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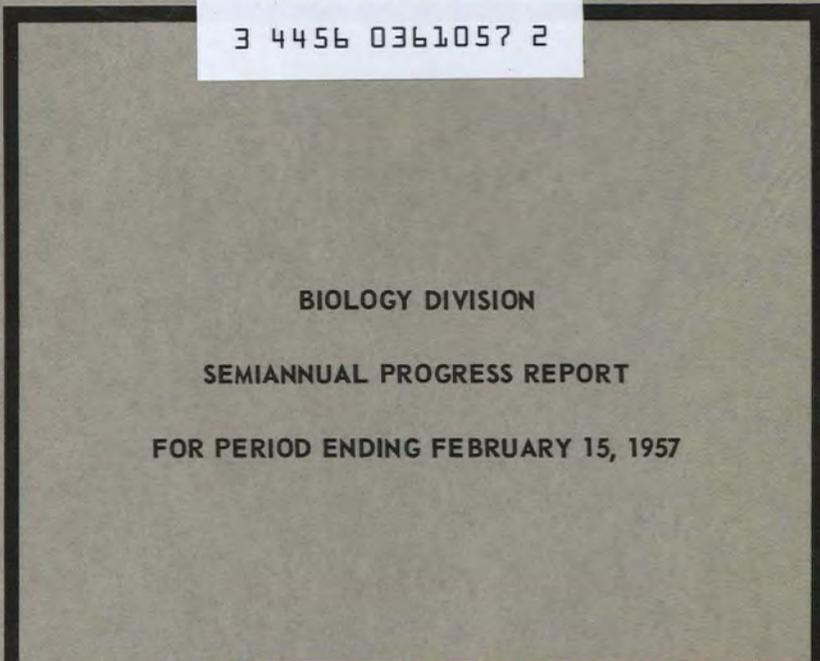
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BIOLOGY DIVISION  
SEMIANNUAL PROGRESS REPORT  
FOR PERIOD ENDING FEBRUARY 15, 1957



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**BIOLOGY DIVISION**

**SEMIANNUAL PROGRESS REPORT**

**for Period Ending February 15, 1957**

Alexander Hollaender, Director

Stanley F. Carson, Assistant Director

C. W. Sheppard, Assistant Director

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88. E. F. Oakberg
89. J. R. Totter
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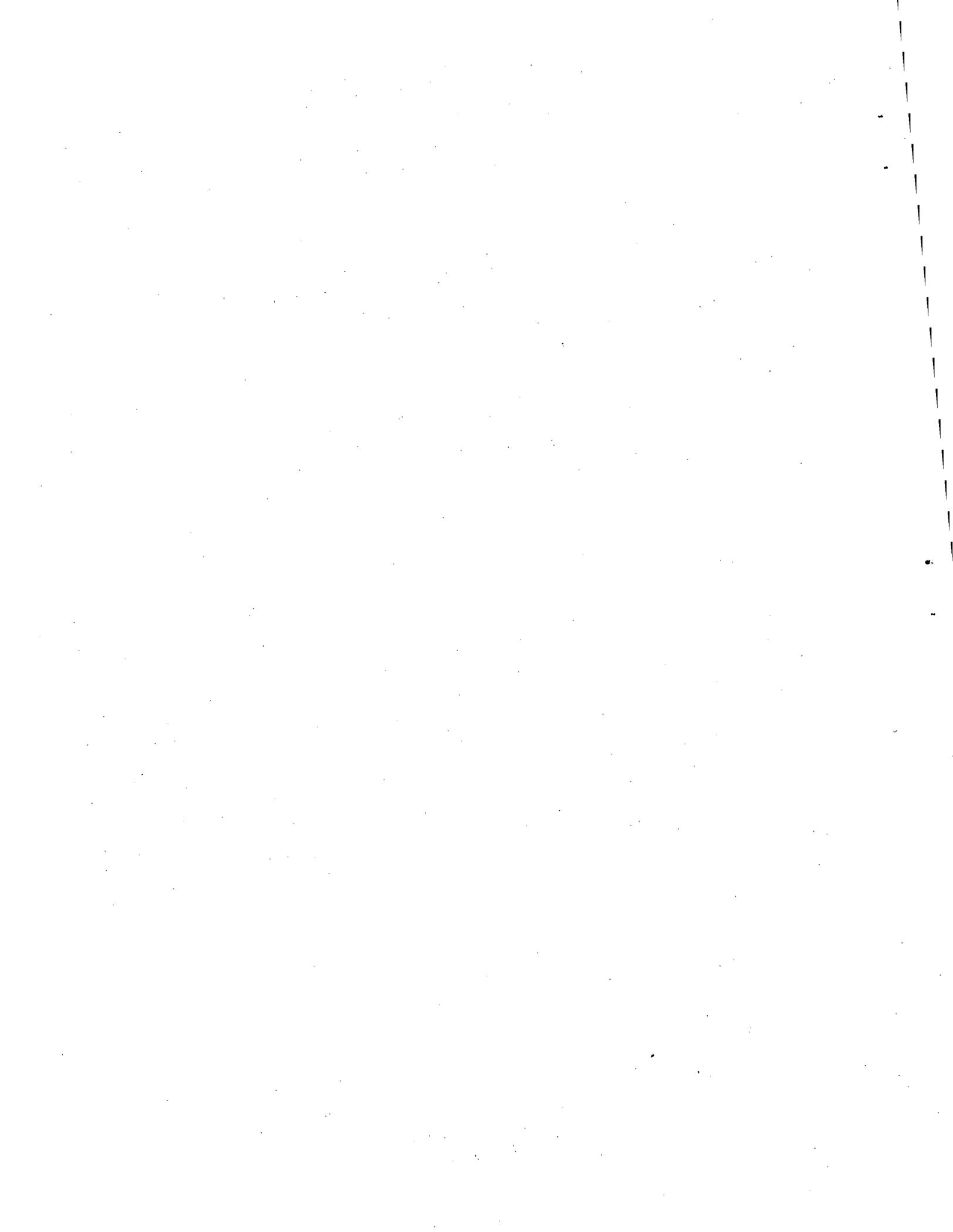
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## BIOLOGY DIVISION SEMIANNUAL PROGRESS REPORT

### PUBLICATIONS AND LECTURES

**Publications.** — The Symposium on Biocolloids, representing the proceedings of the Ninth Annual Biology Research Conference, was sent to press during the period. Forty-five papers, a semiannual report, and 28 abstracts have been published during this six-month period. Eighty-two papers and 33 abstracts are in press.

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Anderson, N. G.	Cell Division. Part I. A Theoretical Approach to the Primeval Mechanism, the Initiation of Cell Division, and Chromosomal Condensation	<i>Quart. Rev. Biol.</i> 31, 169-199 (1956)
_____	Studies on Isolated Cell Components. IX. The Soluble Phase	<i>Exptl. Cell Research</i> 11, 186-196 (1956)
Arnold, W. A., and H. K. Sherwood	Are Chloroplasts Semiconductors?	<i>Proc. Natl. Acad. Sci. U.S.</i> 43, 105-114 (1957)
Astrachan, L., and E. Volkin (with technical assistance of M. H. Jones)	Chromatographic Detection of Differences Between Bacteriophage-related Deoxyribonucleic Acids	<i>J. Am. Chem. Soc.</i> 79, 130-134 (1957)
Christman, J. F., and D. G. Doherty	The Antimicrobial Action of Heparin	<i>J. Bacteriol.</i> 72, 433-435 (1956)
_____	Microbial Utilization of Heparin	<i>J. Bacteriol.</i> 72, 429-432 (1956)
Clifton, K. H., E. Bloch, A. C. Upton, and J. Furth	Transplantable Leydig-Cell Tumors in Mice: Their Physiologic and Pathologic Effects	<i>Arch. Pathol.</i> 62, 354-368 (1956)
Cohn, W. E.	Chromatographic Separation of ATP, ADP, and AMP	<i>Methods in Enzymology</i> (ed. S. P. Colowick and N. O. Kaplan), vol III, p 867-868, Academic Press, New York, 1957
_____	Methods of Isolation and Characterization of Mono- and Polynucleotides by Ion-Exchange Chromatography	<i>Methods in Enzymology</i> (ed. S. P. Colowick and N. O. Kaplan), vol III, p 724-743, Academic Press, New York, 1957
Conger, A. D., and A. H. Johnston	Polyplody and Radiosensitivity (communication to editor)	<i>Nature</i> 178, 271 (1956)
Conte, F. P., G. S. Melville, Jr., and A. C. Upton	Effects of Graded Doses of Whole-Body X Irradiation on Mast Cells in the Rat Mesentery	<i>Am. J. Physiol.</i> 187, 160-162 (1956)

