1958)
Wolff, S.	"Advances in Radiobiology," G. C. deHevesy, A. G. Forssberg, and J. D. Abbatt, Editors, Charles C Thomas, Springfield, Ill., 1957	*Nucleonics* **16**(7), 112–113 (1958) (Book Review)
Wolff, S., K. C. Atwood, M. L. Randolph, and H. E. Luippold	Factors Limiting the Number of Radiation-Induced Chromosome Exchanges. 1. Distance: Evidence from Noninteraction of X-Ray- and Neutron-Induced Breaks	*J. Biophys. Biochem. Cytol.* **4**, 365–372 (1958)
Zelle, M. R., J. E. Ogg, and A. Hollaender	Photoreactivation of Induced Mutation and Inactivation of *Escherichia coli* Exposed to Various Wave Lengths of Monochromatic Ultraviolet Radiation	*J. Bacteriol.* **75**, 190–198 (1958)
Zill, L. P., and N. E. Tolbert	The Effect of Ionizing and Ultraviolet Radiations on Photosynthesis	*Arch. Biochem. Biophys.* **76**, 196–203 (1958)

Scientific Society Lectures and Traveling Seminars. – The number of lectures given by Biology Division members during this half-year represents an increase of 157% over the number given in the preceding half-year. Considerably more than half the lectures were presented before professional societies; several represent long symposium offerings.

AUTHOR(S)	TITLE	PLACE PRESENTED
Adler, H. I.	An Aftereffect of Ionizing Radiation on a Hemin-Dependent Bacterium [*Bacteriol. Proc.*, p 57 (1958)]	Soc. Am. Bacteriol., Chicago
Adler, H. I., and G. E. Stapleton	The Role of Radiation-Produced Hydrogen Peroxide in Inactivation of a Catalase-Negative Bacterium [*Radiation Research* **9**, 84 (1958)]	1st Intern. Congr. Radiation Research, Burlington
Anderson, N. G., and J. F. Albright	Microsomal Peptides and Amino Acids [*Federation Proc.* **17**, 4 (1958)]	Fed. Am. Soc. Exptl. Biol. (Am. Physiol. Soc.), Philadelphia
Arnold, W. A.	Photosynthesis	Am. Chem. Soc. (East Tenn. Sect.), Oak Ridge
Astrachan, L.	RNA Turnover and DNA Synthesis in Bacteriophage-Infected Bacteria [*Federation Proc.* **17**, 183 (1958)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Philadelphia
Atwood, K. C.	The Cellular Lesion in Radiation Injury	Symposium on Genetics in Medical Research, University of Wisconsin, Madison
Atwood, K. C., and S. L. Scheinberg	Examination of Human Erythrocyte Populations for Somatic Mutation at the ABO Locus [*Science* **127**, 1058 (1958)]	Natl. Acad. Sci., Washington, D.C.
Atwood, K. C., and S. L. Scheinberg	Somatic Variation in Human Erythrocyte Antigens	Eleventh Annual Biology Research Conference: Genetic Approaches to Somatic Cell Variation, Gatlinburg, Tenn.

AUTHOR(S)	TITLE	PLACE PRESENTED
Auerbach, C.	Action of Chemical Mutagens	Duke University, Durham
———	The Present State of Research on Chemical Mutagens	Division of Plant Science, University of British Columbia, Vancouver
———	As above	Dept. of Biology, University of Alberta, Edmonton
———	As above	Dept. of Biology, University of Saskatchewan, Saskatoon
———	As above	Dept. of Biology, University of Manitoba, Winnipeg
———	As above	Dept. of Biology, University of Ottawa, Ottawa
———	As above	Dept. of Botany, University of Montreal, Montreal
———	As above	Dept. of Zoology, University of Nova Scotia, Halifax
———	The Problem of Specific Mutations	Biology Laboratories, Harvard University, Cambridge
———	Problems in Chemical Mutagenesis	Dept. of Zoology, University of Indiana, Bloomington
———	As above	Dept. of Botany, University of Wisconsin, Madison
———	As above	Dept. of Zoology, University of Colorado, Boulder
———	As above	Biology Division, California Institute of Technology, Pasadena
———	Work on mutagensis carried out by Dr. Auerbach, her students, and collaborators in Edinburgh during 1957	Dept. of Genetics, University of Indiana, Bloomington
von Borstel, R. C.	Ultraviolet Irradiation Analysis of *Habrobracon* Embryogeny	Alligator Point Embryology Conference, Tallahassee
von Borstel, R. C., and M. L. P. Rekemeyer	Ultraviolet Irradiation of the *Habrobracon* Embryo Nucleus and Cytoplasm During Early Development [*Radiation Research* 9, 199 (1958)]	1st Intern. Congr. Radiation Research, Burlington
Brumfield, R. T.	A Method for Determining the Effects of Varied Environmental Conditions on Cell Growth in *Phleum* Roots [*ASB Bull.* 5, 5 (1958)]	Assoc. Southeastern Biologists, Tallahassee
Carson, S. F., and E. F. Phares	A Bacterial "Carbon Dioxide Enzyme" [*Bacteriol. Proc.*, p 121–122 (1958)]	Soc. Am. Bacteriologists, Chicago
Cohn, W. E.	A General Review of Nucleic Acid Chemistry with Special Reference to the Contributions of Chromatography	Division of Basic Health Sciences, Emory University, Georgia

AUTHOR(S)	TITLE	PLACE PRESENTED
———	Minor Constituents of Ribonucleic Acids [*Federation Proc.* 17, 203 (1958)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Philadelphia
———	As above	Radiotherapeutic Centre, Addenbrooke's Hospital (jointly sponsored by Departments of Radiotherapeutics and Chemistry of Cambridge University), Cambridge
———	As above	Carlsberg Laboratory, Copenhagen
Congdon, C. C.	Early Changes in the Spleens of Lethally Irradiated Mice Given Foreign Bone Marrow [*Radiation Research* 9, 102 (1958)]	1st Intern. Congr. Radiation Research, Burlington
———	Lymphatic Tissue Changes in Lethally Irradiated Mice Given Spleen Cells Intravenously [*Federation Proc.* 17, 433 (1958)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Exptl. Pathol.), Philadelphia
Cormier, M. J.	Glycine Activation by Extracts of *Achromobacter fischeri* [*Federation Proc.* 17, 206 (1958)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Philadelphia
Cosgrove, G. E., E. E. Schwartz, A. C. Upton, and C. C. Congdon	Changes in Irradiated F_1 Hybrid Mice Injected with Parental Spleen Cells [*Federation Proc.* 17, 434 (1958)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Exptl. Pathol.), Philadelphia
Cosgrove, G. E., A. C. Upton, C. C. Congdon, D. G. Doherty, A. W. Kimball, and A. Hollaender	Protection by AET and Bone Marrow Against Late Effects of X Radiation in Mice [*Radiation Research* 9, 103 (1958)]	1st Intern. Congr. Radiation Research, Burlington
Countryman, J. L., and E. Volkin	Metabolic Heterogeneity of Bacterial Nucleic Acids [*Federation Proc.* 17, 206 (1958)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Philadelphia
Darden, E. B., Jr.	Electrophysiological Changes in Irradiated and Excised Skeletal Muscle [*Radiation Research* 9, 105 (1958)]	1st Intern. Congr. Radiation Research, Burlington
Doherty, D. G.	Chemical Protection Against Ionizing Radiation	Lilly Research Laboratories, Indianapolis
Doherty, D. G., and R. Shapira	Chemical Structure and Protection Against Ionizing Radiation [*Radiation Research* 9, 107 (1958)]	1st Intern. Congr. Radiation Research, Burlington
———	Hydrolysis of Carboxyalkylisothioureas by Transamidinase and Arginase [*Federation Proc.* 17, 213 (1958)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Philadelphia
Dolin, M. I.	The Relation Between Cytochrome c Reductase and DPNH Oxidase in *Clostridium perfringens* [*Bacteriol. Proc.*, p 105 (1958)]	Soc. Am. Bacteriol., Chicago
Friedberg, W.	Metabolism of Radioiodine-Labeled Tobacco Mosaic Virus in Mice [*Federation Proc.* 17, 49 (1958)]	Fed. Am. Soc. Exptl. Biol. (Am. Physiol. Soc.), Philadelphia

AUTHOR(S)	TITLE	PLACE PRESENTED
Furth, J., A. C. Upton, and A. W. Kimball	Late Pathologic Effects of Atomic Detonation and Their Pathogenesis	1st Intern. Congr. Radiation Research, Burlington
Gaulden, M. E.	Influence of Nucleolus on Mitosis As Revealed by Ultraviolet Microbeam Irradiation	Dept. of Zoology, University of Chicago, Chicago
Gaulden, M. E., and R. P. Perry	Relation of the Nucleolus to Mitotic Activity As Revealed by Ultraviolet Microbeam Irradiation [*ASB Bull.* 5, 7–8 (1958)]	Assoc. Southeastern Biologists, Tallahassee
Gengozian, N., and T. Makinodan	Analysis of Serum Obtained from Lethally Irradiated Mice Treated with Rat Bone Marrow [*Radiation Research* 9, 118 (1958)]	1st Intern. Congr. Radiation Research, Burlington
———	Development of the Recognition Mechanism in Antibody Production [*Federation Proc.* 17, 512 (1958)]	Fed. Am. Soc. Exptl. Biol. (Am. Assoc. Immunol.), Philadelphia
Goodman, J. W.	Bone Marrow Cell Separation [*Federation Proc.* 17, 438 (1958)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Exptl. Pathol.), Philadelphia
Hollaender, A.	Effects of Radiation on Biological Materials	Istituto di Genetica, Università di Milano, Milan
———	Effects of Radiation on Living Organisms	Army Conference on Basic and Applied Research and Component Development, Washington
———	Opening Remarks, International Congress of Radiation Research	1st Intern. Congr. Radiation Research, Burlington
———	Somatic Effects of Low-Intensity Radiation at Different Levels of Biological Organization	Swiss Acad. Med. Sci., Symposium on Noxious Effects of Low Level Radiations: Physical Elements and Biological Aspects, Lausanne
Kimball, R. F.	Genetic Effects of Radiation	National Science Foundation Institute for High School Teachers, Tennessee Polytechnic Institute, Cookeville
———	Postirradiation Modification of Mutation After Various Radiations [*Radiation Research* 9, 138 (1958)]	1st Intern. Congr. Radiation Research, Burlington
Kirby-Smith, J. S.	The Application of Magnetic Resonance Techniques to Biological and Medical Research	Medical College of St. Bartholomew's Hospital, London
———	Basic Physical and Clinical Mechanisms in Radiobiology	Genetics Institute, University of Pavia, Pavia
———	Chromosome Breakage in *Tradescantia* at High Dose Rates	Medical College of St. Bartholomew's Hospital, London
———	As above	Radiation Research Visiting Club or First Meeting of the New (Society) Association for Radiation Research, Hammersmith Hospital, London

AUTHOR(S)	TITLE	PLACE PRESENTED
Kirby-Smith, J. S., and G. W. Dolphin	Chromosome Breakage at High Dose Rates [*Radiation Research* 9, 139 (1958)]	1st Intern. Congr. Radiation Research, Burlington
———	As above [*Brit. J. Radiol.* 31, 339 (1958)]	Radiobiological Meeting of the British Institute of Radiology, London
Kølmark, G.	Patterns of Interaction Between X Rays and Chemicals As Studied by Reverse-Mutation Rates in *Neurospora* [*Radiation Research* 9, 140 (1958)]	1st Intern. Congr. Radiation Research, Burlington
Kroeger, H.	Investigations on Paired Structures and Symmetric Deformities of Transplanted Wing-Imaginal Discs in *Ephestia*	Alligator Point Embryology Conference, Tallahassee
Lindsley, D. L.	Lethals in *Drosophila melanogaster* Whose Expression Is Determined by the Y Chromosome	Institute for Cancer Research, Philadelphia
Lindsley, D. L., C. W. Edington, and E. S. Von Halle	The Relation of the Genetic Constitution of *Drosophila* Spermatozoa to Their Sensitivity to X Radiation [*Radiation Research* 9, 145 (1958)]	1st Intern. Congr. Radiation Research, Burlington
Lynn, W. G., and J. N. Dent	Phenylthiourea and Iodine Binding in the Tadpole [*ASB Bull.* 5, 13 (1958)]	Assoc. Southeastern Biologists, Tallahassee
Makinodan, T.	Immunologic Aspect of the Bone Marrow Transplant	National Cancer Institute, Washington
———	Review of Graft-Host Relationship in Irradiated Animals with Bone Marrow Transplants	ORINS Medical Division Clinical Conference, Oak Ridge
Makinodan, T., and B. H. Friedberg	Temporal Relation Between *Secondary* Antigen Injection and X Irradiation on Antibody Response [*Radiation Research* 9, 148 (1958)]	1st Intern. Congr. Radiation Research, Burlington
Makinodan, T., and N. Gengozian	Primary Antibody Response in Mice During the Maximum Depression Period that Follows Varying Doses of X Radiation (read by title)	Fed. Am. Soc. Exptl. Biol. (Am. Assoc. Immunol.), Philadelphia
Makinodan, T., N. Gengozian, and I. C. Shekarchi	Further Studies on Antigen-Host Relationship As Affected by X Irradiation	7th Intern. Congr. Microbiol., Stockholm
Novelli, G. D.	Biochemistry of Protein Synthesis	Dept. of Zoology and Entomology, University of Tennessee, Knoxville
Novelli, G. D., and A. N. Best	An Exchange Reaction Between Inorganic Pyrophosphate (PP) and Adenosine Triphosphate (ATP) in Rat Liver Microsomes [*Federation Proc.* 17, 283 (1958)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Philadelphia
Novelli, G. D., and T. Makinodan	Some Biochemical Properties of the Antigens Responsible for Bone Marrow Transplantation Immunity [*Radiation Research* 9, 161 (1958)]	1st Intern. Congr. Radiation Research, Burlington
Odell, T. T., Jr., and B. Anderson	Chemical Nature of Mucopolysaccharides Extracted from Blood Platelets [*Federation Proc.* 17, 119 (1958)]	Fed. Am. Soc. Exptl. Biol. (Am. Physiol. Soc.), Philadelphia

AUTHOR(S)	TITLE	PLACE PRESENTED
Odell, T. T., Jr., and B. C. Caldwell	Comparative Effects of Bone Marrow Transplantation in Irradiated and Myleran-Treated Rats [*Radiation Research* 9, 162 (1958)]	1st Intern. Congr. Radiation Research, Burlington
Owen, R. D.	Erythrocyte Antigens as Markers for Repopulation by Homologous Erythropoietic Tissues in Irradiated Mice [*Radiation Research* 9, 164 (1958)]	1st Intern. Congr. Radiation Research, Burlington
———	Immunology of Tissue Transplantation and Tolerance	Symposium on Immunity and Virus Infection, Vanderbilt University, Nashville
Popp, R. A., and L. H. Smith	Electrophoresis of Proteins of Irradiated Mouse Chimeras [*Federation Proc.* 17, 454 (1958)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Exptl. Pathol.), Philadelphia
———	Fate of Rat Bone Marrow in Irradiated Mice: Studies of Tissue Proteins [*Radiation Research* 9, 167 (1958)]	1st Intern. Congr. Radiation Research, Burlington
Randolph, M. L.	Detection, Dosimetry, and Biological Action of Fast Neutrons	Depts. of Biology and Physics, M. D. Anderson Hospital, Houston
———	As above	Biophysics Program, Tulane University, New Orleans
———	Survival of Mice Treated with Bone Marrow After Fast Neutron Irradiation	Dept. of Biology, Rice Institute, Houston
———	As above	Texas Medical Center, University of Texas Dental Branch, Houston
———	As above	Dept. of Zoology, Louisiana State University, Baton Rouge
Randolph, M. L., and D. L. Parrish	Measurements of Electron Spin Resonances Induced in Amino Acids by Radiation [*Radiation Research* 9, 170 (1958)]	1st Intern. Congr. Radiation Research, Burlington
Rogers, P., Jr.	Synthesis of Ornithine Transcarbamylase in Protoplasts of *Escherichia coli* [*Federation Proc.* 17, 298 (1958)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Philadelphia
Russell, L. B.	Radiation Effects on the Mammalian Mother and Embryo	Brookhaven National Laboratory, Upton, Long Island
Russell, L. B., and M. K. Freeman	The Influence of Dose-Rate on the Sterilizing Effect of Radiation in Female Mice [*Radiation Research* 9, 174–175 (1958)]	1st Intern. Congr. Radiation Research, Burlington
Russell, W. L.	Radiation-Induced Genetic Damage in Mammals	Conference on Genetics and Radiobiology (under auspices of Committee on Biological Effects of Atomic Radiations of the National Academy of Sciences), St. Louis

AUTHOR(S)	TITLE	PLACE PRESENTED
———	Radiology and Genetics	Statewide Forum on Nuclear Energy (sponsored by the Governor's Advisory Committee on Nuclear Energy for the Commonwealth of Kentucky), Lexington
Russell, W. L., and E. M. Kelly	Comparison Between Mutation Rates Induced by Chronic Gamma and Acute X-Irradiation in Mice [*Science* **127**, 1062 (1958)]	Natl. Acad. Sci., Washington
Russell, W. L., and L. B. Russell	The Genetic and Phenotypic Characteristics of Radiation-Induced Mutations in Mice	1st Intern. Congr. Radiation Research, Burlington
de Serres, F. J.	Recombination and Interference in the *ad-3* Region of *Neurospora crassa*	Cold Spring Harbor Symposia Quant. Biol., Cold Spring Harbor
Schwartz, E. E.	Bone Marrow Transplantation and Chemical Protection in the Radiotherapy of Mouse Leukemia [*Proc. Am. Assoc. Cancer Research* **2**, 343 (1958)]	Am. Assoc. Cancer Research, Philadelphia
Shaw, E. I.	The Existence of Two Types of X-Ray-Induced Mitotic Inhibition [*Radiation Research* **9**, 181 (1958)]	1st Intern. Congr. Radiation Research, Burlington
Shekarchi, I. C., and T. Makinodan	Persistence of Heterologous Bone Marrow in Mice as a Function of X-Ray Dose [*Radiation Research* **9**, 181 (1958)]	1st Intern. Congr. Radiation Research, Burlington
Sheppard, C. W., and E. B. Darden, Jr.	Effect of Dose and Postirradiation Interval for Threshold of Radiation Edema Locally Produced in the Rat Forelimb [*Federation Proc.* **17**, 148 (1958)]	Fed. Am. Soc. Exptl. Biol. (Am. Physiol. Soc.), Philadelphia
Smith, L. H.	Protection of Irradiated Bone Marrow Cells [*Radiation Research* **9**, 185 (1958)]	1st Intern. Congr. Radiation Research, Burlington
Smith, L. H., and J. C. Tohá	Survival of Mouse-Grown Rat Erythrocytes [*Federation Proc.* **17**, 150 (1958)]	Fed. Am. Soc. Exptl. Biol. (Am. Physiol. Soc.), Philadelphia
Sproul, J. A., Jr.	Estimates of Recovery Rate in Mice Exposed to Neutrons and Gamma Rays [*Radiation Research* **9**, 187 (1958)]	1st Intern. Congr. Radiation Research, Burlington
Stapleton, G. E.	1. Direct and Indirect Action of Ionizing Radiations on Living Cells	Institute of Biology and Radiobiology, University of Wyoming, Laramie
———	2. Physiological Effects of Radiations on Living Material	
———	3. Protection and Recovery from the Damaging Effects of Ionizing Radiations on Biological Material	
Stapleton, G. E., and N. A. Sicard	X-Ray Sensitivity of Synchronous Cultures of a Thymineless Mutant of *Escherichia coli* [*Bacteriol. Proc.*, p 34 (1958)]	Soc. Am. Bacteriologists, Chicago

AUTHOR(S)	TITLE	PLACE PRESENTED
Tolbert, N. E., and J. R. Loewenberg	Effect of Choline and Its Analogues on Phosphorus Metabolism in Plants [*Federation Proc.* 17, 323 (1958)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Philadelphia
Upton, A. C.	1. The Delayed Effects of Ionizing Radiation in Man and Animal 2. Mechanisms of Acute Radiation Death and Their Modifications by Experimental Means	Genetics Institute, University of Pavia, Pavia
———	1. Hazards of Ionizing Radiation of Practical Concern to Physicians 2. The Role of Hormonal Factors in the Pathogenesis of Leukemia	Tenth Annual Midwest Cancer Conference, Wichita
———	Recent Observations on the Delayed Effects of Ionizing Radiation in Mammals	Commissariat à l Énergie Atomique, Centre d'Études Nucléaires, Saclay, France
———	Shortening of Life Span by Exposure to Ionizing Radiation	Dept. of Zoology and Entomology, University of Tennessee, Knoxville
———	Studies on the Mechanism of Leukemogenesis by Ionizing Radiation	Ciba Foundation Symposium on "Carcinogenesis: Mechanism of Action," London
Upton, A. C., J. A. Sproul, Jr., and M. L. Randolph	Survival of Mice Under Chronic Exposure to Neutrons and Gamma Rays [*Radiation Research* 9, 197 (1958)]	1st Intern. Congr. Radiation Research, Burlington
Upton, A. C., and F. F. Wolff	Effects of Filtrates of Leukemic Tissue on Induction of Lymphomas by X Rays [*Proc. Am. Assoc. Cancer Research* 2, 353 (1958)]	Am. Assoc. Cancer Research, Philadelphia
———	Inhibition by Splenectomy of Development of Radiation-Induced Myeloid Leukemia [*Federation Proc.* 17, 462 (1958)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Exptl. Pathol.), Philadelphia
Upton, A. C., F. F. Wolff, and G. E. Cosgrove	Strain Differences in the Susceptibility of Mice to Radiation-Induced Myeloid Leukemia and Lymphomas	7th Intern. Cancer Congr., London
Urso, I. S.	Long-Term Survival of Lethally Irradiated Mice Treated with Hematopoietic Tissues from Fetal and Newborn Homologous and Heterologous Donors [*Radiation Research* 9, 197 (1958)]	1st Intern. Congr. Radiation Research, Burlington
Volkin, E.	RNA Metabolism in Bacteriophage Infection	Dept. of Biochemistry, University of Michigan, Detroit
Volkin, E., L. Astrachan, and J. L. Countryman	RNA Metabolism in Normal and T2-Infected *Escherichia coli* B	7th Intern. Congr. Microbiol. Stockholm
Welshons, W. J.	An Analysis of Sex-Linked Dominant and Recessive Mutations in *Drosophila melanogaster*	Dept. of Biology, Spring Hill College, Mobile
———	As above	Dept. of Zoology, University of Texas, Austin

AUTHOR(S)	TITLE	PLACE PRESENTED
_____	As above	Depts. of Zoology, Physiology, and Entomology, Louisiana State University, Baton Rouge
_____	The Analysis of a Pseudoallelic Recessive Lethal System at the *Notch* Locus of *Drosophila melanogaster*	Cold Spring Harbor Symposia Quant. Biol., Cold Spring Harbor
Wolff, S.	Biochemical Aspects of Chromosome Breakage and Rejoining	Dept. of Plant Breeding, Cornell University, Ithaca
_____	Interpretation of Induced Chromosome Breakage and Rejoining	1st Intern. Congr. Radiation Research, Burlington
_____	The Metabolic Aspects of the Mutation Process	Assoc. Southeastern Biologists, Tallahassee

Visiting Lecturers. – The following scientists from research establishments in the United States and abroad have given lectures on the Biology Seminar program during the period of this report.

LECTURER	AFFILIATION	TOPIC
Jack Fox	Sloan-Kettering Institute for Cancer Research, New York	Synthesis of Nucleotides
M. A. Bender	The Johns Hopkins University, Baltimore	X-Ray-Induced Somatic Mutation in Diploid Human Tissue Cultures
A. C. Stevenson	The Queen's University of Belfast, Northern Ireland	Problems of Identifying Effects of Recessive Mutations in Man
P. Siekevitz	The Rockefeller Institute for Medical Research, New York	Protein Synthesis in the Pancreas: A Cytochemical Study
H. Rubin	Division of Biology, California Institute of Technology, Pasadena	Radiation Studies of Cell-Virus Interaction in the Rous Sarcoma
G. Bonnier	Genetiska Institutet, Stockholm Hogskola, Stockholm	1. Selection Pressure in Irradiated Populations of *Drosophila* 2. Genetic Studies at the Stockholm Institute of Genetics
H. Moser	Carnegie Institution of Washington, Cold Spring Harbor, Long Island	Genetics and Radiation Studies with Human Cells
K. Van Holde	Department of Chemistry, University of Illinois, Urbana	Equilibrium Ultracentrifugation
G. Hoecker	Catedra de Biologia, Instituto de Biologia "Juan Noe," Santiago	Studies on Histocompatibility
Claudio Barigozzi	Istituto di Genetica, Università di Milano, Milan	Comparative Cytology of *Drosophila* and Vertebrate Tumors
Elio Borghese	Istituto di Anatomia Umana, Normale e di Istologia, Università di Pavia, Pavia	The Embryonic Development of Lethal WW Mice
D. M. Brown	Chemical Laboratory, Cambridge, England	Some Aspects of the Chemistry of Sugar Phosphate Compounds

LECTURER	AFFILIATION	TOPIC
Timothy Merz	Biology Department, The Johns Hopkins University, Baltimore	The Influence of KCN on X-Ray Breakage of the Chromosomes of *Vicia faba*
George Hodgson	Universidad de Chile, Laboratorio de Fisica Nuclear, Santiago	Dose Response Relationship in Starved Animals Treated with Hemopoietine
A. M. Pappenheimer, Jr.	College of Medicine, New York University, New York	Delayed Hypersensitivity in Antibody Formation
A. Lima-de-Faria	Department of Botany, University of Illinois, Urbana	Chromosome Organization and the Gene Theory
F. J. Bollum	McArdle Memorial Laboratory, University of Wisconsin, Madison	The Synthesis of DNA
Mirko Beljanski	Institut Pasteur, Service de Biochimie Cellulaire, Paris	Activation of Amino Acids and Protein Biosynthesis in Subcellular Material
Benjamin Rasmusen	School of Veterinary Medicine, University of California, Davis	The A, B, C, D, M, and X-Z Systems of Blood Groups in Sheep
K. W. Cooper	Department of Zoology, University of Florida, Gainesville	The Structure of the Heterochromatic Region of the X Chromosome in *Drosophila* in Relation to Segregation
George B. Brown	Sloan-Kettering Institute for Cancer Research, New York	N-Oxides of Purine Derivatives
Oddvar F. Nygaard	Department of Biochemistry, University of Michigan Medical School, Ann Arbor	Effect of X Radiation on Synthesis and Stability of DNA in Various Rat Tissues
Stanfield Rogers	University of Tennessee Memorial Research Center and Hospital, Knoxville	Recent Studies on the Carcinogenic Action of Urethane: Relation to Nucleic Acid Synthesis
R. J. Podolsky	Naval Medical Research Institute, National Naval Medical Center, Bethesda	The Influence of Simple Ions on the Structure of Water
William Firshein	Institute of Microbiology, Rutgers University, New Brunswick	Effects of Nucleic Acid Breakdown Products on Population Changes and Growth of *Pneumococci*
D. Mazia	Department of Zoology, University of California, Berkeley	Recent Studies on the Mitotic Apparatus
J. W. Foster	Department of Bacteriology, University of Texas, Austin	Some Aspects of Hydrocarbon Utilization by Microorganisms
R. Schlögl	Max-Planck Institute for Physical Chemistry, Goettingen	The Biological Aspects of Transport in Charged Porous Membranes
J. Gergely	Massachusetts General Hospital, Boston	Recent Studies on the Contractile Proteins of Muscle
H. Ennis	Harvard Medical School, Cambridge	Cellular Interactions During Development in the Slime Molds
N. Arley	University of Copenhagen, Copenhagen	A Discussion on the Somatic Mutation and Virus Hypothesis of Cancer
E. Bolton	Carnegie Institution of Washington, Washington	Macromolecule Synthesis in *E. coli*

LECTURER	AFFILIATION	TOPIC
J. H. Berrian	Naval Medical Research Institute, Bethesda	Immunogenetics of Mouse Skin Transplantation
B. Amos	Roswell Park Memorial Institute, Buffalo	Isoantigen Systems in the Mouse
W. H. Hildemann	University of California Medical Center, Los Angeles	Some Current Studies of the Homograft Reaction
C. Stormont	School of Veterinary Medicine, University of California, Davis	Studies on Blood Groups in Ruminants
E. P. Cronkite	Brookhaven National Laboratory, Upton, Long Island	Use of Tritium-Labeled Thymidine in Studies on Cell Renewal Systems
A. Roper	Carnegie Institution of Washington, Cold Spring Harbor, Long Island	Nucleocytoplasmic Interactions in *Aspergillus nidulans*
L. Sachs	The Weizmann Institute of Science, Rehovoth	The Possibilities of Directed Immunogenetic Changes in the Cells of Mammalian Tumors
M. Ebert	Hammersmith Hospital, London	The Effect of Inert Gases on the Oxygen-Dependent Radiosensitivity of *Vicia faba* and Ascites Tumor Cells
A. Forssberg	Karolinska Sjukhuset, Radiofysiska Institutionen, Stockholm	Radiation Effects on *Phycomyces blakesleanus*
F. H. Sobels	The Weizmann Institute of Science, Rehovoth	Chemical Steps Involved in the Production of Mutations by X Rays in *Drosophila*
H. Matsushita	Wakayama Medical School, Wakayama	Studies on the Multiplication of Bacterial Viruses
C. L. Smith	University of Cambridge, Cambridge	Some Experiments on the Irradiation of Single Cells and Parts of Single Cells in Tissue Culture with Microbeams of α-Particles
K. G. Zimmer	Kernreaktor, Karlsruhe	Development and Present Status of Hypothesis in Quantitative Radiation Biology

Speakers at Professional Meetings, Autumn 1958. – Papers for presentation at professional societies and international congresses between August 15 and the end of autumn are as follows:

Tenth International Congress of Genetics – R. F. Kimball, Drew Schwartz, Sheldon Wolff, Miguel Mota, E. F. Oakberg, W. J. Welshons, D. R. Krieg, R. D. Owen, D. L. Lindsley (coauthors, C. W. Edington and E. S. Von Halle), F. J. de Serres (coauthor, H. G. Kølmark), R. C. von Borstel, A. D. Conger, W. L. Russell (coauthors, J. Bangham and J. S. Gower), and L. B. Russell (coauthor, W. L. Russell).

American Institute of Biological Sciences – R. E. Canning (coauthor, N. G. Anderson), R. A. Popp, W. St. Amand (coauthor, L. H. Smith), A. H. Haber (two papers, one with N. E. Tolbert), and J. R. Loewenberg (coauthor, N. E. Tolbert).

Fourth International Congress of Biochemistry – Elliot Volkin, G. D. Novelli, and W. E. Cohn.

Second International Conference on the Peaceful Uses of Atomic Energy – Alexander Hollaender (coauthors, C. C. Congdon, D. G. Doherty, T. Makinodan, and A. C. Upton), and W. L. Russell (coauthor, L. B. Russell).

American Chemical Society – D. G. Doherty.

European Travel. – In March of this year, Alexander Hollaender attended a meeting of the Swiss Academy of Medical Sciences and presented a paper. On this trip, he visited in Italy and gave a seminar at the University of Milan. One purpose of this trip was to consult with members of the Comitato Nazionale per le Ricerche Nucleari Committee on the Italian radiobiology program.

Dr. Hollaender will again consult with these committee members immediately after the Geneva Conference. He attends the Second Geneva Conference as a technical advisor in the United States delegation; he also presents a paper in one of the Conference sessions.

A. C. Upton spent several weeks in Europe during the spring and early summer. In England, he attended and presented papers at the Ciba Foundation Symposium on "Carcinogenesis: Mechanism of Action" and the Seventh International Cancer Congress. During his travels, he visited a number of laboratories in England, France, Belgium, and Italy. Lectures were given at Centre d'Études Nucléaires in Saclay and at the University of Pavia.

Elliot Volkin attended three international meetings in Europe this summer – the Second International Symposium on Bacteriophage (in Paris), the International Congress of Microbiology (in Stockholm), and the International Congress of Biochemistry (in Vienna). He gave papers at the last two and participated in discussions at the first. He visited various laboratories in France, Sweden, Belgium, Germany, and Austria.

G. D. Novelli also attended the International Congress of Biochemistry in Vienna and gave a paper. He visited a number of laboratories in Italy and participated, as a discussant, in a symposium at the Third International Congress of the Reticuloendothelial Society in Rapallo, Italy.

W. L. Russell attends the Geneva Conference as a member of the United States delegation, serving as technical advisor. He presents a paper at the Conference. Before returning to this Laboratory, he will consult with investigators in several laboratories in England.

Takashi Makinodan gave a paper at the Seventh International Congress for Microbiology in Stockholm. He also visited laboratories in England, Switzerland, France, Netherlands, and Denmark.

W. E. Cohn attended the Fourth International Congress of Biochemistry in Vienna and presented a paper. He also attended the International Microbiology Congress in Stockholm. While on this trip, he consulted with a number of biochemists in England and Western Europe.

1958 Biology Symposium. Genetic Approaches to Somatic Cell Variation. – The collection of papers and open discussions given at the Eleventh Annual Biology Symposium in Gatlinburg on April 2–5, 1958, will be published as a supplement to the *Journal of Cellular and Comparative Physiology.* The manuscript for this supplement is in press; a November publication is expected. The table of contents follows:

Alexander Hollaender. Introduction

Curt Stern. The Nucleus and Somatic Cell Variation

Boris Ephrussi. The Cytoplasm and Somatic Cell Variation

Albert H. Coons. The Cytology of Antibody Formation. Two figures

C. W. Cotterman. Erythrocyte Antigen Mosaicism

K. C. Atwood and S. L. Scheinberg. Somatic Variation in Human Erythrocyte Antigens. Three figures

George Klein and Eva Klein. Histocompatibility Changes in Tumors. One figure

R. Alexander Brink. Mutable Loci and Development of the Organism. One figure

Theodore S. Hauschka. Correlation of Chromosomal and Physiological Changes in Tumors. Seven figures

C. E. Ford, J. L. Hamerton, and R. H. Mole. Chromosomal Changes in Primary and Transplanted Reticular Neoplasms of the Mouse. Two figures

Marguerite Vogt. Study of a Genetic Change in a Tissue Culture Line of Neoplastic Cells. Four figures

Theodore T. Puck. Growth and Genetics of Somatic Mammalian Cells *in vitro*. Seven figures

General discussion: Molecular Basis of the Cause and Expression of Somatic Cell Variation. One figure

Chairman, E. L. Tatum: Michael Heidelberger, Rollin D. Hotchkiss, W. Eugene Knox, Daniel Mazia, G. David Novelli, and Sol Spiegelman

Werner Braun. Cell Population Dynamics and Somatic Change. Three figures

Claudio Bargozzi. Melanotic Tumors in *Drosophila.* Three figures

Joshua Lederberg. Summary Comment on the Conference

Twelfth Annual Biology Research Conference. – The 1959 Biology Research Conference will be held in Gatlinburg, Tennessee, on April 1–4. The conference will be sponsored by the Biology Division and supported by the Atomic Energy Commission. The title is "Enzyme Reaction Mechanisms," and the papers and discussions will be concerned with basic enzymic mechanisms concerned with the formation and splitting of specific chemical bonds. Members of the Biology Division serving as the organizing committee for this conference are: S. F. Carson, W. E. Cohn, D. G. Doherty, G. D. Novelli, and E. Volkin. The program follows.

WEDNESDAY, APRIL 1

MORNING

1. Reactions Involving the P-O Bond

   Chairman and Introductory Speaker: Sir Alexander Todd, Cambridge, England

   a. *Nucleic Acid Synthesis* – G. Khorana, Vancouver, B. C.

   b. *Inorganic Phosphate Esterification and Transfer* – Mildred Cohn, St. Louis, Mo.

AFTERNOON

2. Reactions Involving the C-C Bond

   a. *Acyl-coenzyme A and Fatty Acids* – F. Lynen, München, Germany

   b. *Carboxylations and Decarboxylations* – M. Calvin, Berkeley, Calif.

EVENING

   c. *Formylation and Methylation*

THURSDAY, APRIL 2

MORNING

3. Reactions Involving C-O and C-N Bonds

   Chairman: C. F. Cori, St. Louis, Mo.

   a. *Glycosides* – S. Hestrin, Jerusalem, Israel

   b. *N-Glycosides*

AFTERNOON

Mountain climbing or tour through Smoky Mountains. In case of inclement weather, the program will continue and Saturday morning will remain open for a tour of the Biology Division of the Oak Ridge National Laboratory.

FRIDAY, APRIL 3

MORNING

Reactions Involving C-O and C-N Bonds (continued)

Chairman: G. R. Greenberg, Ann Arbor, Mich.

c. *Heterocyclic Compounds* – J. M. Buchanan, Cambridge, Mass.

d. *Transamination* – E. E. Snell, Berkeley, Calif.

AFTERNOON

Chairman: J. S. Fruton, New Haven, Conn.

e. *Esters* – J. A. Cohen, Rijswijk, Netherlands

f. *Peptides* – H. Neurath, Seattle, Wash.

SATURDAY, APRIL 4

MORNING

4. Round Table: *Active Sites*

Chairman: P. Handler, Durham, N. C.

C. B. Anfinsen, Bethesda, Md.

Max Brenner, Basel, Germany

J. A. Cohen, Rijswijk, Netherlands

D. E. Koshland, Upton, Long Island, N. Y.

Bone Marrow Conferences. – An informal conference on bone marrow transplantation in animals and man was held during the Federation meetings (April 19, 1958) in Philadelphia. The conference was sponsored jointly by L. M. Tocantins of Jefferson Medical College and ORNL Biology Division personnel. An abstract of the proceedings will appear in *Blood*.

Another conference including work on chemical protection against radiation injury as well as on bone marrow transplantation in man and large animals was sponsored by ORNL Biology Division at the time of the International Congress of Radiation Research in Burlington, Vermont.

Education. – In the summer of 1958 nine undergraduate college students came to the Division for a 10-week training course. Eight of the students were from small colleges in the Southeastern region of the United States; one, from Reed College, Oregon, was here on a National Science Foundation Scholarship. This course was designed to give students who have completed three years of college the experience of working in a laboratory devoted to full-time basic research. The trainees were selected on the basis of scholastic records and interest in scientific research, and they were assigned to groups where the work going on was closely related to their individual interests. The course consisted of work on a specific problem, attendance at Division seminars, observation of the work of others, and frequent informal discussions with research scientists. Following are the schools represented by the 1958 trainees:

Martha Dell Bard – Central State College, Edmond, Oklahoma

Mary Virginia Beard – Randolph-Macon Women's College, Lynchburg, Virginia

Nancy Brassington – Winthrop College, Rock Hill, South Carolina

Mayo Fong – Southwestern at Memphis, Tennessee

William Carrol Heird – Maryville College, Maryville, Tennessee

Vera Marion King – Tuskegee Institute, Alabama

Lloyd Franklin Pennington, Jr. – Spring Hill College, Mobile, Alabama

Natalie Carol Tudor – Longwood College, Farmville, Virginia

In June, two members of the Division attended short training courses at other installations. W. D. Gude took a course in histochemistry at the University of Kansas Medical Center. E. B. Darden, Jr., attended an electron microscope seminar given by RCA at Camden, New Jersey.