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AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Cormier, M. J., and H. H. Rostorfer	Flavin Stimulation of Methemoglobin Reduction in Cell-free Bacterial Extracts	<i>Biochim. et Biophys. Acta</i> 22, 292-299 (1956)
Cormier, M. J., J. R. Totter, and H. H. Rostorfer	Comparative Studies on Different Bacterial Luciferase Preparations	<i>Arch. Biochem. and Biophys.</i> 63, 414-426 (1956)
de Serres, F. J.	Genetic Studies of Purple Adenine Mutants in <i>Neurospora crassa</i>	<i>Microbial Genet. Bull.</i> 14, 8-9 (1956)
	Studies with Purple Adenine Mutants in <i>Neurospora crassa</i> . I. Structural and Functional Complexity in the <i>ad-3</i> Region	<i>Genetics</i> 41, 668-676 (1956)
DiStefano, V., D. E. Leary, and D. G. Doherty	The Pharmacology of $\beta$ -Aminoethylisothiuronium Bromide in the Cat	<i>J. Pharmacol. Exptl. Therap.</i> 117, 425-433 (1956)
Friedberg, W.	The Effect of Reduced Liver and Kidney Catalase Concentrations on the Lethality of X Irradiation in Rats	<i>Proc. Soc. Exptl. Biol. Med.</i> 93, 52-53 (1956)
Furth, J., R. F. Buffett, M. Banasiewicz- Rodrigues, and A. C. Upton	Character of Agent Inducing Leukemia in Newborn Mice	<i>Proc. Soc. Exptl. Biol. Med.</i> 93, 165-172 (1956)
Gregg, J. H., and R. D. Bronsweig	Dry Weight Loss During Culmination of the Slime Mold, <i>Dictyostelium discoideum</i>	<i>J. Cellular Comp. Physiol.</i> 47, 483-487 (1956)
Hollaender, A.	The Biology Division of Oak Ridge National Labo- ratory (A general description of research projects in the Biology Division)	<i>AIBS Bull.</i> 7, 10-14 (1957)
Hollaender, A., and S. F. Carson	Biology Division Semiannual Progress Report for Period Ending August 15, 1956	ORNL-2155
Hollaender, A., and G. E. Stapleton	The Influence of Chemical Pre- and Posttreat- ments on Radiosensitivity of Bacteria, and Their Significance for Higher Organisms	<i>Giba Foundation Symposium on Ionizing Radiations and Cell Metabolism</i> (ed. G. E. Wolstenholme and C. M. O'Connor), p 120-135, J. & A. Churchill Ltd., London, 1956
Horn, E. C.	Ascites Tumor Development. II. Cytotoxicity of Various Antisera Prepared Against Ehrlich Ascites Tumor Cell Components	<i>Cancer Research</i> 16, 595-599 (1956)
Hunt, E. L., and J. N. Dent	Iodine Uptake and Turnover in the Frog Tadpole	<i>Physiol. Zool.</i> 30, 87-91 (1957)
Kimball, A. W.	Approximate Confidence Intervals for Specific- Locus Mutation Rates (A Statistical Treatment - Mammalian Genetics)	<i>Am. Naturalist</i> 90, 369-376 (1956)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Kimball, R. F., and N. Gaither	Behavior of Nuclei at Conjugation in <i>Paramecium aurelia</i> . II. The Effects of X Rays on Diploid and Haploid Clones with a Discussion of Dominant Lethals	<i>Genetics</i> 41, 715-728 (1956)
Kirby-Smith, J. S.	The Measurement and Properties of Ionizing Radiation	<i>Physical Techniques in Biological Research</i> (ed. G. Oster and A. W. Pollister), vol II, p 57-110, Academic Press, New York, 1956
Makinodan, T., N. Gengozian, and C. C. Congdon	Agglutinin Production in Normal, Sublethally Irradiated, and Lethally Irradiated Mice Treated with Mouse Bone Marrow	<i>J. Immunol.</i> 77, 250-256 (1956)
Maizel, J. V., A. A. Benson, and N. E. Tolbert	Identification of Phosphorylcholine as an Important Constituent of Plant Saps	<i>Plant Physiol.</i> 31, 407-408, (1956)
Odell, T. T., Jr., and B. Anderson	Isolation of a Sulfated Mucopolysaccharide from Blood Platelets of Rats	<i>Proc. Soc. Exptl. Biol. Med.</i> 94, 151-156 (1957)
Pittenger, T. H.	Synergism of Two Cytoplasmically Inherited Mutants in <i>Neurospora crassa</i>	<i>Proc. Natl. Acad. Sci. U.S.</i> 42, 747-752 (1956)
Randolph, M. L., M. Slater, and D. L. Parrish	O-Ring Mounting of Bellows	<i>Rev. Sci. Instr.</i> 27, 878-879 (1956)
Rostorfer, H. H., and J. R. Totter	The Reduction of Methemoglobin by Phenylhydrazine Under Anaerobic Conditions	<i>J. Biol. Chem.</i> 221, 1047-1055 (1956)
Schwartz, D., and C. E. Bay	Further Studies on the Reversal in the Seedling Height Dose Curve at Very High Levels of Ionizing Radiations	<i>Am. Naturalist</i> 90, 323-326 (1956)
Shaw, E. I.	A Glutamic Acid-Glycine Medium for Prolonged Maintenance of High Mitotic Activity in Grasshopper Neuroblasts	<i>Exptl. Cell Research</i> 11, 580-586 (1956)
Tolbert, N. E., and L. P. Zill	Excretion of Glycolic Acid by Algae During Photosynthesis	<i>J. Biol. Chem.</i> 222, 895-906 (1956)
Upton, A. C., K. W. Christenberry, G. S. Melville, J. Furth, and G. S. Hurst	The Relative Biological Effectiveness of Neutrons, X Rays, and Gamma Rays for the Production of Lens Opacities: Observations on Mice, Rats, Guinea Pigs, and Rabbits	<i>Radiology</i> 67, 686-696 (1956)
Upton, A. C., C. C. Congdon, and A. Hollaender	Mechanisms of Delayed Death from Total-Body Irradiation	<i>Atompraxis</i> 1, 1-5 (1957)
Upton, A. C., and T. T. Odell, Jr.	Utilization of $S^{35}$ -labeled Sulfate in Scorbutic Guinea Pigs: Uptake in Healing Wounds, Megakaryocytes, and Blood Platelets	<i>Arch. Pathol.</i> 62, 194-199 (1956)

**BIOLOGY PROGRESS REPORT**

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Volkin, E., and L. Astrachan	The Absence of Ribonucleic Acid in Bacteriophage T2r+	<i>Virology</i> 2, 594-598 (1956)
	Intracellular Distribution of Labeled Ribonucleic Acid After Phage Infection of <i>Escherichia coli</i>	<i>Virology</i> 2, 455-462 (1956)
Wolff, S., and H. E. Luippold	Inaccuracy of Anaphase Bridges as a Measure of Radiation-induced Nuclear Damage (Communication to editor)	<i>Nature</i> 179, 208 (1957)
	Obtaining Large Numbers of Metaphases in Barley Root Tips	<i>Stain Technol.</i> 31, 201-206 (1956)
	The Production of Two Chemically Different Types of Chromosomal Breaks by Ionizing Radiations	<i>Proc. Natl. Acad. Sci. U.S.</i> 42, 510-514 (1956)
Zill, L. P.	The Anthrone Reagent and Its Application to the Determination of the Heptuloses	<i>Anal. Chem.</i> 28, 1577-1579 (1956)

**Scientific Society Lectures and Traveling Seminars.** - Of the 68 lectures given by members of the Biology Division during the half year, the greater number have been traveling lectures. A total of 29 papers were presented at the ten professional societies listed.

AUTHOR(S)	TITLE	PLACE PRESENTED
Adler, H. I.	Objective Technics in Bacterial Nuclear Cytology	Soc. Am. Bacteriol. (Ky.-Tenn. Br.), Oak Ridge
Anderson, N. G.	Labile Colloidal Complexes of the Cytoplasm	9th Ann. Biol. Research Conf. on Biocolloids, Gatlinburg
Atwood, K. C.	Genetic Effects of Ionizing Radiations on Lower Plants	Am. Inst. Biol. Sci. (Am. Soc. Plant Physiol.), Storrs, Conn.
von Borstel, R. C.	Genetic Effects of Radiation	Dept. of Microbiology, Vanderbilt University, Nashville
von Borstel, R. C., and M. L. Pardue	On the Nature of Radiation-induced Dominant Lethal Mutations in <i>Habrobracon</i> and <i>Drosophila</i> [ <i>Genetics</i> 41, 665 (1956)]	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), Storrs, Conn.
Brown, J. R. C.	Observations on the Reaction of Cell Fractions of the Thymus Gland to Enzymatic Digestion and to Changes in Osmotic Pressure [ <i>Science</i> 124, 936 (1956)]	Natl. Acad. Sci., University of Maryland, College Park
Bruce, A. K.	Alterations of Potassium Exchange in Yeast Following X Irradiation	Dept. of Physiology, University of Illinois, Urbana
	Changes in Potassium Leakage from Yeast After X Irradiation	Soc. Am. Bacteriol. (Ky.-Tenn. Br.), Oak Ridge
Cohn, W. E.	Ion-Exchange Chromatography	University of Marburg, Germany*

\*Information not available until after last semiannual report (ORNL-2155) was issued.

AUTHOR(S)	TITLE	PLACE PRESENTED
_____	Nucleic Acid Chemistry	Stazione Zoologica, Naples, Italy*
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_____	As above	Birkbeck College, University of London, England*
_____	As above	University of Durham, Scotland*
_____	As above	University of Glasgow, Scotland*
_____	As above	Free University of Berlin*
Congdon, C. C.	Experimental Treatment of Irradiation Injury	Dept. of Zoology, University of Tennessee, Knoxville
_____	Mechanism of Acceleration of Regeneration of Hematopoietic Tissues	6th Intern. Congr. Intern. Soc. Hematol., Boston, Aug. 26-Sept. 1, 1956
Conger, A. D., M. L. Randolph, and A. H. Johnston	Chromosomal Aberration Production by X Rays and by Monochromatic 2.5-Mev and 14-Mev Neutrons [ <i>Genetics</i> 41, 639 (1956)]	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), Storrs, Conn.
de Serres, F. J.	The Origin of Prototrophs in Crosses of Purple Adenine Mutants in <i>Neurospora crassa</i> [ <i>Genetics</i> 41, 639-640 (1956)]	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), Storrs, Conn.
Doherty, D. G.	Chemical Protection of Biological Systems from Ionizing Radiation	Division of Basic Health Sciences, Emory University, Emory University, Ga.
Edington, C. W.	A Nonlinear Frequency-Dose Relation for Recessive Lethals Induced by X Rays in <i>Drosophila</i> [ <i>Genetics</i> 41, 640 (1956)]	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), Storrs, Conn.
Faberge, A. C.	Forecasting the Rate of Induced Marker Loss for Maize Endosperm [ <i>Genetics</i> 41, 642 (1956)]	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), Storrs, Conn.
Gaulden, M. E.	DNA Synthesis and X-Ray Effects at Different Mitotic Stages in Grasshopper Neuroblasts [ <i>Genetics</i> 41, 645 (1956)]	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), Storrs, Conn.
_____	Effects of Radiation on Mitosis as Observed in Living Cells	Cancer Research & Chemistry Dept., University of Florida, Gainesville
Hollaender, A.	The Effects of Pre- and Posttreatment on the Radiation Sensitivity of Microorganisms	5th Intern. Radiobiol. Conf., Stockholm

BIOLOGY PROGRESS REPORT

AUTHOR(S)	TITLE	PLACE PRESENTED
_____	Studies on Counteracting Radiation Effects on Mammals	Cancer Training Program of the School of Medicine, Duke University, Durham
_____	Studies on the Mechanism of Radiation Protection and Recovery	Dept. of Zoology Seminar, Duke University, Durham
Hunt, G. E.	C <sup>14</sup> O <sub>2</sub> Incorporation in $\gamma$ -Hydroxyglutamic Acid in Two Species of Phlox	Tenn. Acad. Sci. (Botany Section), Murfreesboro
Jagger, J., and R. Latarjet	Action Spectra for Photo restoration in <i>E. coli</i> B/r	Soc. Am. Bacteriol. (Ky.-Tenn. Br.), Oak Ridge
Khym, J. X., R. Shapira, and D. G. Doherty	Intramolecular Rearrangements of S, 2-Aminoethylisothiuronium Bromide Hydrobromide (AET)	Southwide Chem. Conf. (South-eastern & Southwestern Sections of the Am. Chem. Soc.), Memphis
Kimball, R. F.	Modification of the Genetic Effects of X Rays by Treatment After Irradiation (Intern. Genet. Symp., 1956, Science Council of Japan, p 65)	Intern. Genet. Symposia, Tokyo and Kyoto
Kimball, R. F., N. Gaither, and S. M. Wilson	Postirradiation Modification of X-Ray-Induced Lethal and Deleterious Mutations in <i>Paramecium aurelia</i> [ <i>Genetics</i> 41, 650 (1956)]	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), Storrs, Conn.
Kirby-Smith, J. S.	Application of Paramagnetic Resonance to Radiation Biology	Depts. of Biology & Zoology, University of Texas, Austin
Lindsley, D. L.	Successful Transplantation of Homologous Tissues into Irradiated Mammals	Dept. of Biological Sciences, Florida State University, Tallahassee
_____	As above	Dept. of Biological Sciences, University of Georgia, Athens
_____	As above	Dept. of Biology, Emory University, Emory University, Ga.
Novelli, G. D.	The Activation of Amino Acids in the Biosynthesis of Proteins	Dept. of Nutrition and Biochemistry, College of Agriculture, University of Nebraska, Lincoln
_____	As above	Faculty Seminar in Fundamental Biology, University of Colorado Medical Center, Denver
_____	As above	All departments of biological sciences, University of Texas, Austin
_____	As above	Dept. of Chemistry, Florida State University, Tallahassee
_____	As above	Dept. of Biochemistry, Medical School, University of Florida, Gainesville

AUTHOR(S)	TITLE	PLACE PRESENTED
_____	The Role of Coenzyme A in Nutrition and Metabolism	Dept. of Cancer Research and Animal Husbandry, University of Florida, Gainesville
_____	As above	Dept. of Biochemistry, Medical College, University of Nebraska, Omaha
_____	As above	9th Ann. Scientific Meet., Detroit Institute for Cancer Research, Detroit
_____	Studies on Protein Synthesis	School of Medicine, Western Reserve University, Cleveland
Odell, T. T., Jr.	Establishment of Transplants of Homologous Bone Marrow in Rats Following Total-Body Irradiation	University of Louisville Medical School, Louisville
_____	As above	Dept. of Zoology and Entomology, University of Tennessee, Knoxville
_____	Production, Life Span, and Fate of Blood Platelets: Studies with Radioisotope Labeling Techniques	6th Intern. Congr. Intern. Soc. Hematol., Boston
Phares, E. F., and M. V. Long	A Coupled Malic-Lactic Dehydrogenase from <i>Veillonella gazogenes</i> (Abstracts of papers, 130th Natl. Meet. Am. Chem. Soc., 1956, p 62C)	130th Natl. Meet. Am. Chem. Soc., Atlantic City
Pittenger, T. H.	Complementary Cytoplasmically Inherited Mutants in Neurospora [ <i>Genetics</i> 41, 656 (1956)]	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), Storrs, Conn.
Russell, L. B.	Dominant Lethals Induced at a Highly Sensitive Stage in Mouse Oögenesis [ <i>Anat. Record</i> 125, 647-648 (1956)]	Am. Inst. Biol. Sci. (Am. Soc. Zool.), Storrs, Conn.
Russell, L. B., and M. H. Major	A High Rate of Somatic Reversion in the Mouse [ <i>Genetics</i> 41, 658 (1956)]	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), Storrs, Conn.
Russell, W. L.	Lack of Linearity Between Mutation Rate and Dose for X-Ray-Induced Mutations in Mice [ <i>Genetics</i> 41, 658-659 (1956)]	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), Storrs, Conn.
Schwartz, D., and C. B. Murray	Cytological Studies on the Breakage-Fusion-Bridge Cycles in Maize Endosperms ( <i>Abst. Intern. Genet. Symp.</i> , 1956, Science Council of Japan, p 66)	Intern. Genet. Symposia, Tokyo and Kyoto
Shapira, R., D. G. Doherty, and J. X. Khym.	Mercaptoalkylguanidines as Potential Radiation-Protective Agents [ <i>Southern Chemist</i> 16, 130 (1956)]	Southwide Chem. Conf. (Southeastern & Southwestern Sections of the Am. Chem. Soc.), Memphis
Tolbert, N. E.	Glycolate-Bicarbonate Shift Across Plant Cell Walls	Depts. of Agricultural and Biological Chemistry, Bacteriology, Botany, and Plant Pathology, The Pa. State University, University Park

**BIOLOGY PROGRESS REPORT**

AUTHOR(S)	TITLE	PLACE PRESENTED
_____	Physiology and Identification of Compounds for Phosphorus Storage and Transport	Dept. of Biochemistry, Purdue University, Lafayette
Upton, A. C.	Pathological Effects of Ionizing Radiations	Vanderbilt University, Nashville
Welshons, W. J.	The Production of Wild Type Alleles from Heterozygotes of Notch and Split in <i>Drosophila</i> [ <i>Genetics</i> 41, 665-666 (1956)]	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), Storrs, Conn.
Wolff, S.	Biochemical Aspects of Chromosome Break Rejoining	Mount Vernon Hospital, Northwood, England
_____	Biochemical Aspects of Chromosome Break Rejoining	Dept. of Zoology and Entomology, University of Tennessee, Knoxville
_____	As above	University of Colorado Medical Center, Denver
_____	Chromosome Breakage and Reunion	Dept. of Plant Sciences, University of Oklahoma, Norman
_____	As above	Chemistry Dept., University of Texas, Austin
_____	Recent Studies on Chromosome Breakage and Rejoining	5th Intern. Radiobiol. Conf., Stockholm
Wolff, S., and H. E. Luippold	Inaccuracy of Measuring X-Ray-Induced Chromosomal Damage by Scoring at Anaphase [ <i>Genetics</i> 41, 666-667 (1956)]	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), Storrs, Conn.

**Visiting Lecturers.** - The following scientists have served as visiting lecturers during the past six-month period.

LECTURER	AFFILIATION	TOPIC
G. S. Hodgson	Universidad de Chile, Santiago	Studies in Radiation Biology
R. H. Mole	Radiobiological Research Unit, AERE, Harwell, England	The Experimental Assessment of Damage from Chronic Irradiation
F. D'Amato	Botanical Institute, University of Pisa, Italy	Chromosome Noncongression During Mitosis Induced by Thallium Acetate
G. E. Magni	Instituto di Genetics, Università di Pavia, Italy	Genetic Analysis of the Radiosensitivity of the Yeast Cell
H. K. Weston	Laboratory Director in Pathological Research, Parke, Davis & Co., Detroit	Myleran Marrow Damage in Rats: A Quantitative Chemical and Histologic Evaluation

LECTURER	AFFILIATION	TOPIC
R. Latarjet	Director, Institut du Radium, Paris	1. Radiobiological Studies on a Transforming Agent of Pneumococcus 2. Compared Actions of Radiations and of Organic Peroxides on Some Bacterial and Viral Functions
Z. M. Bacq	University of Liège, Belgium	Search for Useful Tests in Mice After Exposure to Small Amounts of Ionizing Radiation
A. Gilles	University of Louvain, Belgium	Cytogenetic Work at the Carnoy Institute, University of Louvain, Belgium
M. Angot	University of California, Scripps Institute of Oceanography, La Jolla	Carbon-14 Techniques to Assay the Productivity of the Ocean
H. B. Fell	Strangeways Research Laboratory, Wort's Causeway, Cambridge, England	Organ Culture and Some Physiological Problems
H. R. Wolfe	Department of Zoology, The University of Wisconsin, Madison	Factors Affecting Antibody Production in Chickens
H. Fernandez-Moran	Director, Venezuelan Institute for Neurology and Brain Research, Caracas	IVNIC Biophysics and Nuclear Research Center in Caracas, Venezuela
E. Witschi	Department of Zoology, State University of Iowa, Iowa City	Sex Chromatin and Sex Chromosomes
D. W. Barnes	Radiological Research Unit, AERE, Harwell, England	Informal Discussion of Radiobiology Work at Harwell.
A. Heyndrickx	Department of Toxicology, University of Ghent, Belgium	The Influence of U.V. Radiation, Detergents and Electrolysis upon the Sulfhydryl and Disulfide Groups of Cancerous and Normal Serum of Man
R. K. Appleyard	Acting Secretary, Scientific Committee on the Effects of Atomic Radiation, United Nations, New York	Defective Lyogeny in <i>E. coli</i>
E. K. Plyler	National Bureau of Standards, Washington, D.C.	Solar Radiation
N. H. Giles	Yale University	<i>Neurospora</i> Studies
R. Markham	Biochemistry Department, Molteno Institute, Cambridge, England	Plant Viruses
R. S. Bandurski	Department of Botany and Plant Pathology, Michigan State University, East Lansing	Enzymatic Pyrophosphorolysis of ATP by Group VI Anions
W. Stricks	Department of Chemistry, Oglethorpe University, Oglethorpe University, Ga.	Electrometric Determination and Reactivity of Sulfhydryl and Disulfide Groups in Biological Materials

## BIOLOGY PROGRESS REPORT

**Bone Marrow Conferences.** – Two round-table discussions on the use of bone marrow in connection with recovery from radiation damage and in chemical protection have been held by the Biology Division. These conferences were attended by investigators who have been closely associated with this program. Representatives of the following laboratories were present: Veterans' Administration Hospital of Yale University School of Medicine, Harvard Medical School, Mary Imogene Bassett Hospital of Columbia University, The Jefferson Medical College of Philadelphia, University of Tennessee School of Medicine, National Cancer Institute, Children's Cancer Research Foundation (Boston), Parke, Davis & Co., ORINS Medical Division; and, of course, our own Laboratory. Several members of the Division of Biology and Medicine of the U.S. Atomic Energy Commission were also here for the meetings.

These conferences serve a very useful purpose in keeping the different investigators abreast of developments in the field and in coordinating the work of the several projects engaged in this branch of research. Another conference is scheduled for May.

**Speakers at Professional Meetings, Spring 1957.** – Thirty-three papers are scheduled for presentation at professional society meetings before June 15. The societies and speakers are listed.

*National Biophysics Conference* – A. J. Fairbanks, R. P. Perry, and C. W. Sheppard

*American Physical Society (Southeastern Section)* – J. S. Kirby-Smith (coauthor, M. L. Randolph) and M. L. Randolph

*Tenth Annual Biology Research Conference* – T. Makinodan, C. C. Congdon, and G. D. Novelli (coauthor, J. A. De Moss)

*American Association for Cancer Research* – A. C. Upton (coauthors, F. F. Wolff and J. Furth)

*Federation of American Societies for Experimental Biology* – L. Astrachan (coauthor, E. Volkin), R. H. Bradford (coauthors, R. Shapira and D. G. Doherty), W. E. Cohn, C. C. Congdon (coauthors, T. Makinodan and N. Gengozian), M. J. Cormier (coauthor, J. R. Totter), J. W. Davis (coauthors, A. N. Best and G. D. Novelli), T. Makinodan (coauthor, I. Shekarchi), T. T. Odell, Jr. (coauthor, B. C. Caldwell), C. W. Sheppard, L. H. Smith (coauthors, T. Makinodan and C. C. Congdon), N. E. Tolbert, A. C. Upton (coauthors, R. F. Buffett, J. Furth, and D. G. Doherty), and L. P. Zill

*American Association of Anatomists* – J. N. Dent (coauthor, C. W. Sheppard)

*Association of Southeastern Biologists* – B. Limbaugh (coauthor, M. E. Gaulden)

*Pan-American Cancer Cytology Congress* – M. E. Gaulden

*Society of American Bacteriologists* – H. I. Adler, M. I. Dolin, E. F. Phares (coauthors, R. J. Koeppel and S. F. Carson), and G. E. Stapleton (coauthor, D. H. Woodbury)

*AIBS Conference on "Basic Problems of Biological Aging"*. – N. G. Anderson and A. C. Upton

*Second International Congress of Photobiology* – J. Jagger and N. E. Tolbert (two papers: 1. coauthor, R. Rabson; 2. coauthor, F. R. Gailey).