A. C. Upton gave a lecture to the sixty-third class of the Radioisotopes Techniques School of ORINS in May.

W. A. Arnold again spent the summer at the Institute for Muscle Research, Woods Hole, Massachusetts.

The biophysics course started near the end of the last period was completed in April. M. L. Randolph was the course leader; J. Jagger, E. B. Darden, and Sohei Kondo were also instructors.

CYTOLOGY AND GENETICS

CYTOGENETIC EFFECTS OF RADIATION

R. F. Kimball
K. C. Atwood
C. Auerbach
R. T. Brumfield[1]
A. D. Conger
H. G. Kølmark
D. R. Krieg
M. Moutschen-Dahmen[2]
D. Schwartz
F. J. de Serres
S. Wolff
N. Gaither
H. E. Luippold
H. J. Luippold
K. H. McGrath
M. P. Morgan
A. H. O'Bier, Jr.[1]
M. E. Picinich[3]
A. P. Teasley
S. M. Wilson

Starvation and Recovery from the Mutagenic Effects of X Rays on *Paramecium aurelia*

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

Introduction. – It was reported earlier[4] that starvation after irradiation decreases the number of lethal and deleterious mutations induced by X rays in *Paramecium aurelia*, and this finding was interpreted on the hypothesis that any agent that slows growth and division gives greater opportunity for spontaneous recovery from the initial radiation injury. The present report concerns two questions about the recoverable process: (1) Can recovery occur in paramecia that are not growing rapidly? (2) How long does radiation damage remain reparable when irrevocable establishment of the mutations is avoided by preventing progress through the interdivision interval?

Results and Discussion. – The methods of irradiation and detection of mutations have been given in a number of previous reports and will not be repeated here. The quantity measured is the frequency of normal exautogamous clones, which is inversely related to the number of mutations. In earlier experiments, well-fed animals were put under starvation conditions after irradiation. This procedure delays division but does not prevent it. Therefore, in the present work starved animals were irradiated and then held various times after irradiation in starvation medium before transfer to culture medium. The results of the main experiments are plotted in Fig. 1. A variance-equalizing transformation of the frequency of normal clones is plotted on the ordinate since standard errors were more easily calculated for the data in this form. For comparison with other reports, the percentage frequency of normal clones for the 0-hour group was 34% and for the 22-hour group, 47%. The value for the former group is approximately the same as for well-fed paramecia whose growth was inhibited by streptomycin for several hours after irradiation.[4,5] The cause was probably the same since preirradiation starvation delays growth and division of paramecia after they are transferred to culture medium, to about the same extent as exposure to streptomycin.

The second thing to note in Fig. 1 is that mutation is decreased by retaining the animals in the starvation medium for a time after irradiation. A priori, there are two possible explanations: (1) recovery from radiation damage occurs in starved

[1]Consultant.

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[4]R. F. Kimball, *Proc. Intern. Genet. Symposia, 1956, Cytologia*, supplemental volume, p 252–55 (1957).

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

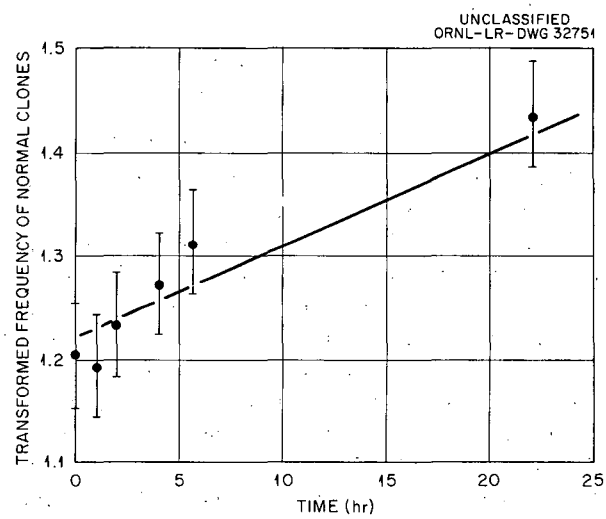

Fig. 1. Frequency of Normal Exautogamous Clones Plotted Against the Time Between Irradiation and Transfer to Culture Medium. The frequencies have been transformed by a variance-equalizing transformation.

individuals during retention in starvation medium, or (2) no recovery occurs until the paramecia are put into culture medium, but the added time in starvation medium increases the time available for recovery after transfer to culture medium. If the latter were the case, additional starvation before irradiation should be as effective as starvation afterward. The following experiment was done to test this. Two groups of starved paramecia, one (A) starved two days more than the other (B), were irradiated; and half (1) of each group was transferred to culture medium immediately afterward, half (2) a day later. The percentage frequencies of normal exautogamous clones were A1, 27%; A2, 43%; B1, 26%; B2, 41%. Thus two additional days of preirradiation starvation were without effect, and hypothesis (2) can be rejected.

Finally, the data in Fig. 1 show that the radiation damage that leads to mutation remains reparable for many hours when irreversible establishment of mutation is avoided by continued starvation. The data do not allow a decision between the hypothesis that recovery occurs at a constant rate throughout the 22-hour period and the hypothesis that the rate of recovery is high in the first few hours and declines thereafter; but recovery certainly continues through a period of between 5 and 10 hours if it does not continue indefinitely.

Free Radicals Produced in Cereal Embryos by Ionizing Radiation

A. D. Conger M. L. Randolph[6]

Introduction. – Barley seeds, barley embryos, and wheat embryos (ground wheat germ) were irradiated with X and gamma rays and examined after irradiation for the presence of radicals (unpaired electrons) by electron paramagnetic spin resonance methods. Experiments were designed to determine if there was any similarity between the effect of various environmental conditions during and after irradiation on the production and decay of radicals detected after irradiation, and the effect of the same environmental conditions on the amount of biological "aftereffect" as detected by biological radiation damage criteria. There is a considerable body of information on biological aftereffects from experiments on irradiated barley seeds.

Results. – Radicals could be detected in 100-mg wheat germ samples after a dose of about 10 kr, but larger doses from 50 to 1000 kr customarily were delivered to give more adequate radical signals. Production and decay of radicals were considerably influenced by the environmental conditions of irradiation and storage. The presence of moisture and/or oxygen greatly enhanced decay of the radical signal. Under whatever conditions of irradiation and storage, decay was most rapid at first and became progressively less rapid with increasing time. Observations were made from 3 minutes to 30 days after irradiation; in this time, the signal from "moist" germ (8.5% water content, by weight) had diminished to about 20% of the initial value, that from "dry" (1.5% water content) germ to about 50%. About 2 times as many radicals are detected after irradiation of dry germ as of wet. It appears that the production of radicals is greater when oxygen is present than when absent, but decay is more rapid in the presence of oxygen.

About 10^{11} radicals per milligram dry weight of wheat germ, irradiated dry in air, are found after a dose of 1 kr of Co^{60} gamma rays; this amounts to about one radical per 500 ev absorbed. The values are only approximate and would be considerably higher for rapid irradiation and measurement.

The radical signal detected in irradiated wheat germ was broad and showed no specific secondary peaks. It was similar to the signals seen in irradiated proteins. For equal weights and doses, a water-soluble protein fraction of wheat germ gave a signal about 3 times as large as whole germ, alcohol-soluble protein and residue fractions about the same, and a carbohydrate fraction about 1.3 times as large; a fats and oils fraction gave no detectable signal. The wheat fractions and barley embryos gave radical signals indistinguishable from that of whole germ; the carbohydrate fraction alone was but slightly different.

Discussion. – Although no relationship is proved, some remarkable similarities are seen between the effect of various environmental and storage conditions – dry and moist, anoxic and oxygenated, etc. – on the decay of radicals detected after irradiation and the aftereffects in seeds treated similarly.

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Effect of Postirradiation Storage on the Growth of Barley Seedlings

S. Wolff

Introduction. – It has been known for some time[7] that X irradiation of superdry barley seeds induces a greater reduction of seedling growth than does the irradiation of "dry" seeds with higher moisture contents. More recently it has been noted that the postirradiation storage conditions of the seeds before germination has a profound effect on the seedling height measurements.[8] In particular, postirradiation storage prior to germination seems to increase the damage in superdry but not in normally dry seeds.

The present experiments were undertaken to see: (1) if other effects of radiation (chromosome aberrations) responded similarly to postirradiation storage; (2) if the depression in seedling height is correlated to nuclear damage as measured by aberrations; and (3) if the storage only increased damage.

Results. – Seeds of Himalayan barley were stored either in the laboratory or in a desiccator over anhydrous calcium chloride. The water content of those in the laboratory (designated N for normal) was found to be 9.0%. Those seeds stored in the desiccator (designated D) had a water content of 1.8%. The D seeds and N seeds were irradiated simultaneously with 30 kr of gamma rays from a Co^{60} distributed source. The intensity was approximately 1 kr per minute. Thirty-five seeds of each type were immediately planted on moist filter paper in petri dishes and grown under a bank of fluorescent bulbs for eight days. For cytological studies, 15 other seeds were separately planted at the same time. The remaining seeds were then stored to be planted at intervals. The four classes of treatments plus storage, therefore, were D seeds stored D, D seeds stored N, N seeds stored D, and N seeds stored N.

The results of the seedling height studies are shown in Fig. 2, in which the ordinate represents the height of the seedling as a per cent of the height of nonirradiated control seedlings. The storage for the point listed as four days under normal (N) conditions was actually in the moist atmosphere of a petri dish that had moistened filter paper inside the cover. This was done to see if a rapid increase of moisture content affected radiation-induced damage. Seeds stored longer than four days in these series were kept in the normal laboratory atmosphere.

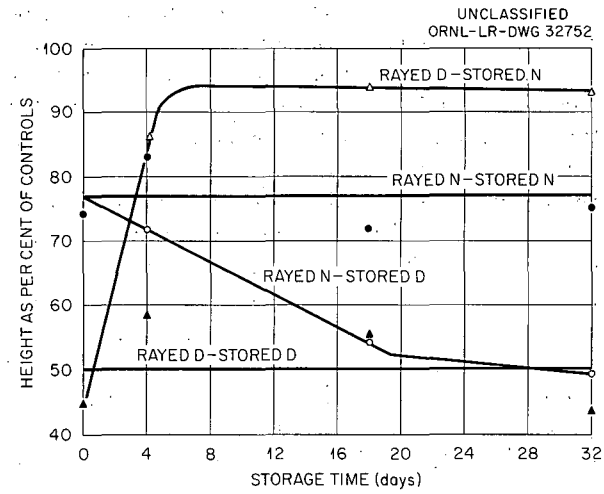

Fig. 2. Seedling Heights After Postirradiation Storage.

A repeat experiment has shown similarly shaped curves. The results of the cytological studies made at metaphase of the first mitotic division of the root tips in germinating seeds are seen in Table 1. For simplicity's sake only the per cent normal cells is recorded. However, all classes of aberrations showed similar patterns.

Discussion. – The seedling height curves illustrate that D seeds stored D show no change in seedling height. The N seeds stored N also showed no change. However, D seeds that were stored under conditions that increased their moisture content (i.e., N) showed a decrease in radiation effects following storage. They even grew more than did the N seeds. The N seeds that were stored in the desiccator showed an increased amount of damage with time. It therefore appears that moisture content after irradiation can have a profound effect on the amount of damage that becomes manifest. However, the effect of storage is not a universally detrimental effect, since superdry seeds seem to recover if allowed to take up moisture before germination.

The cytological results from experiment 1 do not show storage effects that can be correlated

[7] R. S. Caldecott, *Radiation Research* 3, 316–30 (1955).

[8] H. J. Curtis *et al.*, *Radiation Research* 8, 526–34 (1958).

Table 1. Aberration Yields After Postirradiation Storage

Experiment No.	Days Postirradiation Storage	Per Cent Normal Cells			
		N Seed		*D* Seed	
		Stored *N*	Stored *D*	Stored *N*	Stored *D*
1	0	84.0		71.3	
	4	79.7	84.0	78.7	74.7
	18	86.3	79.3	69.0	43.0
	32	67.7	59.7	67.7	40.3
2	0	74.3		58.0	
	3	83.3	80.0	66.7	73.7
	30	81.0	75.6	81.7	68.0

with the effects on seedling height. The aberration yield from N seeds kept D is not significantly increased, nor is there a significant decrease in yield from D seeds kept N. Furthermore, the absolute numbers of aberrations cannot be correlated with seedling height. In experiment 1, which corresponds to Fig. 2, it is found that N seeds stored for 32 days under N conditions have about the same number of aberrations as do D seeds planted immediately. Yet the seedling heights of the two groups are markedly different. Also, the D seeds kept N in experiment 1 all have the same numbers of aberrations but show a great difference in seedling height after storage.

It appears that the damage expressed by retarded growth of seedling is not correlated to nuclear damage as measured by chromosome aberrations. Investigations are being made of the hypotheses that correlate the damage of ability to grow with the production of long-lived radicals by radiation.

The Presence of A_2 Erythrocytes in A_1 Blood

K. C. Atwood

Small proportions of O or B erythrocytes have been found in the blood of normal A or AB individuals, respectively, by means of the isotope dilution method[9] and direct isolation.[10,11] This form of blood group admixture, automosaicism, is distinguishable from true chimerism in that the absence of one agglutinogen is independent of unrelated agglutinogens, whereas in the chimera it is accompanied by unrelated differences signifying the foreign origin of a portion of the hematopoietic tissue. The origin of automosaicism is unsolved; it may represent merely a nonuniform development of the cell phenotype, or it may reflect an underlying genetic heterogeneity of the erythropoietic system. Somatic crossing over as a basis for such heterogeneity has been suggested by Goudie[11] and would lead to homozygosis of the opposing alleles. The exceptional non-A cells in AB individuals would therefore be phenotypically B, and those in heterozygous A individuals would be phenotypically O. It is of interest in this connection to learn that A_1 and A_1B individuals possess exceptional cells bearing the agglutinogen of subgroup A_2.

Suitable reagents for the recognition of the subgroup in isotope dilution experiments are provided by the seed extracts of *Dolichos biflorus*, specific for A_1, and *Phaseolus limensis*, which agglutinates both A_1 and A_2 cells.[12] The method, described in detail elsewhere,[9] requires a series of agglutinations in which the first batch of cells added to the reaction mixture is labeled with Cr^{51}, and all of the succeeding additions are unlabeled. At

[9] K. C. Atwood and S. L. Scheinberg, submitted to *Science*.

[10] K. C. Atwood and S. L. Scheinberg, *Symposium on Genetic Approaches to Somatic Cell Variation, J. Cellular Comp. Physiol.* **52**, Suppl. (in press).

[11] R. B. Goudie, *Lancet* **272**, 1333 (1957).

[12] W. C. Boyd and R. M. Reguera, *J. Immunol.* **66**, 333 (1949).

each stage the cells are agglutinated in a pan and transferred to a separatory funnel. After a brief settling time, the main agglutinated mass is withdrawn leaving a supernatant that contains free cells and small clumps. The supernatant is sampled for determination of Cr^{51} activity and returned to a pan for the next stage, which begins with the addition of further cells and agglutinin. The labeled agglutinable cells are finally exhausted, and the activity of successive supernatants becomes constant. The samples are centrifuged and washed so that only the Cr^{51} bound to cells is counted. Each sample is corrected for the cumulative dilution of the system, and the ratio of the corrected final activity to that of the initial reaction mixture is the proportion of inagglutinable cells. In the present experiments the *Dolichos* and *Phaseolus* agglutinations were adjusted to the same titer, 1:128, with A_1B cells. The initial reaction mixtures contained 5 ml of washed labeled cells, 20 ml of saline, and 10 ml of agglutinin. Their activities ranged from 1.8 to 3.5×10^6 counts/min/ml. The stage additions were 3 ml of agglutinin and 3 ml of a 66% suspension of A_1 or A_1B carrier cells. Agglutinations were carried out at 4°C. A well-type scintillation counter was used, with a background of 250 counts/min.

In Fig. 3 the corrected sample activities are plotted against the stage in two experiments with the same A_1 donor. The proportion of phenotypically O cells is just under 1 per thousand in the experiment with *Phaseolus* anti-A. With *Dolichos* anti-A_1 the sum of O and A_2 cells is about 3.5 per thousand. Substitution of anti-A for anti-A_1 in the stages following the seventh produced a decline to the level of the O cells. Figure 4 shows a similar pair of experiments on an A_1B blood, indicating just above 1 B cell per thousand and 1 A_2B cell per hundred. Again the addition of *Phaseolus* lectin after equilibration to *Dolichos* resulted in a further decline, but the experiment was terminated when the volume of the system became limiting before a second equilibrium was reached. Another A_1B blood is analyzed in Fig. 5; it contains about 1 B cell per two thousand and 7 A_2B cells per hundred.

Cells inagglutinable by anti-A_1 were obtained from A_1B blood by direct isolation, for comparison with known A_2B cells in ordinary typing procedures. The technique of isolation described in

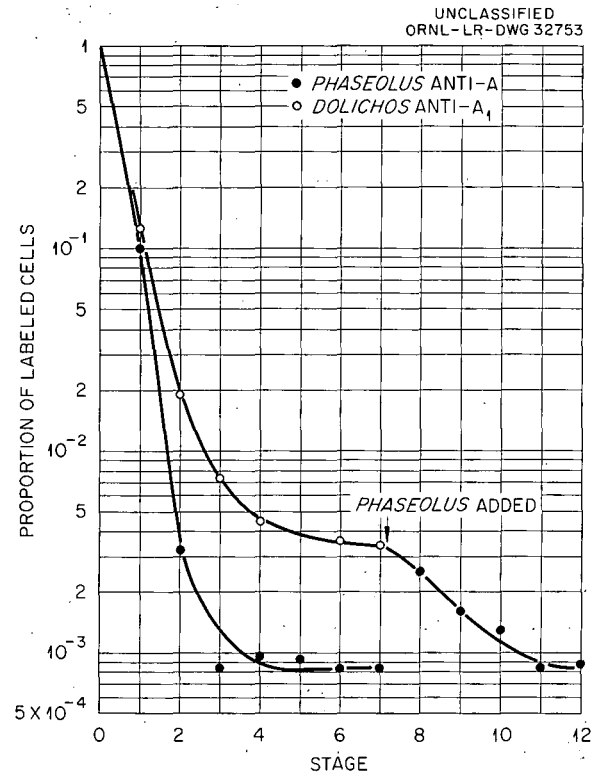

Fig. 3. Isotope Dilution Curves with *Phaseolus* and *Dolichos* Lectins on Cells of A_1 Donor S.W. In the experiment begun with *Dolichos* anti-A_1, *Phaseolus* anti-A was added at each stage following the seventh.

detail elsewhere[10] is designed to minimize entrapment of inagglutinable cells in the pellets formed by centrifugation in the presence of agglutinin. Washed cells are first agglutinated in a large pan, and a preliminary separation is accomplished by gravity in a separatory funnel. The supernatant, which still contains mainly agglutinable cells, is then concentrated by centrifugation over high-density fluorocarbon oil. The cells are deposited in a thin layer at the interface, and the layer is subsequently broken into free cells and flakes which are easily separable. The process is repeated on the free cells until no further agglutination is detectable. Significant losses of inagglutinable cells are incurred, but the preparations are fairly pure.

One hundred fifty ml of cells from donor K.A. yielded 0.4 ml of cells inagglutinable by *Dolichos* anti-A_1. These were thoroughly washed in saline, adjusted to a 2% suspension, and tested with the

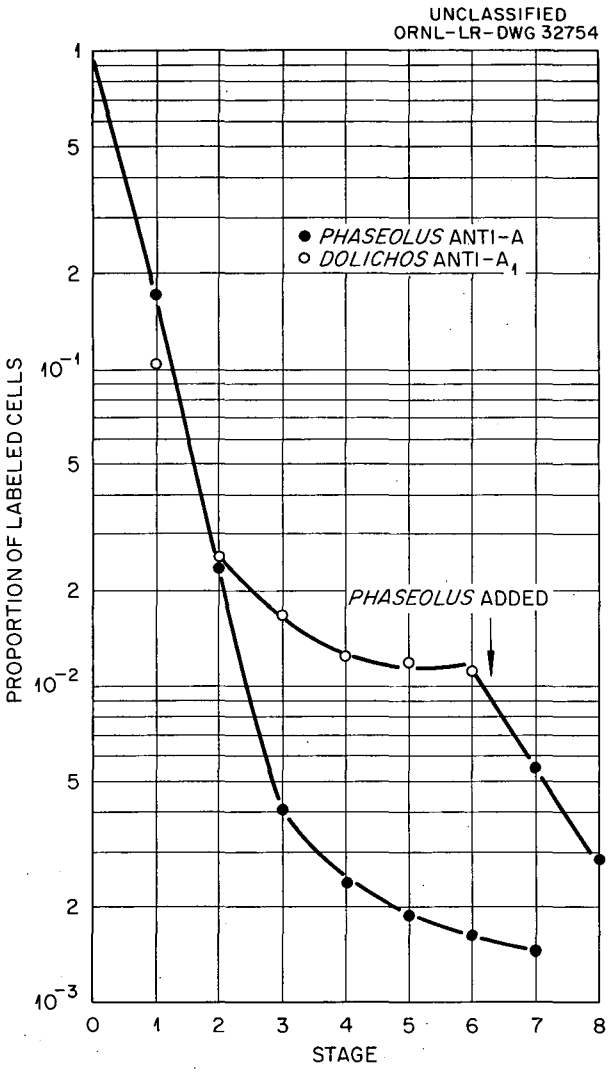

Fig. 4. Isotope Dilution Curves with *Phaseolus* and *Dolichos* Lectins on Cells of A_1B Donor K.A.

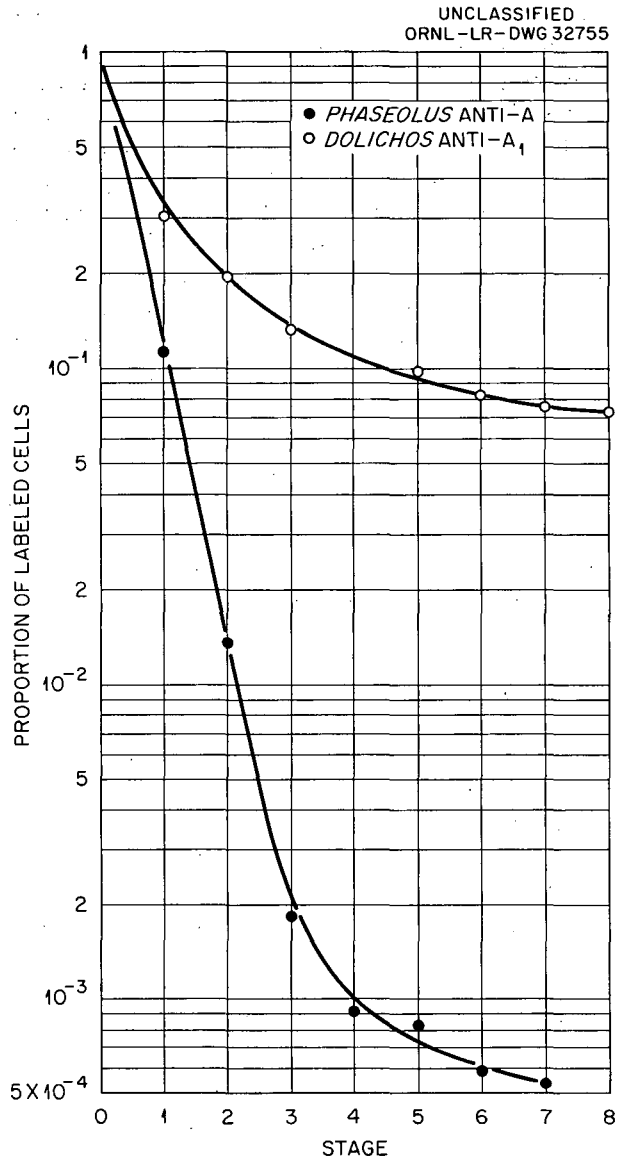

Fig. 5. Isotope Dilution Curves on Cells of A_1B Donor M.E.G. It is noteworthy that the *Dolichos* and *Phaseolus* lectins had the same titer against M.E.G. cells in the standard test, despite the large difference in the proportion of cells inagglutinable by these reagents.

reagents shown in Table 2. Two drops of reagent and one of cell suspension were mixed in a small tube and centrifuged to a firm pellet. The tube was tapped to resuspend the pellet and scored as follows: pellet unbroken, 4; several fragments, 3; many fragments, 2; small fragments only, 1; microscopic agglutination, ±; no agglutination, 0. The C.D.S. (Certified Donor Service) reagents are commercial typing sera. The cells selected with *Dolichos* anti-A_1 are compared with unselected cells of the same donor, with known A_2B and B cells, and with cells selected with *Phaseolus* anti-A. By these criteria the cells selected with anti-A_1 are phenotypically A_2B, whereas those selected with anti-A are phenotypically B. The resemblance of the selected cells to those of known type extends even to the cross-reaction with anti-H. In a similar experiment with A_1B donor M.E.G., 170 ml of cells yielded 6 ml inagglutinable by *Dolichos* anti-A_1. The ease of isolation in this case was entirely consistent with

Table 2. Comparison of the Reactions of Exceptional Cells Selected from A_1B (Donor K.A.) with Those of Unselected Cells, and Known A_2B and B Cells

Reagent	Unselected Cells	Selected with *Dolichos* Anti-A_1	Selected with *Phaseolus* Anti-A	A_2B (Donor L.R.)	B (Donor C.M.)
Dolichos anti-A_1	4	0	0	0	0
C.D.S. anti-A_1	3	0	0	0	0
Phaseolus anti-A	4	2–3	0	1–2	0
C.D.S. anti-A	4	1–2	±	2	0
Ulex anti-H	±	2	3	2	2–3
C.D.S. anti-B	4	4	4	4	4

the result of the isotope dilution experiment on the same donor. The selected cells were used to absorb C.D.S. anti-A, and produced a reagent indistinguishable from C.D.S. anti-A_1. Parallel absorptions with unselected M.E.G. cells exhausted C.D.S. anti-A, as expected. In summation, the individuals tested not only possess some cells lacking in A agglutinogen but a larger number in which A_1 is lacking and A_2 present.

Clearly, the exceptional cells of subgroup A_2B cannot be produced by somatic crossing over or by other processes that lead to homozygosis. Even the separation of an ostensible A_1A_2 cistron cannot be invoked, since neither A_1 nor A_2 is ever found in coupling with B. The proportion of cells having A_2 alone seems to be quite uncorrelated with that of cells in which A is absent, suggesting that the processes giving rise to the two classes are independent. Should this be substantiated, it may narrow the range of speculation as to the origin of the exceptional cells. At present it is possible that they represent somatic mutation in the proliferating marrow, and equally possible that they are phenocopies, or, as Koller[13] has expressed it, "serological freaks."

Factors Influencing the Frequency of Recombination in Crosses of *ad-3* Mutants in *Neurospora*

F. J. de Serres

Introduction. – In experiments reported previously,[14,15] linkage relations of 11 *ad-3A* mutants were studied with markers in adjacent regions. It was found that this group of mutants gave identical linkage relations in crosses with a strain carrying markers at the closely linked *hist-2* and *nic-2* loci. However, when the same strains of these 11 mutants were crossed to *hist-2 ad-3B nic-2* strains and linkage relations in the *ad-3A–ad-3B* region were determined, the estimates of recombination varied markedly and were found to be dependent on whether the *ad-3A* and *ad-3B* mutants crossed were related or unrelated in origin.[16] Estimates of the frequency of recombination in the *ad-3A–ad-3B* region from crosses where the mutants are related in origin differed from crosses where the mutants are unrelated in origin by a factor of 3 to 6. Since the same strains of these 11 *ad-3A* mutants had given identical linkage relations with the *hist-2* and *nic-2* markers in a previous cross, it was of considerable interest to determine whether the variation in the frequency of recombination observed in the *ad-3A–ad-3B* region was correlated with a proportional variation in the adjacent regions *hist-2–ad-3A* and *ad-3A–nic-2* in the same cross.

[13] P. C. Koller, *Nature* 181, 1706 (1958).

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

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

[16] F. J. de Serres, *Biol. Semiann. Prog. Rep. Feb. 15, 1958*, ORNL-2481, p 6–8.

Results. – One mutant was selected from each of the two groups of *ad-3A* mutants; these (mutants A2 and 38701) were used for more extensive analyses of the crosses with the marked *ad-3B* strains, *hist-2 ad-3B* (B10-R2) *nic-2* and *hist-2 ad-3B* (35203) *nic-2*, used previously.[14,15] Since mutant B10-R2 is a temperature-sensitive mutant that grows at wild-type rate at low temperatures, recombination in the *hist-2–ad-3A* and *ad-3A–nic-2* regions could be measured by plating the crosses at 25°C. To estimate the frequency of recombination in the same regions in the crosses with the marked strain of 35203, experiments were performed to obtain a spontaneous reverse mutation of this mutant to wild type. Thus both marked chromosomes could now be used as a *hist-2 nic-2* double mutant in crosses with the *ad-3A* mutants. The revertant of the marked strain of 35203 used in the present analysis was *hist-2 ad-3B*$^+$ (35203-R1) *nic-2* which grows at wild-type rate when supplemented only with histidine and niacin. All crosses were plated at least twice to test for reproducibility of the results presented in Table 3. The linkage relations in the *hist-2–ad-3A* and *ad-3A–nic-2* regions for mutants A2 and 38701 in crosses with the *hist-2 nic-2* strain are given for comparison. The estimates for the *ad-3A–ad-3B* region in the crosses with mutant 35203 were derived from the nonreverted strain *hist-2 ad-3B* (35203) *nic-2*.

The data in Table 3 show clearly that the same *ad-3A* mutants can exhibit quite different linkage relations with the same markers at closely linked loci in different crosses. Both mutants A2 and

Table 3. Frequency of Recombination in the *hist-2–ad-3A–ad-3B–nic-2* Regions in Crosses of *ad-3A* Mutants 38701 and A2 with Various Marked Strains

Cross	Recombination Frequency		
	Region I *hist-2–ad-3A*	Region II *ad-3A–ad-3B*	Region III *ad-3A–nic-2*
hist-2 ++ *nic-2* / + A2+ +	1.89 ±0.14		2.99 ±0.18
hist-2 ++ *nic-2* / + 38701+ +	2.00 ±0.14		2.90 ±0.18
hist-2 +B10-R2 *nic-2* / + A2+ +	1.11 ±0.09	0.14 ±0.02	2.43 ±0.13
hist-2 +B10-R2 *nic-2* / + 38701+ +	2.63 ±0.19	0.38 ±0.02	2.63 ±0.19
hist-2 +35203-R1 *nic-2* / + A2+ +	1.91 ±0.08	0.52 ±0.12	2.69 ±0.10
hist-2 +35203-R1 *nic-2* / + 38701+ +	1.56 ±0.07	0.13 ±0.04	0.32 ±0.03

38701 showed the same linkage with *hist-2* and *nic-2* markers in a cross with the double mutant strain *hist-2 nic-2*. However, when the composition of the *hist-2 nic-2* chromosome is changed by the insertion of *ad-3B* mutants derived from different wild-type strains, crosses of these altered chromosomes with A2 and 38701 show marked changes in the frequency of recombination in the *hist-2–ad-3A* and *ad-3A–nic-2* regions. In the crosses of mutant 38701 there is a tenfold difference in the estimates of the frequency of recombination in the *ad-3A–nic-2* region. It is also evident that the marked differences in the estimates for the *ad-3A–ad-3B* region are not accompanied by proportional changes in adjacent regions. In the crosses with B10-R2, the ratios of the estimates for regions I, II, and III are 2.4, 2.7, and 1.1, respectively. In the crosses with 35203, the ratios of the estimates for these three regions are 1.2, 4.0, and 8.4, respectively.

Discussion. – In the *ad-3A* × *ad-3B* analyses,[16] there was a marked correlation between the origin of the mutants and the frequency of recombination in the *ad-3A–ad-3B* region. The differences in the frequency of recombination in the same region in different crosses were attributed to regional differences in the structure of homologous chromosomes derived from different wild-type strains. In the synthesis of the marked *ad-3B* strains from crosses of the *ad-3B* mutants to the *hist-2 nic-2* strain, we do not know where the crossovers occurred in the *hist-2–ad-3B* and *ad-3B–nic-2* regions. However, if the frequency of recombination in a given region is dependent upon the extent and type of heterogeneity in that region, the disproportionality in the changes in the frequency of recombination in each of the regions under consideration may be more readily understood. If the interpretation of previous analyses[16] is correct, heterogeneity in structure in a given region of paired homologous chromosomes leads to an increased frequency of recombination, and homogeneity to a decreased frequency of recombination. The reduction in the frequency of recombination in the *hist-2–ad-3A* and *ad-3A–nic-2* regions can be accounted for, since the insertion of B10-R2 and 35203 in the *hist-2 nic-2* chromosome results in a decrease in the heterogeneity in these regions in the crosses of A2 × B10-R2 and 38701 × 35203-R1. In the crosses of 38701 × B10-R2 and A2 × 35203-R1 only the type of heterogeneity has been changed, which in these two crosses leads to similar or slightly higher frequencies of recombination in these regions.

Influence of pH on the Interaction Between MEG and X Rays As Studied by Genetic and Toxic Effects in *Neurospora*

G. Kølmark

Introduction. – It has been reported earlier that the chemical AET·Br·HBr in experiments with *Neurospora* failed to give any appreciable protection against the genetic and toxic effects caused by irradiation with X rays in *Neurospora*.[17] On the contrary, in some of the experiments an increase in mutagenic and toxic effects of X rays was noticed when conidia were treated with AET before and during the X-ray treatment. Since AET as a prototype and several other similar compounds are being investigated for protective effect against X rays in animals,[18] it is of interest to find the conditions where AET becomes toxic and mutagenic when given alone or in combination with X rays. The strain and general procedure are the same as in the earlier report. AET when dissolved will undergo chemical transformation to other substances depending on pH in the solution.[19] At a pH range from *ca.* 7 to 12, it is rapidly converted to MEG. MEG has been shown to be the active protective agent against ionizing radiation in experiments with mice.[19] For experiments with animals, the pH range during treatment is naturally a very narrow one, and the solution is in general applied in buffer at pH 7. In *Neurospora* the influence of pH can be studied over a wide range, and some effects are reported here.

Results. – In one experiment conidia were treated for 60 minutes before irradiation in 5 ml of 0.0125 *M* MEG. Before irradiation the conidia were spun down and 4 ml of the supernatant was removed with a pipette. The actual pH during treatment was measured on this sample. Conidia were resuspended and irradiated in the remaining 1 ml with 45,000 r of X rays. The resulting survival

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

[18] R. Shapira, D. G. Doherty, and W. T. Burnett, Jr., *Radiation Research* 7, 22–34 (1957).

[19] J. X. Khym, R. Shapira, and D. G. Doherty, *J. Am. Chem. Soc.* 79, 5663–66 (1947).

and mutation rates are shown in Fig. 6. The survival of conidia in buffer at pH 7.1 has arbitrarily been chosen as 100%. This corresponded to 56.2×10^6 viable conidia. MEG in this concentration has a toxic influence when added to the buffer at pH lower than 5 and higher than 7. In the pH interval from 5 to 7, MEG in this concentration is nontoxic. Still, as seen from the X-ray survival curves, there is no point in the pH range where survival after X-ray treatment in the presence of MEG is as high as in its absence. Based on initially viable conidia, MEG + X rays are killing an excess of 6 to 8% more conidia than X rays alone in the pH range 6 to 7. Thus no protection against the killing effect of X rays could be demonstrated even under conditions where MEG is nontoxic when given alone.

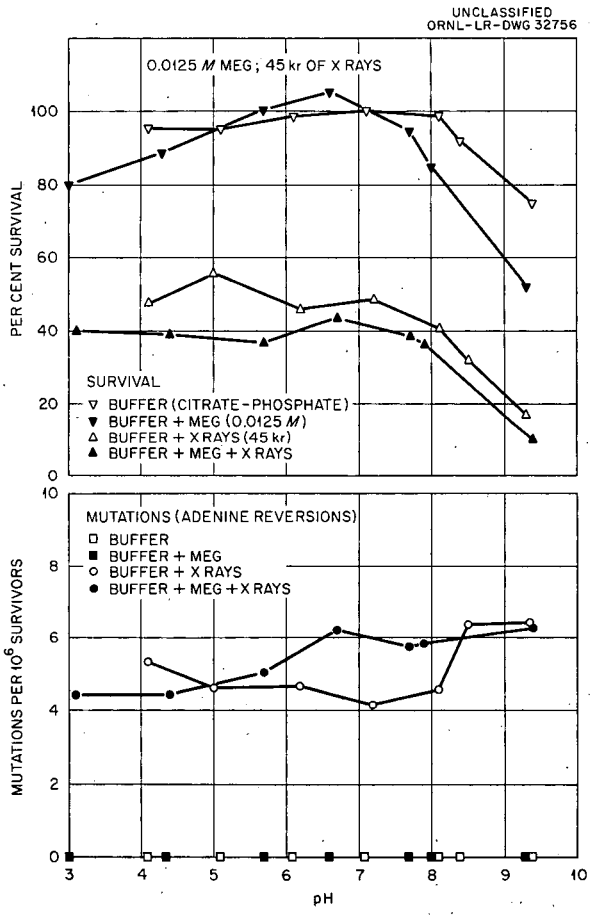

Fig. 6. Influence of pH on the Interaction Between 0.0125 M MEG and 45 kr of X Rays. Number of viable conidia treated at each point on curve, 56.2×10^6.

Looking at the adenine reversions, recorded as mutations per 10^6 survivors in the graph, it is seen that MEG per se does not raise the number of mutations above the spontaneous rate found in the control series.

When MEG is present during irradiation, it results in an increased number of mutations in the pH range between 5 and 8. This increase amounts to a maximum of *ca.* 45% over the rate obtained for X rays alone.

Discussion. – If the present results are considered in relation to experiments with animals, it is important to know that MEG exhibits a negligible cell toxic effect around pH 7. Furthermore, it is nonmutagenic by itself. On the other hand, the beneficial effect on survival found in experiments with animals after X-ray treatment in the presence of MEG is not found in *Neurospora.*

The increase in mutation rate found after the combined treatment in the present system, of course, is a rather specific one, as the adenine reversions can be considered as point mutations in just one locus. It remains to be seen whether this effect can be generalized in other reverse mutations and also in forward-mutation experiments.

A Temperature Mutant in Maize

D. Schwartz

This report deals with a newly found recessive gene which in the homozygous condition results in a high degree of chromosome breakage in maize endosperm. The breakage results from stickiness of sister chromatids during anaphase separation. This gene, designated st^e, is allelic to *st* on chromosome 4 (ref 20). The *st* allele causes stickiness and breakage in the sporophyte, giving rise to chlorophylless striations on the leaves; in the gametophyte, producing high pollen abortion; and in the endosperm, resulting in scarring and some variegation. The st^e allele differs from *st* in that the chromosome breakage is limited to the endosperm. Some stickiness is observed at anaphase 1 of meiosis; however, this does not result in breakage, since no excessive pollen abortion is found. The st^e/st^e sporophytes are completely normal in appearance. The endosperm is highly scarred, and in the appropriate genotype, heterozygous for endosperm markers, a high degree

[20] G. W. Beadle, *Z. indukt. Abstammungs- u. Vererbungslehre* 63, 195–217 (1933).

of variegation is observed. The st^e stickiness appears to involve sister chromatids, whereas with *st* the stickiness usually involves the whole nucleus. The st^e allele is dominant over *st*, with the st^e/st heterozygote being indistinguishable from st^e/st^e.