**Tenth Annual Biology Research Conference.** – Advance reservations indicate that the Biology Conference, which will be held in Gatlinburg on April 8–10, 1957, will be well attended. These conferences are sponsored by the Biology Division and supported by the Atomic Energy Commission. C. C. Congdon and T. Makinodan assisted in arranging the program for the "Symposium on Antibodies: Their Production and Mechanism of Action," which follows:

MONDAY, APRIL 8

MORNING

Chairman: William H. Taliaferro, University of Chicago

*General Introduction and Discussion of Antibody Formation:* William H. Taliaferro, Department of Microbiology, University of Chicago

*Characterization of the Antibody Response:* Frank J. Dixon, Jr., University of Pittsburgh School of Medicine

AFTERNOON

- Chairman: M. R. Irwin, Department of Genetics, University of Wisconsin
- Antibody Reactive Sites and Soluble Complexes:* S. J. Singer, Sterling Chemistry Laboratory, Yale University
- Size of the Reactive Sites on Antibody:* Elvin A. Kabat, College of Physicians and Surgeons, Columbia University

EVENING

*Discussion: Radiation Immunology with Special Emphasis on Bone Marrow Transplantation*

- T. Makinodan, Oak Ridge National Laboratory
- C. E. Ford, Radiological Research Unit, Harwell, England
- J. F. Loutit, Radiobiological Research Unit, Harwell, England
- C. C. Congdon, Oak Ridge National Laboratory
- D. W. van Bekkum, Medisch Biologisch Institute, Rijswijk, Netherlands

NOTE: On Sunday, April 7, at 8:00 P.M., a special meeting will be held for a discussion on biological effects of radiation as background for the bone marrow transplantation discussion on Monday evening. All conference participants who arrive in time are invited to attend.

TUESDAY, APRIL 9

MORNING

- Chairman: A. M. Pappenheimer, Jr., New York University College of Medicine
- Recent Concepts of Protein Synthesis in Relation to Antibody Formation:* Ray D. Owen and Richard Schweet, California Institute of Technology
- The Activation of Amino Acids and Concepts of the Mechanism of Protein Synthesis:* G. D. Novelli and J. A. DeMoss, Biology Division, Oak Ridge National Laboratory
- The Diversity of Antibodies:* David W. Talmage, Department of Medicine, University of Chicago

AFTERNOON

EXCURSION - GREAT SMOKY MOUNTAINS (In case of inclement weather, the program will continue, leaving Wednesday afternoon open for a tour of the Biology Division, Oak Ridge National Laboratory.)

WEDNESDAY, APRIL 10

MORNING

- Chairman: Michael Heidelberger, Institute of Microbiology, Rutgers University
- Passive Transfer of Immune Reactions by Way of Cells:* N. Avrion Mitchison, Department of Zoology, University of Edinburgh
- The Cellular Basis for Antibody Formation:* Robert W. Wissler, Division of Biological Science, University of Chicago

AFTERNOON

- Chairman: Alexander Haddow, Chester Beatty Research Institute, London
- Hypersensitivity:* Elmer L. Becker, Department of Immunochemistry, Walter Reed Army Medical Center
- Some Immunological Aspects of Bone Marrow Transplantation:* T. Makinodan, Biology Division, Oak Ridge National Laboratory

## **BIOLOGY PROGRESS REPORT**

**Education.** – Educational activities for this period have been devoted to teaching. G. E. Stapleton gave a course in radiobiology at Vanderbilt University during the fall quarter. R. F. Kimball presented a refresher course in genetics at the Radiological Society of North America in Chicago. R. C. von Borstel presented a four-week series of lectures for the Radiation Science course in the Department of Biology, Florida State University, Tallahassee.

CYTOLOGY AND GENETICS

CYTOGENETIC EFFECTS OF RADIATION

R. F. Kimball	M. A. Sicard <sup>4</sup>
K. C. Atwood	S. Wolff
A. D. Conger	N. Gaither
R. R. Cowden <sup>1</sup>	H. E. Luippold
M. Mota <sup>2</sup>	H. J. Luippold
T. H. Pittenger	C. B. Murray
D. Schwartz	I. L. Sandler
F. J. de Serres <sup>3</sup>	S. M. Wilson

Effect of Posttreatment with Streptomycin on X-Ray-Induced Mutation in *Paramecium aurelia*

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

**Introduction.** — In the previous report,<sup>5</sup> it was shown that postirradiation exposure to streptomycin appreciably decreases the amount of mutation detected in *Paramecium*. It was also shown that the decrease was not an artifact of certain kinds of selection. The hypothesis was made that part of the mutational process is not completed until the middle of the interdivision interval and is subject to spontaneous reversal before that time. It was suggested that streptomycin acts to delay the completion of the mutational process and thereby allows more time for recovery. The present report gives additional data that are consistent with this hypothesis and also presents data that eliminate the one remaining possibility of a selection artifact.

**Results.** — Figure 1 summarizes results of experiments in which the concentration of streptomycin, the duration of the exposure to streptomycin, or the time between irradiation and the exposure to streptomycin were varied. Curve A shows the influence of concentration of streptomycin. Concentrations much greater than 3 mg/ml are not practicable because they kill too many animals. Curve B shows the influence of time in streptomycin when the exposure is started shortly after irradiation, and curve C shows the influence of the time

between irradiation and the start of the streptomycin exposure. The mutational process remains modifiable longer when the animals are in streptomycin (curve B) than when they are not (curve C), as would be predicted by the theory.

One possible selection artifact was not considered in the last report. *Paramecium aurelia* normally has two diploid nuclei, which form eight meiotic products at autogamy. One of these products is selected to form the new nuclei and the rest disintegrate. It has been shown<sup>6</sup> that this selective process is influenced by the genetic constitution of the meiotic products; consequently it was possible, though not very likely, that streptomycin acted to favor the selection of products free of mutations. A test was made by comparing the

<sup>6</sup>R. F. Kimball and N. Gaither, *Genetics* 40, 878-889 (1955).

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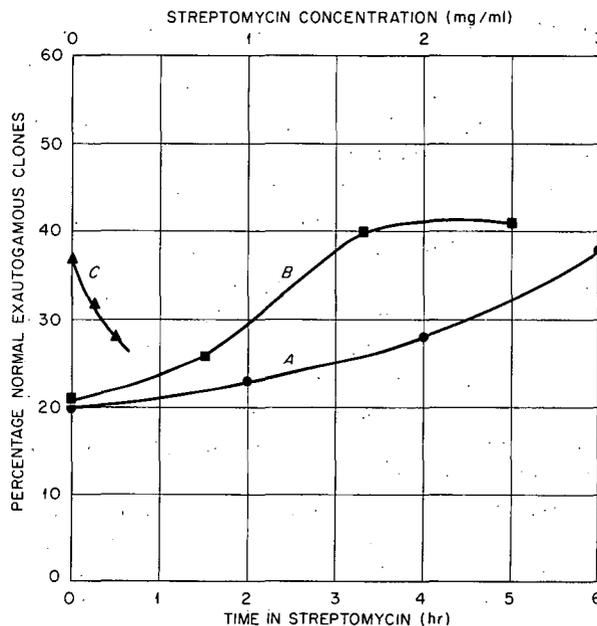


Fig. 1. Posttreatment with Streptomycin. Curve A, relation to concentration of streptomycin (3-hr exposure); curve B, relation to time in streptomycin (3 mg/ml); curve C, relation to time between irradiation and putting into streptomycin (3 mg/ml for 4 hr). Control (no streptomycin) was 23.6%.

<sup>1</sup>USPHS Fellow.

<sup>2</sup>ICA-NRC Fellow.

<sup>3</sup>Research Associate.

<sup>4</sup>Visiting investigator from abroad.

<sup>5</sup>R. F. Kimball, N. Gaither, and S. M. Wilson, *Biol. Semiann. Prog. Rep.* Aug. 15, 1956, ORNL-2155, p 13.

effect of streptomycin on mutation in a clone with one diploid nucleus with the effect of streptomycin on mutation in a clone with two diploid nuclei. The clone with one nucleus produced only four meiotic products, so that the opportunities for selection were less, and streptomycin would have been expected to be less effective if it acted through this form of selection. Actually, the effect of streptomycin was the same as for the clone with two nuclei. Therefore the last remaining possibility for a selection artifact has been eliminated, and it can be concluded with considerable confidence that streptomycin modifies the mutational process itself.

#### A Study on the Mechanism of Acquired Radioresistance in Cancer

A. D. Conger                      H. J. Luippold

It is a fairly common clinical and laboratory observation that tumors subjected to repeated irradiations gradually develop "acquired radioresistance," that is, the tumors require a larger dose for equal regression or show less regression for an equal dose as the radiation treatments continue. Three possible explanations for the effect are commonly proposed: (1) the initial irradiations induce in a tumor cell a mutation to resistance, which is selected for by the later irradiations, (2) some cells in the original population possess a spontaneous mutation for resistance, which is selected for, or (3) the initial irradiations induce complex changes ("tumor bed changes") in the host which interact to make the tumor behave subsequently as though more resistant. The first explanation for ordinary tumor experiments seems highly unlikely, since it requires the induction of the resistant mutation in a cell which survives and which is subsequently favored in proliferation; the second explanation is less unlikely, since only the last two requirements need be met.