The mutant phenotype of $st^e/st^e/st^e$ endosperms was found to be expressed only when the plants were grown in the field in the summer. Plants of identical genotype grown in the greenhouse in the winter were of normal phenotype. The mutant phenotype was again expressed when the normal-appearing progeny seeds of the greenhouse crop were grown in the field the following summer. Thus the phenotypic differences reflect differential gene expression rather than mutation or any other permanent change.

The winter greenhouse growing conditions differ from those in the field in two main respects: shorter day and lower temperature. The st^e/st^e plants were grown in a light chamber that simulated the long day condition of the field (16 hours) and the low temperature of the greenhouse (20°C) in order to distinguish between a photoperiod and a temperature effect. The kernels produced were completely normal in phenotype. A sib plant was grown in the light chamber under the same conditions except that after pollination a heating pad was wrapped around the ear shoot, which raised the daytime temperature in that region of the plant to approximately 32°C. All of the kernels produced on this plant showed the extreme mutant phenotype. These experiments established that the st^e allele is temperature sensitive, with the mutant phenotype expressed only at the high temperature.

In collaboration with Dr. Harry Highkin of the California Institute of Technology a number of sib st^e/st^e plants were grown in the phytotron at identical photoperiods (16 hours daylight) but at four different temperatures (20°C day–14°C night, 23°C day–17°C night, 26°C day–20°C night, and 30°C day–24°C night). The results indicated that there is a direct correlation between temperature and the expression of the mutant phenotype, rather than a threshold effect.

The *st* allele as described by Beadle is not temperature sensitive. He reported no difference in the phenotype of plants grown in the greenhouse and in the field.

Ultraviolet-Induced Back-Mutation of Bacteriophage T4*r*

D. R. Krieg

Introduction. – After irradiation of extracellular bacteriophage T4 with ultraviolet light (UV) from a low-pressure mercury arc, progeny phage produced by multiple infection of unirradiated *Escherichia coli*, strain B, bacteria are found to include an increased frequency of genetic variants.[21] Further investigations of this phenomenon may be considered under three headings: the origin of the variants, the dose-response relationship, and the role of multiplicity reactivation in the yield of variants.

Evidence That the Variants Originate by Induced Mutation. – An increased frequency of r^+ phage was found only after treatment of T4r_{II} mutants which are capable of spontaneous reversion.[21] Although it was demonstrated that the conditions of treatment and subsequent growth did not increase the relative frequency of r^+ phage among a mixture of wild-type and r_{II} mutants,[21] there had not been a direct test of the possible selective enrichment of the r^+ revertants of the treated population. This test has now been performed, and it shows that the phenomenon cannot be attributed to selection.

A stock of T4r_{59} was irradiated with a dose of UV about 25 times the mean lethal dose (25 phage-lethal hits), and a progeny phage population with an increased frequency of revertants was obtained from a multiple infection. A stock prepared from this progeny population was irradiated with a similar dose of UV and again used for a multiple infection. As a result of this second treatment, the frequency of revertants per 10^8 phage increased from 52 ± 16 to 113 ± 3. This increase does not differ significantly from the increase (from 0.5 ± 0.6 to 52 ± 6 per 10^8) obtained with the original stock in an experiment run parallel with the second treatment. Since the increased frequency of revertants was independent of the frequency prior to treatment, the possibility of selection was ruled out, and the increased frequency is attributable to induced mutation.

Dose Dependence of Induced Mutation. – The revertant frequency was determined for progeny of

[21] D. R. Krieg, *Biol. Semiann. Prog. Rep. Feb. 15, 1958*, ORNL-2481, p 12.

$T4r_{59}$ exposed to various doses of UV from 8 to 40 phage-lethal hits. Without exception, a higher frequency of induced revertants was found at each higher dose. The exact shape of the dose-response curve has not yet been established, but the present data indicate that the yield of induced revertants from a given increment of dose is greater at the higher doses employed.

Role of Multiplicity Reactivation in Induced Phage Mutation. – After exposure of bacteriophage to the doses of UV employed in these studies, almost all of the phage particles are "inactivated," i.e., unable to reproduce on single infection of a bacterium. The progeny examined in these studies resulted from multiple infection, in which the inactivated particles can interact to produce viable phage. This phenomenon, called multiplicity reactivation, is attributed to the action and recombination of undamaged substituents among the lethally damaged phage.[22,23]

The question may be asked whether the proportion of induced mutants in a progeny population is simply a function of the UV dose or whether it is also influenced by other aspects of the treatment. Pertinent to this question are the results of an experiment in which a single preparation of irradiated phage was used for several parallel infections at different mean multiplicities ranging from 3 to 13 phage per bacterium. At the lower multiplicities, the number of progeny phage produced was much smaller, but they contained a higher proportion of induced revertants. This suggests that the yield of induced mutants is not only directly related to the UV dose but also inversely related to the efficiency of multiplicity reactivation. An attempt to clarify the basis of these relationships is in progress.

Effect of Localized Ultraviolet Irradiation on Timothy Roots

R. T. Brumfield A. H. O'Bier
M. E. Picinich

Two curvatures are induced in the growing point of the seedling root of timothy when exposed to ultraviolet unilaterally. A curvature (positive) toward the source appears soon after irradiation in the basal portion of the growing point. Later a curvature away from the source (negative) occurs in cells nearer the apex. Thus ultraviolet inhibits the growth of cells in the basal region while those near the apex are stimulated. Experiments were undertaken using localized exposures to determine whether or not these effects are on cells *in situ* or whether there is a transmission of the stimulus from the point of irradiation to cells some distance removed.

Preliminary experiments with roots grown on the surface of cover slips in a film of water showed that irradiation of the tip alone induced both the inhibitory effects and the stimulatory ones. In the research reported here, the roots were grown in an atmosphere saturated with water vapor. Irradiation of 150-μ sections (18.7 ergs/mm^2/sec, 4 min) of a region extending from 300 μ behind the apex to 1200 μ behind it consistently gave positive curvatures which were somewhat proximal to the section irradiated. This region is that generally referred to as the "region of elongation" where no cell divisions occur. Raying the apical 300 μ of the root, the "meristematic region," and the root cap gave very inconsistent and unpredictable results. Sometimes strong positive curvatures were induced, sometimes strong negative curvatures, and sometimes there was no response. The factors responsible for the variable effects of radiation on the meristematic region are being investigated.

INSECT CYTOLOGY AND GENETICS

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J. G. Carlson[24] P. A. Smith
A. R. Whiting[24] B. J. Stevens
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Haploid Intersexual Females in *Habrobracon*

R. C. von Borstel P. A. Smith

Introduction. – Unfertilized *Habrobracon* eggs develop into normal haploid males. Half of the males in a given stock are genotypically different from the other half in their sex allele constitution, for example, *xa* and *xb*, but are phenotypically

[22] S. E. Luria and R. Dulbecco, *Genetics* 34, 93–125 (1949).

[23] R. H. Epstein, submitted to *Virology*.

[24] Consultant.

[25] Research Participant.

[26] Graduate student, University of Tennessee.

[27] Summer employee.

indistinguishable. Femaleness is determined by heterozygosity of the sex alleles in fertilized eggs, *xa/xb*. Homozygosity of the sex alleles, *xa/xa* or *xb/xb*, following fertilization, gives rise to diploid males. There is, therefore, a complementary action of the two sex alleles dominant over the recessive maleness of either sex allele alone, and females are induced by this complementary action. The mechanism of sex determination in *Habrobracon* was worked out in detail by Whiting.[28]

Different types of sex intergrades have been observed from time to time in *Habrobracon*. These vary from weak feminization of males or masculinization of females to true gynandromorphs, individuals that are a composite of true haploid male and diploid female parts. The gynandromorphs usually come from eggs in which two meiotic products become pronuclei and one of them combines with a sperm. Occasional gynandromorphs arise from fertilization of an egg with two sperms, one of which combines with the egg nucleus. The gynandromorphs most commonly have a female abdomen and male thorax and head, or the reverse. Bilateral and fractional gynandromorphism also occurs. The various types of sex intergrades occurring in *Habrobracon* have been reviewed by Whiting and Starrells,[29] and the multiple causes of gynandromorphism have been discussed recently.[30]

Opportunities occasionally arise to analyze further the sex-inducing mechanisms in *Habrobracon*; in this case an interesting sex-intergrade mutant occurred.

Results. – The mutant was obtained in an experiment following irradiation of sperm. When F_1 females were tested unmated to determine heterozygosity for any mutations, one of the virgin females had 11 normal sons and 13 sex intergrades that resemble gynandromorphs with male heads and thoraxes and female abdomens. The intergrades showed linkage with the ivory allele (ivory-eyed males had been irradiated and crossed with wild-type females). This mutation had occurred twice previously, and Whiting and Starrells suggested that it might have been caused by a translocation involving the sex loci. Their hypothesis was essentially tested with this new recurrence, since hatchability of the eggs from the mother of the intergrades was recorded. If the mother had been heterozygous for a translocation, egg hatchability would have been depressed.[31] Hatchability was close to 100%. Whiting and Starrells also suggested that the brothers of the intergrades should be tested, since some of them might carry the genetic system necessary for producing the intergrades. In their case the males had been discarded. With this recurrence the males were all tested – no intergrades were produced in the F_2 generation.

Discussion. – The exact nature of these sex intergrades is unknown. With the negative test for translocations, the hypothesis of transposition of the sex alleles appears to be ruled out. Also, with each occurrence of this mutation, the cross was fortunately such that the mutation could be examined for linkage to the ivory locus. Linkage was observed each time. Since the sex alleles are not linked with the ivory gene, it seems unlikely that a transposition of sex alleles would occur to the same location the three times the "mutation" has occurred. The possibility exists that the mutation allowed true gynandromorphism to take place by letting two of the meiotic nuclei fuse and develop along with the haploid meiotic product rather than the fusion of the meiotic nuclei and later disintegration that normally occurs. In this circumstance fusion nuclei homozygous for the sex alleles should also occur unless the sex alleles were closely linked to the centromere. Males with diploid male abdomens did not occur, and there is no other reason to suspect that the location of the sex alleles is close to the centromere. It therefore seems most reasonable at present to assume that one genetic locus is responsible for the occurrence of this sex intergrade, and it also would appear that the intergrade is truly haploid.

[28] P. W. Whiting, *Genetics* 28, 365–82 (1943).

[29] P. W. Whiting and R. Starrells, *Am. Naturalist* 84, 467–75 (1950).

[30] R. C. von Borstel, in *The Beginnings of Embryonic Development*, A. Tyler *et al.* (eds.), p 175–99, AAAS, Washington, D.C., 1957.

[31] R. C. von Borstel and M. L. P. Rekemeyer, *Biol. Semiann. Prog. Rep. Aug. 15, 1957*, ORNL-2390, p 32–33.

Studies on *Lilium longiflorum*

H. V. Crouse

Irradiation of Meiotic Metaphase Chromosomes. – Previous studies on lily[32] showed that all the X-ray aberrations induced at first meiotic metaphase are half-chromatid exchanges. Scoring of the X-ray effects was done at anaphase, 2 hours after treatment, at which time some of the exchanges revealed themselves in the form of bridges. With dosages of 10, 15, and 30 r the frequency of half-chromatid bridges per cell was found to vary linearly with dose. Half-chromatid exchanges between chromatids separated at anaphase II instead of anaphase I were expected to result in bridge formation at the second division; preliminary study showed that this was indeed the case.

Extensive study of the second meiotic division in cells X-rayed at first metaphase has now been undertaken at four different doses: 5, 10, 15, and 20 r. For this analysis no anthers were used which contained stages older than MI; and anthers from the same plant were fixed at the end of 2 hours (for division I study) and also at the end of 16 hours (for division II study). Between the time of irradiation and fixation anthers were cultivated in Bonner's solution at 22°C. The results of these experiments may be summarized as follows:

1. The number of half-chromatid bridges per cell at AI and at AII varies linearly with dose; and the number of such bridges *per chromatid* is approximately the same at the two divisions.

2. The chromatids at second anaphase have nonstaining "gaps" along their length; the frequency of such "gaps" with dose has yet to be determined (material fixed but not scored). This will be an important determination because the section of chromatid distal to such a "gap" may represent a chromatid fragment produced by X-ray breakage at first metaphase of two half-chromatids belonging to the same chromatid. That this is the the correct interpretation seems likely from another line of evidence: chromosomes irradiated at second meiotic metaphase and examined 2 hours later at AII show no such "gaps."

Preliminary comparative study has been made on the relative sensitivity of MI and MII chromosomes. Cells given 20 r at MI show approximately 0.67 half-chromatid bridge per cell when scored 2 hours later. The same dose applied at MII produces only 0.25 half-chromatid bridge per cell when the scoring is done at AII 2 hours following irradiation.

Agents Affecting the Response of Meiotic Chromosomes to X Rays. – Cells in MI were irradiated *in vacuo* (doses of 5, 10, 15, and 20 r) to determine whether there is an "oxygen effect" on half-chromatid exchange. All anthers were fixed after 2 hours with a view to determining the frequency of half-chromatid bridge formation at AI. The scoring of these cells is far from complete, but preliminary observations suggest there is an "oxygen effect": more half-chromatid bridges are found at AI when the irradiation is done in air.

Experiments were carried out to determine whether chloramphenicol can appreciably alter the frequency of half-chromatid exchanges. Anthers were floated prior to irradiation in a solution of chloramphenicol (300 μg per ml) for various intervals of time ranging from 15 to 90 minutes. Postirradiation treatments were also used. Unirradiated anthers floated in the same chloramphenicol solution served as a control. Fixations were made with a view to scoring half-chromatid bridges at AI. None of this material has yet been scored.

Effect of Nitrogen Mustard on MI Chromosomes. – Anthers suitable for first division study were floated in freshly prepared solution (0.1%) of methyl bis(β-chloroethyl)amine hydrochloride for intervals of time varying from 1 hour to 3 hours. The cells were scored at AI. No half-chromatid bridges were observed, nor was there any other detectable cytological effect.

Studies on *Sciara coprophila*

H. V. Crouse

Egg and Embryo Studies. – Cytological studies were undertaken on oogenesis and cleavage for two reasons: (1) to determine whether a long X′ inversion, with break points very close to either end of the sex chromosome, included the centromere or was paracentric; (2) to observe the normal process of "limited" and sex chromosome elimination so that similar studies could be undertaken on material in which the X chromosome had been reciprocally translocated with one of the autosomes.

[32] H. V. Crouse, *Science* 119, 485 (1954).

The method worked out by von Borstel and Lindsley[33] for handling and staining insect eggs was used. Excellent results were obtained with Kahle's fixative followed by the Feulgen reaction. Gallocyanin proved unsatisfactory for *Sciara* eggs; for later embryonic stages it was fair but inferior to the Feulgen reaction.

The results of these cytological studies may be summarized as follows: (1) Dicentrics and acentrics are formed at meiosis in the X′ inversion material, but they are far less numerous than the dicentrics observed by Carson[34] in inversion material in *S. impatiens*. This oocyte study is useful in localizing the centromere on the sex chromosome in the salivary gland nuclei, where normally there is no chromocenter. (2) The elimination of both the "limited" chromosomes and the sex chromosomes during cleavage was observed and found to agree with DuBois' original description.[35] The actual elimination of the sex chromosome from the germ line (as described by Berry,[36] for *S. ocellaris*) has not been seen; this observation is far more difficult in *S. coprophila*, where the germ cells contain, in addition to the nine regular chromosomes, a variable number of "limited" chromosomes.

Induction of X Translocations. – A number of reciprocal translocations involving the X chromosome were obtained by irradiating adult males (4000 to 4500 r of X ray). The criterion for detecting the translocations was the occurrence in F_2 female families of exceptional patroclinous males. According to earlier studies such males result from irregular disjunction of sex chromosomes during oogenesis.[37] This method of diagnosis was found to be useful but not infallible; inversions and certain "invisible" changes on the X chromosome produced low percentages of exceptional patroclinous males.

Of 33 tentative "translocations" five proved to be viable X translocations, transmissible through both the male and female germ line, and potentially valuable for the cytogenetic analysis of sex chromosome behavior in this genus. The five translocations have been designated T1, T5, T7, T18, and T26. The following data have been collected on these translocations: (1) The points of translocation have been fairly accurately localized on the salivary gland chromosomes; from this study it is known that the translocation chromosomes in all five cases are distinguishable at metaphase on the basis of shape; thus all of the translocations will be useful in localizing the control of the precocity phenomenon observed regularly in *Sciara* at the second spermatocyte division. (2) The amount of aberrant disjunction at oogenesis shown by each translocation has been examined and found to be characteristic for each particular translocation. (3) The effect of each translocation on male developmental rate, viability, and fertility has been studied. Cytological examination of spermatogenesis has not yet been undertaken on any of the translocation material. (4) Preliminary tests have been run to determine whether any of the translocations is viable in homozygous condition and whether individuals heterozygous for two different translocations can be obtained. (5) In the case of T7 exceptional patroclinous sons have been derived from normal and from T7 males; the mothers in both types of cross were female-producing females heterozygous for T7. When normal males are used, the patroclinous sons are completely sterile; when T7 males are used, the patroclinous sons are partially fertile. Yet, after chromosome elimination during embryogeny, the two kinds of males are identical in genome; chromosomally they differ in that one is heterozygous for T7 and the other contains an untranslocated X and autosome.

Induction of Mutations in Oocytes. – Newly emerged adult females were found to be only partially sterilized by doses of X rays as high as 10,000 r. Among 30 F_1 male progeny examined from such an experiment, one wing variant was obtained, which, upon testing, proved to be an autosomal dominant.

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[33] R. C. von Borstel and D. L. Lindsley, *Stain Technol.* (in press).

[34] H. L. Carson, *Genetics* 31, 95–113 (1946).

[35] A. M. DuBois, *Z. Zellforsch. u. mikr. Anat.* 19, 595 (1933).

[36] R. O. Berry, *J. Morph.* 68, 547 (1941).

[37] H. V. Crouse, *Anat. Rec.* 94, 386 (1946).

DROSOPHILA GENETICS

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An Analysis of ABO Blood Group Combinations in Mother-Child Pairs

E. Novitski R. D. Owen

Introduction. – Data collected in Australia on the blood groups of mother-child pairs have been shown to be inconsistent with expectations based on the established system of inheritance of the blood groups.[42] The reason for the departure of the observations from the expectations is not known; a simple comparison of the two indicates that certain classes are in excess and others are deficient. It is difficult to evaluate the merits of any hypothesis designed to account for these deviations, for the reason that any assumption about the source of the deviations automatically affects the expectations because those expectations are themselves based on gene or genotypic frequencies derived from the data. The Oracle was programed to test for possible disturbing influences in the data by making the appropriate corrections in the expectations demanded by any hypothesis and by evaluating, with the method of maximum likelihood, the extent to which the data fit the model being tested compared with the fit obtained with other models.

The particular hypotheses tested included the assumptions (1) that abnormal segregation in heterozygotes, either male or female, was altering the frequencies of the zygotic types; (2) that of the fourteen possible mother-child combinations, individual classes were depressed in frequency, or that either children, or mothers, or both, of any of the four blood groups were depressed in frequency (viability effects); and (3) that consistent misclassification occurred of some one blood group for any of the other three, in either children, mothers, or both, or of any one mother-child combination for any other. In all there is a total of 250 simple hypotheses which can be tested individually, or in any combination with each other.

Results. – When the five genotypic frequencies are estimated, there remain eight degrees of freedom, and the best estimates of those frequencies, when algebraically introduced into the expectations for the fourteen mother-child combinations, give a chi-square of 33.62, indicating a very poor fit. The additional assumption that abnormal segregation is altering the observations does not lead to any improvement of the fit, nor has this assumption proved helpful in combination with other hypotheses. It has been rejected from further consideration.

Table 4 shows the factors by which the theoretical expectations of each of the fourteen classes would have to be multiplied to give a best fit, along with the standard errors of those estimates, and the resulting chi-square. Table 4 also shows the extents to which misclassification would have to occur for all possible combinations of the blood groups.

While there are a number of assumptions which reduce the chi-square appreciably, the most persistent effect appears to be that of the AB class, which is depressed in frequency. It appears in Table 4 as a significant depression in AB children of A mothers and as a deficiency of all AB individuals by misclassification. It is surmised that all these are related in that there is a deficiency of AB individuals, so that any hypothesis that will tend to increase the theoretical expectation of this class will provide a much better fit to the data. The greatest single reduction in chi-square biologically acceptable comes from the assumption that both mothers and children of group AB are being misclassified as B.

The arbitrary decision was made to assume that AB for B misclassification was a feature of the data and then to check the other classes individually to determine whether they reduced the heterogeneity in combination with this misclassification. In any one of three cases the chi-square is reduced to the point where one would expect as great or greater heterogeneity more than 50% of the times by chance alone (for 6 D.F., $P > 0.50$ when $\chi^2 < 5.35$). These cases include an 11% excess of A children of A mothers, a 17% depression of O children from A mothers, and an 8% depression of A children of O mothers.

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[38] American Cancer Society Fellow.

[39] Visiting investigator from abroad.

[40] Consultant.

[41] Summer employee.

[42] R. L. Kirk *et al.*, *British J. Prev. and Soc. Med.* 9, 104–11 (1955).

Table 4. Estimates of Viability and Misclassification Factors for Each Mother-Child Combination

Blood Group		Viability Factor	
Mother	Child	Estimate	Chi-Square
A	A	1.112 ±0.036	23.8
A	B	1.219 ±0.090	26.7
A	AB	0.758 ±0.059	20.5
A	O	0.782 ±0.046	17.8
B	A	1.159 ±0.093	30.3
B	B	1.125 ±0.065	30.2
B	AB	0.877 ±0.073	30.9
B	O	0.817 ±0.066	27.9
O	A	0.944 ±0.027	29.4
O	B	0.987 ±0.050	33.5
O	O	1.077 ±0.044	29.9
AB	A	1.027 ±0.083	33.5
AB	B	1.051 ±0.088	33.3
AB	AB	0.947 ±0.122	33.4

Misclassification	Estimate	Chi-Square
A as B	0.992 ±0.003	25.64
A as AB	1.013 ±0.003	19.63
A as O	1.038 ±0.010	19.24
B as A	0.991 ±0.015	32.91
B as AB	1.050 ±0.015	22.84
B as O	1.094 ±0.029	21.94
AB as A	0.872 ±0.030	16.89
AB as B	0.870 ±0.027	12.62
AB as O	0.957 ±0.036	32.61
O as A	1.004 ±0.009	33.38
O as B	0.993 ±0.004	30.94
O as AB	1.013 ±0.007	30.76

Discussion. – An advantage of computer analysis of problems of this sort is that a greater diversity of hypotheses can be subjected to machine computation and examined for their biological implications after many possibilities have been evaluated. In ordinary analyses the tendency to stop when an acceptable conclusion is reached often leaves many possible tests undone; some of these unknown answers may in fact be more significant than those accepted from more superficial analyses. Further, the rejection of hypotheses as inadequate when a large number are tested is in itself of some biological interest.

The present study has not yet quite arrived at the point of complete evaluation of the disturbances under consideration and their interactions, and it would therefore be premature to state a definitive biological conclusion here. It may be noted, however, that trends at hand in the analysis make biological sense. For example, selection against A children borne by O mothers on the well-known basis of A-B-O maternal-fetal incompatibility, combined with weaker reactivity of AB infant blood leading to infant misclassification, perhaps particularly through missed reactions with anti-A, may well provide an adequate interpretation of the data. Other reasonable biological bases for deviation may also be defined, and it remains quite possible that phenomena previously unsuspected may be pointed out by this analysis, to be established later by direct serological test procedures.

Intracranial Injections of Isologous Adult Bone Marrow and Spleen in Newborn Anemic (WW) Mice

D. L. Lindsley B. M. Blanton

Several types of inherited anemia have been described in the mouse. The dominant spotting genes, W and W^v, cause a macrocytic type of anemia. The WW type, first described by Little,[43] is a black-eyed, pale individual which develops to birth but dies at that time or shortly thereafter, while the heterozygotes (Ww) and their recessive (ww) offspring have normal hair pigmentation and normal erythrocyte counts. E. S. Russell, through inbreeding of a C57BL strain, has been able to increase the live birth rate and survival time to 20.7% and 11.6 days, respectively.[44] Russell and other investigators[45] have been successful in keeping a limited number of these severely anemic mice alive for a normal life span either by periodic transfusions or by intraperitoneal injections of embryonic liver into irradiated recipients.

[43] C. C. Little, *Am. Naturalist* 49, 727–40 (1915).

[44] E. S. Russell, *Genetics* 41, 658 (1956).

[45] J. W. Gowen and E. H. Gay, *Nature* 66, 289–300 (1932).

There is the possibility that newborn WW mice also could survive if inoculated intracranially with blood-forming tissues from adult ww donors. Six breeding pairs of the heterozygotes (Ww) and six ww mice were obtained from Russell for the purpose of testing this possibility.

Procedure used in these injections was as follows: within twelve hours after birth the anemic mice were inoculated intracranially with either spleen tissue cells or bone marrow cells. The count of the former was at least 10×10^6 cells per injection and of the latter, 2×10^6 cells per injection. Unfortunately, a number of the first litters were lost due to inexperience in this technique and/or the delicacy of the recipients.

Thirteen litters with seventy-six live births have been recorded within this two-month period. Fourteen (18.4%) of this number were judged to be anemic on the basis of their phenotype. Ten of these died soon after injection, one survived nine days, one survived fourteen days, and two are living at the present time (one born July 24 and the other August 8). The older mouse shows some indication of a successful implant. It has survived twenty-three days, twice the average life span for WW mice, and its present condition suggests that it may have a normal life expectancy. The average weight of the WW mice at birth was 1.5 grams compared to 3.0 grams for the Ww and ww litter mates. At present this survivor weighs 7.5 grams, while its litter mates average 9.0 grams. E. S. Russell[46] has published that the average erythrocyte count of newborn WW mice is 1.2×10^6 and that of the Ww and ww is 4.8×10^6. The erythrocyte level of this survivor is 2.6×10^6. This mouse, of course, is still severely anemic since the level of her litter mates is 7.6×10^6.

Although conclusions as to the success or failure of this experiment cannot be based on this single surviving mouse, the results indicated here are sufficiently suggestive to warrant further investigations along these lines.

Further Analysis of Y-Covered, Sex-Linked, Recessive Lethals in *Drosophila melanogaster*

D. L. Lindsley M. S. Moore

Introduction. – We previously reported (ORNL-2481, p 16–18) that the expression of approximately 20% of sex-linked lethals produced at 4000 r is suppressed by the Y chromosome and consequently missed by the Muller-5 technique. A sample of thirty-two Y-suppressed lethals has been divided into three groups: (1) six *bobbed* deficient lethals, (2) ten bb^+ X/Y fertile lethals, and (3) seventeen bb^+ X/Y sterile lethals.

Results. – We postulate that the lethals which are not in the proximal heterochromatin are variegation position effects, which have long been known to be much more extreme in X/0 than in X/Y males. To test this hypothesis the recovery of each bb^+ lethal in X/Y males with two different enhancers of variegation [*M(2)S10* and *E-Var 7*] was determined. It was found that *M(2)S10* reduced the recovery of five lethals, *E-Var 7* reduced the recovery of two, while the recovery of six others was reduced by both enhancers of variegation. Furthermore *y 1*/Y; *E-Var 7* males were found in several cases to exhibit exaggerated variegated phenotypes.

The salivary analysis of the lethal-bearing chromosomes is in progress and has been completed only for the bb^+, X/Y fertile class. Of the ten examined, five are pericentric inversions with one break in the proximal heterochromatin. The position of the distal break is as follows: No. 59 in region 3F-4A, No. 132 in region 4E, No. 146 in region 4D, No. 227 in region 1E-2A, and No. 231 in region 1C-1D. Two are insertions of what appears to be heterochromatic material into region 9A in No. 47 and into region 1B in No. 306. Number 11 is a T(1;4) broken proximally in chromosome 4 and in region 15A in the X. Number 139 is a pericentric inversion with the euchromatic break between 3C6 and 3C7. This inversion variegates for *rst* and *w* and requires two Y's for survival. Number 459 is a complex rearrangement in which the X seems to be broken in region 3D and in the right arm; chromosome 2 seems to be broken in the centromere and in region 49 or 50. It looks as though the middle piece of the X has closed to form a ring deficient for the terminal region, and the middle piece of chromosome 2 has been capped distally by X^R and proximally by the distal piece of X, and remaining fragments of chromosome 2 (i.e., $2R^D$ and 2L) have rejoined. If this is correct it indicates that the centromere region of chromosome 2 has been split into two functional centromeres.

Discussion. – Suppression by the Y chromosome of bb^+ lethals has led to the supposition that they are variegation position effect lethals. This is

[46] E. S. Russell, *J. Hematol.* 6, 892–905 (1951).

supported by four additional characteristics of these lethals: (1) all so far examined are rearrangements in which a heterochromatic region is brought into contact with euchromatin of the X; (2) where testable, females homozygous for the lethal may survive in the absence of the Y chromosome; (3) under suitable genetic conditions, many exhibit variegated phenotypes; and (4) in many cases the survival as lethal/Y males is decreased by enhancers of variegation.

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

G. E. Brosseau, Jr.

Introduction. – In an attempt to obtain a clearer picture of the genetic organization of heterochromatic genes, an analysis of the fertility region of the Y chromosome was undertaken. Y chromosomes were irradiated, and Y's that had lost their ability to confer fertility on males carrying them were isolated. These sterile Y chromosomes were then investigated genetically.

Results. – The sterile Y chromosomes were tested for allelism by combining them two at a time in a male carrying a normal X chromosome (an $X/Y^{st}/Y^{st}$ male). These males were then tested for fertility. If the males are sterile it is assumed that the two Y chromosomes carry a common deficiency, while if they are fertile then the chromosomes are deficient at different loci. In the case of the short arm of the Y, two fertility factors were revealed by this method. Table 5 gives the results of the crosses involving deficiencies for factors on the long arm of the Y. Deficient Y's that behaved the same in all crosses are grouped in the same column. All members of a group were intersterile. These data indicate that there are seven fertility factors on the long arm of the Y (designated k_1–k_7). The genotype of each deficiency is given below the appropriate column of the table. These data also indicate that the genes are probably linearly ordered. Evidence that k_1 is the most proximal of the genes is obtained from tests with detachment products from an attached-X chromosome, where the detachment involves an exchange with the Y chromosome. Some of the detachments carry a duplication for some of the fertility factors, the most proximal factor being the most likely to be present. Deficiency No. 13 is the only one of those tested that is covered by all of the detachments carrying fertility genes, and since No. 13 is deficient for only k_1, k_1 must be the most proximal factor.

The analysis of the sterile Y's was complicated by the fact that reciprocal combinations of a given pair of sterile Y's did not always behave identically. This was found to be due to the presence of an unmarked fertile Y in the test males (all of the sterile Y's carry the marker y^+ at the end of the long arm). It has been possible to demonstrate that these fertile Y's are the result of gonial exchange between the sterile Y and another marked fertile Y that is used in making up the test males. This type of gonial exchange apparently occurs with a much higher frequency than other types of gonial exchanges, e.g., between autosomes.

Two other properties of the sterile Y's remain to be completely analyzed. Some of the Y's exhibit a high degree of sterility when combined in a male with a normal marked Y. This property has been designated "dominant" sterility. This sterility is less extreme when the attached X-Y is used instead of the normal Y. In this respect the sterility is similar to that observed in males carrying three normal Y's vs two normal Y's and the attached X-Y.[47] The other property is that of partial fertility. A partially fertile culture is one in which the number of progeny from the test mating is vastly smaller (usually around two to five flies) than that from other fertile test matings. This type of fertility generally shows nonreciprocality and is reproducible. About one male in fifty is partially fertile, the remainder being completely sterile. The cause of this type of fertility is not yet known, but it occurs in males carrying sterile Y's that are deficient for at least two common genes.

Spermiogenesis has been examined in males carrying a normal X and a sterile Y. In all cases spermiogenesis appears to be normal; however, no motile sperm are ever produced.

Discussion. – The genetic organization of the Y chromosome is particularly interesting in that the Y is wholly heterochromatic. The evidence presented here indicates that the fertility region of the Y is composed of a linear array of unitary factors, and in this respect it is not different from the way in which we think euchromatic genes are organized. It has not been possible to make a distinction between the fertility genes phenotypically, except for the cases of "dominant" sterility.

[47] K. W. Cooper, *Genetics* 41, 242–64 (1956).

Table 5. Results of Tests of Allelism Between Y Chromosomes Deficient for One or More of the Fertility Factors on the Long Arm*

		No. of Sterile Y Obtained from the Female												
		13, 25, 29, 33	37	41	12, 23	3, 28, 34	36	11, 20, 27	38	7	8, 14, 18, 19, 24, 39	10, 32	1, 5, 9, 15	4
No. of Sterile Y Obtained from the Male	13, 25, 29, 33	X	F	F	F	F	F	F	F	F	F	–	F	–
	37	F	X	F	–	F	F	F	F	F	F	–	–	–
	41	F	F	X	–	F	F	–	–	F	–	–	–	–
	12, 23	F	–	–	X	F	F	–	–	–	–	–	–	–
	3, 28, 34	F	F	F	F	X	–	F	F	–	–	F	–	–
	36	F	F	F	F	–	X	F	–	–	–	F	–	–
	11, 20, 27	F	F	–	–	F	F	X	–	–	–	–	–	–
	38	F	F	–	–	F	–	–	X	–	–	–	–	–
	7	F	F	F	–	–	–	–	–	X	–	–	–	–
	8, 14, 18, 19, 24, 39	F	F	–	–	–	–	–	–	–	X	–	–	–
	10, 32	–	–	–	–	F	F	–	–	–	–	X	–	–
	1, 5, 9, 15	F	–	–	–	–	–	–	–	–	–	–	X	–
	4	–	–	–	–	–	–	–	–	–	–	–	–	X
	Fertility Factor													
	k_1	–	+	+	+	+	+	+	+	+	+	–	+	–
	k_2	+	–	+	–	+	+	+	+	+	+	–	–	–
	k_3	+	+	–	–	+	+	–	–	+	–	–	–	–
	k_4	+	+	+	–	+	+	–	–	–	–	–	–	–
	k_5	+	+	+	+	+	+	–	–	–	–	±	–	–
	k_6	+	+	+	+	+	–	+	–	–	–	+	–	–
	k_7	+	+	+	+	–	–	+	+	–	–	+	–	–

*F = fertile; – = sterile; X = all intergroup combinations were sterile; + = indications of fertility.

In this latter regard, three identical deficiencies (Nos. 11, 20, and 27) all show this property. While gonial recombination has been observed for most of the sterile Y's, it has not yet been observed for members of the group deficient for k_1 (Nos. 13, 25, 29, and 33). This observation suggests that this factor may be spatially separated from the rest of the genes, lying much closer to the centromere. Recombination has also been observed in Y short.

In addition to providing evidence as to the genetic organization of heterochromatic genes, the chromosomes that have been produced provide valuable tools for the analysis of the heterochromatic content of special chromosomes such as

compound X's and detachments of attached X chromosomes.

Determination Mosaics from Combined Implanted Imaginal Discs in *Ephestia*

H. Kroeger

Introduction. – Stern[48,49] showed that in genetic mosaics the different phenotypic effects of certain mutations can be brought about by different responses of the genotypically different cells to the same unaltered general organization. This has been called the prepattern of the developing system; it was of great interest to know whether, besides genetically differing ones, tissues which differ in their developmental determination may react in the same way.

Results. – The development of a microsurgical method by which it was possible to combine different pieces of tissue in microscopic dimensions made it feasible to combine fore- and hindwing tissue of imaginal discs of *Ephestia* as well as wing tissue with those of the genital discs. The compound organs were subsequently implanted into the body cavity of a host and their development was studied.

The first combination yielded 25 survivors out of 65 implants. In 12 of these the borderline between the fore- and hindwing tissue crossed the region of those sclerites that form a comparative morphologic unit. In all these cases there were no intermediate structures formed; each part developed in its gross morphology according to its situation on the implant but in its details according to the determinative state of the respective tissue.

The combination of the wing with genital-disc tissue did not lead to joint fields consisting of two types of tissues; each part of the joint implant developed morphogenetic fields of its own exclusively on one type of tissue. However, the mounting of genital-disc tissue prevented the wing disc from regenerating another symmetric wing.

Discussion. – These results show that distinctly diverse structures may be brought about by an alteration of the competence of certain tissues to respond to the same prepattern. Since the failure to produce joint fields consisting of tissues as different as those from wing and genital discs may be due to incompatibilities between these tissues, it is possible that the diversity of structures all over the body is produced by the different response of tissues, which have become diversified during development, to one kind of prepattern. This type of developmental mechanism has been proved here for the more closely related organs such as fore- and hindwing.

The identical way in which mutated and differently determined tissues express their properties suggests that the determination process leads to changes in the genetic content of cells similar to some extent to those brought about by mutational events.

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[48] C. Stern, *Caryologia*, Suppl. to vol 6, p 355–69 (1954).

[49] C. Stern, *Wilhelm Roux' Arch. Entwicklungsmech. Organ.* 149, 1–25 (1956).

MAMMALIAN GENETICS AND DEVELOPMENT

GENETIC AND DEVELOPMENTAL EFFECTS OF RADIATION IN MICE

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Radiation-Induced Mutation Rates in Female Mice

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Information on radiation-induced mutations in female mice has remained far below the level attained for males as early as 1951.[6] The reason has been that adult female mice given acute X-ray doses comparable to those used in our experiments with males become permanently sterile after bearing only one or two litters. Thus, in order to obtain, in experiments with females, a total number of offspring equivalent to that from experiments with males, a much larger number of animals has to be irradiated.

In spite of this difficulty it was feasible, and seemed important, to conduct an experiment that would at least go far enough to determine whether females showed any indication of a much higher sensitivity to mutagenesis than males. Such an experiment, using acute X irradiation, was consequently carried out at an early date, 1949–51. More recently, a much larger investigation has been started with chronic gamma irradiation, following the demonstration that the sterilizing effect on females can be minimized by administering the dose at a low intensity.[7,8] The present paper will discuss mutation rate data from both the acute X-ray and chronic gamma-ray experiments, the latter of which is still in progress.

The data from the acute X-ray experiment are presented in Table 6. This investigation was carried out in two parts. In the experiment proper (1950, 1951), adult wild-type F_1 hybrid females from a cross of 101 × C3H strains were exposed to 400 r of X radiation (250 kvp; 15 ma; inherent filtration 3 mm of Al; hvl 0.4 mm of Cu; dose rate

[1]Consultant.

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[4]Summer employee.

[5]Biology Student Trainee.

[6]W. L. Russell, *Cold Spring Harbor Symposia Quant. Biol.* 16, 327–36 (1951).