**Results.** — A clonal line of the Ehrlich mouse ascites tumor was used in an experiment particularly designed to maximize the likelihood of inducing radioresistance. A "continuously irradiated line" was carried by weekly transfer in mice exposed to  $\text{Co}^{60}$  gamma radiation 23 hr/day for nine weeks; the average dose rate was 134 r/day for 63 days, a total dose 8440 r. Growth, or survival, in the "irradiated line" was only  $10^{-9}$  to  $10^{-10}$  as much as in the unirradiated "control line" for the nine-week course of the irradiation; radiation selection

had been very severe, as desired. At the end of the irradiation, the irradiated line was allowed to recover until the per cent of divisions which were normal (had no chromosomal bridges or fragments) was the same as the control line, about 90% normal. The radiosensitivity of the control and irradiated lines was then compared for two radiation effects. Lots of control-line and irradiated-line tumor were x-rayed in vitro with doses of from 50 to 600 r and injected into recipient mice; cytological damage (per cent of divisions with chromosomal bridges and/or fragments) was observed, in samples drawn from the mice, at increasing time intervals after irradiation. The cytological experiment was done twice, and in both experiments the decline in per cent normal divisions as a function of dose was the same for the control and the previously irradiated lines. Radiation killing of the two lines was compared by irradiating lots of tumor in vitro, injecting a measured number of tumor cells into recipient mice, and then observing the amount of growth — increase in cell number — occurring in a five-day growth period; cell number on the fifth day is roughly proportional, within limits, to the number of viable cells inoculated initially. Cell survival — number of cells per animal — as a function of dose was the same, within the limits of error, for the two lines. It can be said that in this experiment, designed particularly to maximize the likelihood of causing "acquired radioresistance" in a tumor, such resistance has not been caused.

**Discussion.** — On the basis of what is already known about cellular radiosensitivity, genetics, and radiation selection in a cell population, it would seem highly unlikely, though not impossible, that the acquired radioresistance found in the ordinary laboratory or clinical experience arises from mutation induction and selection, or from mutation selection, in the tumor cell population itself. Radioresistance was not caused in the experiment just described, designed with these points in mind to maximize the likelihood of causing radioresistance. This negative evidence, coupled with the known positive evidence that a change in an irradiated host causes a change in the apparent radiosensitivity of a tumor, suggests that the explanation for most cases of acquired radioresistance of a tumor is not a change in the intrinsic (genetic) radioresistance of the tumor itself, but is due to a change in the irradiated host.

## Modification of the Chromosome Aberration Yield in *Tradescantia* by Postirradiation Anoxia

S. Wolff

**Introduction.** — Shortly after the discovery of the "oxygen effect" whereby x irradiation of *Vicia faba* chromosomes under anoxic conditions produced fewer aberrations than did similar irradiation in air,<sup>7</sup> the phenomenon was re-examined in *Tradescantia*, the classical material for chromosome cytology.<sup>8</sup> The purpose of these latter studies was to determine whether or not the decreased aberration yield was caused by less original breakage of the chromosomes or by more restitution of the same number of breaks. Giles and Riley<sup>8</sup> concluded from these studies that only breakage was affected, that the recovery processes were not affected, and, what is probably more important, that postirradiation anoxia cannot modify the aberration yield.

A more critical approach to the problem led Wolff and co-workers<sup>9-11</sup> to discover that in the seed of *Vicia faba* two independent effects of the radiation existed, one on breakage and the other on the recovery process. It was observed that post-irradiation anoxia did indeed have an effect on the *time* (although not the *amount*) of rejoining.

Since the experimental design of the previous *Tradescantia* experiments would not have allowed the detection of any effect on the time of recovery and, consequently, any modification of the aberration yield caused by such an effect, the present experiments were performed on *Tradescantia* in an attempt to see if the effects noticed in *Vicia* are general and, more particularly, to see whether or not postirradiation treatment of *Tradescantia* chromosome breaks can modify aberration yield.

**Results.** — In Table 1 may be seen the results of dose fractionation experiments performed on interphase cells of *Tradescantia* microspores.

Between the two doses, anoxia was produced either by placing the inflorescences in a vacuum in the dark or by passing nitrogen over the material after a preliminary evacuation of the air.

**Discussion.** — If the two doses of radiation are separated by 1 hr during which the inflorescences are in air, the two-hit aberration yield is only the sum of the aberrations produced by each dose alone. This indicates that the breaks induced by the first dose have rejoined before the second dose was administered. If, however, the material was placed under anoxic conditions between the two doses (this included placing the *Tradescantia* in the dark to prevent the formation of oxygen by photosynthesis), then the first breaks remained open for the hour interval and were able to rejoin with the breaks of the second dose. This resulted in an aberration yield greater than the additive amount of the two half doses.

These results are comparable to the effects noted in *Vicia* seed wherein postirradiation anoxia can influence the aberration yield by affecting the time that the breaks stay open. This particular quantitative effect can only be noted in dose fractionation or dose intensity experiments in which it may be seen whether or not breaks are open and capable of rejoining at various times after their formation. It cannot be observed by single-dose experiments carried out at constant intensity. This is why it was not seen in the earlier *Tradescantia* experiments. The conclusions to be drawn from these studies are that the effects of radiation on *Tradescantia* chromosome breakage and reunion are similar to those described for *Vicia* chromosomes. This, consequently, indicates that previous conclusions about the oxygen effects in *Tradescantia* were partially erroneous in that postirradiation presence or absence of oxygen dose have an effect on the recovery process, and that, in addition to the effect noted on the breakage of the chromosomes, there is another effect on the time that the breaks remain capable of rejoining.

<sup>7</sup>J. M. Thoday and J. Reed, *Nature* 160, 608 (1947).

<sup>8</sup>N. H. Giles and H. P. Riley, *Proc. Natl. Acad. Sci. U.S.* 36, 337-344 (1950).

<sup>9</sup>S. Wolff and K. C. Atwood, *Proc. Natl. Acad. Sci. U.S.* 40, 187-192 (1954).

<sup>10</sup>S. Wolff and H. E. Luippold, *Science* 122, 231-232 (1955).

<sup>11</sup>S. Wolff, in *Proceedings of the Fifth International Radiobiology Conference*, Stockholm, Sweden (in press).

## Cytochemical Observations on the Nucleoli of Planorbis Snails

R. R. Cowden

**Introduction.** — In view of the general confusion among cytologists concerning the composition and function of the nucleolus, a series of investigations was undertaken, using the ovotestes of *Planorbis*

BIOLOGY PROGRESS REPORT

Table 1. Effect of Postirradiation Anoxia on the Rejoining of Chromosome Breaks in *Tradescantia*

Dose I in Air (r)	Treatment for 1-hr Interval	Dose II in Air (r)	2-Hit Aberrations per Cells Scored		2-Hit Aberrations per 100 Cells	Expected Yield If Doses I and II Are Additive
			Exp 1	Exp 2		
0	Air in light	0	0/150		0.0	
150	Air in light	0	17/116	51/350	14.6	
150	Air in light	150	81/237	103/300	34.2	29.2
150	Vacuum in dark	0	13/100	50/300	15.7	
0	Vacuum in dark	150	22/150	43/300	14.5	
150	Vacuum in dark	150	75/150	124/250	49.7	30.2
0	Vacuum in dark	0	1/200		0.5	
300	Vacuum in dark	0		106/200	53.0	
	Vacuum 3 min., then air in dark		0/150		0.0	
150	Vacuum 3 min., then air in dark	0	52/300		17.3	
0	Vacuum 3 min., then air in dark	150	35/200		17.5	
150	Vacuum 3 min., then air in dark	150	123/300		41.0	36.1
150	Vacuum 3 min., then N <sub>2</sub> in dark	0	63/300		21.0	
0	Vacuum 3 min., then N <sub>2</sub> in dark	150	34/300		11.3	
150	Vacuum 3 min., then N <sub>2</sub> in dark	150	154/300		51.3	32.3
0	Vacuum 3 min., then N <sub>2</sub> in dark	0	0/150		0.0	

snails. In these animals, cells of both sexes are available within the same tissue, so that simultaneous cytochemical studies may be carried out on the giant nucleoli of the growing oocytes and on the more conventional sized nucleoli of the spermatogenic series.

**Results.** - Sections subjected to DNase (see Appendix for alphabetical list of abbreviations) digestion and subsequent staining with azure B bromide,<sup>12</sup> which is a specific stain for RNA, indicated that most of the nucleolar RNA was located on the periphery of the nucleolus. This

<sup>12</sup>M. H. Flax and M. H. Himes, *Physiol. Zoöl.* 25, 297 (1952).

staining was extinguishable by prior treatment with RNase. Further, the same perinucleolar region was Feulgen positive, indicating the presence of DNA. In addition to the sectioned material, nuclei isolated by two different methods were used: nuclei isolated by exhaustive teasing in 1% citric acid and nuclei mechanically isolated in 0.65% NaCl. These isolated nuclei were stained by the Feulgen method followed by Deitch's<sup>13</sup> naphthol yellow S, which is specific for free amino groups. The Feulgen reaction for DNA was not affected by either isolation procedure, but in the citric-acid-isolated nuclei, the nucleoli were not stained by

<sup>13</sup>A. D. Deitch, *Lab. Invest.* 4, 324 (1955).

naphthol yellow S. The nucleoli of the mechanically isolated nuclei were stained by naphthol yellow S. Evidently the proteins of the nucleolus are removable with 1% citric acid, but are not completely removed by 0.65% NaCl.

**Discussion.** — In view of recent investigations by various procedures and various workers,<sup>14</sup> Caspersson's hypothesis<sup>15</sup> that the nucleolus is the site of RNA synthesis is no longer tenable. Before any consideration of nucleolar function may be undertaken, the morphological relationships between the two classes of nucleic acids and proteins must be more clearly understood. Some of these relationships have been studied in Planorbis snails, and these results are in line with those of Austin<sup>16</sup> and Vincent.<sup>17</sup> Since the material under investigation could have represented a special case, further investigations are now in progress on nucleoli of other cell types.

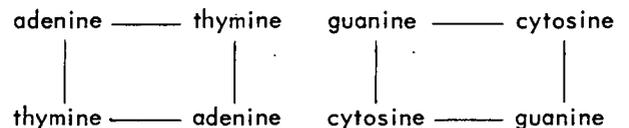
#### Replication and Synapsis of Deoxyribonucleic Acid

D. Schwartz

C. B. Murray

The assignment of the role of genetic determinant to DNA requires that its structure satisfy restrictions imposed by genetic as well as physical and chemical studies. Specific pairing between homologous regions of DNA molecules is required for bacterial transformation and for recombination in bacteriophage. In the former, purified DNA enters the bacterial cell and replaces or displaces some homologous DNA on the bacterial chromosome. In the latter, interchanges occur at precise homologous regions between phage DNA molecules. These processes require specific pairing between the macromolecules, at least for small distances. Synapsis of this kind is not feasible without some modification of the Watson-Crick helical structure during the pairing process. The outside of the helix is undifferentiated throughout its length. Differentiation or specificity exists only in the sequence of paired bases which lie on the inside

of the helix. Synapsis must therefore occur between pairs of bases lying on the inside of the one helix with corresponding pairs of bases which lie on the inside of a homologous region of a second DNA molecule. This can occur only if the two DNA helices pair to form a four-stranded configuration. The specificity for the 2 × 2 pairing of the bases exists through H bonding (Fig. 2). With the distances between the sugar-phosphorus chains in the four-stranded configuration held constant, a pair of bases has the specificity to form H bonds with only one of the two possible pairs in only one of the two possible arrangements:



There is not sufficient room in the Watson-Crick helix to accommodate four chains. Thus in the process of pairing, the helices are forced to uncoil somewhat, increasing the pitch and separating the bases along the fiber axis such that the distance between adjacent base levels becomes greater than 3.4 Å. The uncoiling process can be visualized as being forced to completion by collision of water molecules which form a monomolecular layer sandwiched in between the planes of paired bases. At this state, in the synapsed region, the bases are 7 Å apart and the chains are fully extended and completely uncoiled, lying parallel to each other. In homologous sectors synapsis can proceed for a considerable distance along the molecules with the associated uncoiling in the paired regions. Two levels of this four-stranded structure (eight bases) have been built with the Courtauld space-filling-type atomic models.