[7]L. B. Russell and M. K. Freeman, *Anat. Rec.* 128, 615–16 (1957).

[8]L. B. Russell and M. K. Freeman, *Radiation Research* 9, 174–75 (1958) (abstr.).

Table 6. Mutations at Specific Loci in Female Mice Exposed to Acute X Radiation

Experiment	Dose (r)	Number of Offspring	Number of Mutations at Locus						
			a	*b*	*c*	*d*	*p*	*s**	*se*
1949	300	498	–	–	–	–	–	–	–
	400	760	–	–	–	–	–	–	–
	500	48	–	–	–	–	–	–	–
1950–51	0	5845	–	–	–	–	–	–	–
	400	1729	–	–	–	–	–	1	–

*See text for qualification concerning 1949 experiment.

96 r/min) and mated to males of our T stock which is homozygous for seven recessive marker genes. Only one mutation at any of the specific loci was obtained in 1729 offspring of irradiated females. No mutations were found in 5845 young born to control, unirradiated females (Table 6, 1950–51 experiment). In a still earlier pilot study, no specific locus mutations had been found (1949 experiment). At this earlier time the test stock had not been fully developed, and some of the test males used in this experiment, although homozygous for the marker genes at six of the loci, may not have been homozygous at the seventh (*s*) locus. The mutation rate of 12×10^{-8} per roentgen, per locus, obtained by combining the data of the 1950–51 and the 1949 series, and its 95% confidence limits of 0.3×10^{-8} and 68×10^{-8}, may, therefore, be slightly biased, but since this bias is small compared with the sampling error, it will be ignored in the following discussion.

Although the estimate of the mutation rate is based on only one mutation and, therefore, has the wide confidence interval given above, the results obtained were nevertheless adequate to answer the question posed: they ruled out the possibility of females having a much greater sensitivity to radiation-induced mutagenesis than that found in males. Thus, the upper 95% confidence limit of the female mutation rate is less than three times the acute X-ray-induced mutation rate of 25×10^{-8} per roentgen, per locus, originally reported in males,[6] or the rate of 28×10^{-8} later found in males exposed to 300 r.[9] Therefore, it could be concluded that calculation of the average risk of equal exposure of both sexes on the basis of the far more reliable mutation rate obtained in males would almost certainly not underestimate the hazard from acute X rays by more than a factor of 2. Of course, such a calculation is actually more likely to overestimate the hazard, since the point estimate for females is below that for males and the lower 95% confidence limit of the female rate is very much below that. However, it is obvious that a calculation based on the correct male rate can never overestimate by more than a factor of 2, no matter how low the true rate in females is.

The limited experiment on females was, then, sufficient to show that if the more reliable mutation rate in males is used to calculate risks, any error introduced by not allowing for a difference between the acute X-ray-induced rates in males and females is likely to be well within the limits of other uncertainties in the estimation of genetic hazards in man. Consequently, the obtaining of more accurate information on specific locus mutation rates in females was postponed until recently, and the use of the specific locus method for the exploration of the many other factors that enter into the estimation of genetic hazards[9,10] was pursued in males.

In the interval between the early specific locus experiments in females and the present ones to be described below, various other genetic effects in females were investigated in experiments that do not require large numbers and are not hampered by radiation-induced sterility. These studies[11–14] dealt with chromosome aberrations, particularly dominant lethals and translocations, which had been virtually unexplored in the female. They had been the subject of much work in the male, where it was shown that they are probably not a major hazard because the incidence, following irradiation of spermatogonia (the cell stage that is important in human hazards), was found not to be significantly different from that in controls. Dominant lethals could conceivably have turned out to be a hazard in the female, where oogonia are not present in the adult,[15] and all germ cells are in primary oocyte stage. However, it was shown that death due to dominant lethals occurs very early in embryonic development.[11] Translocations, which might also have proved to be a hazard in females, were shown to be induced with only low frequency.[14]

Concurrently with the work on chromosome aberrations, experiments were conducted to determine whether there were conditions of radiation

[9] W. L. Russell and L. B. Russell, *Proc. Second Intern. Conf. Peaceful Uses Atomic Energy, Geneva, 1958* (in press).

[10] W. L. Russell, L. B. Russell, and E. F. Oakberg, Chap. 8 in *Radiation Biology and Medicine* (ed. W. D. Claus), Addison-Wesley Publ. Co., Inc., Reading, Mass., 1958, p 189–205.

[11] L. B. Russell and W. L. Russell, *Cold Spring Harbor Symposia Quant. Biol.* 19, 50–59 (1954).

[12] L. B. Russell and W. L. Russell, *Progress in Radiobiology* (ed. by J. S. Mitchell, B. E. Holmes, and C. L. Smith), p 187–92, Oliver and Boyd, Edinburgh, 1956.

[13] L. B. Russell, *Anat. Record* 125, 647–48 (1956).

[14] L. B. Russell and L. Wickham, *Genetics* 42, 392 (1957).

[15] E. F. Oakberg, *Proc. Tenth Intern. Congr. Genet.* vol 2, p 207, 1958.

that would minimize the sterilizing effect and so provide a more practical method for measuring specific locus mutation rates in females. Results from such investigations on fertility, taken together with the studies of dominant lethals and ovarian histology, aid not only in the design of experiments but also in the interpretation of work on genetic effects of radiation. Early experiments indicated that the radiation effect on female productivity was lessened by increasing fractionation of X-ray dose.[7,16] More recent work shows that chronic gamma irradiation is even less sterilizing and that there is clearly an inverse relationship between dose rate and total young subsequently produced.[8] An example of the relative effectiveness of acute X rays, fractionated X rays, and chronic gamma rays is provided by the following data from the females used in our experiments: after receiving 300 r acute X rays, a female produces, on the average, 7.6 young before sterility sets in; with the same total dose given in five 10-r fractions per week, she produces 13.7 young; if about the same weekly dose (47.2 r per week) is, instead, administered continuously as chronic gamma radiation, females produce on the average 72.7 and 59.1 young for total doses of 256 and 342 r, respectively. A dose distribution that is compatible with long-continued fertility is not only of practical advantage in experiments but also tests the situation that is potentially most hazardous in humans, namely, that in which a high total dose is absorbed with minimal interference in transmission of genetic damage to descendants. On the basis of these results and considerations, the present specific locus experiment was started.

In this experiment (101 × C3H)F_1 young adult females were exposed to 258 r of gamma radiation from a Cs^{137} source at an intensity of 86 r per week. After removal from the radiation field they were mated with T stock males and the offspring were examined for mutations at the seven specific loci. The results to date, including those from control females, are given in Table 7. Only two mutations have occurred, one at the *b* locus and the other, presumably a deficiency, involving simultaneous loss of the phenotypic effects of the closely linked *d* and *se* loci.

[16] R. Rugh and J. Wolff, *Fertility and Sterility* **7**, 546–60 (1956).

Table 7. Mutations at Specific Loci in Female Mice Exposed to Chronic Gamma Radiation

Dose (r)	Number of Offspring	Number of Mutations at Locus							
		a	*b*	*c*	*d*	*p*	*s*	*se*	*d se*
0	29,739	–	–	–	–	–	–	–	–
258	22,234	–	1	–	–	–	–	–	1

The induced mutation rate of 5×10^{-8} per r per locus (95% confidence interval: 0.6×10^{-8} to 18×10^{-8}) is significantly lower than the rate of 28×10^{-8} per r per locus already cited for irradiation of males at the comparable dose level of 300 r of X rays delivered at 83 r per minute.[9] In addition to sex, there are two other obvious differences between these experiments on males and females, namely, intensity and quality of radiation. The weight of genetic evidence from other organisms indicates that neither of these factors is likely to be of much importance, although the difference in quality between the X and gamma radiation used might be expected to have a slight effect. Thus, on the basis of the evidence considered so far, it would be tempting to conclude that the low mutation rate obtained in the experiment with females is due to sex. However, preliminary data from males exposed to chronic gamma radiation from the same Cs^{137} source also show a low mutation rate[17,9] which, as far as it goes, is not significantly different from the female mutation rate.

Two alternative hypotheses may be formulated on the basis of these findings. According to the first hypothesis, the low mutation rate with chronic gamma irradiation is due to the same factor in males and in females. This could be either the lower intensity or the quality of radiation, although the weight of earlier genetic evidence is against both factors. According to the second hypothesis, the low rates in males and females are due to different causes. Thus, the rate in males irradiated with chronic gamma rays may be low due to indirect factors, to be discussed below, while in females, where such indirect factors cannot be easily invoked, the low rate may be characteristic of the sex or of the oocyte stage.

[17] W. L. Russell and E. M. Kelly, *Science* **127**, 1062 (1958).

Possible indirect factors that may affect the mutation rate measured following irradiation of spermatogonia have been discussed elsewhere.[9,10] They may be summarized briefly by saying that it is possible that the low mutation rate observed in chronic gamma experiments with males may have resulted from death of sensitive cells in the testis. The marked variation in sensitivity of spermatogonia to killing by irradiation,[18,19] and the observed actual reduction in mutation rate in an experiment with a high dose (1000 r) of acute radiation,[20,9] may both be taken as support for such a hypothesis. If this is the answer, then the low mutation rate observed in the chronic irradiation experiments with males would be a peculiarity of the levels of intensity and dose used. With males at lower doses or intensities, where the killing of spermatogonia or other interference with the dynamics of spermatogenesis was reduced to on unimportant level, one would expect, on this hypothesis, a mutation rate as high as, or even higher than, that found with acute irradiation. As has been pointed out elsewhere,[9,17] since this hypothesis is at least plausible, it would be incautious to conclude at the present time that chronic gamma irradiation of males will, at all doses and intensities, be less effective than acute X irradiation.

However, it seems unlikely that an indirect factor of the type discussed for males would apply to the present data on females. Since oogonia do not occur in the adult ovary,[15] the chronic irradiation will be received by oocytes, which, furthermore, for the bulk of the dose will be in the remarkably uniform dictyate state. These oocytes are, therefore, quite different from spermatogonia, which are going through rapid cycles of mitosis and consequently might be expected to show marked differential sensitivity to radiation. Thus, it is reasonable to predict that the low mutation rate per roentgen observed in the present data may hold for other doses and intensities of chronic gamma radiation in females. It also seems reasonable to assume that this information can be carried over to humans.

Summary. – Mutation rates in females have been measured by the specific locus method in two experiments.

A relatively small experiment with acute X rays, at a dose which rapidly sterilizes females, was nevertheless sufficient to show that, if the average risk of equal exposure of both sexes to acute X rays is calculated on the basis of the far more reliable rate determined in males, the hazard will almost certainly not be underestimated by more than a factor of 2.

An experiment with chronic gamma rays, which allowed almost normal fertility of the females, has to date given a rate of 5×10^{-8} per r per locus with a 95% confidence interval of 0.6×10^{-8} to 18×10^{-8}. This rate is significantly below that of 28×10^{-8} per r per locus found in males irradiated with a similar dose of acute X radiation. Possible reasons for the lower rate are discussed. Whatever the explanation turns out to be, the results indicate that the mutation rate in women exposed to chronic gamma radiation may be less than that estimated from our earlier yardstick, namely, the mutation rate obtained from acute X irradiation of male mice.

Effects of Chronic Gamma Irradiation on the Testis

E. F. Oakberg E. B. Blalock

Experiments on the histological effects of irradiation from a Cs^{137} source have been initiated in order to obtain information required for interpretation of the effects of chronic gamma irradiation on fertility[21] and induction of mutations.[22]

At present, data have been completed for the group receiving 282 rad at a rate of 0.521 rad/hr. Mice were killed 0, 6, 12, 18, 24, 48, and 72 hours and 5 and 10 days after removal from the field. Histological procedures used were the same as described previously.[23]

Spermatids and spermatocytes were greatly reduced in number in all mice, resulting in tubule shrinkage and decrease in testis weight to about 50% of control from 0 through 72 hours. Further decreases to 41% at 5 days and 32% of control at 10 days were observed.

[18] E. F. Oakberg, *Radiation Research* 2, 369–91 (1955).

[19] E. F. Oakberg, *J. Exptl. Zool.* 134, 343–56 (1957).

[20] W. L. Russell, *Genetics* 41, 658–59 (1956).

[21] W. L. Russell and E. M. Kelly, *Biol. Semiann. Prog. Rep. Feb. 15, 1958*, ORNL-2481, p 21.

[22] W. L. Russell and E. M. Kelly, *Science* 127, 1062 (1958).

[23] E. F. Oakberg, *J. Exptl. Zool.* 134, 343–56 (1957).

The number of Sertoli cells was used to correct spermatogonial counts for the bias introduced by tubule shrinkage. At the end of irradiation, type A spermatogonia were 39% of control; they remained at this level through 48 hours, then increased to 51% at 72 hours, 55% at 5 days, and to 70% of control at 10 days. Thus the number did not fall as low as after 300 r of acute X rays.

Since spermatogonia are in the intermediate and type B stages for only about 48 hours, they would have to be extremely sensitive for the dose received during these stages to result in the reduction of cells observed. Other possibilities are that cells damaged earlier in the cycle of spermatogonial multiplication degenerate as they reach late type A or intermediate stages, or that the rate of differentiation of type A spermatogonia into intermediate spermatogonia has been retarded by chronic irradiation.

The incidence of necrotic type A cells was 1.6% in controls, and with the exception of the 1.9% observed at 24 hours, ranged from 5.6% to 11.4% in the irradiated mice. Thus in terms of permanent survival, the experimental/control ratios for normal spermatogonia given above are maximum estimates. Furthermore, it is interesting that the rate of degeneration does not return to control levels as rapidly as after a comparable acute exposure.

Mitosis of spermatogonia was occurring at all intervals, and some cells in all stages of development were present, indicating that spermatogenesis was occurring but at a much reduced rate. In addition to the change in number of differentiating cells, more subtle effects on spermatogenesis were suggested by the greatly increased frequency with which different spermatid stages were observed in the same tubule cross section.

Recombination Experiments Between Pseudoallelic Recessive Lethals at the *Notch* Locus

W. J. Welshons E. J. McCrone

Previous work on the *Notch* locus has demonstrated the pseudoallelic relationship of three recessive visible mutants with two or more dominant *Notch* mutants.[24,25] Furthermore, it has been demonstrated that two of the dominant *Notches*, which may be thought of as recessive lethal mutations, are separable by crossing over and can be referred to as two mutants comprising a pseudoallelic recessive lethal system.[25] A third dominant *Notch* (N^{22}) mutant arose spontaneously on a chromosome which already carried the pseudoallelic mutant *facet* (*fa*). Crossover experiments localized this mutant to right of the pseudoallelic loci *facet* (*fa*), *facet-notchoid* (fa^{no}), and *split* (*spl*),[25] so that one could tentatively localize the three dominant *Notches* with reference to *split* in a linear order as follows: $Notch^{264\text{-}40}$ ($N^{264\text{-}40}$), *split* (*spl*), $Notch^{22}$ (N^{22}), and $Notch^{Co}$ (N^{Co}). In order to establish this tentative linear order of dominant *Notches* with reference to each other it would be necessary to construct heterozygotes of $N^{264\text{-}40}/N^{22}$ and N^{22}/N^{Co} and obtain by crossing over N^{+} chromosomes that were also recombinant for closely linked markers on either side of the *Notch* locus.

In order to perform this experiment, females which were $y\ w^a\ fa\ +\ N^{22a}$ +/+ $w^a\ +\ N^{264\text{-}40}\ +\ rb$; $Dp\ w^+\ N^+$ were mated to $w^a\ fa^{no}\ spl\ rb$ males. If the linear order of two of the *Notches* is correctly represented above, it should be possible to recover the recombinant chromosomes $y\ w^a\ fa\ +\ +\ rb$ in the next generation. Eight such recombinants were found out of a total of 72,556 tested chromosomes (0.02% recombination). It can, therefore, be concluded that N^{22} is localized to the right of $N^{264\text{-}40}$. In order to test for the localization of N^{22} to the left of N^{Co}, the heterozygous females $y\ w^a\ fa\ N^{22}\ +$ +/+ $w^a\ +\ +\ N^{Co}\ rb$; $Dp\ w^+\ N^+$ were crossed to $w^a\ fa^{no}\ spl\ rb$ males. One would expect to find $+\ w^a\ +\ +\ +\ +$ recombinants in the next generation; however, no recombinants have yet been found in approximately 97,394 tested chromosomes. Thus, these two *Notches*, both of which are known to be localized to the right of the recessive mutant *split*, are inseparable to date, and might possibly be allelic.

Although it is possible that N^{22} and N^{Co} might eventually be separated by crossing over, the fact that no reversions of N^{22} have yet been detected, while several reversions of $N^{264\text{-}40}$ and N^{Co} have been found,[25] suggests that N^{22} might be a minute deficiency localized between the pseudoalleles *split* and $Notch^{Co}$. With the genetic tools available for the study of this locus, it should be possible to gather pertinent information concerning this possibility.

[24] W. J. Welshons, *Proc. Natl. Acad. Sci. U.S.* 44, 254 (1958).

[25] W. J. Welshons, *Cold Spring Harbor Symposia Quant. Biol.* (in press).

MICROBIAL PROTECTION AND RECOVERY

RADIATION PROTECTION AND RECOVERY IN BACTERIA

A. Hollaender A. S. Angel
G. E. Stapleton A. M. Perry[1]
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Radiation-Induced Hydrogen Peroxide Sensitivity in *Escherichia coli* H_7–

H. I. Adler G. E. Stapleton

Introduction. – *Escherichia coli* H_7 grown without hemin (H_7–) is sensitized to H_2O_2 by ionizing radiation.[3] That is, survivors of any dose of radiation are more rapidly inactivated by H_2O_2 than are unirradiated controls. This sensitization phenomenon is of basic interest because it may help toward an understanding of the means by which ionizing radiation inactivates cells. The experiments described in this report are directed toward an understanding of the factors that influence the development and maintenance of the H_2O_2-sensitive state. In every case, incubation and irradiation were at 25°C.

Results. – If H_7– cells are irradiated to the 2% survival level in the presence of $Na_2S_2O_4$ (104 kr) and then exposed to 2×10^{-5} *M* H_2O_2, the rate of the aftereffect is greater than that observed for cells irradiated to this same survival level in air (26 kr) (Fig. 7). The "protected" cells are actually very sensitive to H_2O_2. Apparently, the damage that leads to development of the sensitive state accumulates as a function of dose even in the presence of a protective agent.

If irradiated H_7– cells are held in the absence of H_2O_2, there is very little inactivation. Cells stored in phosphate buffer and reintroduced into H_2O_2 at intervals after irradiation demonstrate full sensitivity. There is no decay of the sensitive state by cells stored in phosphate buffer up to one hour (Table 8).

The medium in which cells are grown influences the degree of their sensitivity displayed after irradiation. If glucose is autoclaved separately and then added to peptone-phosphate water used for growth of the H_7– cells, the rate of inactivation in H_2O_2 is about one-third that observed for cells grown in medium in which the glucose is not autoclaved separately. A role of chelating agents in the sensitization phenomenon is implied by these data since such agents are known to be formed when glucose is autoclaved in the presence of phosphate.

If irradiated H_7– cells that have reacted with H_2O_2 are plated on a medium containing hemin, survival is considerably better than if they are plated in the absence of hemin. The dose-reduction factor is approximately 2. This observation indicates that the inactivation brought about by reaction with H_2O_2 is not immutable and, like the prompt inactivation brought about by irradiation, is influenced by plating conditions.

Observations of the aftereffect for long post-irradiation periods are complicated by secondary reactions but are nevertheless necessary for an understanding of the phenomenon. There are certain inherent difficulties in making observations on suspensions of washed cells in phosphate

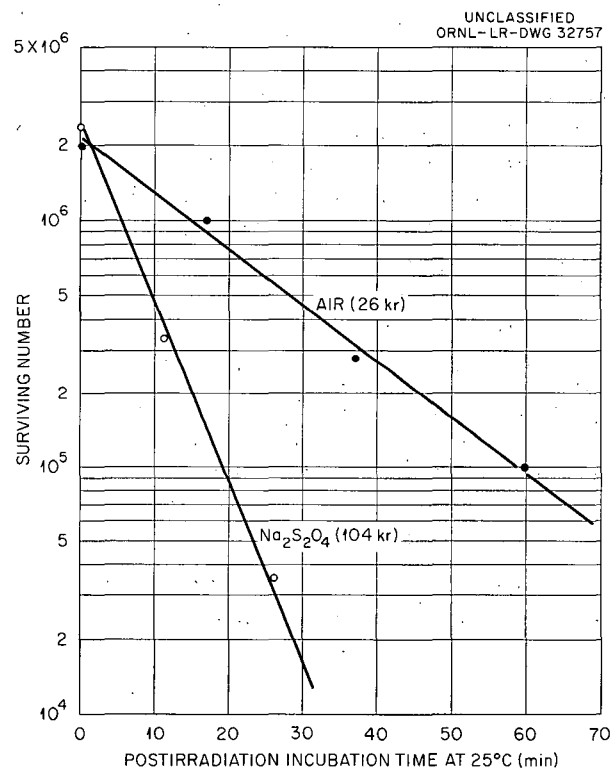

Fig. 7. Aftereffect for Protected and Unprotected H_7– Cells.

[1]Summer employee.

[2]Biology Student Trainee.

[3]H. I. Adler and G. E. Stapleton, *Biol. Semiann. Prog. Rep. Feb. 15, 1958*, ORNL-2481, p 28.

Table 8. Lack of Decay of Sensitization to Lethal Action of H_2O_2 (at 25°C)

Holding Time (min) Between Irradiation and Treatment with H_2O_2	Number of Cells Surviving at Various Reaction Times with H_2O_2		
	0	20 min	40 min
0	1.8×10^7	4×10^6	1.5×10^6
20	1.65×10^7	5×10^6	1.7×10^6
40	2.0×10^7	1.7×10^6	5.0×10^5
60	1.75×10^7	2.0×10^6	7.0×10^5

buffer over periods of hours at 25°C. Nevertheless, it is possible to demonstrate that unirradiated cells are relatively resistant to H_2O_2 for at least two hours, whereas survivors of an X-ray dose are rapidly inactivated (Fig. 8).

Discussion. – We are not able, at present, to explain the mechanism by which the catalase-negative cells of H_7 are rendered sensitive to H_2O_2. We have, however, elaborated some of the factors that must be carefully controlled in order to make meaningful quantitative studies of the phenomenon. We have established that (1) sensitivity accumulates as a function of dose even in the presence of a protective agent, (2) it is retained for relatively long periods after irradiation, and (3) it can be influenced by manipulation of the growth conditions before and after irradiation.

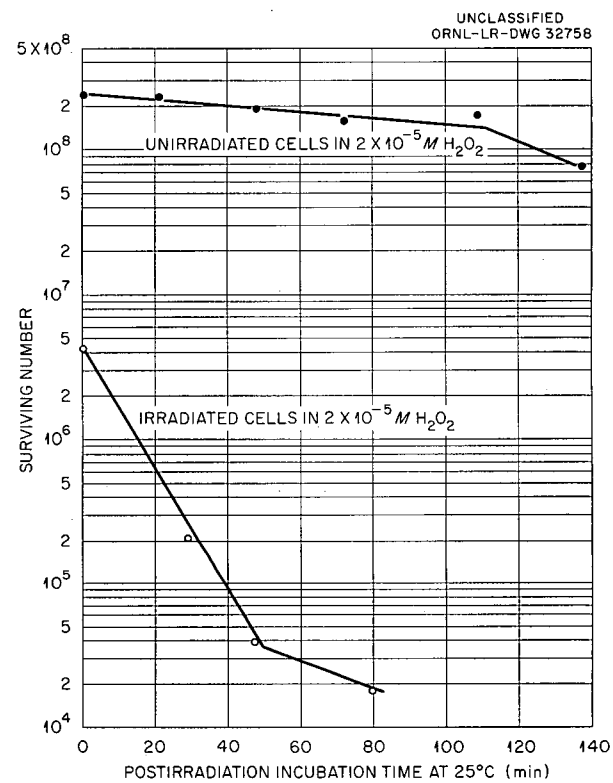

Fig. 8. H_2O_2 Sensitivity of Unirradiated and Irradiated H_7– Cells.

Nutritional Studies on *Escherichia coli* H_7

G. E. Stapleton H. I. Adler A. M. Perry

To date this strain of *E. coli* has not been successfully cultured on a chemically defined medium.[4,5] For a number of physiological and

[4]M. Beljanski, *Compt. rend.* 240, 374 (1955).

[5]H. I. Adler, *Biol. Semiann. Prog. Rep. Aug. 15, 1957*, ORNL-2390, p 46.

mutation studies it is necessary to know the absolute nutritional requirements of the bacterium. It is equally important to establish whether the absolute requirements of the organism are different in the presence and absence of hemin.

The following medium was adapted from Ravel's medium,[6] which supports growth of *Lactobacillus*.

Component	Final Concentration (mg/ml)
K_2HPO_4	12.5
KH_2PO_4	12.5
$MnSO_4 \cdot 4H_2O$	0.0004
$MgSO_4 \cdot 7H_2O$	0.08
NaCl	0.0004
$FeSO_4 \cdot 7H_2O$	0.0004
NH_4Cl	0.60
Sodium acetate	1.2
Glucose	10.0

[6] J. M. Ravel *et al.*, *J. Biol. Chem.* 206, 391 (1954).

The preceding components make up the basal medium; the complete medium contains, in addition, 15 amino acids, vitamins, and purines and pyrimidines.

Strain H_7 grows well in the complete medium but will not grow in the basal medium. In the presence of hemin, the organism will grow in the basal medium if cysteine is supplied. Cysteine may act by establishing the correct oxidation-reduction potential for initiation of growth. In the absence of hemin, this organism has more-elaborate requirements. Apparently, no vitamin or purine and pyrimidine supplementation is needed, but several amino acids are required. Whether or not the absolute amino acid requirements are those expected by the fermentative nature of H_7 grown without hemin will have to await further critical experiments.

It is interesting to consider the kinds of genetic information one could obtain with relative ease with such a system; e.g., (1) H_2O_2 production of mutations in a sensitized cell, (2) the frequency of back mutations to independence of the several amino acids, and (3) reversion from hemin dependence to independence.

MAMMALIAN RECOVERY

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C. C. Congdon
D. B. Duda
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L. H. Smith
I. S. Urso

The Major Efferent Lymphatic Vessels and Trunks in the A_1 Mouse

D. B. Asher

Introduction. – To understand regional immune phenomenon requires knowledge of the lymph vessel connections between lymph nodes. Antigens from skin homotransplants, for example, are thought to go first to the regional lymph node, then to the next node in the chain. In this study a 27-ga. needle attached by a polyethylene tube to a motor-driven syringe containing pontamine blue dye was placed in nearly every lymph node of the mouse. The motor was started, and the dye was slowly injected into the lymph node. Forty-five etherized mice were studied but not every lymph node in each animal. The course of the dye as it left the node was observed, and dissections of the lymphatic system were made to show where the dye went.

Results and Discussion. – Figure 9 shows the most commonly encountered major efferent lymphatic

[1]Summer employee.
[2]Biology Student Trainee.
[3]Consultant.

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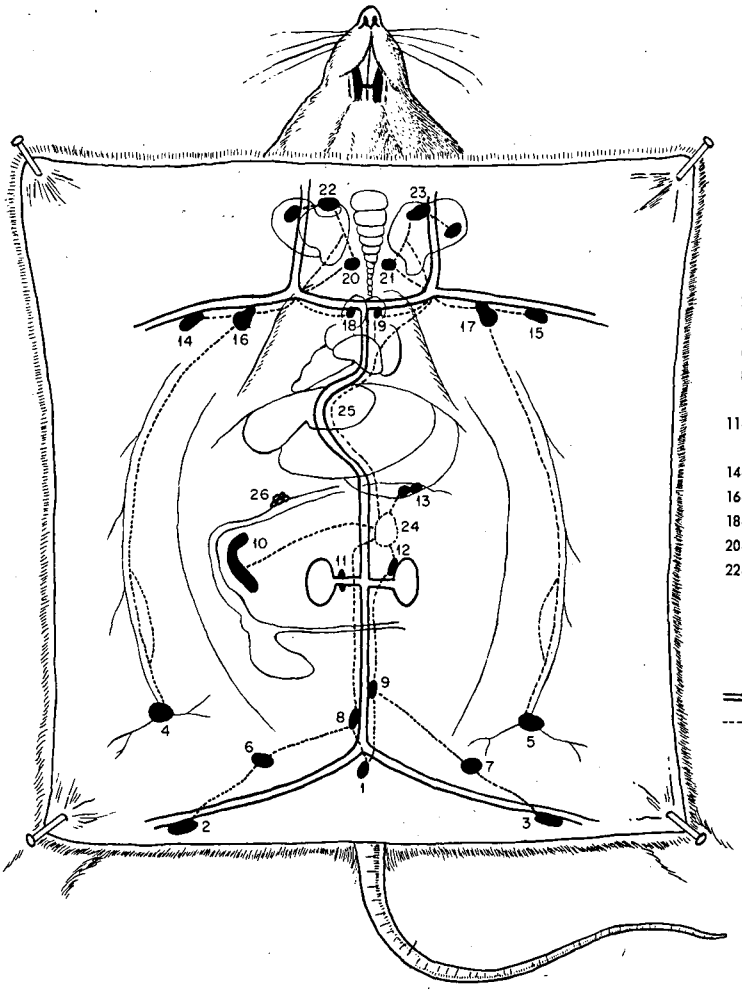

Fig. 9. Gross Anatomy of Lymphatic System of the Adult Mouse. Diagrammatic sketch showing lymph nodes and their major connections in the A_1 mouse (redrawn and modified from Dunn).

vessels. The Peyer's patches had no efferent lymphatic vessels. It is also known that they have no afferent lymphatic vessels. This is possibly the reason why this lymphatic structure does not regularly participate in the foreign bone marrow reaction as do other lymphatic tissues.

Effect of Cell Dose of 101 Bone Marrow on 30-Day Survival of Irradiated (C57BL × 101)F_1 Mice

M. D. Bard

Introduction. – Data obtained by van Bekkum and Vos[4] indicated that the bone marrow cell dose requirements in their irradiated F_1 mice receiving parental marrow were equivalent to cell dose requirements in isologous experiments. When the parent was irradiated and given F_1 marrow, the cell dose requirements for 30-day survival were much greater, as were those in homologous bone marrow experiments. In the present work a parent-to-F_1 combination was studied that could subsequently be used for red blood cell repopulation because of the favorable genetic circumstances.[5] Such circumstances are desirable in bone marrow cell separation experiments.[6]

Results. – Figure 10 shows the 30-day survival versus the number of 101 bone marrow cells injected intravenously into (C57BL × 101)F_1 mice after 900 r. Sixty-five irradiated mice were used for each cell dose.

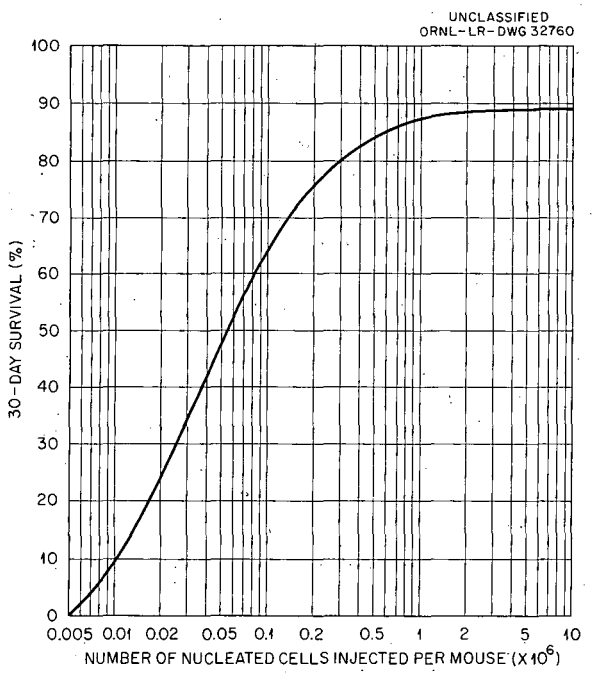

Fig. 10. Effect of 101 Bone Marrow on 30-Day Survival of Lethally Irradiated (C57BL × 101)F_1 Mice.

Discussion. – These results confirm in part the findings of van Bekkum and Vos[4] on a different parent-to-F_1 genetic combination. The survival versus cell dose curve in the present work is essentially the same as that seen earlier in the isologous (C57BL × 101)F_1 bone marrow experiment.[6]

Effect of Partial Removal of Lymphatic Tissue on the Skin Homograft Reaction in Normal Mice and on the Heterologous Bone Marrow Reaction in Lethally Irradiated Mice

R. R. Bigelow

Introduction. – Studies by Gengozian *et al.*[7] of the immune status of mice surviving the foreign bone marrow reaction for extended periods of time indicated a poor immune response to sheep red blood cells. Autopsy studies on the mice showed greatly reduced amounts of lymphatic tissue. The sequel to the foreign bone marrow reaction in the node is usually fibrous atrophy. This and other information suggested that surgical removal of lymphatic tissue might influence immune response. Skin homografting was used as a test system because of its great sensitivity.

Results. – In a preliminary experiment the effect of splenectomy on long term survival of lethally irradiated mice given rat bone marrow was determined. Nineteen mice splenectomized two to six hours before irradiation and marrow injection showed no improvement in long term survival. Neither was there improvement in long term survival in ten mice splenectomized eight days before treatment.

In another preliminary experiment more extensive lymphatic tissue removal was carried out followed by skin homografting one week after surgery. Lymph node removal was facilitated by giving the mice an intravenous injection of pontamine blue to stain the nodes prior to surgery.

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

[5] R. D. Owen, *Radiation Research* 9, 164 (1958) (abstr.).

[6] J. W. Goodman, *Biol. Semiann. Prog. Rep. Aug. 15, 1957*, ORNL-2390, p 53–54.

[7] N. Gengozian *et al.*, *Proc. Natl. Acad. Sci. U.S.* 44, 560 (1958).

Ten mice were splenectomized, and immediately thereafter their popliteal, inguinal, brachial, axillary, and superficial cervical lymph nodes were removed. The average weight of all lymph node tissue removed was 56 mg. In two normal mice weighing 25 and 29 grams the total weight of stainable lymphatic tissue excluding thymus and spleen but including Peyer's patches was 122 mg and 251 mg, respectively. Ten operated (101 × C3H)F_1 mice were given skin isografts and LAF_1 homografts seven to nine days after surgery. The homografts sloughed at the same time as nonoperated controls, and the isografts all remained intact. No gross evidence of local regeneration of excised lymph nodes was observed.

Discussion. – The removal of spleen and one-half or less of the total lymph node tissue mass, including those lymph nodes that drain the skin graft site, failed to lengthen the survival of skin homografts put on about one week after surgery. These studies suggest either that the tissue transplantation antigens got to residual lymphatic tissue through the blood stream or that the interrupted lymphatic vessels quickly rejoined to allow the antigens access to residual lymphatic tissue in the lymph node chain.

Early Changes in the Spleens of Lethally Irradiated Mice Given Foreign Bone Marrow

C. C. Congdon

The numbers of homologous and heterologous (rat) bone marrow cells needed to promote 30-day survival of lethally irradiated (950 r) mice are considerably higher than when isologous bone marrow cells are used. There is a tissue reaction in the white pulp of the spleen of mice treated intravenously with foreign bone marrow that may be related to the prompt destruction of many of the injected foreign bone marrow cells. The reaction does not occur with isologous bone marrow.

The reaction in the bone marrow-treated mice consists of a swelling of the white pulp at 24 hours after irradiation when compared to the irradiation control animals. The swelling is great enough in some instances to cause a change in spleen weight.

Infiltration of the white pulp by granulocytes and mononuclear cells was present at 24 hours. The reticular cells of the white pulp were swollen. At 48 hours the reticular cells were very prominent and often had basophilic material in the cytoplasm. At 72 hours the granulomatous reaction was subsiding, and at 96 hours the white pulp was essentially similar to that seen in X-ray control spleens. In some mice many plasma cells were present in the white pulp at the end of the reaction.

Rat plasma and serum did not produce the reaction under the conditions tested. Rat liver, guinea pig spleen and bone marrow, hamster bone marrow, and horse serum produced the reaction. At low X-ray exposures (500–600 r) the reaction after rat bone marrow was prolonged, but at high exposures (2500 r and greater) the reaction did not occur.

The evidence suggests that this is a host-determined response to rat and other foreign antigens.

Tissues Capable of Conferring Adoptive Immunity to Rat Bone Marrow Transplantation in the Irradiated Mouse

D. B. Duda

Introduction. – There are two ways of interfering with the therapeutic action of rat bone marrow on supralethally irradiated mice. One method is to presensitize the mice to rat tissue antigens.[8] The other consists in injecting isologous mouse lymph node or thymus into the treated mice.[9] The latter technique is called adoptive immunity.[10] Spleen cells are also very effective in producing adoptive immunity. In the present study the ability of many tissues to confer adoptive immunity was demonstrated. Since blood cells are very effective in conferring adoptive immunity it is conceivable that blood contained in the organs may be responsible rather than the fixed components of the effective tissues. The actual mechanism of action has not been determined.

Results. – Table 9 gives the data obtained in these experiments on (C57BL × 101)F_1 mice given a standard dose of rat bone marrow after exposure to 900 r. The isologous test tissues were injected intraperitoneally.

Discussion. – In addition to the mechanisms of action mentioned in the introduction there is the

[8] T. Makinodan, N. Gengozian, and J. F. Albright, *Ann. N. Y. Acad. Sci.* (in press).

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

[10] N. A. Mitchison, *J. Cellular Comp. Physiol.* 50, Suppl. 1, 247–64 (1957).

Table 9. Tissues Capable of Conferring Immunity

Isologous Tissue Tested	Amount Injected Intraperitoneally (mg)	No. of Mice Dead at 30 Days over No. Irradiated and Injected with Rat Bone Marrow
None		22/40
Lymph node	10	15/15
	20	10/10
Spleen	1	10/10
	5	15/18
	10	9/9
Thymus	10–17	14/14
	20–26	16/16
Blood	0.05 ml	5/5
	0.1 ml	10/10
	0.25–0.5 ml	25/25
Liver	10	10/10
	20	7/7
	80	4/4
Lung	10	8/10
Thigh muscle	10	9/10
Bone marrow	r.15	9/27

possibility of isologous tissues inducing recovery of injured antibody-forming cells somewhat like the effect reported by Taliaferro[11] from yeast autolyzates on antibody response in irradiated rabbits.

Red Blood Cell Repopulation by Plethorized Marrow

J. W. Goodman

Introduction. – Bone marrow nearly completely devoid of maturing red blood cell elements was produced in mice by artificial polycythemia. It was as effective on a nucleated cell dose basis as whole normal bone marrow in promoting survival of lethally irradiated mice. A study of the red blood cell repopulation of recoverying mice given plethorized marrow was made by agglutination and hemolytic techniques.[12]

[11]W. H. Taliaferro, *J. Cellular Comp. Physiol.* 50, Suppl. 1, 1–26 (1957).

[12]R. D. Owen, *Radiation Research* 9, 164 (1958) (abstr.).