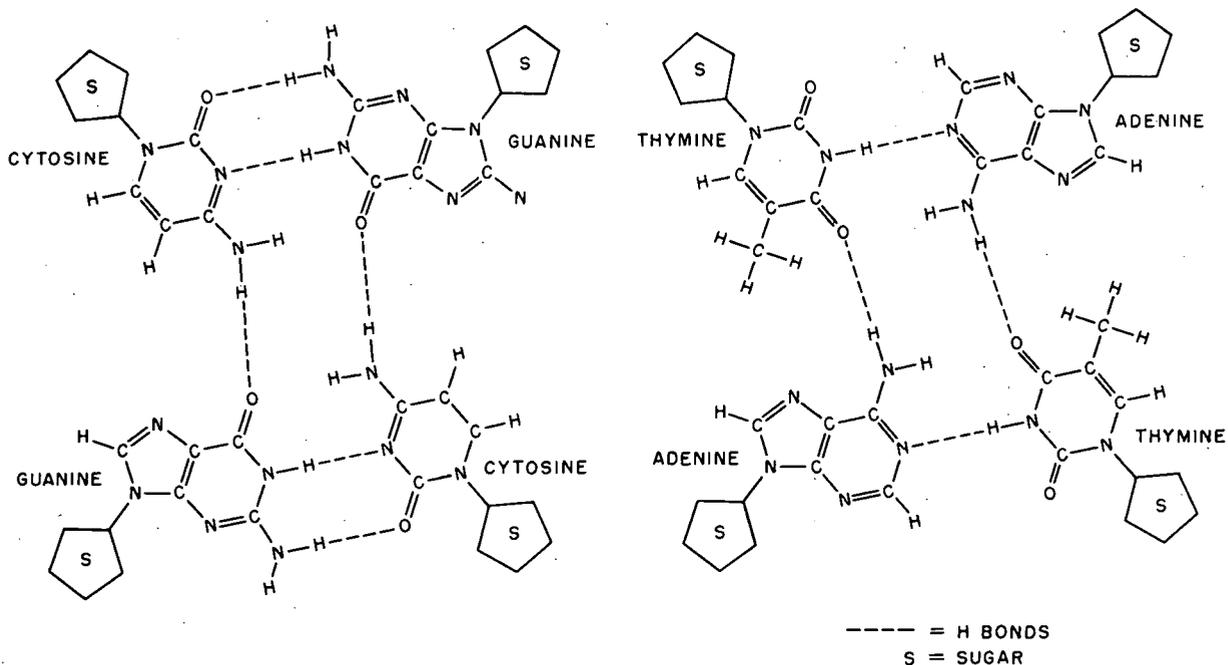
The 2 × 2 pairing between bases at each level requires that the bases pivot slightly on the N-H—N axis, thus separating the 6-keto and 6-amino groups of each pair. In the guanine-cytosine tetrad (Fig. 2), this configuration is maintained by the formation of an H bond between the 2-keto and 2-amino groups of cytosine and guanine, respectively, which are brought into close proximity. In the thymine-adenine tetrad, steric hindrance of the methyl group is responsible for this configuration. The methyl carbon can only approach to within 3.4 Å of the 7-position nitrogen of adenine. Keeping this distance between these points, the formation of the appropriate H bonds between the four bases orients the bases into a

<sup>14</sup>H. Swift, *et. al.*, "The Cytology of Nuclear RNA," in *Cellular Mechanism in Differentiation and Growth*, ed. by D. Rudnick, Princeton U. Press, Princeton, N. J., 1956.

<sup>15</sup>T. O. Caspersson, *Cell Growth and Cell Function, A Cytochemical Study*, 1st ed., Norton, New York, 1950.

<sup>16</sup>C. R. Austin, *Exptl. Cell Research* 4, 249 (1953).

<sup>17</sup>W. S. Vincent, *Intern. Rev. Cytol.* 4, 269 (1955).

Fig. 2. The  $2 \times 2$  Pairing of Bases.

configuration similar to that of the guanine-cytosine tetrad. The N-H---N distances<sup>18</sup> fall in the range between 2.96 and 3.37 Å. The postulated pivoting of the bases to form the new configuration requires N-H---N distances near the upper limit.

This model can also be used for DNA replication, with each two-stranded molecule serving as the template for the formation of another like molecule. Pairs of mononucleotides can be positioned and H-bonded to their complementary pair at each level. Polymerization of the nucleotides to form the two new chains would cause the original strands to uncoil and the four chains to lie parallel. They are now free to separate  $2 \times 2$ , and each recoils to form the two-stranded helical configurations.

The four-stranded structure is only a temporary condition existing during the process of replication. There are two possible planes of cleavage; one puts two old and two new strands together, the other has each daughter DNA composed of an old and a new strand.

<sup>18</sup>L. Pauling and R. B. Corey, *Arch. Biochem. and Biophys.* 65, 164-181 (1956).

The model offers a possible clue for the absence of uracil in DNA. Since the steric hindrance of the methyl group in thymine is essential to maintain the desired base tetrad configuration, only those uracil derivatives in which the methyl group is substituted for by something of about equal size should be incorporated, as are 5-bromo-, 5-iodo-, and 5-chlorouracil.

The proposed scheme for synapsis reconciles the Watson-Crick DNA model with Levinthal's model of bacteriophage heterozygotes. The formation of linear H bonds between the bases in the four-stranded structure requires that the "old and new" base pairs do not lie on the same plane.

#### Correlated Categories of Mutants

K. C. Atwood

I. L. Sandler

**Introduction.** — The proportion of *Neurospora* mutants that are irreparable, that is, that show no response to nutritional supplements, is much higher among mutants obtained by the heterokaryon method than among those obtained by the selection

of temperature-sensitive types. It was suggested<sup>19</sup> that certain irreparable mutants cannot have temperature alleles. This suggestion has been criticized on the ground that special features of the heterokaryon method, for example, the genetic background of the stocks, may affect the apparent reparability of the mutants. Accordingly, experiments are being done to test the correlation between temperature sensitivity and reparability under identical conditions. Mutants obtained on minimal medium at 35°C by the heterokaryon method were tested at 25°C. The same series of mutants was tested for reparability on complete medium.

**Results.** — Two hundred fifty-one ultraviolet-induced mutants have been found; 11 of these are reparable. Five of the mutants are temperature sensitive; of these, two are reparable.

**Discussion.** — The suggested correlation is probably correct, but larger numbers are needed. The importance of this study is that some enzymes that catalyze reparable functions are known to possess thermolabile analogues; hence, if the correlation is borne out it will suggest that many of the irreparable functions are of a fundamentally different character, since they involve gene products that have no thermolabile analogues.

### Instability of Disomic Nuclei in *Neurospora*

T. H. Pittenger

**Introduction.** — In haploid organisms such as *Neurospora*, stable diploids would provide an extremely useful tool in many types of genetic experiments. Repeated attempts by many investigators to obtain stable diploids by using the techniques found so successful with *Aspergillus* have resulted in failure. From crosses of linked mutants, however, it is possible to recover in the progeny phenotypically wild-type strains which segregate for the original parental mutants when crossed to standard wild-type strains.<sup>20,21</sup> Such strains are believed to originate as nondisjunctional disomics but are not stable during somatic divisions, since some homokaryotic mutant conidia can be recovered from them. The somatic reduction is being investigated to learn more concerning the

instability of the disomic nuclei with the hope that such information may be useful in understanding and overcoming the failure to obtain stable diploids.

Since the disomic nuclei break down sometime during somatic division to form a heterokaryon composed of two different genetically distinguishable haploid nuclei whose proportions can be accurately determined, an analysis was made of the nuclear ratios present in mycelium derived from two or more individual hyphae just emerged from opposite ends of ascospores containing disomic nuclei. It was hoped that such an analysis would reveal something of the time of the breakdown and whether it was reciprocal in nature.

**Results.** — Eighty phenotypically wild-type ascospores from a cross of linked mutants, *a al-2,nic-2* × *A lys-3*, were analyzed, but only ten were unisexual disomics of the desired type. The proportions of *lys-3* and *al-2,nic-2* nuclei in the mycelium derived from hyphae isolated from the opposite ends of disomic ascospores onto agar slants supplemented with lysine and nicotinamide were determined. The proportions for the *lys-3* nuclei (columns A and B represent the opposite ends of the ascospores) were as follows:

A	B
1.00	0.94
0.99	0.57
0.99	0.68
0.96	0.68
0.53	0.42
0.53	0.42
0.53	0.07
0.28	0.0
0.28	0.01
0.06	0.01

Although a wide range (0–100%) exists in the proportion of *lys-3* nuclei that can be found in the hyphae emerging from a disomic spore, there is a high positive correlation between the proportion of *lys-3* nuclei found in the hyphae from the opposite ends of the spores, and this is similar to the correlation between the nuclear proportions in different hyphae from the same end of the same spore.

**Discussion.** — Mitosis of disomic nuclei of *Neurospora* is clearly abnormal, since such nuclei give rise during somatic division to haploid nuclei, but it is impossible to deduce from the present

<sup>19</sup>K. C. Atwood and F. Mukai, *Proc. Natl. Acad. Sci. U.S.* 39, 1027 (1953).

<sup>20</sup>M. B. Mitchell, T. H. Pittenger, and H. K. Mitchell, *Proc. Natl. Acad. Sci. U.S.* 38, 569–580 (1952).

<sup>21</sup>T. H. Pittenger, *Genetics* 39, 326–342 (1954).

experiments what mechanism is operative in this somatic reduction. The wide range of nuclear ratios between different spores suggests a non-reciprocal reduction; however, the data are still compatible with the assumption that reduction of the disomic gives equal numbers of both nuclear types but that inequality later results from unknown factors.

Since the reduction obviously must precede any inequality in the proportion of the two nuclear types, it is concluded that reduction takes place during nuclear division inside the ascospore. The high correlation between nuclear proportions in the hyphae from opposite ends of the spore suggests that these proportions are established in the spores prior to the emergence of the hyphae.