Results. – The homologous bone marrow experiment was done [LAF_1 marrow into lethally irradiated $(C3H \times 101)F_1$ mice] with normal and plethorized donor material from the femur and tibia. Plethora was produced by six injections per mouse over an eight-day period of at least 0.2 ml per injection of whole, heparinized, isologous blood. Marrow was taken for use in doses of 10 million cells per recipient (unless otherwise noted) on the day following the last blood injection, and tissues, including the sternum from each animal, were taken for histological examination. Isologous and X-ray control groups were included in each experiment.

In all cases recipients of normal marrow showed 100% donor (LAF_1) cells by day 18, and in one experiment by day 14. Cell doses within the range of 5 to 50 million normal nucleated marrow cells were found not to alter the rate of repopulation.

Plethorized marrow recipients reached the 100% donor (LAF_1) level, at a much slower repopulation rate, by day 35.

Individual microhematocrit values were obtained on the treated mice. Recipients of plethorized marrow had consistently higher hematocrits than did corresponding recipients of normal marrow. This difference in hematocrit values progressively decreased as repopulation proceeded, but normal hematocrits had not been reached by most of the survivors tested at 60 days.

The grafted homologous erythropoietic systems persisted in all cases tested (including three survivors tested at 116 days), and no reversions to host red blood cell type were seen.

Discussion. – A very rapid red blood cell repopulation seems to be of no advantage in recovery to the homologously treated irradiated mouse. Those animals given plethorized marrow repopulate very slowly but survive equally well. Moreover, the slower the repopulation, the less acute the depression of hematocrit within the first month after irradiation. The mechanism for this is not understood. Host cells seem to disappear abnormally fast in the recipient of normal marrow, although the animal is quite anemic; whereas host cells persist in the less anemic, slowly repopulating recipient of plethorized marrow.

Serotonin Levels in Tissues from X-Irradiated Mice Given Isologous or Heterologous (Rat) Bone Marrow

G. W. Royal

Introduction. – Serotonin (5-hydroxytryptamine), a vasoconstrictor, is widely distributed in animal tissues. In high concentrations it is a toxic substance and has been shown to be involved in anaphylaxis.[13] The present study was made to determine if levels of this metabolite in mice that received supralethal, total-body exposure to X rays were significantly different from those in animals treated with isologous or rat bone marrow.

A modification of the isolation procedure introduced by Udenfriend,[14] followed by a colorimetric measurement suitable for assays of 1–16 μg of serotonin, was used. The weighed tissues were (1) homogenized in cold borate buffer, pH 10, (2) extracted with normal butanol and freed of protein by NaCl saturation, (3) acidified with HCl, (4) esterified with *n*-butanol, (5) simultaneously extracted with ethyl acetate and formate buffer, pH 4.5. The formate buffer layers were used for colorimetric measurements. The decoloration of a solution of alkaline potassium ferricyanide is proportional, within workable limits, to the amount of serotonin in each sample. Optical density measurements were made at 410 mμ on a Coleman Junior spectrophotometer.

Results. – For each tissue, isologous bone marrow (IBM) treatment either maintained or elevated the serotonin level when compared with the normal. Rat bone marrow (RBM) reduced the level below that of the normal (Table 10). Experiments at one- and two-week intervals showed that serotonin levels in each of the five tissues were as low as those found in the rat bone marrow animals at three weeks.

Discussion. – BDF_1 females that receive a supralethal dose of X radiation experience a significant drop in serotonin level within the first week of the postirradiation period, regardless of type of bone marrow treatment. Those mice that receive isologous bone marrow, however, apparently resume the metabolic pathway compatible

[13] T. Waalkes *et al.*, *J. Clin. Invest.* **36**, 1115–20 (1957).

[14] S. Udenfriend, H. Weissbach, and C. Clark, *J. Biol. Chem.* **215**, 337–44 (1955).

Table 10. Serotonin Levels in Tissues from BDF_1 Female Mice Three Weeks After Irradiation and Bone Marrow Treatment

Treatment	Body Weight (g)	Serotonin Levels (μg per gram body weight)					
		Brain	Lung	Liver	Kidney	Spleen	Total
900 r + IBM	16.5	1.9	2.7	16.1	3.7	1.9	26.3
None	20.7	2.3	1.5	9.9	1.9	1.6	17.2
900 r + RBM	15.2	0.5	0.7	4.2	0.6	0.5	6.5

with maintenance of a normal serotonin level in brain, lung, liver, kidney, and spleen. The capacity for this resumption seems lacking in mice that receive rat bone marrow. Additional studies have been started in an effort to determine why the rat bone marrow treatment affects the postirradiation serotonin level in this manner.

Erythrocyte Survival Studies in the Rat

L. H. Smith

Introduction. – Estimates of erythrocyte survival in the rat varying from 4 to 100 days were obtained by isotope or hemodynamic methods.[15] Using a strain of rats that segregate for C and D red cell antigens,[16] simultaneous Cr^{51} labeling and differential agglutination[17] techniques were used to obtain estimates of erythrocyte survival in the rat.

Results. – The disappearance curves of radioactivity and C-type cells are shown in Fig. 11.

[15] C. E. Hall, J. B. Nash, and O. Hall, *Am. J. Physiol.* 190, 327–29 (1957).

[16] R. D. Owen, *Genetics* 33, 623–24 (1948).

[17] T. T. Odell, Jr., *et al.*, *Ann. N. Y. Acad. Sci.* 64, 811–25 (1957).

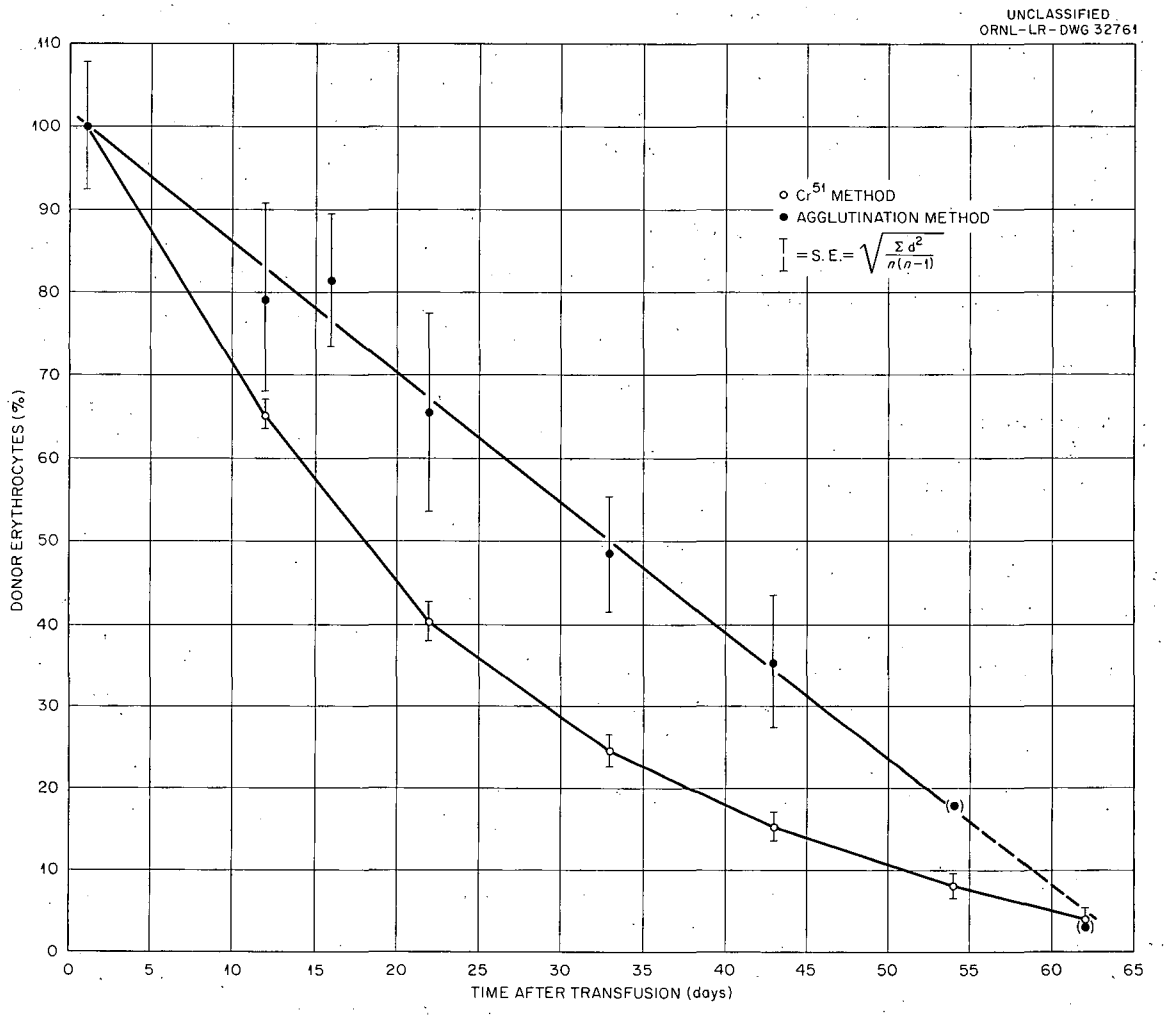

Fig. 11. Survival of Rat Erythrocytes Simultaneously Determined by Agglutination and Cr^{51} Labeling Techniques. Ordinate values for the Cr^{51} curve were calculated as a percentage of the 24-hour radioactivity of labeled cells; values for the agglutination techniques refer to the percentage (× 2.98) of donor erythrocytes in circulation. Agglutination points in parentheses are estimates based on observations in the counting chamber rather than on numerical counts. Counting of C and D cells in artificial mixtures showed that 2% C cells was just detectable; when agglutination was stronger but still not countable (<10%) the value of 5% was arbitrarily assigned.

According to the results from the Cr^{51} method, the erythrocytes disappeared in a curvilinear manner, while the curve obtained from the agglutination technique indicated a linear disappearance of the C erythrocytes. The extinction points, however, were essentially the same (viz. ~62 days) for both methods.

Discussion. – It has been pointed out that a linear disappearance curve will be obtained in the absence of factors that produce random destruction of erythrocytes.[18] According to the present data, therefore, random destruction of rat erythrocytes normally is not operative, and the curvilinear disappearance of Cr^{51}-labeled erythrocytes is probably associated with *in vivo* elution of the isotope from the cells. The results indicate that the extinction point obtained from the Cr^{51} method provides a reliable estimate of the life span of rat erythrocytes.

Long-Term Survival of Lethally Irradiated Mice Given Fetal and Newborn Tissues from Homologous and Heterologous Donors

I. S. Urso

Introduction. – Additional data on the long term survival of homologous and heterologous recipients of fetal and newborn tissues were obtained.[19]

Results. – Cumulative mortality is shown in Tables 11 and 12. A histological study was made on serially killed mice at frequent intervals 1–60 days after treatment with fetal or adult homologous blood-forming tissues. Fetal-liver-treated mice showed nearly normal recovery of lymphatic tissues. Adult bone marrow treatment resulted in extreme atrophy. Transplanation of homologous donor red blood cell percursors was demonstrated by hemagglutination and lytic methods[20] in homologous fetal liver and homologous one-day newborn liver recipients tested at 2–4 months after treatment.

[18] G. S. Eadie and I. W. Brown, Jr., *Blood* 8, 1110–36 (1953).

[19] I. S. Urso, *Biol. Semiann. Prog. Rep. Feb. 15, 1958*, ORNL-2481, p 34.

[20] R. D. Owen, *Biol. Semiann. Prog. Rep. Feb. 15, 1958*, ORNL-2481, p 40–43.

Table 11. Effect of Homologous (101 × C3H)F_1 and (101 × C3H)F_2 Donor Blood-Forming Tissue on the Mortality of Irradiated (900 r) LAF_1 Mice

Cell doses, 30×10^6 to 65×10^6 unless otherwise indicated

Donor Tissue	Number of Mice	Mortality (%) at Day		
		30	60	90
Adult bone marrow	112	53	86	93
Fetal liver	119	10	22	28
Fetal spleen*	11	18	45	45
Newborn spleen (1 day)**	36	19	25	28
Newborn spleen (2–6 days)	53	64	92	94
Newborn liver (1 day)	32	9	19	25
Newborn liver (2–4 days)	33	39	79	82

*0.7×10^6 to 12×10^6 cells.
**7×10^6 to 17×10^6 cells.

Table 12. Effect of Heterologous (Sprague-Dawley) Donor Blood-Forming Tissue on the Mortality of Irradiated (950 r) (101 × C3H)F_1 Mice

Cell doses, 80×10^6 to 180×10^6 unless otherwise indicated

Donor Tissue	Number of Mice	Mortality (%) at Day		
		30	60	90
Adult bone marrow	101	50	76	81
Fetal spleen*	10	80	100	100
Fetal liver	161	57	83	88
Newborn spleen**	25	68	80	84
Newborn liver (1 day)	29	34	79	83

*8×10^6 to 50×10^6 cells.
**10×10^6 to 60×10^6 cells.

Heterologous experiments with larger numbers of mice did not show improvement in survival with injected fetal or newborn tissues beyond that obtained with adult rat bone marrow. Newborn one-day liver gave good survival at 30 days, but mortality increased as in the other heterologous groups at 60 and 90 days.

Discussion. – The survival results presented here are in agreement with other reports[21–23] that fetal blood-forming tissues circumvent to some extent the foreign bone marrow reaction. In some genetic combinations, however, fetal blood-forming tissues were less effective.[24]

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[21] C. C. Congdon and I. S. Urso, *Am. J. Pathol.* 33, 749–67 (1957).

[22] D. W. H. Barnes, P. L. Ilbery, and J. F. Loutit, *Nature* 181, 488 (1958).

[23] D. E. Uphoff, *J. Natl. Cancer Inst.* 20, 625–32 (1958).

[24] R. D. Owen (this report).

RADIATION IMMUNOLOGY

T. Makinodan

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Primary Antibody Response to a Closely Related Heterologous Antigen After Varying Doses of X Radiation, and Its Significance to Related Biological Phenomena

T. Makinodan N. Gengozian

Introduction. – Previous studies[3-6] on rate of recovery of the immune mechanism of mice after exposure to varying doses of X radiation and AET-irradiation, and irradiation–bone-marrow treatment led to the following hypothesis: There could exist either multiple types of antibody-producing cells or an antibody-producing cell with the ability to recognize varying degrees of antigenicity; i.e., homologous, closely related heterologous, and distantly related heterologous antigens. The degree of destruction by X rays would increase in the order of mention. A study on the antibody response of mice to sheep red blood cells (RBC), a distantly related heterologous antigen, during the maximum depression period after varying doses of X radiation was recently reported.[7] A comparable study with rat RBC, a more closely related heterologous antigen, was undertaken to gain a better insight to the relative radiosensitivity of the recognition mechanism of antibody-producing cells.

Results. – The immune status of these mice injected with RBC antigen 24 hours after X irradiation was based on the following criteria: (1) induction period, (2) primary rate of appearance of circulating antibodies, (3) mean peak titer, and (4) mean total titer. The data obtained and reported previously for the sheep RBC antigen[7] have been tabulated along with data of the present study with rat RBC antigens to facilitate analysis of the radiosensitive mechanism of antibody-producing cells with respect to the two different antigens. In terms of the four criteria stated above, normal adult mice respond to sheep RBC antigen equally as well as to rat RBC antigen (Table 13). After varying doses of X radiation to the total body there is a curvilinear relation between radiation dose and antibody response, and the degree of difference between the two antigens is dependent on the index of antibody response. In terms of the induction period, no difference between the two antibody responses is seen up to the 500-r level; but beyond this level there is a definite lag in the induction period of the antirat response. The primary rate of appearance of circulating antibodies was not strikingly different at three of the five X-ray dose levels. Significant differences were noted at the 300- and 500-r levels in the mean peak titer, and at the 100-, 300-, 500-, and 710-r levels in the mean total titer. Except for the slight difference in the primary rate of appearance of circulating antibodies at the 100- and 300-r levels, the difference in immune response was in favor of the more distantly related sheep antigen and increased with increasing X-ray dose.

Transplantation of IBM immediately after irradiation did not affect the induction period when either antigen was employed.

Discussion. – The results are compatible with the hypothesis presented earlier; the radiosensitive mechanism of antibody-producing cells of normal adult mice can differentiate and respond to closely and distantly related antigens with equal effect, but irradiation damage to the mechanism for recognizing and responding to the more closely related antigen is greater than to that for a distantly related antigen.

The use of RBC antigens, admittedly a heterogeneous population of immunochemically distinct

[1] Research Participant.

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

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

[5] T. Makinodan, I. C. Shekarchi, and C. C. Congdon, *J. Immunol.* **79**, 281 (1957).

[6] T. Makinodan, *J. Cellular Comp. Physiol.* **50**, Suppl. 1, 327 (1957).

[7] T. Makinodan and N. Gengozian, *Biol. Semiann. Prog. Rep. Feb. 15, 1958*, ORNL-2481, p 37; *J. Immunol.* (in press).

Table 13. Antisheep* and Antirat RBC Response of Mice During Maximum Depression Period After Varying Doses of Total-Body X Radiation

Treatment	Induction Period (days)		Primary Rate of Appearance of Circulating Antibodies (log_2 titer per day)		Mean Peak Titer (log_2 titer) LSD(5%) = 1.72		Mean Total Titer (log_2 titer) LSD(5%) = 0.603	
	Antisheep	Antirat	Antisheep	Antirat	Antisheep	Antirat	Antisheep	Antirat
None	2	2	2.2	2.4	8.7	8.7	8.01	7.81
100 r	3	3	1.7	2.4	7.8	7.1	7.41	6.56
300 r	4	4	1.1	1.6	8.0	5.6	6.97	4.76
400 r	5	5						
500 r	6	8	0.5	0.4	7.8	3.4	5.73	1.83
550 r		12						
550 r–IBM		12						
600 r	8	15						
600 r–IBM	8							
710 r	11	18	0.2	0.2	3.6	2.0	2.33	0.86
710 r–IBM	11							
790 r–IBM	15							
800 r–IBM		21						
850 r–IBM	16							
950 r–IBM	17	24				1.3		0.40
1100 r–IBM	19	26						

*Data for antisheep response presented previously.[7]

antigens, and the mouse as the test animal have provided data from which we could predict with accuracy certain biological phenomena in irradiation and bone marrow transplantation research. Thus, based on data derived from such studies, the following could be predicted: (1) a 100% killing effect results from rat bone marrow transplantation to (C3H × 101)F_1 mice exposed to 710 r, a 30-day LD_{25} dose,[4,8,9] (2) the immune status of long-term chimeric survivors after irradiation and bone marrow treatment is subnormal,[4,6,10] (3) the dose of X ray that permits transplantation of foreign bone marrow is directly dependent on the genetic relationship of donor and host,[6,11] and (4) the immunological efficiency of the very young depends on genetic relation between antigen and host.[12]

[8]N. Gengozian and T. Makinodan, *Cancer Research* 17, 970 (1957).

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

[10]N. Gengozian *et al.*, *Proc. Natl. Acad. Sci. U.S.* 44, 560 (1958).

[11]I. C. Shekarchi and T. Makinodan, *Radiation Research* 9, 181 (1958) (and this report).

[12]N. Gengozian and T. Makinodan, *Federation Proc.* 17, 512 (1958).

Analysis of Serum Obtained from Lethally Irradiated Mice Treated with Rat Bone Marrow

N. Gengozian T. Makinodan

Introduction. – Lethally irradiated mice may be protected from radiation death by injection of rat bone marrow after irradiation; however, among these mice there occur a marked secondary body weight loss and high incidence of mortality three

to six weeks after treatment. From previous immunologic studies on irradiated mice treated with bone marrow[3-6,8,9] it was concluded that this "foreign bone marrow reaction" occurring in 950 r–RBM mice was caused by a delayed immunologic response of the irradiated host against the antigens of the foreign graft, resulting in a chronic *in vivo* antigen-antibody reaction and its subsequent disease syndromes. The alternative possibility, that the transplanted rat hematopoietic tissue is producing antibodies against the antigens of the mouse and leading to this *in vivo* reaction, has been proposed by others. Recent support for the latter thesis has been the reports by Weyzen and Vos[13] and Grabar *et al.*[14] claiming the presence of rat globulin proteins, presumably antibodies, in the serum of 950 r–RBM mice as indicated by immunologic tests. The present study is an analysis of serum of 950 r–RBM mice for rat and mouse proteins by *in vitro* immunologic tests using specific antisera to mouse and rat protein.[15]

Results. – Fifty serum samples were obtained from 950 r–RBM mice over a period of 12 to 55 days after treatment. Specific mouse antirat serum (sensitivity, 0.015 mg rat protein) and rat antimouse serum (sensitivity, 0.180 mg mouse protein) were employed in interfacial tests in the individual serum samples. As shown in Table 14, only one sample contained a detectable amount of rat protein although the mean value of total protein globulin in these sera ranged from 27.81 to 35.06 mg per ml. Tests for mouse protein were positive on samples chosen randomly from this group, including that which had shown a positive test for rat protein. Using mouse antirat globulin sera and rat antimouse globulin sera, double serum-agar diffusion tests on pools of these samples were completely negative for rat-type protein and positive for mouse-type protein.

Serum samples were obtained from twelve 950 r–RBM mice before and after immunization with sheep RBC antigens 200–300 days after irradiation and bone marrow treatment. Although the majority of these mice produced titratable antibodies to the sheep RBC, all but one of the interfacial tests for rat protein were negative. Three of these mice showed an absolute increase of protein globulin in the sera after immunization, yet the interfacial and double serum-agar diffusion tests failed to show the presence of rat protein. Tests with rat antimouse serum for mouse protein showed normal reactivity.

Tests for rat protein were performed on serum samples obtained from 950 r–RBM mice which had received a supralethal dose of X radiation (1000–2000 r) and were bled at various intervals thereafter. The sera of seven of these heterologous (rat) chimeras were negative for rat protein before this X-ray treatment; however, after destruction of the foreign graft in this manner, four of the seven serum samples showed the presence of

[13] W. W. H. Weyzen and O. Vos, *Nature* 180, 288–89 (1957).

[14] P. Grabar *et al.*, *Transplantation Bull.* 5, 58 (1958).

[15] N. Gengozian and T. Makinodan, *Radiation Research* 9, 118 (1958).

Table 14. Interfacial Tests with Specific Antisera on Sera from Mice Treated with 950 r and Rat Bone Marrow

Time After Treatment (days)	Interfacial Tests* with		Milligrams of Protein Globulin per Milliliter of Serum (mean value)
	Mouse Antirat Serum (4^6)**	Rat Antimouse Serum (4^4)	
12	0/10	5/5	30.3
22	0/10	5/5	27.8
34	0/10	5/5	32.8
44	1/10	5/5	33.2
55	0/10	5/5	35.1

*Samples with positive test/samples tested.

**Reciprocal of serum dilution.

rat-type protein as indicated in interfacial tests with mouse antirat globulin sera.

Discussion. – The use of specific mouse antirat serum in *in vitro* immunologic tests indicates the absence of appreciable amounts of rat protein in the serum of 950 r–RBM mice at various intervals after treatment. The ability to induce the presence of rat proteins in the serum of heterologous (rat) chimeras by destruction of the graft by X irradiation invalidates the contention of others[13,14] that these proteins are necessarily rat globulin proteins synthesized by the foreign transplant. Thus, the rat proteins in the serum of these animals may be derived from the active destruction of the transplanted foreign hematopoietic tissue by the host's immune mechanism. This hypothesis is in accord with the host versus graft hypothesis presented earlier.

Persistence of Heterologous Bone Marrow in Mice as a Function of X-Ray Dose

I. C. Shekarchi T. Makinodan

Introduction. – Based on previous immunologic studies in X-irradiated mice,[3–7] Makinodan has presented the hypothesis that there are either multiple types of antibody-producing cells or an antibody-producing cell with the ability to recognize varying degrees of antigenicity; i.e., homologous, closely related heterologous, and distantly related heterologous antigens. The degree of destruction of the recognition factor by X irradiation would be in the same order. The present experimental study was designed to show that the amount of X radiation required to permit transplantation of bone marrow is dependent on the genetic relationship of donor and recipient; i.e., the more foreign the bone marrow the greater the X-ray dose required.

Results. – Twelve-week-old (C3H × 101)F_1 mice were given total-body X irradiation ranging from 300 to 1500 r. After irradiation they were injected intravenously with 140 × 10^6 bone marrow cells from rat, guinea pig, hamster, or rabbit. Two or more mice were sacrificed at daily intervals after treatment; blood and bone marrow smears and spleen imprints were obtained and stained for alkaline phosphatase cells. Detection of foreign transplanted cells was based on the fact that mouse granulocytes are alkaline phosphatase negative whereas the foreign donor granulopoietic cells used in this study are alkaline phosphatase positive. With this technique, it was found that foreign bone marrow persists in normal nonirradiated mice for one day (Table 15). After rat bone marrow treatment alkaline phosphatase-positive staining cells were found in mouse spleen 2 days after 300 r, 3 days after 400 r, 4 days after 500 r, 5 days after 600 r, and in the survivors, 10 days after 710 r. After hamster bone marrow treatment, positive staining cells were found 5 days after 600 r, 6 days after 700 r, 8 days after 800 r, and, in survivors, 35 days after 950 r. After guinea pig bone marrow treatment, positive staining cells were found 1 day after 950 r, 3 days after 1150 r, and 4 days after 1300 r. After rabbit bone marrow treatment, positive cells were found 1 day after 1300 r and 4 days after 1500 r.

Active transplantation leading to protection from X-radiation death was obtained with rat bone marrow when X-ray doses of 800 to 1500 r were used and with hamster bone marrow with X-ray doses of 950 to 1500 r (Table 15). With guinea

Table 15. Persistence of Donor Cells in Mouse Spleen After Various Doses of X Radiation and Bone Marrow Treatment

X-Ray Dose (r)	Persistence Time (days)			
	Donor Bone Marrow			
	Rat	Hamster	Guinea Pig	Rabbit
0	1	1	<1	<1
300	2			
400	3	1		
500	4[a]	1		
600	5[a]	5[a]		
700		6[a]		
710	>10[b]			
800	>10[c]	8[a]	<1	
950	>10[c]	>10[c]	<1	<1
1150	>10[c]	> 5[b]	3	<1
1300	> 6[b]	> 4[b]	4	1
1500	> 4[b]	> 3[b]		4
2000	> 3[b]			4

[a]Temporary transplantation.
[b]Short-term survivors.
[c]Prolonged transplantation.

pig and rabbit bone marrow increased survival was not obtained with the high X-ray doses used.

Discussion. – It was found that rat, hamster, guinea pig, and rabbit bone marrow persisted in normal nonirradiated mouse tissue for not more than one day after injection. Persistence of such foreign cells longer than two days after irradiation and bone marrow treatment would be indicative of the depressed immunologic status of the host. The results show that the more distant the phylogenetic relationship of donor and host the higher the X-ray dose required to permit persistence and active transplantation of the foreign hematopoietic cells. These findings are in accord with the hypothesis presented above.

Erythrocyte Repopulation After Injection of Homologous Fetal and Newborn Liver into Irradiated Mice

R. D. Owen

Introduction. – Cellular antigens are convenient markers for following the course of repopulation by erythrocytes of donor type in homologous bone marrow experiments with mice.[16] Indications that homologous hematopoietic tissues of fetal origin may have advantages over those derived from adult donors in this system[17] suggested a study of repopulation by cells from fetal liver. Cell suspensions were pooled from the livers of a total of 31 C57BL/10D2 mice: 9 newborns, several hours old; 15 fetuses of about 20 days gestation; and 7 fetuses of 16–17 days gestation. Forty male (C3H × 101)F_1 mice 12 weeks old were given 950 r and injected intravenously, each with 0.5 ml of the liver cell suspension containing 35×10^6 nucleated cells. The recipients were individually earmarked and distributed five to a pan. Each mouse was weighed at weekly intervals.

Results. – The average weights of survivors, plotted as percentages of the initial average weight, and the cumulative mortality curve are given in Fig. 12. In contrast to certain combinations of fetal donor and adult recipient strains (see ref. 17) but in agreement with others, delayed mortality was high in this combination. The average weight of all survivors fell to 72.5% of the initial average weight six weeks after the radiation and treatment, then rose to exceed the initial average weight after the twelfth week. Two still survive, 220 days after the treatment; the average weights of these long-term survivors are also plotted in Fig. 12, as percentages of the initial average weight of the entire population. The long-term survivors were considerably heavier than the average at the start; unlike the group as a whole they regained their initial weight two weeks after the treatment, but they then entered a decline remarkably parallel to that experienced by the entire group, with a sharp reversal and recovery after the sixth week.

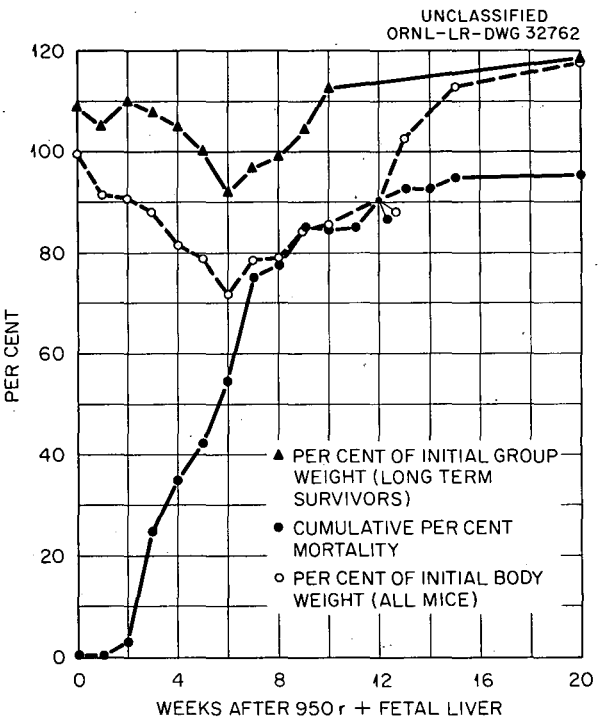

Fig. 12. Cumulative Mortality and Changes in Body Weight of (C3H × 101)F_1 Mice Injected with a Suspension of C57BL/10D2 Fetal and Newborn Liver Cells After 950 r.

Hemolytic tests for the course of repopulation are summarized in Fig. 13. Values for five mice were averaged for each point, except that the final point at 130 days is based on only two; different mice were bled for successive points. Two of the mice failed to show a transplant; they are not included in the averages. The repopulation curve with a donor-specific reagent (serum OL, C3H × 101-anti-LAF_1) is very similar to that found for adult bone marrow.[16] The calculated

[16] R. D. Owen, *Biol. Semiann. Prog. Rep. Feb. 15, 1958*, ORNL-2481, p 40–43.

[17] I. S. Urso (this report).

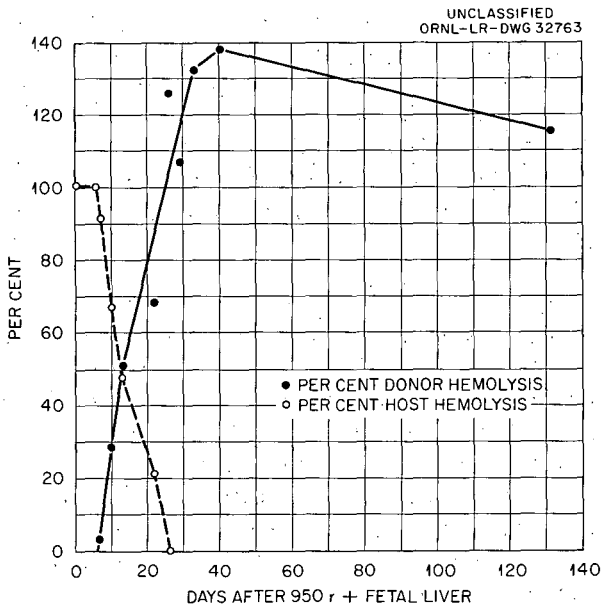

Fig. 13. Hemolysis of Erythrocyte Suspensions from (C3H × 101)F_1 Mice Injected with a Suspension of C57BL/10D2 Fetal and Newborn Liver Cells After 950 r.

percentage of host cells shown by the broken line falls at a rate compatible with the independent calculations of repopulation by donor cells (solid line), and reaches 0% by 26 days. Although the observations on host cells appear to be nicely consistent with other aspects of the data, they are to be taken with some reservation. The reagent for host cells (serum OQ, BDF_1-anti-C3H × 101) is not completely specific, but cross-reacts weakly with donor cells, and the necessity of correcting for the amount of this hemolysis in the repopulating mice introduces an uncertainty of unknown magnitude. An indication that the curve for host cell disappearance may indeed be misleading here was the persistence of agglutination reactions in tests for host cells beyond the time when lytic tests indicated that such cells were no longer present. It is, of course, possible that these agglutinations were of donor cells, more sensitive than normal cells of the donor type to the weak cross-reactive antibody in this reagent. It is also possible that these were truly host cells, persisting in the "nonlysable" fraction that escapes detection by the hemolytic technique with a reagent that produces only partial hemolysis of a normal cell population of the positive type.

Discussion. – The *H-2* antigens are believed to be absent on the red cells of mice at birth, and to appear on the third day, developing to adult levels by the fifth day of postnatal life.[18] The superiority of fetal hematopoietic tissues over adult bone marrow for homologous postirradiation protection and recovery, in particular strain combinations, might conceivably depend in part on failure of the graft to differentiate in the direction of normal and effective antigenicity, when its source is fetal liver and its environment is a foreign, irradiated adult. In the present experiment, however, this is not the case; repopulation by cells of donor specificity was very similar, quantitatively and qualitatively, to that observed for adult bone marrow. The fact that mortality and body weight changes were also similar to those found for homologous bone marrow may appear to reduce the significance of this finding for the interpretation of combinations in which fetal liver has an advantage over adult marrow. Other combinations, however,[17] also demonstrate that the *H-2* antigens of cells of fetal liver origin differentiate autonomously and normally according to their genotypes in the irradiated homologous adult host.

Effect of Subcutaneous Injection of Serotonin in the Ears of Mice on Localization of Intravenously Injected Trypan Blue

G. C. Royal, Jr.

Introduction. – The bioassay of serotonin is based primarily on smooth muscle contraction. A simplified *in vivo* method for measuring this compound is suggested by the present work. Lewis[19] and Ramsdell[20,21] reported infiltration of trypan red and trypan blue in edematous tissues. The dyes seem to function as an indicator of toxic action on the capillary endothelium.[20] In this study the possibility of detecting tissue damage in mice by trypan blue infiltration after serotonin injection was investigated.

Results. – Table 16 shows the area of trypan blue diffusion in the ears of albino (C57BL × A)F_2 mice that received varying concentrations of

[18] G. Hoecker, Discussion in *J. Cellular Comp. Physiol.* 52, Suppl. (in press).

[19] P. M. Lewis, *J. Exptl. Med.* 23, 669 (1916).

[20] S. G. Ramsdell, *J. Immunol.* 15, 305 (1928).

[21] S. G. Ramsdell, *J. Immunol.* 16, 133 (1928).

serotonin. Measurements of two dimensions of dye spread at each ear site obtained from two animals were averaged. Levels of serotonin greater than 0.4 μg seldom produced a response greater than 55 mm^2.

Table 16. Effect of Varying Levels of Serotonin on the Degree of Trypan Blue Diffusion in the Ears of (C57BL × A)F_2 Albino Mice*

Concentration of Serotonin Injected into Ear (μg)	Average Area of Dye Spread (mm^2)
0.0125	3
0.025	8
0.05	13
0.1	22
0.2	54
0.4	53

*Procedure:

Compounds injected in a volume of 0.02 ml.

Trypan blue administered in a standard dose of 0.025 ml of a 1% solution per gram of body weight.

The dye injection should follow the serotonin administration during a 5-minute period of time.

Reactions are read 15 minutes later.

Tests were also performed on the following analogs to serotonin: L-tryptophan, D-tryptophan, 5-hydroxytryptophan, tryptamine, 5-hydroxy-3-indoleacetic acid, 3-indoleacetic acid, and indole. At approximately an equal molar basis (±0.28) none of these compounds modified the permeability of the capillary endothelium under the conditions of this method.

Discussion. – Injection of serotonin into the ears of (C57BL × A)F_2 albino mice causes a change in the permeability of capillary walls near the site of administration as judged by trypan blue infiltration. As little as 0.0125 μg of this compound could be detected. Various analogs of serotonin yielded negative tests in this bioassay, indicating some specificity of the reaction. The analogs used are not generally regarded as vasoconstricting agents.

Preliminary tests have indicated that this bioassay will also demonstrate responsiveness of mice to histamine. On a molar basis, however, histamine reaction was found to be much less sensitive than that of serotonin. Such was also found to be the case in rat inflammatory studies by Gözsy and Kátó.[22]

[22] B. Gözsy and L. Kátó, *Science* **125**, 934 (1957).

PATHOLOGY AND PHYSIOLOGY

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Accentuation of Foreign Bone Marrow Mortality by Preimmunization of the Marrow Donor Against the Recipient[6]

G. E. Cosgrove E. E. Schwartz[7]
E. S. Ledford

Introduction. – It has been noted that preimmunization of animals used as donors for splenic homogenates results in increased mortality in the irradiated recipients of such homogenates.[8] The effect of preimmunization of bone marrow donors has therefore been studied.

Results. – Young adult F_1 mice exposed to lethal X radiation followed by inoculation of viable bone marrow cells from parental strain donors showed increased mortality if the donor was preimmunized against tissue of the homologous parental strain several weeks before use (Table 17).

Discussion. – Preimmunization of the bone marrow donor against homologous parental strain antigens apparently stimulates the immunologic potency of the cells grafted into the F_1 recipient, giving rise to an intensified immunological reaction of the graft against the host. The nature of the cellular change in the marrow involved in this

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[8] E. E. Schwartz, A. C. Upton, and C. C. Congdon, *Proc. Soc. Exptl. Biol. Med.* **96**, 797 (1957).

Table 17. Effects of Nonimmunized and Immunized 101 and C3H Parental Strain Bone Marrow on Survival of Lethally Irradiated F_1 Recipients

Recipient Strain	Donor Strain	Immunological Status of Donor*	Cumulative Mortality** at Day			
			30	60	90	120
(101 × C3H)F_1	(101 × C3H)F_1	Nonimmunized	2/35	2/35	2/35	2/35
(C3H × 101)F_1	(C3H × 101)F_1	Nonimmunized	0/20	1/20	1/20	
(101 × C3H)F_1	101	Nonimmunized	2/10	4/10	4/10	4/10
(C3H × 101)F_1	101	Nonimmunized	5/69	10/69	11/69	12/61
(C3H × 101)F_1	101	Preimmunized 1 week	1/39	6/39	6/39	6/39
(C3H × 101)F_1	101	Preimmunized 3 weeks	2/20	14/20	16/20	16/20
(101 × C3H)F_1	101	Preimmunized 4 weeks	10/18	18/18	18/18	18/18
101	(101 × C3H)F_1	Nonimmunized	2/10	3/10	3/10	3/10

*Immunization was carried out by the intraperitoneal or intravenous injection of homogenates of C3H lymphoid tissues.

**Number dead/number treated.

effect and the mechanism whereby the heightened immunologic reaction leads to death of the recipients are, however, not clear.

Possible Role of Viral Agents in the Pathogenesis of Radiation-Induced Leukemia

F. F. Wolff T. Mack

Introduction. – The importance of filterable agents in the etiology of spontaneous murine lymphomas is now well established.[9] It is important, therefore, to determine whether such agents are also involved in the induction of leukemia by ionizing radiation. Accordingly, filtrates of tissue from irradiated mice developing leukemia are being assayed for leukemogenic potency in irradiated and nonirradiated isologous recipients.

Results. – Whereas inoculation of the brain tissue of nonleukemic RF mice did not augment the incidence of myeloid leukemia in irradiated RF recipients, filtrates of brain tissue from donors with myeloid leukemia greatly enhanced the development of this disease in the one experiment analyzed thus far (Fig. 14). Preliminary data suggest that filtrates prepared from irradiated mice developing thymic lymphomas similarly enhance the induction of lymphomas.

Discussion. – These results, although inconclusive, strongly suggest that mice developing radiation-induced leukemia harbor filterable leukemogenic agents similar to those present in animals with spontaneous leukemia. Further experiments are being performed to determine how consistently such agents are present in animals with radiation-induced leukemias and whether the agents are viruses, free nucleic acid, or of some other nature.

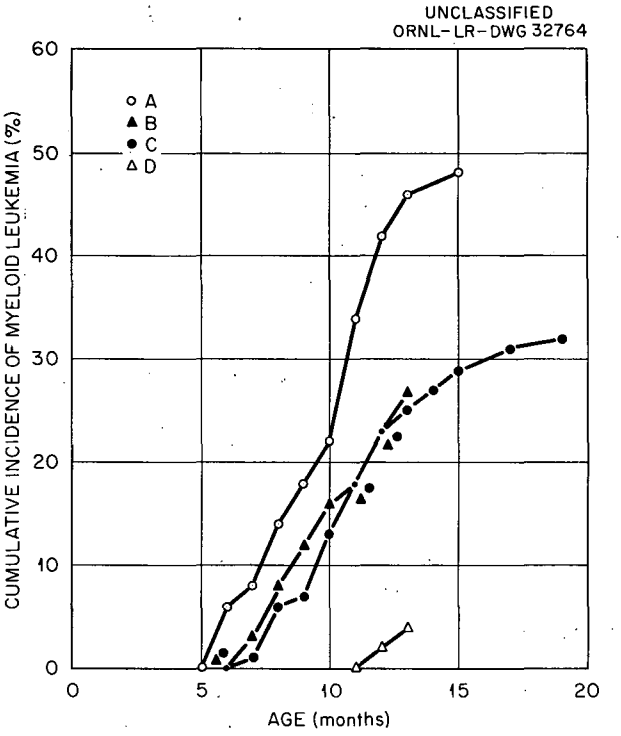

Fig. 14. Enhancement of Leukemia Induction in Irradiated RF Mice by Inoculation of Cell-Free Filtrate of Leukemic Tissue. The mice in each group were males, 10 weeks old at the time of treatment. Curve A, 450 r whole-body X radiation followed by intravenous injection of cell-free filtrate of brain tissue from three RF mice with radiation-induced myeloid leukemia (49 mice in group). Curve B, 450 r plus filtrate of nonleukemic isologous brain tissue (94 mice in group). Curve C, 450 r plus Tyrode's solution (84 mice in group). Curve D, nonirradiated recipients of nonleukemic brain tissue (45 mice in group).

Comparative Results of Bone Marrow Injection in X-Irradiated and Myleran- and Chlorambucil-Treated Rats

T. T. Odell, Jr. B. A. Caldwell

Introduction. – Successful homotransplantation of bone marrow from C rats into X-irradiated D rats was previously demonstrated. Transplantation was rarely successful, however, when C marrow was injected into D rats treated with the marrow depressant Myleran,[10] even though recovery of Sprague-Dawley rats from a lethal dose of Myleran was promoted by the injection of isologous marrow.[11] Since Myleran depresses primarily myelopoiesis, whereas chlorambucil (CB 1348) depresses primarily the lymphatic system, which is believed to be the principal site of the immune mechanism, it was plausible that homotransplantation might be more successful with chlorambucil than with Myleran. This experiment was carried out to test this possibility.

Results. – CB 1348[12] dissolved in 50% dimethylacetamide in water was injected into either the

[9] L. Gross, *Cancer Research* 18, 371 (1958).

[10] Myleran (1,4-dimethanesulfonoxybutane) kindly supplied by Burroughs Wellcome & Co., Inc., Tuckahoe, New York.

[11] J. E. Weston *et al.*, *Federation Proc.* 16, 377 (1957).

[12] We are grateful to A. Haddow and associates of the Chester Beatty Research Institute in London for supplying us with the CB 1348 used in these studies.

saphenous or jugular vein. No rats died after a single dose of 7 mg/kg or less, but all rats died within 24 hours after 10 mg/kg or more, and 2 of 24 animals died after 8.5 mg/kg. Two injections of 6 mg/kg each 24 hours apart were fatal to 1 of 13 rats; similarly, two injections of 7 mg/kg were fatal to 2 of 4, and two injections of 8 mg/kg were fatal to 8 of 8 animals. No deaths were encountered later than 24 hours after injection of the drug.

A total of 25 DD rats have been injected with bone marrow from normal CC rats at varying intervals after treatment with various doses of CB 1348 (Table 18). In the first group, donor-type red cells were found in the circulation 13 days after marrow injection in 4 out of 5 rats; they made up 14% to 23% of the recipients' circulating red cells on the 21st day and then declined until none were present in any animals by 84 days. No donor-type red cells were found in any other rats, including the 6 given the same schedule of treatment as the first group.

Table 18. Injection into Rats of CB 1348 Followed by Homologous Bone Marrow

Dose of CB 1348 (mg/kg of body wt)	No. of Doses	No. of Rats	Time of Marrow Injection* (hours)	Presence of Donor Cells
8.5	1	5	48	+
8.5	1	6	48	–
8.5	1	5	24	–
6.0	2**	9	3–4	–

*Time elapsed since last injection of CB 1348. (All the drug-injected animals survived the period of observation.)

**Injections separated by 24 hours.

Discussion. – It is clear that chlorambucil as used in these experiments is at least no more effective than Myleran in preparing rats for transplantation of homologous bone marrow, although theoretic considerations suggest that it might be. It is possible that further trials with multiple doses of the drug and various time intervals would disclose a successful method of promoting homotransplantation with this agent.

Genetic Differences in Hemoglobin as Markers for Bone Marrow Transplantation in Mice

R. A. Popp G. E. Cosgrove, Jr.
R. D. Owen[13]

Introduction. – The serological markers presently used for typing mouse erythrocytes are based on differences at the *histocompatibility-2* (*H-2*) locus.[14,15] Owen[16] has found that this system can also be used to distinguish cells of donor origin from those of the recipient in irradiated mouse chimeras when the donor and recipient are of distinguishable *H-2* genotypes. An independent method for distinguishing erythrocytes is potentially useful, however, in the study of bone marrow transplantation, particularly when the *H-2* markers cannot be used. The following preliminary report demonstrates that the erythrocytes of mouse chimeras can also be typed by use of inheritable differences occurring in mouse hemoglobin.

Results. – The mice used as donors and recipients in this experiment were strains C57BL/S, 101, and (C57BL/S × 101)F_1. The erythrocytes of C57BL/S are of $H\text{-}2^b$ genotype and have a single type of hemoglobin, whereas erythrocytes of 101 mice appear to be of $H\text{-}2^k$ genotype and have a diffuse type of hemoglobin pattern which consists of three electrophoretically separable fractions (Popp and W. St. Amand, unpublished data). The erythrocytes of (C57BL/S × 101)F_1 mice also have diffuse hemoglobin. Donor bone marrow or splenic suspensions were injected intravenously and intraperitoneally, respectively, into X-irradiated recipients. The blood samples used for starch gel electrophoresis[17] and serological typing were obtained by severing a tail vein. The comparative results obtained in the 20 mice examined to date are presented in Table 19. The data show that when bone marrow or splenic suspensions from a donor with one type of hemoglobin are transplanted into irradiated mice which differ at the *H-2* locus and have another type of hemoglobin, the hemoglobin in the recipients circulating

[13]Radiation Immunology Group.

[14]D. B. Amos, P. A. Gorer, and Z. B. Mikulska, *Proc. Roy. Soc.* (London) **B144**, 369 (1955).

[15]P. A. Gorer, S. Lyman, and G. D. Snell, *Proc. Roy. Soc.* (London) **B135**, 499 (1948).

[16]R. D. Owen, *Radiation Research* 9, 164 (1958).

[17]O. Smithies, *Biochem. J.* 61, 629 (1955).

Table 19. Comparison of Serotype and Hemoglobin Type in X-Irradiated Mouse Chimeras

Recipient	Number of Mice	Dose of X Rays (r)	Number of Nucleated Donor Cells				Days After Treatment	Serotype		Hemoglobin Type	
			Bone Marrow		Spleen			Recipient (%)	Donor (%)	Recipient	Donor
			C57BL/S	(C57BL/S × 101)F_1	C57BL/S	(C57BL/S × 101)F_1					
			$\times 10^6$	$\times 10^6$	$\times 10^6$	$\times 10^6$					
101	2	850	15				43	100		X	
(C57BL/S × 101)F_1	2	900	15				93	100		X	
	2	900	15				93		100		X
	1	900	15				142	3	97		X
C57BL/S	1	900		15			42		100		X
(C57BL/S × 101)F_1	1	400			30		93	100		X	
	1	400			30		93	93	7	X	
	1	400			30		93		100		X
	1	500			30		21	100		X	
	1	500			30		21	64	36	X	X
	1	500			30		21	62	38	X	X
	1	500			30		21	80	20	X	
	1	600			30		93	100		X	
C57BL/S	3	400				120	35	100		X	
	1	400				120	35	97	3	X	

erythrocytes characterized by starch gel electrophoresis corresponds with the serological type of the red blood cells.

Discussion. – The electrophoretic method has not yet been refined sufficiently to detect small quantities (20% or less) of the single type of hemoglobin when 80% or more of the red blood cells are estimated by serological methods to be of diffuse hemoglobin type. Nevertheless, both the hemoglobin type and the red cell serotype appear to be under the autonomous control of the genotype of the nucleated cell from which the erythrocyte was derived. The origin (host or recipient) of the circulating red blood cells can therefore be inferred from the hemoglobin type as well as from the serotype. After transplantation of bone marrow cells between strains of mice with identical *H-2* genotypes but different hemoglobin types, circulating red blood cells of donor or recipient origin can be distinguished even though serological markers are not available in such instances.

Relative Biological Effectiveness of 14-Mev Neutrons and Co^{60} Gamma Rays for Cataract Induction in Mice

K. W. Christenberry M. L. Randolph[18]
J. A. Rutherford

Introduction. – Although earlier studies have demonstrated that neutrons, in general, have a high relative biological effectiveness (RBE) for damaging the lens of the eye,[19] the RBE of 14-Mev neutrons for this effect was investigated to determine the influence of neutron energy in cataract formation.

Results. – Lens opacities, as viewed with the slit lamp, developed at a rate and to an extent that varied with the dose of gamma rays (Fig. 15) or of 14-Mev neutrons (Fig. 16). A multitude of RBE values for these radiations, administered at

[18] Biophysics Section.

[19] A. C. Upton *et al.*, *Radiology* 67, 686 (1956).

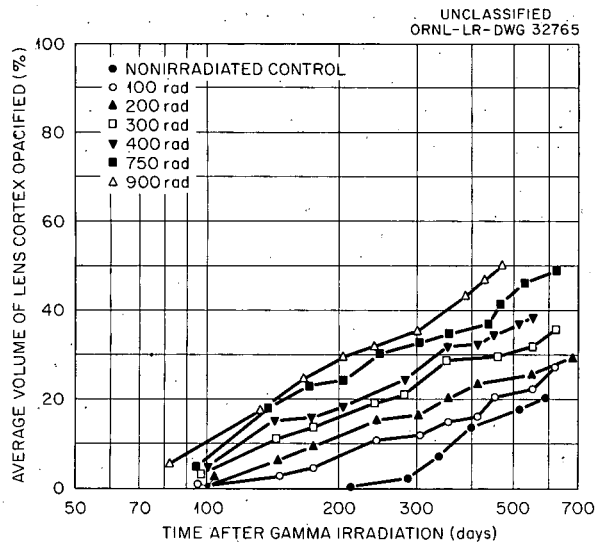

Fig. 15. Lens Opacities in RF Mice Exposed to Co^{60} Gamma Rays. The mice were females irradiated at 10 weeks of age at a rate of 3–5 rad/min.

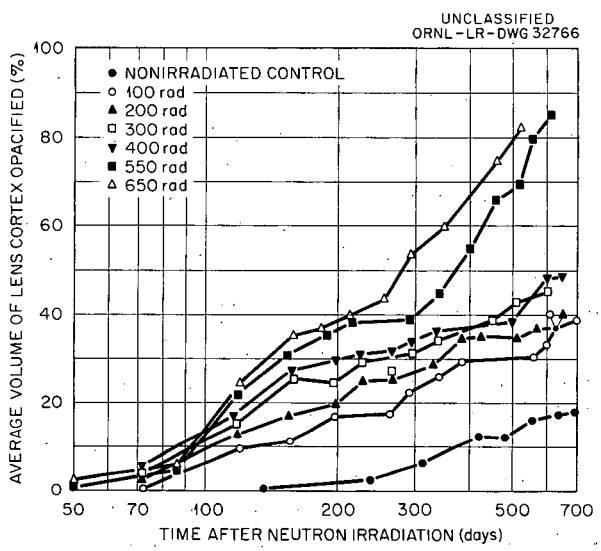

Fig. 16. Lens Opacities in RF Mice Exposed to 14-Mev Neutrons from a Cockcroft-Walton Generator. The mice were females irradiated at 10 weeks of age at a rate of 3–5 rad/min.

the same dose rate, can be obtained from the data by comparisons of the doses required to induce various degrees of lens opacity at various times after irradiation. Crude, preliminary examination indicates that the neutrons are two to three times as effective as gamma rays on the average, and that the RBE tends to decrease slightly with increasing dose. Successive fractional doses of neutrons administered over periods up to 70 days apart were essentially as effective as the same total dose administered in a single exposure.

Discussion. – The RBE of 14-Mev neutrons compared to Co^{60} gamma rays for cataract induction is lower than that of neutrons of lesser energy,[19] in keeping with the usual relation found between RBE and linear energy transfer (LET);[20,21] however, the RBE for cataractogenesis exceeds the RBE of the same radiations for 30-day lethality.[22] The high degree of additivity of successive doses of neutrons in the production of lens damage conforms to earlier observations and contrasts with the less markedly cumulative effects of successive doses of gamma rays.[23]

Determination of Platelet Life Span in Rats by Daily Injection of S^{35}-Labeled Sulfate

T. T. Odell, Jr. B. Anderson

Introduction. – We have previously estimated platelet life span in rats by two methods: (1) the length of time that injected platelets labeled with an isotope remain in the circulation has been observed (five days); (2) animals have been given a dose of a radioactive isotope that labels platelets, and the time lapse between the midway points on the ascending and descending limbs of the radioactivity curve of the labeled platelets has been measured (four to five days). Each method has advantages and disadvantages. A major criticism of the former is that it requires considerable handling of platelets during their removal from donors and preparation for injection into recipients, thereby possibly subjecting them to injury which could shorten their life span. The second method is open to difficulties of interpretation. It seemed that the following method might avoid many criticisms of the two: a radioactive label was injected daily in order to produce a constant pool of label in the animal, and the platelet activity was determined at intervals until it was no longer rising,

[20] E. F. Riley *et al.*, *Radiology* 67, 673 (1956).

[21] J. B. Storer *et al.*, *Radiation Research* 6, 188 (1957).

[22] J. A. Sproul and M. L. Randolph, *Radiation Research* 7, 452 (1957).

[23] K. W. Christenberry *et al.*, *Biol. Semiann. Prog. Rep. Aug. 15, 1957*, ORNL-2390, p 66.

thus indicating that one complete platelet population had been labeled and newly labeled platelets entering the circulation were replacing those being removed.

Results. – The results of two such experiments are shown in Fig. 17. Platelet activity increased for six days following the first injection of S^{35}-labeled sulfate and then leveled off. The plateau indicates that new labeled platelets entering the circulation were replacing labeled platelets that were being removed from circulation, and therefore that the survival time of the average rat platelet in the circulatory system was between five and six days.

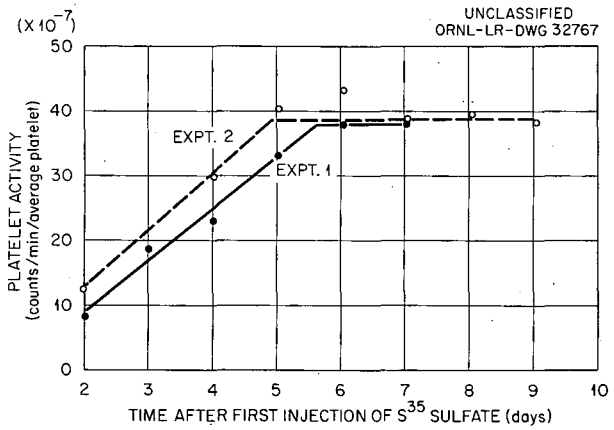

Fig. 17. Specific Activity in Platelets of Rats Injected Daily with S^{35}-Labeled Sulfate. Each point represents the average of two animals.

Discussion. – The method used in this study should give an accurate estimate of platelet life span, and it avoids many pitfalls of other methods. A disadvantage for human patients is that it requires the injection of larger amounts of radioactivity than may be thought desirable under ordinary circumstances.

Localization of Injected Particulate Material in the Mouse Spleen

W. Friedberg L. H. Smith[24]
W. D. Gude

Introduction. – There have been reports that injected C^{14}-tobacco mosaic virus[25] and S^{35}-sulfanilazoglobulin[26] are preferentially fixed in the red pulp of the spleen, little or no radioactivity being detected in the white pulp. In view of these findings and the importance of the spleen in transplantation of hemopoietic cells, it seemed of interest to characterize the sites of localization of cells in the spleen shortly after their injection.

Results. – Labeled cells were prepared by giving multiple injections of tritiated phenylalanine or tritiated thymidine to donor rats and mice. Suspensions of bone marrow or spleen cells labeled in this way were injected into normal and irradiated mice. Four hours after injection, the recipients were killed, and autoradiograms were prepared from spleen sections. This time interval was chosen to minimize cell multiplication and yet give the cells time to be cleared from the lung. Labeled cells were seen in significant numbers in both the red and white pulp of the spleen.

Discussion. – Although injected cells were found in both red and white pulp, it will be necessary to examine many more sections before drawing conclusions as to the relative distribution of isologous and heterologous cells in each location.

A Method for Staining the Interendothelial Cement Substance of Small Blood Vessels and Capillaries

E. B. Darden, Jr. W. D. Gude

Introduction. – Methods available for specific staining of interendothelial cement have been largely directed to that of isolated large blood vessels. In response to a need for studying this material in capillaries *in situ*, the method of McGovern[27] has been modified and adapted to a microinjection staining procedure.

Results. – A 0.5% solution of indigo tetrasulfonate and a solution consisting of a 1:1 mixture of 0.075% azure A and 0.2% methylene blue[28] are freshly prepared in physiological saline and filtered. The first solution is introduced into an artery of the vascular network of excised fresh tissue to be studied by means of a capillary drawn to a small-diameter tip. The capillary is connected by small-bore polyethylene tubing to a tuberculin syringe. A few minutes after the perfusion the second

[24] Mammalian Recovery Group.

[25] F. Gavosto and A. Ficq, *Nature* 172, 406 (1953).

[26] F. Haurowitz, p 5, in *Serological Approaches to Studies of Protein Structure and Metabolism* (ed. by W. H. Cole), Rutgers University Press, New Brunswick, New Jersey, 1954.

[27] V. J. McGovern, *J. Pathol. Bacteriol.* 69, 283 (1955).

[28] P. B. Samuels, B. M. Samuels, and D. R. Webster, *Lab. Invest.* 1, 50 (1952).

solution is similarly introduced, causing a reddish purple color to appear, tracing the vascular tree. The tissue is then fixed in 10% formalin, dehydrated, and either cleared for preparation as whole mounts or embedded in paraffin and sectioned at about 20 μ for examination at high magnification.

Discussion. – This technique has enabled satisfactory delineation of the interendothelial cement in capillaries and small arterioles and venules. The method is currently being used in a study of acute vascular injury after massive localized X irradiation.

CELL PHYSIOLOGY

N. G. Anderson
J. F. Albright[1]
J. C. Nichol[2]
M. M. Lowry[2]
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M. L. Anderson[4]
R. E. Canning
L. F. Pennington, Jr.[5]

Ultracentrifugal Analysis of Soluble Tissue Proteins

N. G. Anderson R. E. Canning

Introduction. – In the analytical ultracentrifuge rat liver soluble proteins show several fairly well defined peaks. This suggests that tissue proteins tend to fall into a number of general classes, or that the peaks observed are caused by the presence of large amounts of a few specific proteins that obscure the broad distribution of the majority of cell proteins. As a first approach to this problem, a comparative study of the ultracentrifuge patterns of the readily soluble proteins of several rat organs was undertaken to see whether the discontinuous distribution of sedimentation coefficients observed in liver obtains in other tissues.

Results. – Rapidly prepared extracts from perfused rat liver, brain, kidney, and testis were dialyzed against pH 7.5, 0.1 ionic strength phosphate buffer and analyzed in the ultracentrifuge at 56,100 rpm at 20°C. Enlarged tracings of the schlieren diagrams were divided into Gaussian curves, and the sedimentation coefficient and area for each curve were determined. The results are shown in Table 20. Considerable differences were seen in the percentages and sedimentation coefficients for the more rapidly sedimenting ($S_{20,w}$ greater than 4) components. Surprisingly, the largest peak in each case had a sedimentation coefficient of approximately 4, which accounted for a third of the protein in testis, and half in kidney and brain. The 17 S component of testis is of considerable interest since the macroglobulins of rat plasma have the same sedimentation coefficient. The origin of this blood component is unknown.

Discussion. – The ease with which the ultracentrifuge patterns of such complex mixtures can be divided into normal distribution curves suggests that most readily soluble tissue proteins do indeed fall into certain general classes and that the individuality of the patterns is not attributable to the superimposition of two or three proteins present in large amounts on a broad sedimentation coefficient distribution curve. Two Oracle codes that will allow diffusion and concentration effects to be eliminated and a more-exact distribution of sedimentation coefficients to be calculated have been completed.

[1]USPHS Fellow.

[2]Research Participant.

[3]Summer employee.

[4]Consultant.

[5]Biology Student Trainee.

Some Studies of the Soluble Components of Rat Liver Mitochondria

J. F. Albright M. M. Lowry

Introduction. – Serological analyses of intact mitochondria have revealed that these organelles possess species- and organ-specific antigens. However, it has not been possible to show that there are antigens localized on mitochondria that are not also found in the other cellular fractions. Analyses of the distribution of cellular enzymes lead to the conclusion that there are fraction-specific substances. The failure to demonstrate these substances serologically might be caused by several factors, chief among which is the antigenic complexity of intact mitochondria, which might lead to extensive cross reactions of anti-mitochondrial sera with components in the other cell fractions. The work reported herein consists of first attempts at fractionating rat liver mitochondrial proteins for the immediate purpose of obtaining one or more purified mitochondria-specific substances. The principal target for the attack has been a protein, mitochrome, which has been reported to be localized in mitochondria.[6]

Results. – Mitochondria were isolated from rat liver breis by differential centrifugation. Extracts were made of the mitochondria in various ways. The results show that, in terms of mitochondrial nitrogen solubilized, the most satisfactory procedure was to perform the extraction at pH 9 to

[6]B. D. Polis and H. W. Shmukler, *J. Biol. Chem.* **227**, 419–40 (1957).

Table 20. Sedimentation Coefficients and Percentage Composition of Soluble Organ Proteins

Preparation	Curve 1		Curve 2		Curve 3		Curve 4		Curve 5		Curve 6		Curve 7		Curve 8		Per Cent Protein in Preparation
	$S_{20,w}$	Per Cent	$S_{20,w}$	Per Cent	$S_{20,w}$	Per Cent	$S_{20,w}$	Per Cent	$S_{20,w}$	Per Cent	$S_{20,w}$	Per Cent	$S_{20,w}$	Per Cent	$S_{20,w}$	Per Cent	
Kidney																	
1			>11	4	11.7	7.5			7.2	19.3	5.3	3.7	4.1	63.8	1.7	1.7	1.46*
2	>17	7.4	17.4	7.9	11.4	10.5	7–11	2.6	7.1	13.3	5.5	5.8	3.7	51.0	2.0	1.6	1.44*
3			10–19	4.5	11.0	6.1	7–10	1.1	6.9	18.9	5.5	2.5	3.3	65.0	1.02	1.8	1.22
4			10–20	7.2	10.9	4.8			6.3	18.2	4.8	4.1	3.8	65.7			1.28
5	>16	7.2	16.4	7.6	14.2	8.0	7–14	1.4	8.2	16.0	5.2	1.9	3.5	57.1	2.0	0.8	1.99*
Average		2.9		6.2	11.8	7.4		1.0	7.1	17.1	5.3	3.6	3.7	60.5	1.7	1.5	
Testis																	
1	23–35	4.2	24.3	5.0	16.5	8.1	6–16	23.5	6.2	15.1			4.0	38.2	2.1	5.9	1.75*
2	23–34	5.3	23.1	4.1	18.9	7.3	6–16	19.4	6.9	18.1			4.1	37.8	2.4	8.1	1.38
3	23–34	2.4	21.4	5.5	18.8	6.9	7–18	18.1	7.1	19.8			4.1	38.1	2.1	9.2	1.32*
4	>20	6.7	20.6	5.9			7–20	17.7	6.2	17.7			4.0	45.3	2.0	8.0	1.29
5	21–36	5.0	21.5	5.2	17.4	8.6	6–17	22.5	6.0	15.9			4.0	36.3	2.2	6.5	1.74*
Average		4.7	22.2	5.1	17.9	7.7		20.2	6.5	17.3			4.0	39.1	2.1	7.5	
Liver																	
1	21.4	1.2	18.2	2.8			10.4	5.2	7.8	26.9	5.7	4.8	3.3	56.0	~1	3.0	2.65*
2			19.3	4.6			12.1	8.0	7.6	26.5	6.3	3.1	3.7	54.6	~1	3.1	1.31
3							11.7	3.7	8.0	27.8	6.0	3.7	3.2	63.5	~1	1.2	1.08
Average	21	0.4	18.7	2.8			11.4	5.6	7.8	27.1	6.0	3.9	3.4	58.0	~1	2.4	
Brain																	
1			~22.7	5.0			15.7	19.7	9.1	7.4	7.0	13.2	4.2	46.1	1.6	3.6	0.56
2			>14.3	1.9			14.7	17.0	9.3	10.8	7.5	15.2	4.2	52.0	1.6	3.0	0.74*
3			>14.3	3.0			15.2	24.3	9.5	7.9	7.0	12.5	4.1	48.0	2.1	4.4	0.44
Average				3.3			15.2	20.3	9.3	8.7	7.1	13.6	4.2	48.7	1.8	3.7	

*Data obtained from area measurements on ultracentrifuge patterns; other values from nitrogen determinations.

10. Approximately 70% of the mitochondrial nitrogen was taken into solution by extracting with 0.1 *M* ethanolamine buffer at pH 9.0.

The ethanolamine extract was examined in the analytical ultracentrifuge. One major and two minor components were found. The sedimentation constants of these components were calculated:

	S_{20}
Major	3.62
Minor 1	13.61
Minor 2	8.32

An electrophoretic analysis of the mitochondrial extract, in Veronal buffer, ionic strength 0.1, pH 8.6, is shown in Fig. 18. At least four components are present. Further analyses over a wide pH range are required before an accurate estimate of the number of different components can be made.

Mitochrome was isolated from the mitochondrial extracts by isoelectric precipitation. It was purified by repeated isoelectric precipitations and paper curtain electrophoresis. The resulting purified protein was examined in the analytical ultracentrifuge and was found to be quite heterodisperse. This protein, therefore, either is highly impure or exists in solution in a state of varying degrees of polymerization. Analyses to clarify this point are under way. Moreover, serological studies have been initiated to determine if mitochrome is antigenic and if it behaves as a single antigen.

Discussion. – Further studies of soluble mitochondiral components will eventually lead to correlation of physical, chemical, and serological data. It should then be possible to determine: (1) to what extent mitochondrial substances are also to be found in other cellular components, and whether or not there are mitochondria-specific substances, (2) where among the major protein fractions soluble mitochondrial enzymes are to be found.

Among other studies that such information should make more meaningful is that of the extent to which mitochondria undergo differentiation concomitantly with over-all cellular differentiation. It is possible that mitochondria participate in cellular differentiation in an even more direct manner than that of providing energy.

Countercurrent Dialysis

N. G. Anderson L. F. Pennington, Jr.

Introduction. – The amino acid and peptide mixture extracted from rat liver microsomes[7] is difficult to resolve since many of the peptides are not separated from the amino acids by conventional chromatographic procedures. Countercurrent dialysis has therefore been investigated as a means of making such a separation before chromatography.

The rate of escape of a solute from a cellophane dialysis sac is a function of the particle size, charge, concentration difference between the inside and outside of the sac, and the agitation of the inside and outside fluids. Previous work[8,9] has shown that large differences in the escape rates of amino acids and peptides exist, but no systematic apparatus suitable for making such separations has been developed.

Results. – The requirements for countercurrent dialysis are (1) that a series of rocking or otherwise agitated dialyzers are arranged so that the solute that has escaped from the first dialysis sac is reconcentrated and introduced into the sac of a second dialysis cell, and (2) that this process is serially repeated through several stages. The material remaining in the sac at each stage is concentrated and returned to the inside of the sac of the preceding stage. Dialysis cells were constructed which supported vertically a length of $^{8}/_{32}$-in. cellophane tubing in a glass tube with a cross-sectional area six times that of the dialysis sac.

The device constructed agitates seven dialysis cells by means of cell supports oscillating through 30° at rates variable from 0 to 150 cycles per minute. The cell holders are connected as a continuous gear chain and are designed to also oscillate dialysis cells in beakers or flasks (see Fig. 19).

In these studies the rate of solute escape is measured in a closed constant-volume system, whereas in previous studies the external solution either has been changed at frequent intervals or has been very large in proportion to the volume in the dialysis sac. The escape rates of glycine,

[7] N. G. Anderson and J. F. Albright, *Biol. Semiann. Prog. Rep. Feb. 15, 1958*, ORNL-2481, p 53.

[8] L. C. Craig, T. P. King, and A. Stracher, *J. Am. Chem. Soc.* 79, 3729 (1957).

[9] L. C. Craig and T. P. King, *J. Am. Chem. Soc.* 78, 4171 (1956).

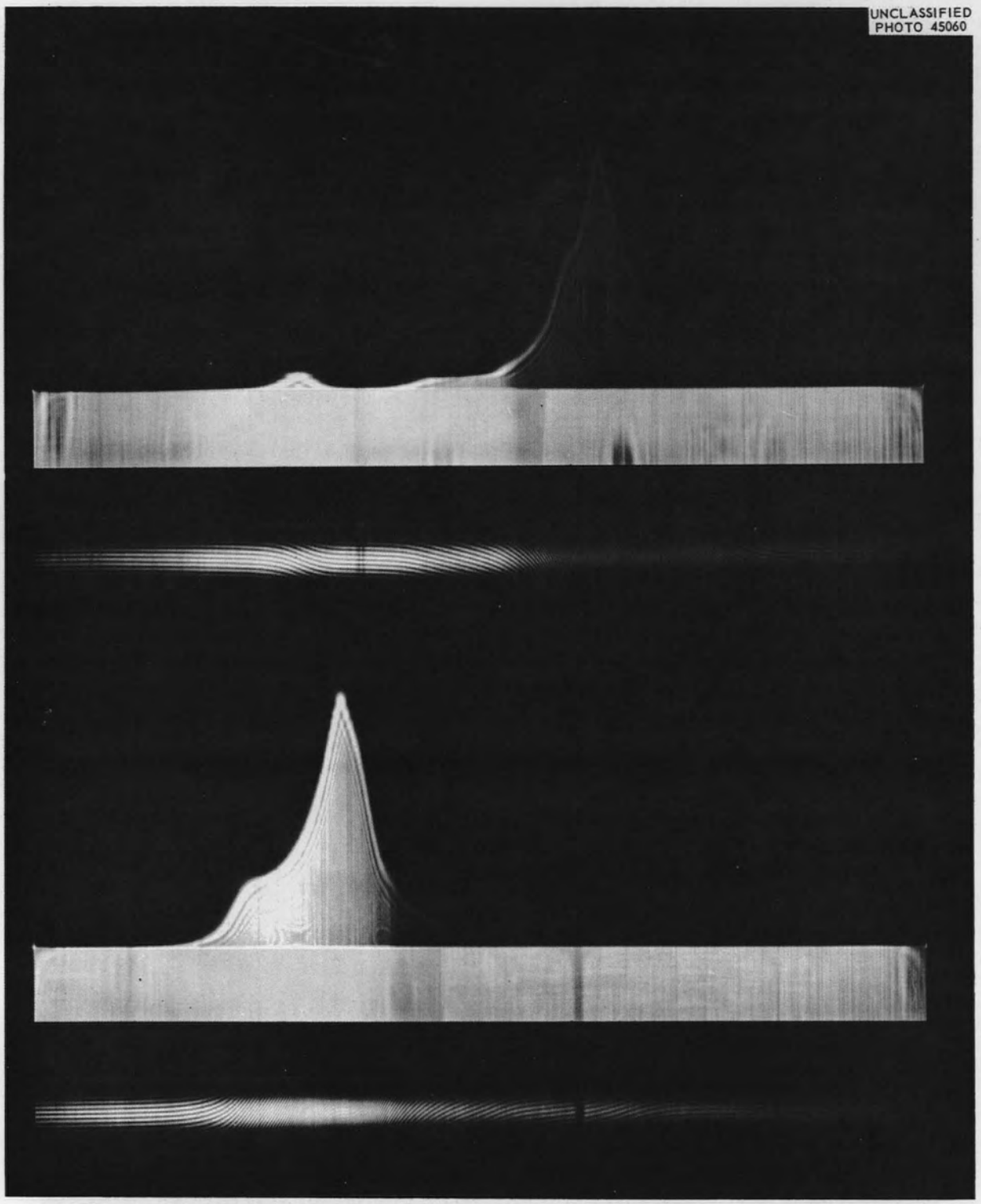

Fig. 18. Electrophoretic Pattern of Mitochondrial Extract. Veronal buffer, pH 8.6, 0.1 ionic strength, 100 minutes. Top: ascending limb, migration to left.

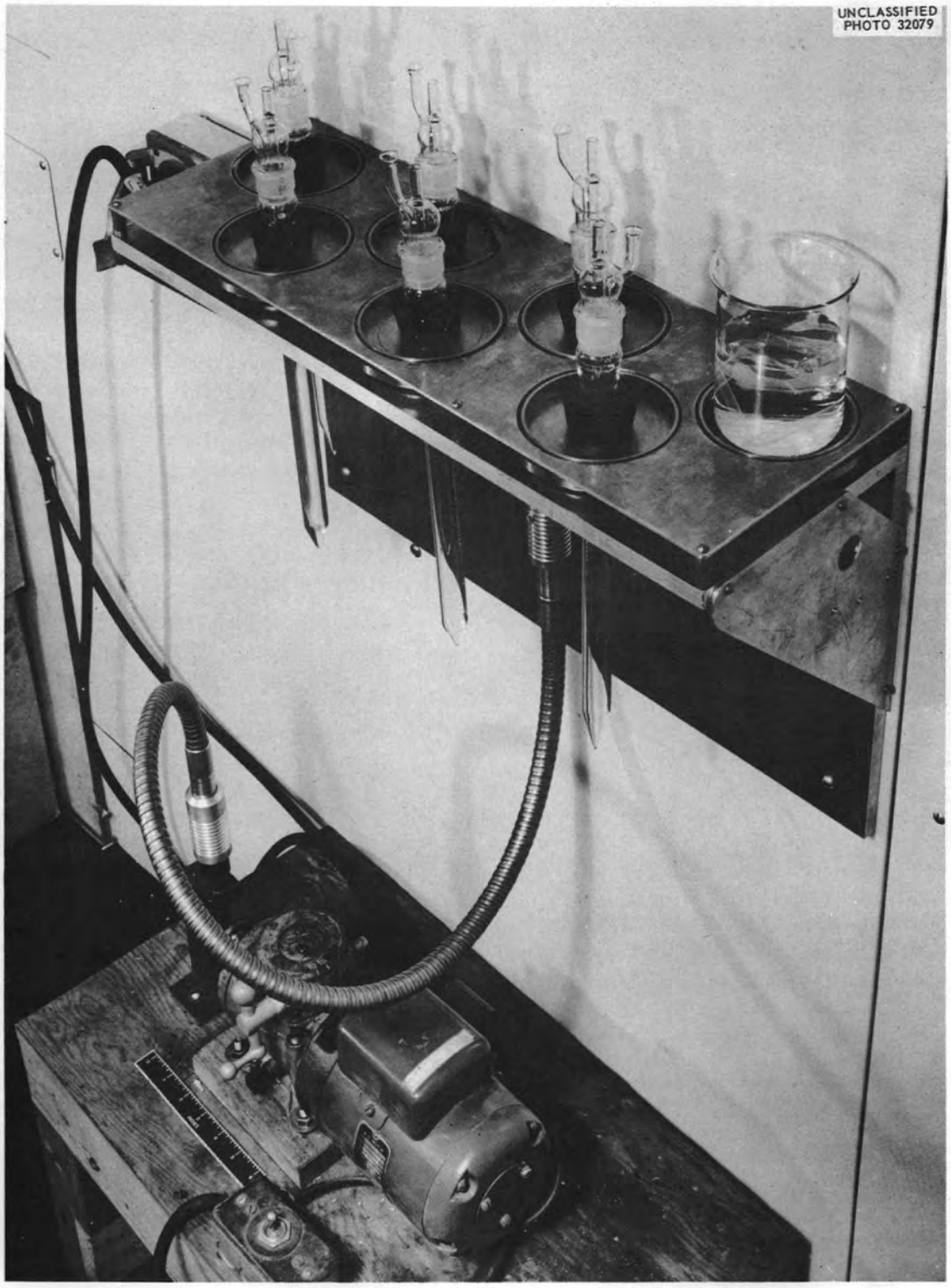

Fig. 19. Apparatus for Continuous Countercurrent Dialysis.

phenylalanine, glutamic acid, glycylglycine, glycylglycylglycine, lysine, and methionine have been measured. The concentration of most amino acids inside the sac is reduced to one-half the original concentration in approximately 30 minutes, whereas peptides require considerable additional time.

Discussion. – The oscillation of the glass dialysis cell and dialyzing sac about a vertical axis provides a maximum movement of fluid across the membrane surface, preventing the formation of concentration gradients in the liquid on either side of the dialyzing membrane. The compact method of mounting allows samples to be readily removed for concentration by evaporation and reintroduced into the sac of the next cell.