#### Analysis of a Viable Duplication Covering the *ad-3B* Locus in *Neurospora crassa*

F. J. de Serres

**Introduction.** — Previous studies<sup>22</sup> have shown that the order of loci and map distances in the *ad-3* region of *Neurospora crassa* with reference to the centromere are as follows: centromere—0.5 unit—*hist-2*—2.0 units—*ad-3A*—0.1 unit—*ad-3B*—3.0 units—*nic-2*. From a cross of an *ad-3B* mutant of x-ray origin (B12), however, the proportion of histidine-requiring progeny approached 50% of the total adenine-independent progeny instead of the expected 3%. Since the asci from this cross showed a marked 6:2 and 4:4 abortion pattern, it appeared likely that mutant B12 was associated with a chromosome rearrangement which segregated so that a large proportion of the progeny carried a duplication covering the *ad-3B* and *nic-2* loci and were thus histidine-requiring. Since strains which give rise to viable duplications permit the study of gene heterozygotes for loci in the region covered by the duplication, a cytogenetic analysis was initiated to characterize the chromosome rearrangement in mutant B12 and to determine the size of the region on the right arm of linkage group I (LGI) covered by the duplication.

**Results.** — A genetic analysis on serial ascospore isolates from a cross of mutant B12 with a *hist-2-nic-2* strain indicated that two chromosome rearrangements were present in the original isolate of

mutant B12, one closely linked and another which segregated independently of the mutant phenotype. The analysis was confined to a study of the rearrangement linked to the mutant phenotype. Crosses of B12 with markers on the right arm of LGI showed that the duplication covered the *ad-3B* and *nic-2* loci, but covered neither the *ad-3A* locus to the left nor the *crisp*, *thi-1* or *al-2* loci located (in that order) to the right of the *ad-3B* locus. Such evidence suggested that a chromosome segment carrying the *ad-3B* and *nic-2* loci had been deleted from the right arm of LGI and inserted in some other position in the genome.

Adenine-independent isolates were recovered from a cross of B12 with a marked *ad-3A* strain at a frequency which indicated that the distance from *ad-3A* to the proximal break point is about 0.1 map unit. Genetic data from the crosses of B12 with LGI markers combined with counts of mature asci, made to determine the ratio of asci with a 6:2 abortion pattern to those with either eight ripe spores or a 4:4 abortion pattern, suggest that the deleted segment is inserted about 25 crossover units from the centromere on some chromosome other than the one carrying LGI.

Crosses of strains carrying the duplication for the *ad-3B-nic-2* region to normal strains are highly sterile; many perithecia are formed, but asci fail to mature, and only a few ascospores, if any, are found in individual perithecia. Cytological examination of asci from such crosses has shown that meiosis rarely proceeds beyond the early prophase stages of the first meiotic division. However, genetic analyses of the ascospores from such a cross indicate that both normal strains and strains carrying the duplication are formed in those instances where meiosis is completed.

**Discussion.** — The analysis of the chromosome rearrangement associated with the mutant phenotype in mutant B12 provides further evidence for the validity of the separation of the *ad-3* region into two separate loci. Previous evidence, which indicated that the loci were separable by crossing over, is augmented by the present analysis, which indicates that the loci are separable by means of chromosome rearrangements. In addition, the fact that strains heterozygous for *ad-3B* and *nic-2* are wild type in phenotype provides conclusive evidence for the dominance of the wild-type alleles in the heterozygote, as has been found for the heterokaryon.

<sup>22</sup>F. J. de Serres, *Biol. Semiann. Prog. Rep.* Feb. 15, 1956, ORNL-2060, p 17.

**INSECT CYTOLOGY AND GENETICS**

M. E. Gaulden

R. C. von Borstel	R. P. Perry <sup>24</sup>
J. G. Carlson <sup>23</sup>	M. L. Pardue
A. R. Whiting <sup>23</sup>	B. A. Limbaugh
R. L. Amy <sup>23</sup>	A. P. Teasley

**Lethal Effects of X Rays on the Neuroblasts and Embryos of the Grasshopper *Chortophaga viridifasciata***

B. A. Limbaugh M. E. Gaulden

**Introduction.** — Previous work on the neuroblasts of the 14-day-old grasshopper embryo has shown the pronounced effect of as little as 1 r of x rays in producing mitotic inhibition.<sup>25</sup> In view of the extreme sensitivity of the mitotic process in neuroblasts to x rays, experiments have been performed to determine the sensitivity of these cells and the embryos to the lethal effects of x rays.

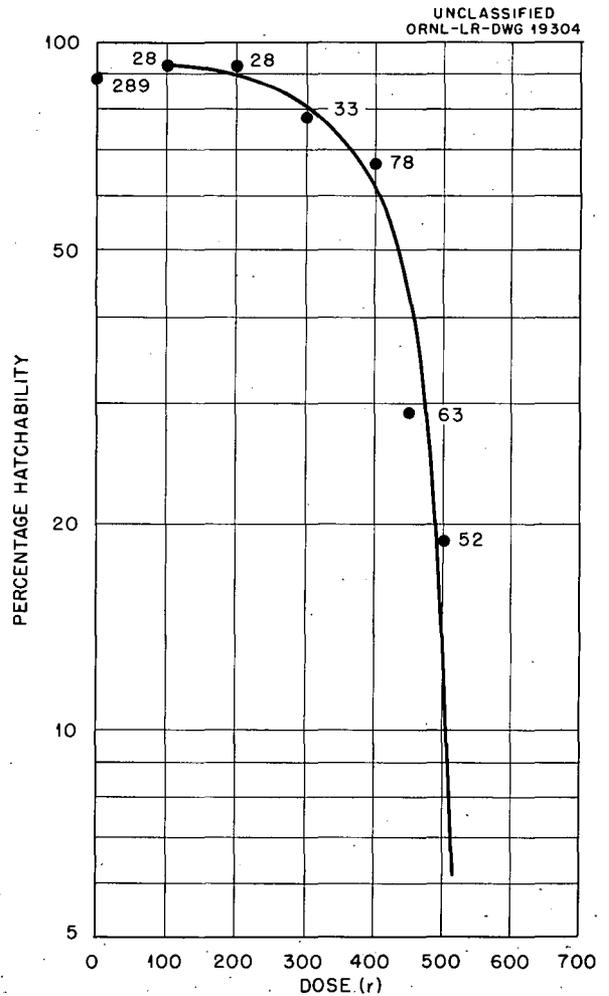
**Results.** — All embryos used in the present experiments were 14 days old (room temperature) at the beginning of observations and/or at the time of irradiation. When failure of the embryo to hatch after irradiation is taken as a criterion of lethality, the LD<sub>50</sub> (30 days) to the embryo lies between 400 to 450 r; the LD<sub>100</sub> (33 days) is approximately 600 r (Fig. 3). The maximum hatchability of 89% in the controls was reached at 27 days. The total number of embryos irradiated is indicated for each dose in Fig. 3. At 600 to 800 r, the embryo develops to the point of hatching but is unable to emerge from the chorion. Between exposures of 800 to 8000 r, the embryos develop to stages which are comparable, morphologically, to at least 20 to 21 days of normal development. Between doses of 8000 to 17,000 r, the appendages of the embryos continue development for a maximum of 2 to 3 days, after which time degeneration of the embryo sets in. At doses of 17,000 r and above, no further development in terms of gross morphological development could be detected.

Failure of the neuroblasts to recover from mitotic inhibition was used as a criterion of lethal effects

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<sup>24</sup>Research Associate.

<sup>25</sup>M. E. Gaulden, *Acceleration of Mitotic Rate in Unirradiated Cells and Prevention of Mitotic Retardation in X-rayed Cells by Hypertonic Culture Medium.* In manuscript.



**Fig. 3. Per Cent Hatchability of Grasshopper Embryos at X-Ray Doses 0–500 r. The number of eggs tested is indicated at each point.**

of x rays on these cells. Irradiated and unirradiated embryos were made into hanging-drop preparations as previously described.<sup>26</sup> Figure 4 shows the ratio of the number of mid-mitoses (prometaphase, metaphase, and anaphase) in irradiated embryos to the number in control embryos after doses of x rays from 3 to 8000 r. The data for 3 r are from an earlier experiment<sup>25</sup> and are shown for comparison with the effects of the high doses. It can be seen that as the dose of x rays is increased, the duration of mitotic inhibition increases

<sup>26</sup>M. E. Gaulden and K. L. Kokomoor, *Proc. Soc. Exptl. Biol. Med.* 90, 309–314 (1955).

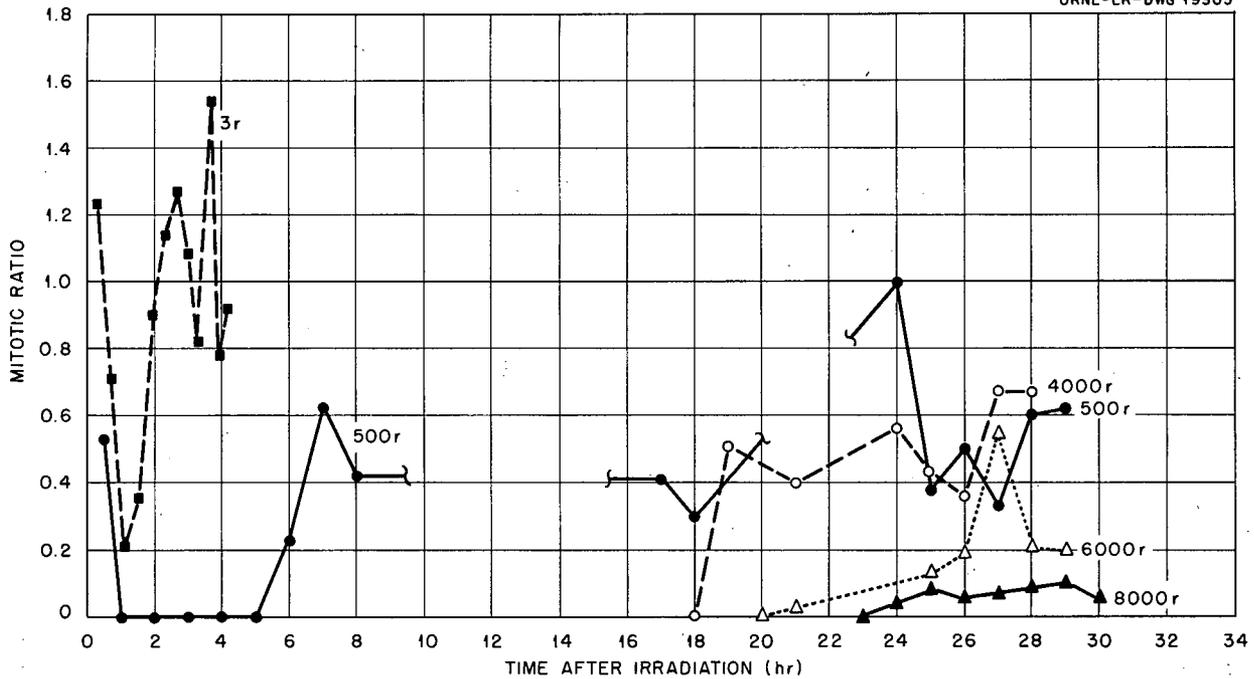


Fig. 4. Effects of 3-8000 r of X Rays on Mitotic Activity in Neuroblasts of Grasshopper Embryos.

and the number of cells recovering from inhibition decreases so that after 8000 r very few cells can continue mitosis. Above a dose of 8000 r, no dividing cells were observed in the cultures. However, after doses of 10,000 to 12,000 r, an occasional dividing cell was observed at 48 to 72 hr in some fixed and stained preparations of embryos. Such cells could not be definitely identified as neuroblasts.