Countercurrent dialysis appears to be a gentle method for separating molecular species on the basis of the rate of their movement through a porous membrane.

An Apparatus for Desalting Amino Acids

J. C. Nichol

Introduction. – The presence of salts in amino acid preparations, such as protein hydrolyzates or tissue extracts, that are to be analyzed chromatographically is objectionable for a variety of reasons.[10,11] Several desalting procedures have been developed, but to date no one completely satisfactory method is available.

The desirability of a simple general method of desalting micro quantities of amino acids in this Laboratory led us to devise and test the following procedure, which takes advantage of the volatility of ammonium formate.[12]

The electrode compartments of a Durrum-type paper electrophoresis apparatus are filled with solutions of 0.1 ionic strength ammonium formate buffers that have pH's above and below the isoelectric points of any amino acids which may be present. The ends of a 1 × 10-in. strip of Whatman No. 1 filter paper are placed in the electrode compartments, the paper being supported at its mid-point about 5 cm above the buffer level by means of a glass rod. Some 20 to 30 μl of the solution to be desalted are placed across the mid-point of the strip, a glass cover is placed on the apparatus, and the buffer solutions are allowed to migrate up the strip to complete the liquid junction. A potential of about 50 volts is applied overnight. The amino acids migrate to the region of their isoelectric pH on the strip, whereas the salts are displaced from the strip by ammonium formate. Upon termination of electrophoresis, the ends of the strip in the buffer are cut off, and the strip is eluted with water. The eluate is vacuum-dried overnight at 40°C (a Laboratory Glass and Instruments Corporation Evapo-Mix evaporator is used in our experiments).

Results. – 1. All the amino acids examined (glycine, L-alanine, L-glutamic acid, L-cysteine, L-methionine, DL-phenylalanine, L-arginine, L-lysine, L-histidine, L-tryptophan, and L-tyrosine), when applied in 1 *N* NaCl solutions, were rapidly concentrated in a band 1 to 2 cm wide (as revealed by ninhydrin spraying) at 0 to 3 cm from the point of application, depending on their isoelectric points and the pH gradient in the strip. There was no indication of spreading of the bands with prolonged electrophoresis.

2. Quantitative tests on salt removal and amino acid recovery were made using 30-μl samples of 1% DL-phenylalanine in water and in aqueous NaCl. Some typical results are shown in Table 21. It will be observed that the electrolyte level, expressed as milligrams of NaCl, is reduced to about 5% or less of its original value (solutions 1, 2, and 3), and that roughly comparable electrolyte values in the reconstituted eluates are obtained in solutions 4 and 5, which contained no NaCl. At least part of the residual electrolyte is attributable to traces of conducting material eluted from the filter paper (solution 6), and part may be the result of traces of unsublimed ammonium formate.

The method shows promise for the removal of the major portion of the electrolytes from amino acid preparations and is being investigated further.

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[10] R. J. Block, E. L. Durrum, and G. Zweig, *A Manual of Paper Chromatography and Paper Electrophoresis*, p 52, 119, Academic Press, Inc., New York, 1958.

[11] H. G. Cassidy, *Fundamentals of Chromatography*, p 189, Interscience Publishers, Inc., New York, 1957.

[12] C. H. W. Hirs, S. Moore, and W. H. Stein, *J. Biol. Chem.* 195, 669 (1952).

Table 21. Salt Removal and Amino Acid Recovery Data

Original Solution	NaCl in Original Solution (mg)	Electrolyte Present[a] in Vacuum-Dried Eluate (as mg NaCl)	Per Cent[b] Recovery of Phenylalanine
1. Phenylalanine in 5 *N* NaCl	8.8	0.12	
2. Phenylalanine in 1 *N* NaCl	1.76	0.09	100
3. Phenylalanine in 1 *N* NaCl	1.76	0.06	95
4. Phenylalanine in H_2O	None	0.11	95
5. Phenylalanine in H_2O	None	0.12	100
6. H_2O[c]	None	0.05	

[a]Calculated from conductance measurements on vacuum-dried eluate reconstituted with 10 ml of H_2O.
[b]Calculated from absorbance measurements at 260 mμ.
[c]Untreated strip eluted with water.

BIOCHEMISTRY

NUCLEIC ACIDS, ENZYMES, AND RADIATION-PROTECTIVE COMPOUNDS

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L. Astrachan
J. L. Countryman[1]
F. J. Finamore[2]
J. W. Hastings[2]
T. W. McKinley, Jr. (part time)
J. X. Khym
M. H. Jones
D. E. Emmer
A. J. Bandy
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Minor Constituents of Nucleic Acids

W. E. Cohn
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D. G. Doherty (part time)

The identity of the so-called "fifth nucleotide" of ribonucleic acid has been tentatively established as 5-ribosyluracil. The principal evidence pointing to such an unusual structure is as follows.

Among the products of periodate oxidation of the nucleoside (1 mole is consumed) is one that, after reduction and hydrolysis, shows the chromatographic behavior expected of 5-glycolyluracil. In ion exchange it follows 5-hydroxymethyluracil; on paper chromatography in butanol-water it lies intermediate in position between the starting material (or the original oxidation product) and 5-hydroxymethyluracil. In paper electrophoresis, it follows thymine rather than uracil or 5-hydroxymethyluracil. When this product is further oxidized by periodate (again 1 mole is consumed) and again reduced, the product behaves in all three systems as does 5-hydroxymethyluracil and the spectra (in acid and in alkali) are identical. The reactions are formulated as shown in Fig. 20. Only the ready hydrolysis (step 3) remains unexplained in this sequence.

The nuclear magnetic resonance spectrum of the nucleotide was observed by members of the staff of Varian Associates in Palo Alto, California, together with those of uridine and related substances. The tracings sent us were interpreted both by the California group and by H. W. Morgan of the ORNL Physics Division. In contrast to uridine, the unknown shows the following: (1) no C_5 proton (shown by disappearance of its own characteristic peak, as well as by absence of splitting of the C_6 proton peak) and (2) substitution of C for H on C_5 (shown by decrease in shift of −102 to −71 cps). It appears similar to uridine in the $-CH_2-$ region, affording further evidence that the sugar is similar to that in uridine

[1]Research Associate.
[2]Research Participant.

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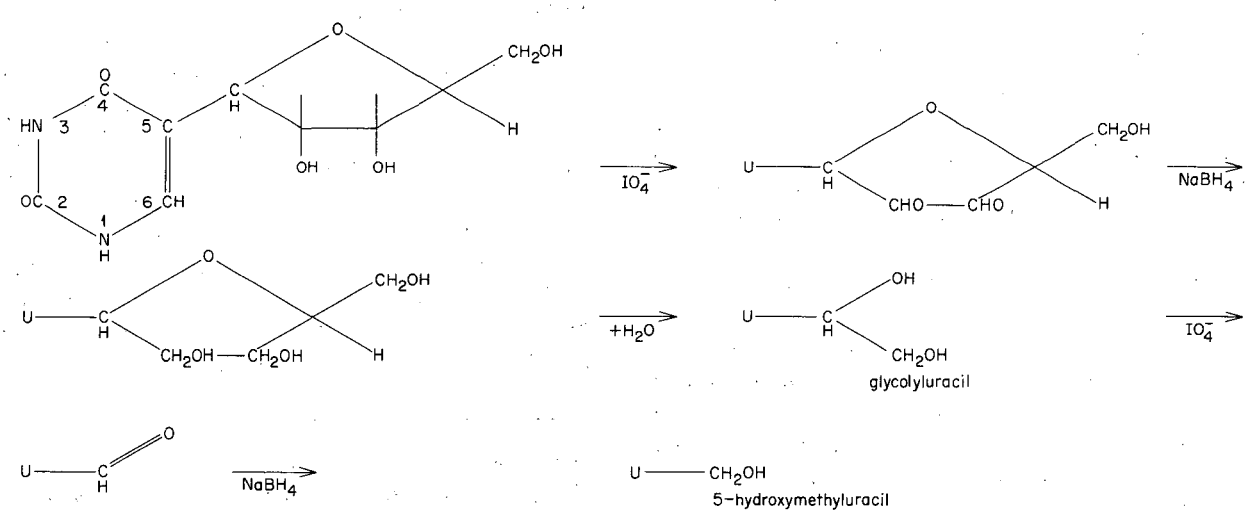

Fig. 20. Proposed Reactions for Formation of 5-Hydroxymethyluracil from the "Fifth Nucleotide."

(see below for other evidence bearing on this point).

Evidence that the sugar is ribose comes from the following:

1. The ion-exchange behavior and paper chromatographic behavior, with and without borate, of the nucleoside are always very similar to those of uridine, indicating the same high degree of borate complexing (characteristic of ribofuranosides).
2. Periodate oxidation occurs to the same degree and at the same rate as does that of uridine.
3. Elemental analysis indicates C/N/P = 9/2/1.
4. Isomerism in the nucleotide form is analogous to that observed in the major nucleotides.
5. The nuclear magnetic resonance spectrum (see above) indicates a sugar structure similar to that of uridine.

Additional direct evidence is being sought to prove the structure of the pyrimidine ring, the point of attachment, and the exact structures assumed by the sugar side chain in each of the three nucleoside forms. Three of the latter are known, two being produced from the "normal" form by acid treatment. All three are regarded as isomers since each can be isolated as an intact nucleotide (two of them in *a* and *b* forms) and all show, after dephosphorylation, a positive but diminished degree of borate complex formation. The decrease in borate complex formation gives rise to the postulate that a sugar rearrangement into pyranose and straight-chain forms occurs in acid. Hydrogenation does not affect the pyrimidine ring but apparently produces still another variety of sugar side chain.

Tentative proof that this nucleotide occurs in normal internucleotide linkage comes from the following: (1) isolation of isomeric forms of the nucleotide that give identical nucleosides upon dephosphorylation, which indicates the formation of the cyclic phosphate intermediate expected in polynucleotide hydrolysis; (2) appearance of the nucleotide in both the mononucleotide and polynucleotide fractions after hydrolysis with ribonuclease, behavior characteristic of pyrimidine nucleotides that are randomly distributed along polynucleotide chains; (3) appearance of the nucleoside upon hydrolysis with rattlesnake venom, indicating the intermediate existence of the 5′ nucleotide form.

All other nucleotides so far identified as nucleic acid constituents are N-ribosyl (or deoxyribosyl) derivatives. This is the first case of a C-ribosyl derivative in such a situation. The significance of the finding is as yet not known, but it lends point to the elucidation of the unusual chemical features so far discovered.

RNA Phosphorus Metabolism in *Escherichia coli* Infected with Bacteriophage T7

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

Metabolic turnover in some fraction of the ribonucleic acid (RNA) of *Escherichia coli* B infected with T2r+ has been demonstrated previously. Brief exposure of the infected cells to $P^{32}O_4$ results in a pattern of labeling of the RNA nucleotides that differs from the host RNA nucleotide composition but resembles the molar proportions of the analogous deoxynucleotides in T2 deoxyribonucleic acid (DNA). The T2 DNA is characterized by a relatively high content of deoxyadenylic acid and thymidylic acid,[3] and isotope incorporation into the RNA of the T2-infected cell results in relatively high specific activities of adenylic and uridylic acids. We have now investigated RNA activity in *Escherichia coli* infected with the virulent bacteriophage T7. Such a comparison is of interest since bacteriophage T7 DNA has a nucleotide composition differing widely from that of T2, and T7 does not contain the base 5-hydroxymethylcytosine.[4] In addition, the T7-infected cell uses relatively little extracellular material for DNA synthesis, i.e., there is little, if any, net DNA synthesis.[5,6]

The T7 stock used in the present experiments was analyzed for base composition. Bases were obtained by acid hydrolysis and separated by paper chromatography. Also, nucleotides (plus nucleosides) were liberated by enzymic hydrolysis and separated by ion-exchange chromatography. The analyses are in close agreement with those obtained by Lunan and Sinsheimer[4] and show that T7 DNA has nearly equal amounts of adenine plus thymine and of cytosine plus guanine. The (A + T)/(G + C) ratio is about 1.11.

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[3] G. R. Wyatt, *Biochem. J.* 48, 584–90 (1951).

[4] K. D. Lunan and R. L. Sinsheimer, *Virology* 2, 455–62 (1956).

[5] L. W. Labaw, *J. Bacteriol.* 62, 169–73 (1951).

[6] F. W. Putnam *et al.*, *J. Biol. Chem.* 199, 177–91 (1952).

Logarithmically dividing cultures of *E. coli* B were infected with T7 and subsequently allowed to assimilate $P^{32}O_4$, either continuously or for only a brief period. Samples were removed at regular intervals and analyzed for nucleic acid content and radioactivity. The manner of labeling of RNA is similar to RNA labeling in the T2-infected system in that the maximum extent of incorporation in peptone broth culture is attained within ten minutes after isotope is supplied. The progressive labeling of DNA is, by comparison with the T2 system, very slight and is simply a reflection of the low utilization of medium nutrients for DNA synthesis. When only a brief period of incorporation is permitted, P^{32} enters the RNA and then leaves it after isotope is removed from the medium. This occurs in the absence of any measurable net synthesis (or loss) of RNA. A metabolic turnover of some RNA therefore exists in the T7 system just as in the T2 system. However, in the T2 system, the rate of incorporation of P^{32} into DNA is faster than the rate of loss of isotope from total RNA. In the T7 system, during the entire course of infection, more P^{32} leaves RNA than the total P^{32} entering DNA; and the initial rate of loss of P^{32} from RNA exceeds the rate of accumulation of P^{32} into DNA at any time interval.

The distribution of P^{32} into the constituent mononucleotides of the RNA is not uniform; rather, adenylic and uridylic acids have, on the average, about 1.2 times the specific activities of the other two nucleotides. This pattern of RNA nucleotide labeling differs from that obtained with uninfected bacteria or T2-infected bacteria. As in the T2 system, however, the RNA mononucleotide specific activity ratios bear a striking resemblance to the molar composition of the analogous deoxynucleotides in T7 DNA.

On the Heterogeneity and Structure of Ovarian Egg Ribonucleic Acid

F. J. Finamore E. Volkin

Introduction. – These experiments were designed to elucidate the structure and nucleotide composition of ovarian egg RNA in an attempt to establish a firm basis for the study of the function of RNA in embryonic development.

The ribonucleic acids were isolated from mature frog ovaries by use of the phenol extraction procedure and were purified to rid the preparation of an unknown phosphorus-containing contaminant. The purification involved precipitating the contaminant by adjusting the nucleic acid preparation to 0.03 *M* with respect to $MgCl_2$ at pH 6.

Results. – It has been found that total RNA of mature ovaries is composed of at least two distinct types of RNA: one that is soluble in cold 1 *M* perchloric acid (soluble RNA) and another that is insoluble in the same concentration of perchloric acid (insoluble RNA). Each comprises roughly 50% of the total RNA.

A study of the mononucleotide composition of the two types of RNA by column chromatography after alkaline hydrolysis has shown that they differ significantly in that soluble RNA contains relatively less cytidylic and guanylic acids but more adenylic and uridylic acids than does insoluble RNA (Table 22). This distinguishing feature of soluble RNA is curious because on alkaline hydrolysis the soluble RNA yields 5′-adenylic acid and 5′-uridylic acid in addition to the expected 2′,3′ mononucleotides, whereas insoluble RNA yields only the normal 2′,3′ mononucleotides after similar treatment.

Table 22. Composition of Soluble and Insoluble RNA's

Acid	Soluble RNA		Insoluble RNA	
	(μmoles)	(%)	(μmoles)	(%)
Cytidylic	2.91	23.6	3.67	30.6
Adenylic	3.10	25.1	2.16	18.0
Uridylic	2.85	23.0	1.48	12.3
Guanylic	3.48	28.3	4.69	39.1

A striking similarity of both the soluble and insoluble RNA's is that each is *not* quantitatively hydrolyzed to mononucleotides on prolonged treatment with alkali. About 90% of each RNA has been recovered as mononucleotides and the remaining 10% as a mixture of di-, tri-, and tetranucleotides.

Discussion. – These results indicate that the RNA of mature ovarian frog eggs is a mixture of at least two distinct types of RNA. The fact that 5′-adenylic and 5′-uridylic acids are liberated on alkaline hydrolysis of soluble RNA, in addition to the normal constituents, and the fact that both soluble and insoluble RNA are not quantitatively

hydrolyzed to mononucleotides on prolonged treatment with alkali indicate that structural peculiarities exist in each type.

The Mechanism Involved in Persistent Diurnal Rhythms

J. W. Hastings L. Astrachan

This investigation is concerned with the physicochemical basis for cellular rhythmicity. The marine dinoflagellate *Gonyaulax polyedra* is the organism being used in the study. From previous studies,[7,8] it is known that, if the organism is kept under natural illumination, the bioluminescence is about 60 times as great during the night as during the day. This variation is known to be correlated with similar variations in the amount of extractable enzyme and substrate involved in luminescence.[9] There is particular interest in this phenomenon because these variations continue even when the organism is kept under absolutely constant conditions of light and temperature. The period of the rhythm under constant conditions is close to but not exactly 24 hours, and it is believed that the mechanism involved may be related to a "biological clock."

The present study has been directed to discover the nature of the biochemical processes associated with the rhythm. It has been found that there is an apparent persistent rhythm in photosynthesis. The rate of $C^{14}O_2$ fixation, measured at saturating light intensity, is about 5 to 6 times as great during the middle of the day as during the night. This variation continues in cells kept under constant conditions, indicating that the phenomenon is not the direct result of light-dark changes. Similar differences in the rate of phosphorus incorporation at different times of day have been observed.

Since maximum photosynthesis and maximum luminescence occur at different times, it is considered that variations in nucleic acids might be involved in the mechanism responsible for rhythmicity. A variety of extraction and purification procedures have been used to obtain nucleic acid preparations from *Gonyaulax* cells. The preparations of highest purity and best yield have been obtained by extracting broken cell preparations with hot sodium chloride. RNA and DNA extracted in this way account for about 1%–2% of the dry weight of the cells. Analyses of nucleic acid fractions from cells harvested at different times of day will be made in order to determine possible changes in the rate of synthesis and/or base composition of RNA and DNA.

Structure and Activity in Radiation-Protective Sulfhydryl Compounds

D. G. Doherty J. D. Bales, Jr.
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Since the aminoalkylisothioureas may be administered as such or rearranged to the corresponding mercaptoalkylguanidine and given, the relative effectiveness of the two forms by various routes was examined for several compounds.[10] AET and APT are equally effective orally whether given as the isothiourea or as the mercaptoalkylguanidine, an $LD_{50}/30$ of 1500 r being obtained for 101 × C3H mice. However, both compounds are less effective when given intraperitoneally as the isothiourea, $LD_{50}/30$ = 1300 r, than as the mercaptoguanidine, $LD_{50}/30$ = 1450 r. In contrast, APMT is more effective when given as the isothiourea than as the mercaptoguanidine, regardless of the route of administration. Curiously, the chloride salt of APMT is less effective and slightly more toxic chemically in an equivalent molecular weight dose, in comparison with the bromide salt, when given orally, $LD_{50}/30$ = 1000 r vs 1100 r for the bromide, but is more active intravenously with an $LD_{50}/30$ of 1250 r. Chemical studies on the two salts reveal no differences in their behavior that might explain this anomaly.

In view of the increased activity on a molar basis at 900 r and decreased chemical toxicity of APMT, a series of variously substituted aminoalkyl-N′-alkylisothioureas were prepared and examined for protective activity. The corresponding AET compound S,2-aminoethyl-N′-methylisothiourea dihydrobromide, AEMT, did not show a decreased toxicity as does APMT, was ineffective orally, and gave an $LD_{50}/30$ at 90 mg/kg intravenously

[7] B. M. Sweeney and J. W. Hastings, *J. Cellular Comp. Physiol.* 49, 115–28 (1957).

[8] J. W. Hastings and B. M. Sweeney, *Proc. Natl. Acad. Sci. U.S.* 43, 804–11 (1957).

[9] J. W. Hastings and B. M. Sweeney, *J. Cellular Comp. Physiol.* 43, 209–26 (1957).

[10] D. G. Doherty *et al.*, *Biol. Semiann. Prog. Rep. Feb. 15, 1958*, ORNL-2481, p 59–60.

of 1100 r. N′-Ethyl or higher AET's and APT's were completely unprotective. Symmetrical substitution of alkyl groups on both guanido nitrogen atoms yielded compounds that intratransguanylated in the usual way but had little or no radiation-protective activity. In an attempt to increase the fat solubility and delay the excretion, several bis-AET and -APT compounds were prepared with the two AET or APT molecules linked by alkyl chains of varying lengths. All compounds intratransguanylated in a normal manner yielding two moles of –SH per mole of compound, indicating the formation of the corresponding bis-mercaptoguanidines. Initial screening results indicate a somewhat decreased protective activity in comparison with AET and APT, as well as an increased toxicity, especially in the mercaptoguanidine form.

ENZYMOLOGY

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Mechanism of the Carboxyl Activation of Glycine in Extracts of *Photobacterium fischeri*

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G. D. Novelli

Introduction. – Previous reports[5,6] have described the isolation, purification, and properties of an enzyme from *Photobacterium fischeri* that catalyzes a reaction between ATP and glycine that leads to the activation of the carboxyl group of glycine. Results were presented that led us to suggest that glycine activation may proceed via one of two possible mechanisms shown below:

Scheme I

$$E + ATP \rightleftharpoons E\text{-}ADP + P$$
$$E\text{-}ADP + \text{glycine} \rightleftharpoons E\text{-glycine} + P$$

Scheme II

$$E + ATP + \text{glycine} \rightleftharpoons E\text{-glycylphosphate} + ADP$$

The two reactions can be distinguished by the use of carboxyl-labeled O^{18}-glycine. In Scheme I the oxygen from the carboxyl group of glycine might be expected to appear in the terminal phosphate of ADP and certainly not in the orthophosphate. If, however, the reaction proceeds via Scheme II, the O^{18} should appear in the orthophosphate.

Results. – The enzyme (17 mg of protein) (9-fold purified) was incubated with 140 μmoles of ATP, 100 μmoles of $MgCl_2$, 1000 μmoles of glycine-O^{16} or glycine-O^{18}, 20,000 μmoles of salt-free NH_2OH, 2000 μmoles of Tris buffer, pH 7.8, and water to a final volume of 10 ml. After incubation for 1 hr at 37°C, the reaction was stopped by the addition of 5.4 ml of cold 30% perchloric acid (PCA) in 3.1 *M* HCl. The mixture was cooled immediately to 0°C, and after 10 min the protein was removed by centrifugation. The PCA was removed as the potassium salt and the NH_2OH was removed by repeated lyophilization at pH 8.0. The sample was divided into equal parts to permit duplicate determinations. The orthophosphate, after addition of carrier, was precipitated as $MgNH_4PO_4$. The adenylic nucleotides were separated on Dowex 1 columns. The acid-labile phosphates of the nucleotides were liberated by hydrolysis and after carrier additions were precipitated as $MgNH_4PO_4$. The $MgNH_4PO_4$ precipitates were converted to KH_2PO_4, which was then analyzed for O^{18} content as described by Harrison *et al.*[7] The O^{18} analysis indicated that 50% of the expected label from glycine-O^{18} appeared in the orthophosphate. This suggested that the activation was proceeding via Scheme II. Since only half of the expected label appeared in the orthophosphate, it seemed possible that some of the O^{18} in the inorganic phosphate (Pi) was being diluted by a secondary reaction. This possibility was tested by carrying out an experiment identical with the previous one except that glycine was omitted and Pi-O^{18} (0.371 atom % excess O^{18}) was added. The phosphates were worked up as before, and the analysis of the O^{18} content of the Pi revealed an O^{18} content of 0.183 atom % excess. Thus a side reaction is present that can cause a 50% dilution of O^{18} in Pi.

Discussion. – The fact that 50% of the O^{18} from carboxyl-O^{18}-glycine appeared in orthophosphate, together with the experimental verification of the presence of a side reaction that results in a 50% dilution of O^{18} in orthophosphate, establishes that glycine activation in extracts of *P. fischeri* proceeds via Scheme II. This is similar to the mechanism postulated for glutamic acid activation in the glutamine synthetase reaction.[8]

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[5]M. J. Cormier and G. D. Novelli, *Biol. Semiann. Prog. Rep. Feb. 15, 1958*, ORNL-2481, p 61.

[6]M. J. Cormier and G. D. Novelli, *Biochim. et Biophys. Acta* (in press).

[7]W. H. Harrison, P. D. Boyer, and A. B. Falcone, *J. Biol. Chem.* **215**, 23 (1954).

[8]P. D. Boyer, O. J. Koeppe, and W. W. Luchsinger, *J. Am. Chem. Soc.* **78**, 356 (1956); A. Kowalsky *et al.*, *J. Biol. Chem.* **219**, 719 (1956).

Ornithine Transcarbamylase Synthesis by a Uracil-Requiring Mutant of *Escherichia coli*

P. Rogers, Jr. G. D. Novelli

Introduction. – Our previous studies on the synthesis of ornithine transcarbamylase (OTC) by *Escherichia coli* upon release from arginine suppression indicated that the sudden appearance of OTC activity represents synthesis of new protein molecules.[9,10] In order to clarify a possible involvement of RNA in OTC synthesis, we turned to a uracil-requiring mutant. At the time of this report, our data suggest that OTC synthesis can occur in the absence of simultaneous RNA formation.

Results and Discussion. – The uracil-requiring mutant of *E. coli* did not grow in the absence of added uracil, and yet OTC synthesis occurred in both cells and protoplasts without added uracil. In five experiments in which cells were carefully uracil starved prior to release from arginine suppression, an absolute requirement of uracil for OTC synthesis *could not* be demonstrated, although uracil always provided a stimulation.

Pardee[11] has reported that purine and pyrimidine mutants of *E. coli* will not synthesize β-galactosidase in the absence of the required nucleic acid precursor, under conditions where adequate carbon source is available. He interpreted his results to mean that simultaneous RNA synthesis occurred during the induction of β-galactosidase. In order to establish the apparent difference between the OTC synthesizing mechanism and induction of β-galactosidase, an experiment was carried out in which both enzymes were synthesized simultaneously in the same cells. The uracil mutant was grown in minimal salts, glycerol, arginine, and uracil. Log-phase cells were harvested, starved for uracil for 75 min at 37°C, harvested, and resuspended in a medium with glycerol, with lactose, with or without arginine, and with or without uracil. The time course of simultaneous OTC and β-galactosidase synthesis was followed in the same cell suspensions. As was found previously, OTC synthesis occurred even in the absence of added uracil but never in the presence of arginine. In contrast, β-galactosidase was synthesized only when uracil was present in the medium. This experiment was repeated with the same results using cells adapted to utilizing amino acids as a sole carbon source. Thus, these results indicate that OTC was synthesized without new RNA synthesis, which requires uracil, while, in agreement with Pardee, β-galactosidase induction occurred only under conditions favoring RNA synthesis.

In an effort to obtain further information concerning the role of RNA in the synthesis of OTC by suppressed cells, the incorporation of P^{32}-labeled inorganic phosphate into total RNA nucleotides of the uracil mutant was investigated. Cells were starved of their uracil and then allowed to synthesize OTC in the presence and absence of uracil with P^{32} inorganic phosphate in the medium. OTC synthesis again proceeded without uracil at about one-half the rate as in those cells exposed to uracil in the medium. In contrast, in three separate experiments, P^{32} inorganic phosphorus incorporation into total RNA nucleotides was almost entirely dependent on uracil's presence in the medium.

It has been postulated that a specific RNA is formed in conjunction with a specific protein.[12] If this is true, it may be that a small increment of RNA is turned over in the absence of uracil with a selection in favor of OTC formation. It seems more likely, however, that under growing conditions the uracil-requiring mutant is under stress in many directions. Thus any uracil leaking into an available pool from RNA breakdown or from a leakage through the block would be quickly utilized into many other systems simultaneously, leaving OTC synthesis proportional to any general small protein increase. However, since the specific activity of OTC increases some 20- to 100-fold in the absence of uracil, it seems likely that the initial burst of OTC is due to the presence of some preformed synthesizing system. When uracil is added and RNA synthesis proceeds normally, there is an increase in OTC synthesis, presumably because of the formation of new OTC forming sites.

Experiments now under way are directed toward the eventual isolation of the OTC synthesizing system from arginine-suppressed cells and protoplasts.

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[9] J. A. DeMoss and G. D. Novelli, *Biol. Semiann. Prog. Rep. Aug. 15, 1957*, ORNL-2390, p 83.

[10] P. Rogers and G. D. Novelli, *Biol. Semiann. Prog. Rep. Feb. 15, 1958*, ORNL-2481, p 62.

[11] A. B. Pardee, *Proc. Natl. Acad. Sci. U.S.* **40**, 263 (1954).

[12] R. Ben-Ishai, *Biochim. et Biophys. Acta* **26**, 477 (1957).

Isolation and Purification of Ornithine Transcarbamylase from Extracts of *Escherichia coli*

P. Rogers, Jr. G. D. Novelli
H. Maclay

Introduction. – Investigations on the biosynthesis of proteins in cell-free extracts of tissues are hampered by methods for the measurement of specific protein synthesis. Various investigators have used one of two methods to circumvent the problems. One method is to measure the synthesis of some easily assayed enzyme. In this case, however, one is measuring an increase in *activity* of the enzyme. An increase in the number of molecules of enzyme is not the only way that an apparent increase in enzyme activity can occur. Thus this method is not an absolute measurement of increase in protein molecules. The other popular method is to use isotopically labeled amino acids and to measure their appearance in the recovered protein. Amino acid incorporation has several drawbacks. First, it does not distinguish between exchange or addition reactions as opposed to net protein synthesis, and second, since a specific protein is not being examined, one has little notion regarding the system that leads to incorporation.

It seems to us that a reliable measure of protein synthesis would be the study of a specific enzyme combined with the isotope technique. The rate of increase in enzyme activity could then be related to the rate of appearance of isotope in the isolated enzyme. In order to perform such an experiment, it is necessary to isolate a purified enzyme from the cell proteins and form an antiserum against this enzyme. The enzyme activity could then be isolated by a specific precipitation reaction between this antiserum and the enzyme molecules.

In the anticipation that a cell-free system for the study of the synthesis of ornithine transcarbamylase might soon be available, the present project was started to isolate highly purified OTC to be used as an antigen for the preparation of a specific antiserum.

Results. – *Escherichia coli* W. was grown in a minimal salts solution containing 1% glucose and 200 μg/ml arginine. Arginine suppresses the synthesis of OTC. After growth with vigorous aeration the cells were harvested and resuspended in the same medium in 3 times the original concentration and in the absence of arginine. This suspension was incubated anaerobically for 2 hr to allow release of suppression. During this time the specific activity of OTC went up 2½ times and the total units increased 10-fold. These cells were ground with alumina and extracted with phosphate buffer. The crude extracts have been subjected to a number of fractionation procedures in pilot runs in order to work out a fractionation scheme for the isolation of OTC. Heating the extract at 65°C for 5 min gives a 2-fold increase in specific activity with little loss. Precipitation with acetic acid between pH 4.5 and pH 4.3 gives a 4-fold increase in specific activity. Fractionation with cold ethanol up to 62.5% gives a 3-fold increase in specific activity, and fractionation with ammonium sulfate gives a 2-fold purification. Preliminary trials with diethylaminoethylcellulose (DEAE) columns indicate that they will be suitable for further fractionation.

Discussion. – These preliminary trials on the purification of OTC indicate that a variety of fractionation procedures give promise of considerable purification of this enzyme. All of the trials were individual, and we are now starting to combine various steps. It seems that a 50-fold purified preparation will be rather easily obtained. At that point further trials will be necessary.

Induction and Synthesis of β-Galactosidase in Cell-Free Preparations of *Escherichia coli*

G. D. Duda G. D. Novelli

Introduction. – In order to describe in detail the mechanism by which β-galactosidase is induced and synthesized in *Escherichia coli* it is necessary to have a cell-free preparation where the various parts of the system can be analyzed.

Results. – Four different methods for preparation of cell-free systems were studied. These were (1) sonic oscillation in a Raytheon 10-kc sonic oscillator; (2) disruption with glass beads in a Nossal shaker; (3) rupture at –30°C in a Hughes press; and (4) grinding in the cold with Alumina A301. The broken cells were fractionated by differential centrifugation into particulate and soluble components. These were tested alone and in combination, together with additional supplements, for β-galactosidase induction. In none of these preparations could the induction of β-galactosidase be demonstrated.

Since the synthesis of β-galactosidase may be a special case of protein synthesis with unusual

requirements, we felt that a more direct and less specific approach might be successful. Accordingly, the incorporation of C^{14}-labeled amino acids into protein was studied in these same preparations of ruptured cells. The incorporation of amino acids into protein has indeed been observed in these preparations. The incorporation, however, has been small in magnitude and highly variable.

Discussion. – The inability to observe the induction of β-galactosidase synthesis in broken cell preparations suggests that some critical factor is not being controlled in our experiments. This same statement can be applied to the erratic observations on the incorporation of C^{14} amino acids into protein. In the latter case, however, the small positive results suggest that the system is amenable to experimental reinforcement.

The Role of Amino Acids in RNA Synthesis by *Escherichia coli*

M. P. Stulberg G. D. Novelli

Introduction. – Gros and Gros[13] and others have observed that amino acid auxotrophs of *Escherichia coli* approximately double their content of RNA when protein synthesis is largely inhibited in the presence of chloramphenicol. This RNA synthesis occurs *only* in the presence of small amounts of the required amino acid, and thus has implicated amino acids as required factors in RNA synthesis. These studies were initiated to further elucidate this possible role of amino acids in RNA synthesis with the use of O^{18} as a tracer.

Results. – Upon adding O^{18}-leucine to a leucine-depleted culture of a leucine auxotroph of *E. coli* under chloramphenicol inhibition, the RNA content of the cells was increased by 50% without any significant concomitant synthesis of protein. Isolation and analysis of the phosphate oxygen of RNA, acid-soluble organic phosphate, and inorganic phosphate of the cells showed no detectable O^{18} label. However, upon isolating leucine from the cells and incubation media, it was found that more than 50% of the O^{18} label had been removed.

Discussion. – The lack of incorporation of O^{18} from O^{18}-leucine into the phosphate oxygen of the various phosphate fractions examined presents strong evidence against the postulated amino acid–nucleoside phosphate anhydride as an intermediate for both RNA and protein synthesis. However, the loss of O^{18} from leucine indicates that the amino acid did undergo a reaction involving carboxyl oxygen during the observed synthesis of RNA. It appears that under the conditions of this experiment leucine turns over, and this turnover may reflect the manner in which the amino acid participates in RNA synthesis.

Formation of Activated Amino Acids by Whole Cells of *Azotobacter vinelandii*

R. W. Bernlohr G. D. Novelli

Introduction. – It has been shown[14–17] that various preparations of amino acid-activating enzymes convert amino acids into a form that is adsorbable on charcoal, that is unstable to alkali, and that reacts with hydroxylamine to form amino acid hydroxamates. These "activated" amino acids seem to be amino acid–polynucleotide compounds, and have been suggested[17] as intermediates in protein synthesis because the amino acid of such compounds is incorporated into the protein of isolated microsomes. Activated amino acids have been formed only in cell-free preparations, and it is of considerable interest to determine whether similar compounds are formed by intact cells.

Results. – A charcoal-adsorbable, radioactive factor can be isolated from *A. vinelandii* cells that have been incubated in the presence of glycine-C^{14} or leucine-C^{14}. The properties of this factor are similar to those mentioned above, with one exception. Hydroxamate-producing material has been found only in a perchloric acid-soluble fraction, and cannot be precipitated with ethanol or extracted with phenol. Experimentation to isolate this factor which binds amino acids *in vivo* is in progress.

Discussion. – The report of Beljansky and Ochoa[18] casts some doubt concerning the part

[13] F. Gros and F. Gros, *Exptl. Cell Research* **14**, 104 (1958).

[14] M. B. Hoagland, P. C. Zamecnik, and M. L. Stephenson, *Biochim. et Biophys. Acta* **24**, 215 (1957).

[15] P. Berg and E. J. Ofengand, *Proc. Natl. Acad. Sci. U.S.* **44**, 78 (1958).

[16] R. S. Schweet *et al.*, *Proc. Natl. Acad. Sci. U.S.* **44**, 173 (1958).

[17] M. B. Hoagland *et al.*, *J. Biol. Chem.* **231**, 241 (1958).

[18] M. Beljansky and S. Ochoa, *Proc. Natl. Acad. Sci. U.S.* **44**, 494 (1958).

played by the amino acid-activating enzymes during the incorporation of amino acids into protein. In addition, the isolation of the activated amino acids from the perchloric acid supernatant solution[19] differs from previously reported work[14–17] in which the S–RNA–amino acid can be isolated in a pH 5 preparation or by phenol extraction. It is hoped that these anomalies can be clarified and that the elucidation of the mechanism of protein biosynthesis can be approached a little more closely.

Uronic Acid Metabolism by Microorganisms

R. A. McRorie G. D. Novelli

Introduction. – The initial reaction in the utilization of the D-alduronic acids, glucuronic acid and galacturonic acid, is isomerization by an induced enzyme(s) to yield the corresponding D-keturonic acids, fructuronic acid and tagaturonic acid.[20–22] The keturonic acids are subsequently reduced by another induced enzyme(s) to the corresponding L-hexonic acids, gulonic acid and galactonic acid, in the presence of reduced pyridine nucleotides.

Uronic isomerase is present in all organisms that utilize uronic acids, but keturonic reductase is not universally present in these organisms. In addition, adapted organisms are not simultaneously induced to metabolize the homologous pentoses expected from the decarboxylation of the hexonic acids. These results suggested that the metabolism of alduronic acids might proceed through the keturonic acids in the presence of additional enzymes.

Results. – The products of the anaerobic metabolism of glucuronate by *Aerobacter aerogenes* cells were compatible with the hypothesis that the metabolism of fructuronate proceeds through a pathway that is analogous to the glycolytic scheme (Fig. 21). In support of this pathway, the presence

[19] R. W. Bernlohr and G. C. Webster, *Nature* **182**, 531 (1958).

[20] A. J. Wahba, J. W. Hichman, and G. Ashwell, *J. Am. Chem. Soc.* **80**, 2594 (1958).

[21] W. J. Payne and R. A. McRorie, *Biochim. et Biophys. Acta* (in press).

[22] R. A. McRorie, A. K. Williams, and W. J. Payne, *J. Bacteriol.* (in press).

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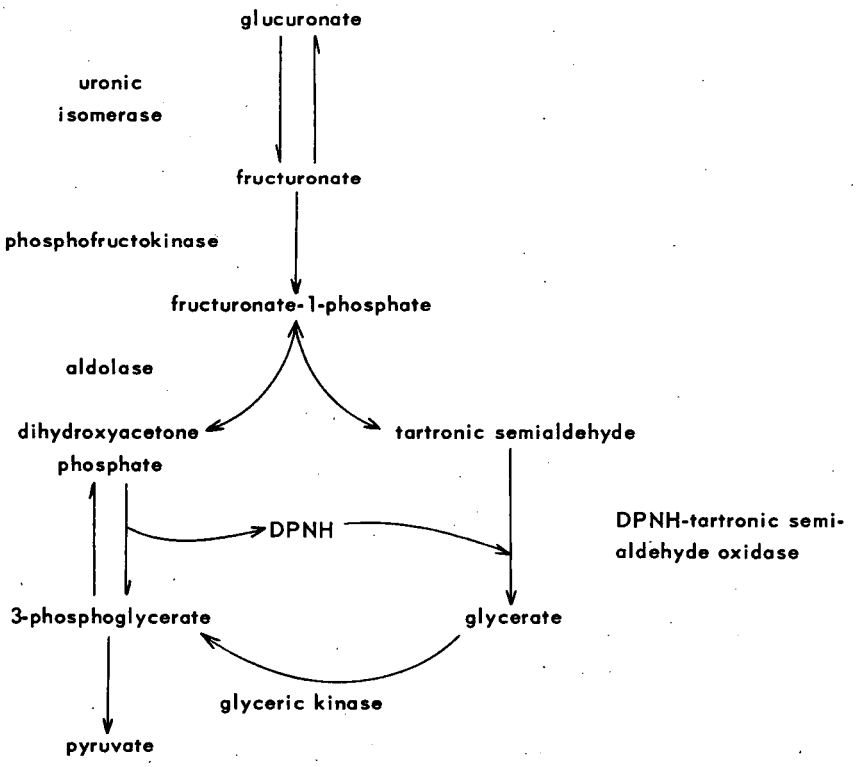

Fig. 21. Proposed Mechanism for Metabolism of Glucuronate.

of fructuronic kinase, fructuronic-1-phosphate aldolase, and DPNH–tartronic semialdehyde activities have been demonstrated in cell-free extracts of both glucose- and glucuronate-grown cells. Work is now in progress to demonstrate the presence of the proposed enzymes, to purify the demonstrated enzymes, and to determine the substrate specificities of the normal glycolytic and uronic acid-induced enzymes.

Synthesis of Nucleic Acids in the Developing Chick Embryo

K. B. Jacobson

Introduction. – The formation of nucleic acids in the rapidly growing chick embryo was established by direct determination. Reddy *et al.*[23] showed that the RNA per embryo increases from about 3 to 42 mg and that the DNA increases from about 2 to 27 mg between the seventh and nineteenth day of incubation. The formation of nucleotide polymers by enzymatic systems from bacteria has been described by Ochoa[24] and Kornberg.[25] Ochoa's group found that ribonucleoside *di*phosphates polymerize, whereas Kornberg's group found that deoxyribonucleoside *tri*phosphates undergo polymerization. The polymers formed resemble RNA and DNA, respectively. Hilmoe and Heppel[26] have demonstrated that guinea pig liver nuclei can convert polyadenylic acid to ADP in the presence of inorganic phosphate. This is the only suggestion that polymerizing enzymes similar to the polynucleotide phosphorylase of Ochoa occur in vertebrates.

Results. – Preliminary investigations have revealed that chick embryos at various stages of development have enzyme systems that apparently can convert ribose-containing derivatives of ribonucleotide triphosphates to deoxyribose derivatives.

Discussion. – The developing chick embryo seems to be a good tissue in which to examine the extent to which the diphosphonucleotide and the triphosphonucleotide mechanisms participate in nucleic acid synthesis.

[23] D. V. N. Reddy, M. C. Lombardo, and L. R. Cerecedo, *J. Biol. Chem.* 198, 267 (1952).

[24] S. Mii and S. Ochoa, *Biochim. et Biophys. Acta* 26, 445 (1956).

[25] V. Z. Littauer and A. Kornberg, *J. Biol. Chem.* 226, 1077 (1957).

[26] R. J. Hilmoe and L. A. Heppel, *J. Am. Chem. Soc.* 79, 4810 (1957).

Biochemical Properties of Antigens Responsible for Bone Marrow Transplantation Immunity

A. N. Best T. Makinodan[27]
G. D. Novelli

Introduction. – In previous communications[28,29] it was reported that preimmunization of mice with various fractions of rat liver homogenate resulted in the failure of injected rat bone marrow to protect the mice against death from lethal doses of X radiation. The present study was initiated to obtain further information regarding the chemical nature of the antigens involved in this reaction.

Results. – We have found that the antigenic activity of rat liver microsomes is stable to the following treatments: storage at –20°C for at least 1 yr; repeated freezing and thawing; and heating for 3 min at 100°C. In a dose study, an injection of as little as 0.015 mg N per mouse has retained full antigenic activity. When rat liver microsomes were subjected to sonic oscillation in a 10-kc Raytheon sonic oscillator for 30 min at 10°C and subsequently centrifuged at 105,000 × *g*, the antigenic activity was found in both the sedimentable and nonsedimentable fractions. The antigenic activity of rat liver mitochondria is stable to treatment with acetone in the cold. When such acetone powders are extracted with buffer, some antigenic material becomes soluble.

Discussion. – Although the chemical nature of the antigenic material is not yet established, several of the observations are consistent with the idea that the material may be polysaccharide. These are (1) heat stability, (2) stability to acetone, (3) the fact that as little as 0.015 mg N per mouse is antigenically active. Under test at the moment are preparations that have been treated with digitonin, lauryl sulfate, deoxycholate, and *n*-butanol. It is hoped that one of these treatments will yield a soluble antigen whose chemical nature could then be more easily ascertained.

[27] Radiation Immunology Group.

[28] T. Makinodan, N. Gengozian, and J. F. Albright, *Biol. Semiann. Prog. Rep. Feb. 15, 1958*, ORNL-2481, p 37.

[29] T. Makinodan, N. Gengozian, and J. F. Albright, *Ann. N.Y. Acad. Sci.* (in press).

Relative Rate of *in vivo* Protein Synthesis in the Livers and Spleens of Lethally Irradiated Mice

A. N. Best C. C. Congdon[30]
G. D. Novelli

Introduction. – Congdon[31] has noted a characteristic reactive change that occurs in the white pulp of the spleen in lethally irradiated mice that have received heterologous bone marrow. The change seems to be most pronounced within 48 hr after irradiation and the injection of bone marrow. One interesting characteristic of this reaction is that, normally, after lethal total-body irradiation there is an approximately 50% loss in the weight of the spleen that is evident within 24–48 hr after irradiation. If, however, the irradiated animals are treated with heterologous bone marrow the spleen weight loss is partly prevented. This observation suggested that the presence of the foreign bone marrow might be stimulating protein synthesis in the spleens of the irradiated mice. If this is true, then the reaction should be characteristic of spleen and not of other organs. This possibility has been tested for by comparing the rate of *in vivo* incorporation of glycine 2-C^{14} into the spleen and liver proteins of normal mice and irradiated mice treated with rat bone marrow.

Results and Conclusions. – A preliminary experiment was performed as follows: Four groups of mice [normal (3); 900 r (4); 900 r + 98 × 10^6 rat bone marrow cells (4); and 900 r + 90 × 10^6 isologous bone marrow cells (2)] were given two intravenous injections of 0.5 μc of glycine 2-C^{14} separated by 30 min. The injections were begun 48 hr after the start of the experiment. The animals were sacrificed 2 hr after the last injection, and the livers and spleens of each group were removed, pooled, and homogenized in 5% trichloroacetic acid (TCA). The protein was extracted with cold TCA to remove acid-soluble compounds, extracted with alcohol, with alcohol plus ether, and finally with ether to remove lipid. The residue was extracted with hot perchloric acid to remove nucleic acids and then washed with alcohol and ether. The radioactivity of the dry protein was measured, and the data were calculated in terms of counts per minute per milligram of protein. When the ratio of the specific activity of the spleen protein to the specific activity of the liver protein was calculated, the results were as follows: normal, 1.16; 900 r, 0.94; 900 r + rat bone marrow, 1.28; 900 r + isologous bone marrow, 0.67. These data suggest that the two groups of animals treated with rat bone marrow showed a stimulation of the rate of protein synthesis in their spleens relative to that of their livers. Since, however, the numbers of animals used in this experiment were small it was felt that a study of the variation of spleen/liver protein synthesis in individual animals using this technique was warranted. Accordingly, ten groups of two normal animals each were studied in a similar fashion. The spleen/liver ratios of such animals varied from 1.05 to 1.59, and thus it seems that the observations recorded in the previous experiment are uncomfortably close to the limits of error of the method. We then worked on an improvement in the technique for counting the protein samples and found that much more reproducible counting results could be obtained by first dissolving the dry denatured protein in hot 90% formic acid. Such solutions could be plated at infinite thinness, and replicate determinations did not vary by more than 3 cpm. When the bone marrow experiment was repeated using the improved counting method, spleen/liver ratios were obtained as follows: normal, 1.13; 900 r + isologous bone marrow, 1.24; 900 r + heterologous bone marrow, 1.19. It seems clear, therefore, that possible differences in protein synthesis in the spleens and livers of these mice cannot be revealed by this method.

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[30] Mammalian Recovery Group.

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

MICROBIOLOGY

TRACER STUDIES ON INTERMEDIARY METABOLISM

S. F. Carson E. F. Phares
M. I. Dolin M. V. Long
N. P. Wood[1]

Enzymes of the Succinic Decarboxylase System

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

Introduction. – The isolation of a bacterial "carbon dioxide enzyme" that functions in the succinic decarboxylase system has been described.[2] The enzyme sediments in the 140,000 × *g* pellet, which consists of three main components with Svedberg units of 11, 20, and 36. The present report concerns further purification of the carbon dioxide enzyme (which is contained in the particulate fraction) and the decarboxylase (which resides in the soluble phase).

Results and Discussion. – A gradient centrifugation of the particulate fraction was carried out in order to determine which size of particles was richest in the carbon dioxide enzyme. A pellet, which was obtained by centrifuging at 140,000 × *g* for 5 hr in the angle head, was washed with water and 15% sucrose. This fraction was recentrifuged through a 10% to 40% sucrose gradient at 140,000 × *g* in the swinging bucket head of the Spinco preparative centrifuge. Thirty per cent of the protein sedimented only very slightly and was devoid of activity (S 11 fraction). About 40% of the protein, representing most of the S 20 fraction, was spread through the lower half of the gradient, and a specific activity peak was observed in the middle of the S 20 fraction. The remaining 30% of the protein was in the pellet and appeared to consist of a mixture of S 20 plus S 36 fractions, which exhibited a specific activity as high as that found in the peak of the S 20 fraction. Gradient centrifugations carried out in the angle head were successful in resolving the two heavier fractions more completely. Pellets, probably composed mainly of the S 36 fractions, had lower specific activity than the adjacent unsedimented layer, indicating that the S 20 fraction is the source of the CO_2 enzyme.

The decarboxylase, which is present in the soluble phase, is so unstable that purification of it has been quite difficult. Extracts lose 25% to 50% of their activity overnight at 0°C. No protective action is contributed by various compounds, such as reducing and chelating agents. In the search for a purification method compatible with this extreme lability, the common fractionation methods have been carried out at −10°C. Glycerol was used to depress the freezing point during all operations except in the cases of salt and solvent precipitation. Some purification seems to be possible by use of this approach, but as yet only zone electrophoresis on starch has produced both purification and resolution from the interfering CoA-transphorase. Hence purification is being carried out by starch electrophoresis on significantly larger scale than heretofore.

Flavoproteins in Electron Transport: DPNH Oxidase of *Clostridium perfringens*

M. I. Dolin

Studies are being continued on the mechanism of flavoprotein catalysis in obligate and facultative anaerobes. The four-electron DPNH oxidase of *Clostridium perfringens* has been shown to be a flavoprotein with both DPNH oxidase and cytochrome c reductase activity.[3] Spontaneous or ultraviolet-induced inactivation of oxidase activity leads to conversion of the enzyme to a cytochrome reductase that has no oxidase activity. The altered enzyme has different physical properties from the original enzyme and can be separated from it. Kinetic studies with altered and native enzyme indicate that, in addition to the loss in oxidase activity, the altered enzyme has undergone a change in catalytic properties at the cytochrome reductase locus. The latter finding suggests that the conversion from native enzyme to cytochrome c reductase involves a change close to the cytochrome reductase locus. The factor that is labile to ultraviolet irradiation or destroyed spontaneously on storage may link the cytochrome reductase locus to the site of oxygen reduction.

[1] Research Participant.

[2] E. F. Phares, S. F. Carson, and M. V. Long, *Biol. Semiann. Prog. Rep. Feb. 15, 1958*, ORNL-2481, p 65.

[3] M. I. Dolin, *Bacteriol. Proc.* 58, 105 (1958).

Studies on the ultraviolet sensitivity of the oxidase reaction indicate that the labile factor decays more rapidly than free flavin.

The Diaphorase of *Streptococcus faecalis*

M. I. Dolin N. P. Wood

The diaphorase of *Streptococcus faecalis*[4] has now been purified to a turnover level of 7500 moles of DPNH oxidized per minute per 100,000 grams of protein, or, on a flavin basis, 8000 moles of DPNH oxidized per minute per mole of bound flavin. A minimum molecular weight of approximately 100,000 may be calculated from the flavin content. The purified enzyme shows a typical flavin spectrum with maxima at 270, 370, and 450 mμ. On the addition of substrate (DPNH) the yellow color of the enzyme is bleached almost completely. There is 90% reduction in extinction at 450 mμ, and no new absorption bands are observed in the visible region. This behavior differs markedly from that of another *S. faecalis* flavoprotein, DPNH peroxidase.[5] In the latter system DPNH combines with the enzyme to form a complex which has absorption bands at 450 mμ and in the region 520 to 600 mμ. If the diaphorase does form a complex with DPNH, either it has no distinctive absorption in the visible region or the concentration of the complex is below the level of detection.

Straub's soluble diaphorase is the only other highly purified diaphorase so far reported.[6] Fluorescent and spectrophotometric properties of the latter enzyme[6] indicate that it differs from the *S. faecalis* diaphorase in mode of flavin binding to the protein and in mechanism of DPNH "activation." Present results suggest that the bacterial enzyme resembles microsomal cytochrome c reductase[7] in catalyzing a stoichiometric reduction of flavoprotein, as shown in the following equation:

$$DPNH + H^+ + FP \rightarrow DPN^+ + FPH_2$$

[4] M. I. Dolin, *Arch. Biochem. Biophys.* **55**, 415 (1955).

[5] M. I. Dolin, *J. Biol. Chem.* **225**, 557 (1957).

[6] N. Savage, *Biochem. J.* **67**, 146 (1957).

[7] P. Strittmatter and S. F. Velick, *J. Biol. Chem.* **228**, 785 (1957).

PLANT PHYSIOLOGY AND PHOTOSYNTHESIS

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J. R. Loewenberg[2,3]
R. Rabson
W. A. Arnold[4]
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Rendering Light-Insensitive Lettuce Seeds Sensitive to Light

A. H. Haber

Introduction. – The germination of some varieties of lettuce seed can be reversibly stimulated by exposure to red (or white) light and inhibited by exposure to far-red light.[6] It has been suggested that the well-known potentiating effect of infrared radiation on X-ray-induced chromatid aberrations was caused exclusively by a far-red component with action spectrum identical to that of the far-red light effective in seed germination, photomorphogenic effects in seedlings, and control of flowering.[7] Moreover, Moh and Withrow have recently concluded that the potentiating effect of far-red light on X-ray-induced chromatid aberrations can be nullified if the exposure to far red is followed by exposure to red light.[8] The work reported below was performed to determine whether a similar interaction between far-red light and X rays could be demonstrated for seed germination. In contrast to previous reports from this laboratory, experiments were performed with a variety of lettuce seeds whose germination is normally *insensitive* to light at the temperatures employed.

Results. – New York lettuce seeds germinate equally well in darkness and under continuous white or far-red light at 21°C. When such seeds were X-irradiated during imbibition, however, their subsequent germination under far-red light was greatly reduced after receiving doses of X radiation that did not significantly affect their germination under white light (Fig. 22). This effect of far-red light on X-irradiated seeds was to reduce the total extent, and not merely the rate, of germination. After having received 1000 r/min of 250-kvp X rays during the first 45 minutes of imbibition, seeds that were transferred to darkness gave 35% germination after 20 hours; seeds similarly treated except for 60 minutes exposure to far red after X irradiation gave 11% germination, while similarly treated seeds given 60 minutes far red followed by 60 minutes white light gave 29% germination. Since the seeds had been exposed to room lighting during and immediately after the X irradiation, certain populations of the seeds had therefore shown reversible inhibition and stimulation of germination by far-red and white light, respectively.

In order to determine whether the induced photosensitivity was peculiar to damage caused by X radiation, seeds were frozen in liquid nitrogen after various periods of imbibition. Their subsequent germination under far-red light was greatly reduced compared to their germination under white light (Fig. 23). Seeds were also germinated in the presence of mannitol or 2,4-dinitrophenol, germination inhibitors for which

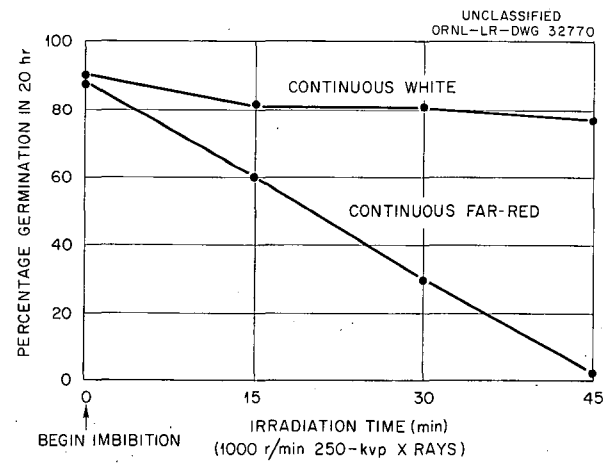

Fig. 22. Effects of X Irradiation and of Subsequent Light Conditions on Germination of New York Lettuce Seeds at 22°C.

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[2]To June 1958.

[3]Research Associate.

[4]On official leave, summer 1958.

[5]Biology Student Trainee.

[6]H. A. Borthwick *et al.*, *Botan. Gaz.* **115**, 205–25 (1954).

[7]R. B. Withrow and C. C. Moh, *Radiation Research* **6**, 491–500 (1957).

[8]C. C. Moh and R. B. Withrow, *Plant Physiol.* **32**, xi (1957).

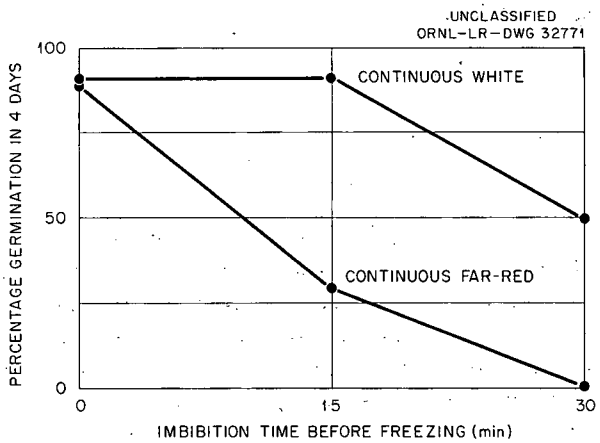

Fig. 23. Effects of Freezing in Liquid Nitrogen and of Subsequent Light Conditions on Germination of New York Lettuce Seeds at 22°C.

known modes of action have been postulated (osmotic effects and uncoupling of oxidative phosphorylation, respectively). Germination in the presence of either of these inhibitors was also light sensitive (Table 23). As with X-irradiated seeds, photoreversibility could be demonstrated with seeds damaged by freezing or moistened with solutions of mannitol.

Discussion. – Neither decreased germination in the experiments here reported nor chromatid aberrations[7] were effected by far-red light alone. Thus the interaction between X rays and far-red light in inhibiting germination was analogous to the interaction of these two radiations in the production of chromatid aberrations. When the seeds were subjected to other treatments in place of X irradiation, however, similar interactions with far-red light were also obtained. These results draw our attention to the question of whether or not the potentiating effects of far-red light are peculiar to damage induced by ionizing radiation in the production of chromatid aberrations.

The behavior of New York seeds that had been subjected to X irradiation, freezing treatment, or germination inhibitors appeared similar to the behavior of varieties of lettuce seed normally considered to be photosensitive. Since lettuce is a cold-weather crop, the previously described induction of photosensitivity in light-insensitive varieties of lettuce by high-temperature treatment[6] should be considered as yet another form of injurious treatment whose effects may be potentiated by far-red light.

Table 23. Effects of Chemical Inhibitors and Light on Germination of New York Lettuce Seeds

Supplement	Percentage Germination in 24 Hours	
	White Light	Far-Red Light
None	92	85
0.1 *M* mannitol	85	7
10^{-4} *M* dinitrophenol	77	17

Some Aspects of Serine Metabolism in Plants

R. Rabson

Introduction. – An earlier report[9] of this work described the incorporation of radioactivity from $C^{14}O_2$ during short-time photosynthesis into serine and alanine. The serine carbon atoms were found to be approximately uniformly labeled, while the carboxyl carbon of alanine was found much more heavily labeled than either the α or β carbons. It has now been found that glyceric acid may arise from serine by a direct pathway when labeled glycolic acid or serine is administered to excised leaves. The possibility that free glyceric acid arises from serine during photosynthesis has also been investigated.

Results. – Free glyceric acid, extracted from corn or barley leaves that were administered serine 3-C^{14} for either 15 or 30 minutes in the dark, had essentially all of the radioactivity in the β carbon. When corn leaves were administered glycolic 2-C^{14} acid for 15 or 30 minutes in darkness, both the serine and glyceric acid extracted from these leaves were heavily labeled in the α and β carbons but had little or no detectable C^{14} in the carboxyl carbon.

When mint leaves were allowed to photosynthesize 20 seconds in the presence of $C^{14}O_2$, the C^{14} distribution in the carbon atoms of serine was found to be approximately uniform. The phosphoglyceric acid (PGA) had most C^{14} label in the carboxyl carbon (about four times as much as either the α or β carbon). Since the labeling pattern in the free glyceric acid appeared to be a

[9] R. Rabson and N. E. Tolbert, *Biol. Semiann. Prog. Rep. Feb. 15, 1958*, ORNL-2481, p 68.

composite of the patterns found in serine and PGA (only about twice as much label in the carboxyl as in the α or β), it may be suggested that glyceric acid is deriving label both from serine, as described below, and from PGA by phosphatase activity. A similar difference in labeling patterns was observed in the PGA and free glyceric acid of barley leaves treated in the same way.

Discussion. – Glyceric acid probably arises from serine via the intermediate hydroxypyruvic acid formed by transamination of serine. Stafford *et al.*[10] have described an enzyme found in green leaf tissue and other plant materials which converts hydroxypyruvic acid to glyceric acid with the participation of reduced diphosphopyridinenucleotide (DPNH). These results suggest a means by which carbon from a pentose phosphate of the photosynthetic carbon cycle could be transferred sequentially into triose phosphate via 2-carbon intermediates, serine, glyceric acid, and by phosphorylation to PGA.

[10] H. A. Stafford, A. Magaldi, and B. Vennesland, *J. Biol. Chem.* **207**, 621 (1954).

BIOPHYSICS

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Seasonal and Sexual Variation in the Pars Distalis of the Newt

J. N. Dent

Introduction. – The cytology of the pars distalis in *Triturus viridescens* had been closely examined,[5] but the changes that occur in it throughout the year had not been carefully followed, and the histochemical procedures that have been so effective in the study of the mammalian gland[6,7] had not been applied to it. With that information in mind the present investigation was undertaken.

Results. – Over a period of two years, monthly collections of *T. viridescens* were made, and, after sectioning, the pituitary glands were stained with aldehyde fuchsin and by the periodic acid–Schiff technique. The pars distalis is made up of acidophils, chromophobes, and two types of basophils – globular basophils that contain acidophilic spheroids and aglobular basophils that do not. Both types of basophils are PAS-positive, indicating that they contain the thyrotrophic and gonadotrophic hormones. The aglobular basophils stain readily with aldehyde, indicating that they are the thyrotrophs.

The globular basophils are more numerous in the female than in the male throughout the year. They increase in numbers during gametogenesis and decrease during the breeding period.

The aglobular basophils stain most heavily when the thyroid gives histological evidence of greatest activity and more lightly during the summer months when the thyroid is quiescent.

Discussion. – The findings outlined above give evidence that the globular basophils of the pars distalis in *T. viridescens* represent the thyrotrophic cells of the newt and that the aglobular basophils are the gonadotrophs.

Action of Goitrogens on the Tadpole Thyroid

W. G. Lynn J. N. Dent

Introduction. – A number of chemical substances are known to inhibit the normal production of the thyroid hormone. These differ in modes of action, relative effectiveness, and duration of effect. In our experiments an autoradiographic method has been used to study the effects of several different types of goitrogens upon the uptake of radioiodine by the thyroid glands of tadpoles of *Hyla versicolor*.

Results. – Tadpoles were treated by being maintained continuously for 15 days in spring water solutions of the following goitrogens at a concentration of 0.01%: phenylthiourea, $KClO_4$, $KClO_3$, KIO_3, KSCN. At the end of the 15-day period, five untreated controls and five animals from each of the experimental groups were put in 50 ml of spring water containing I^{131} (20 μc/ml). After 24 hours the animals were removed to spring water and kept for another 10 days. They were then given a tracer dose of I^{131} and fixed. Autoradiograms were prepared from sections of the thyroid and thymus regions. Other experiments were conducted in which the goitrogen treatment was discontinued 2 days before administration of the high dosage of I^{131} and others in which the treatment was continued throughout the I^{131} administration, the radioiodine being added to the appropriate drug solution.

In untreated control tadpoles, the autoradiograms showed that the first treatment with I^{131} resulted in uptake and retention of sufficient radioiodine to cause complete destruction of the thyroid during the ensuing 10-day period, with the exception of a few cases in which an occasional follicle persisted. Tadpoles given pretreatment with goitrogenic drugs differed markedly in the extent

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[5] D. E. Copeland, *J. Morphol.* 49, 345–63 (1943).

[6] H. D. Purves and W. E. Griesbach, *Endocrinology* 49, 244–64 (1951).

[7] N. S. Halmi, *Endocrinology* 50, 140–42 (1952).

of damage suffered by the thyroid gland, depending on the mode of action and degree of effectiveness of the inhibitor. Study of the sections and their autoradiograms indicated the order of effectiveness when the goitrogen treatment was continued during administration of the high dosage of radioiodine to be: $KClO_4$, KIO_3, KSCN, phenylthiourea, $KClO_3$. When goitrogen treatment was discontinued at the time of exposure to I^{131}, the previous treatment with phenylthiourea proved ineffective in protecting the thyroid against damage but the other drugs showed the same order of effectiveness. Discontinuance of goitrogen treatment 2 days before exposure to I^{131} showed a loss of protective effect by KSCN but the effects of $KClO_4$, KIO_3, and $KClO_3$ persisted.

Examination of the thymus gland, which also has a differential affinity for iodine, indicated that none of the goitrogens prevented damage to this structure. In all cases extensive cellular destruction occurred in the thymus and large fluid-filled spaces appeared in the gland.

Discussion. – As would be expected, it is clear that goitrogens, like perchlorate and thiocyanate – which interfere with the thyroid's ability to accumulate iodine from the blood serum – are most effective in preventing radiation damage to the thyroid under the conditions of this experiment. However, phenylthiourea, which permits concentration of iodine by the thyroid but inhibits its binding with protein, is also effective providing the drug is actually present during the exposure to I^{131}. These experiments indicate that inhibition of thyroid function by phenylthiourea ceases at once when the drug is withdrawn. Inhibition by thiocyanate is also short lived, but the effects of perchlorate, iodate, and chlorate persist for at least three days. Failure of these drugs to protect the thymus against damage may indicate either that the mechanism by which iodine is concentrated in the thymus differs from that of the thyroid or that cellular destruction occurs at a lower dosage level in the thymus than in the thyroid.

Apparent Deviations from the Inverse Square Law for Measurements of Photon Sources with Finite Size Ionization Chambers

S. Kondo M. L. Randolph

Introduction. – That the intensity of radiation from a point source varies as the inverse square of the distance to the source, when allowance is made for secondary effects, is a fundamental law accepted for all radiation measurements. Most dose measurements of photon sources by ionization chambers show appreciable deviation from the inverse square law at very close distances because of their finite size. Previously,[8-11] corrections have been calculated by assuming that the inverse square law applies everywhere within the chamber and integrating over the volume of gas. However, by the Bragg-Gray principle, most of the ionization in properly designed chambers is produced by secondary electrons originating at internal surface layers of the cavity; and hence, integration over the surface should yield more precise correction formulae. We have compared precise measurements with the corrections predicted by volume and surface integrations.

Results. – Our formulation for surface integration goes as follows. Basically the amount of energy, or ionization, dE, produced in the gas by secondary electrons passing through a surface element dA_1 at a distance d from a point source of photons is

$$dE = \text{const.}\ \frac{l\, d\omega \cos\psi_{21}\, dA_1}{d^2}\ , \qquad (1)$$

where l is the distance across the cavity from dA_1 to another surface element dA_2, $d\omega$ is the solid angle subtended by dA_2 from dA_1, and ψ_{21} is the angle between l and the inward normal of dA_1. Several plausible assumptions which cannot be discussed here are implied by Eq. (1). Integration yields:

$$E = \text{const.} \int dA_1 \int dA_2 \frac{\cos\psi_{12} \cos\psi_{21}}{d^2 l}\ , \qquad (2)$$

where ψ_{12} is the angle at dA_2. The deviation factor, ratio of reading predicted by theory to that expected from the inverse square law, is:

$$K = \frac{D^2}{4\pi V^2} \int dA_1 \int dA_2 \frac{\cos\psi_{12} \cos\psi_{21}}{d^2 l}\ , \qquad (3)$$

[8] G. Failla and L. D. Marinelli, *Am. J. Roentgenol. Radium Therapy* 38, 333 (1937).

[9] W. V. Mayneord and J. E. Roberts, *Brit. J. Radiol.* 10, 365 (1937).

[10] R. K. Clark and S. S. Brar, *Nucleonics* 12(8), 28 (1954).

[11] F. H. Attix and V. H. Ritz, *J. Research Natl. Bur. Standards* 59, 293 (1957).

where D is the center-to-center distance from source to chamber and V is the chamber volume. For a spherical chamber of radius a the following analytic result has been obtained:

$$K = \frac{D}{2a} \log \frac{D + a}{D - a} , \qquad (4)$$

but for cylindrical and other shaped chambers, the equations become quite complicated and require numerical integration.

Figure 24 gives a comparison of the predictions of the volume and surface integration methods with experimental values obtained with a very small Co^{60} gamma-ray source moved along the axis of a relatively large cylindrical ionization chamber. Deviations because of back-scattering, absorption, and finite source size are thought to to be negligible.

Conclusions. – The agreement between our theory and experiment is satisfactory and better than with previous theory for a cylindrical cavity with source on its axis. Further studies with other geometries are under way.

Paramagnetic Resonance Studies of Irradiated Amino Acids

M. L. Randolph D. L. Parrish

Introduction. – Because of many inherent complexities, both biological and physical, the study of the role of free radicals in radiobiology must be approached with great caution. In particular,

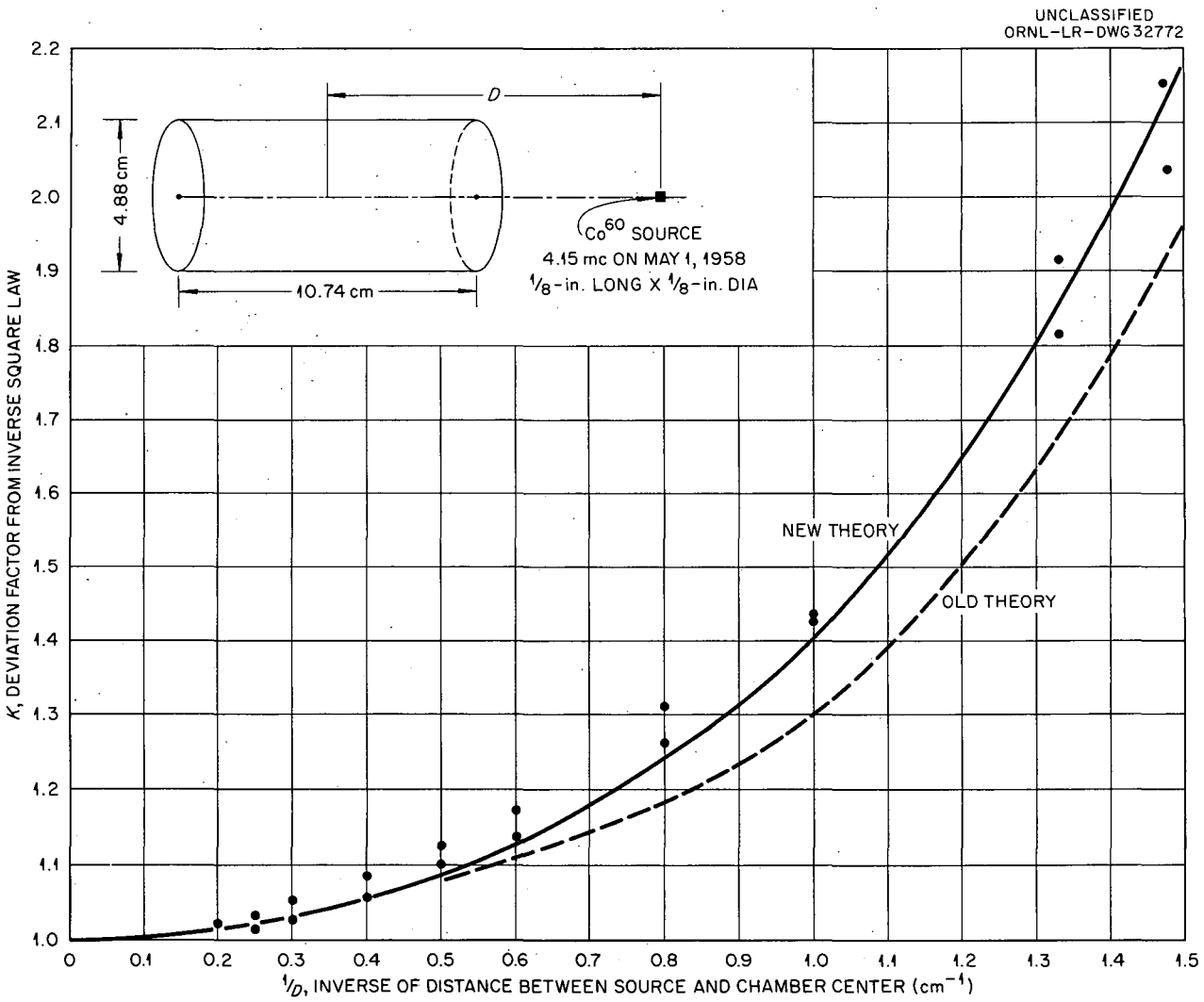

Fig. 24. Comparison of Predicted and Measured Deviations from the Inverse Square Law.

quantitative information concerning the amount of energy expended per radical or uncoupled spin produced is necessary for the interpretation and evaluation of the information so far obtained in this field. Fortunately this problem can be studied quantitatively with minimum ambiguity.

Our work has been directed primarily toward the determination of this value in powdered crystals of amino acids (irradiated with Co^{60} gamma rays in air at room temperature) because of the general significance of amino acids in biology and the stability of the radicals formed.

Our paramagnetic resonance instrumentation, with a free-running klystron immersed in oil, a cylindrical transmission cavity operating in the TE_{101} mode with resonance at 9.45 kmc, bolometer detector and bridge, narrow band 13-cycle chopped amplifier system, and chart recording of the derivative curve, differs significantly from conventional machines only in that the chart recorder mechanically drives a potentiometer that furnishes the input to a "FLEPAC" (four linear element packaged analogue computer),[12] which in turn presents the absorption curve on a second recorder. Thus we plot the absorption curve and its first derivative simultaneously.

Results. – Results from five point dose curves and four point decay curves for six irradiated amino acids are given in Table 24. The values of energy dissipated by Co^{60} gamma rays per radical formed were obtained by frequently repeated calibrations versus standard diphenylpicrylhydrazyl (DPPH) samples. Each magnetic center is here assumed to represent one free radical, and each molecule of DPPH (molecular weight taken as 440 ± 50 to allow for possible DPPH benzine dimer) is assumed to have one free electron. Comparisons of curve shapes with and without DPPH samples run simultaneously have made possible obtaining spectroscopic g values without precise absolute measurements of microwave frequencies and magnetic fields. Four of the patterns clearly showed hyperfine structure with five or seven peaks in the absorption curves. The separation of hyperfine peaks from the central peak and per cent of total absorption by each peak are given in the table. Glycylglycine gave the customary doublet pattern. Leucine gave a four-peak pattern which we tentatively attribute to overlapping of two hyperfine patterns, each with three peaks. Decay measurements indicate half lives of more than twenty days except for proline and leucine. Because of the appreciable decay of leucine during irradiation, no value for the energy dissipated in this material per free radical form is given now. Samples of glycine and glycylglycine stored in air for seven months at room temperatures show about ⅓ and ½, respectively, as many radicals as do samples a few days after irradiation.

Discussion. – If amino acids in solution form radicals about as readily as in the crystalline form, our values of the average energy dissipated per free radical formed suggest that the number of free radicals produced by ionizing radiation in biological tissue is smaller than, but perhaps of the

[12]Kindly loaned to us by E. R. Mann of the Instrumentation and Controls Division of ORNL.

Table 24. Characteristics of Paramagnetic Resonance Spectra Induced in Amino Acids by Ionizing Radiation

Material	Energy Dissipated per Radical (ev)	g for Central Peak	Hyperfine Structure: Central Peak Per Cent of Total Absorption	First Satellite: Per Cent of Total Absorption	First Satellite: H (gauss)	Second Satellite: Per Cent of Total Absorption	Second Satellite: H (gauss)	Third Satellite: Per Cent of Total Absorption	Third Satellite: H (gauss)	Very Rough Half Life (days)
α-Alanine	60	2.0033	36	26.5	±26	5.5	±52.5			>30
Glycylglycine (doublet)	71	2.0007 2.0059			±18					200
Glutamic acid	91	2.0022	42	22	±20	5.5	±41	1.2	±57.5	>30
Glycine	120	2.0025	53.5	22	±22.5	1.3	±45			12–100
L-Proline	340	2.0029	50	22.5	±22.5	2.5	±47			~7
DL-Leucine		1.9987 2.0080	? ?	? ?	±25 ±19.5	? ?				<2

same order of magnitude as, the number of ion pairs. This establishes by direct physical measurement that free radicals can be produced in sufficient numbers to play an important part in radiobiology. The amino acids usually show hyperfine structure; but very complex possibilities, including anisotropy of crystals and multiple overlapping systems of patterns with concentrations which may change with storage time, are indicated by (1) the leucine pattern, (2) the poor agreement of per cent absorption by the various peaks, except for α-alanine, with simple theory, and (3) changes in derivative curve shapes with storage time.

GLOSSARY

ADP	adenosine diphosphate
AET	S,2-aminoethylisothiourea dihydrobromide
APMT	S,3-aminopropyl-N′-methylisothiourea dihydrobromide
APT	S,3-aminopropylisothiourea dihydrobromide
ATP	adenosine triphosphate
DPNH	reduced diphosphopyridine nucleotide
MEG	2-mercaptoethylguanidine

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