Some experiments were performed to determine the mitotic rate of cells which recover from higher doses of radiation. Embryos were given 500 to 2000 r of x rays and made into hanging-drop cultures. Individual cells in irradiated and unirradiated embryos were observed during the recovery period (17 to 25 hr after irradiation). It was found that cells which recover from these doses of radiation divide at approximately the same rate as cells in unirradiated embryos.

**Discussion.** - Although the neuroblasts of the grasshopper are extremely sensitive to the effects of radiation in producing temporary mitotic inhibition, they are fairly "resistant" to its effects in producing permanent inhibition. One roentgen of x rays can cause appreciable mitotic inhibition; yet a dose greater than 8000 r is required to cause

complete cessation of mitotic activity in all cells. Thus it can be seen that neuroblasts can tolerate x rays over a wide range of doses. The dose which prevents hatching of the embryos (600 r) is much lower than the maximum tolerance dose for the neuroblasts.

**DROSOPHILA GENETICS**

- |                                   |                             |
|-----------------------------------|-----------------------------|
| E. Novitski                       | L. M. Sandler <sup>29</sup> |
| G. E. Brosseau, Jr. <sup>27</sup> | G. D. Hanks                 |
| C. W. Edington <sup>28</sup>      | E. S. Von Halle             |
| D. L. Lindsley                    | R. D. Wilkerson             |

**Induction of Dominant Lethals and Sex-linked Recessive Lethals in *Drosophila* by Monochromatic 14-Mev Fast Neutrons**

- |                |                              |
|----------------|------------------------------|
| C. W. Edington | M. L. Randolph <sup>30</sup> |
|----------------|------------------------------|

**Introduction.** - Experiments have already been reported from this laboratory on the induction of

<sup>27</sup>American Cancer Society Fellow.

<sup>28</sup>Temporary employee.

<sup>29</sup>Research Associate.

<sup>30</sup>Biophysics Group.

dominant lethals in *Drosophila* by x rays<sup>31</sup> and 1-Mev fast neutrons<sup>32</sup> and on the induction of sex-linked recessive lethals by x rays, gamma rays, and 1-Mev fast neutrons.<sup>33</sup> Since the Biology Division's linear accelerator is capable of producing monochromatic 14-Mev neutrons at dose rates necessary for genetic studies, experiments were conducted using similar biological techniques to compare the effect of 14-Mev fast neutrons on the induction of these genetic events with the results of the above-mentioned radiation studies.

**Results.** - The weighted linear exponential regression, calculated by the least-squares method, which best fits the experimental data for the frequency of egg hatch (1 - dominant lethals), gave the equation,  $\ln Y = -0.476 \times 10^{-3} D$ , and the weighted linear regression for recessive lethals gave the equation,  $Y = 0.11 - 2.39 \times 10^{-3} D$  (where  $Y$  is the predicted percentage and  $D$  is the dose in kilorep). The egg-hatch dose curves for 1- and 14-Mev neutrons were linear, and for x and gamma rays were curvilinear; therefore the RBE was determined by comparing the doses of each radiation which produced 50% dominant lethals. All dose curves for recessive lethals were linear (except for x rays, in which case the frequency of

induced recessive lethals increases more rapidly with dose than was expected on the basis of linearity). Since this was the case, the RBE was calculated simply by comparing the slopes of the linear regressions for each radiation. No direct comparison involving x rays was made, since the RBE of x rays was dose dependent. The relative effectiveness of 14-Mev fast neutrons as compared with x rays, gamma rays, and 1-Mev neutrons for the induction of dominant and recessive lethals is shown in Table 2.

**Discussion.** - It is obvious from these comparisons that the RBE of the different radiations for the induction of these genetic effects increases as the ion density increases, even within the energy range of fast neutrons. Therefore any RBE comparison which involves fast neutrons is valid only when the energy distribution and, consequently, the approximate average energy of the neutron source are known. It is also of interest to point out here that up to approximately 1825 rep, 14-Mev neutrons are slightly more effective than x rays in the production of recessive lethals but that above this dose these neutrons become increasingly less effective. In every case studied thus far the RBE for the induction of recessive lethals is less than that for the induction of dominant lethals. This reduction in the RBE values for recessive lethals could be the result of: (1) a siphoning off of potential recessive lethals in dominant lethals or (2) an average of different RBE values for several genetic events, each of which expresses itself as a recessive lethal.

<sup>31</sup>W. K. Baker and E. S. Von Halle, *Proc. Natl. Acad. Sci. U.S.* 39, 152-161 (1953).

<sup>32</sup>C. W. Sheppard *et al.*, *Radiation Research* (in press).

<sup>33</sup>C. W. Edington, *Genetics* (in press).

Table 2. Relative Biological Effectiveness of Radiations of Different Ion Density for the Induction of Dominant and Recessive Lethals in *Drosophila*

Radiation	Genetic Effect	
	Dominant Lethals (50% hatch)	Recessive Lethals
Co <sup>60</sup> gamma rays	1.0	1.0
250-kvp x rays	1.27	Varies with x-ray dose; 1.01 at 1000 r, 1.58 at 4000 r
14-Mev neutrons	2.31	1.23
1-Mev neutrons	4.36	1.62

**Induction of Dominant and Recessive Lethals in Mature and Immature Sperm of *Drosophila***

C. W. Edington

**Introduction.** — It has been shown that the sensitivity of sperm in *Drosophila* to the mutagenic action of x rays varies with the stage of development of the sperm at the time of irradiation. Immature sperm (generally thought to be spermatids at the time of irradiation) were reported to be about four times more sensitive than mature sperm.<sup>34,35</sup> In these experiments, however, several long mating periods were utilized, and in some cases the males were allowed to inseminate three or more females in each mating period. Using 24-hr mating periods in which males were mated to virgin females in pair matings (although only two 24-hr mating

periods were checked), Baker and Von Halle<sup>31</sup> observed that the frequency of dominant lethals induced by x rays in the first 24-hr sample was significantly higher than that in the second 24-hr sample. Furthermore, using the same mating procedure, it was shown that fast neutrons induced the same frequency of dominant lethals in both the first and second 24-hr sperm samples.<sup>36</sup> The possibility exists, therefore, that differential sensitivity of sperm may be dependent also on the radiation used. With this in mind, experiments were conducted, using the 24-hr pair mating technique, to determine whether the sperm sensitivity pattern varied when sperm in different stages of development were irradiated with x rays or monochromatic 14-Mev fast neutrons.

**Results.** — The frequencies of recessive lethals and dominant lethals induced by x rays and fast neutrons are shown in Table 3. It is obvious from

<sup>34</sup>K. C. Lüning, *Hereditas* 38, 91-107 (1952).

<sup>35</sup>C. Auerbach, *Z. für indukt. Abstammungs-u. Vererbungslehre* 86, 113-125 (1954).

<sup>36</sup>W. K. Baker and E. S. Von Halle, *Science* 119, 46-49 (1954).

**Table 3. Percentage of Dominant Lethals and Sex-linked Recessive Lethals Induced in Mature and Immature Sperm in *Drosophila***

Sperm Sample	X Rays			Neutrons	
	Recessive Lethals		Dominant Lethals At 4 kilorep	Recessive Lethals At 1 kilorep	Dominant Lethals At 1 kilorep
	At 1 kilorep	At 4 kilorep			
1	3.03	13.7	77.3	1.62	39.3
2	1.84	8.9	67.4	1.19	32.0
3	2.09	7.7	58.9	2.89	32.5
4	2.34	8.4	65.2	4.44	37.8
5	4.35	9.2	78.1	5.44	55.5
6	3.76	12.7	93.4	4.05	69.4
7	7.72	9.6	89.2	4.55	77.9
8	5.68	8.3	94.4	1.87	79.0
9	5.07	9.1	87.5		78.7
10	6.06	9.3	65.7		43.6
11	1.05	9.5		1.41	32.4
12		4.8		0.98	
13		4.1			
14		4.8			
15		0.3			
16		1.7			

these results that similar effects are observed regardless of the radiation used; however, the recessive lethal data in the neutron series are based on too few tests to be significant. The initial decrease in both recessive and dominant lethals is observed for both radiations. In the 1000-r x-ray and neutron series maximum frequency is attained in the fifth or sixth sperm sample and remains at this level through the ninth or tenth sample (postmeiotic and meiotic stages) after the tenth sample a sharp decrease is observed (spermatogonia). In the 4000-r x-ray series, however, the lowest frequency of recessive lethals is not reached until the fifteenth sperm sample. Unfortunately, the 4000-r dominant lethal data which were collected at a different time were not continued for the same period of time.

**Discussion.** — From these data it can be safely concluded that immature sperm at the time of irradiation are more sensitive than mature sperm. The significant decrease in the frequency of both genetic effects in sperm sample 2 as compared with sample 1 could be due to either a difference in mutability of the supposedly mature sperm or to restitution or recovery of the primary radiation effect (chromosome breaks). The difference between the dominant lethal effects observed in the neutron experiments conducted in this investigation and those of Baker and Von Halle<sup>36</sup> are in all probability due to a difference in the fate of the primary effects induced by the more densely ionizing 1-Mev neutrons (Baker and Von Halle) as compared with those of 14-Mev neutrons (i.e., breaks induced by the former are less likely to recover).

#### Localization of the Genetic Factors Responsible for the Kinetic Activity of the Chromosome

E. Novitski

D. L. Lindsley      E. S. Von Halle

**Introduction.** — It was shown earlier<sup>37</sup> that X chromosomes of *Drosophila melanogaster* are characterized by different levels of kinetic activity depending upon the origin of the kinetochore and that three distinct levels of activity can be distinguished genetically. Later work made it seem likely that the control of kinetic activity was not the direct function of the kinetochore, but

rather of the associated heterochromatin.<sup>38</sup> The experiments reported here were designed to test directly the hypothesis that the movement of chromosomes during cell division is controlled, in part at least, by genetic factors on the chromosome itself or that the chromosomes are not passive bodies whose movement depends simply on the formation of the spindle mechanism and the presence on the chromosome of a centromere to which a spindle fiber becomes attached.

The method involved the induction by x rays of new X chromosomes in such a way that the centromere and euchromatic regions of the chromosomes were kept constant, while the heterochromatic region was allowed to vary. This was achieved by detaching attached-X chromosomes in females without a Y chromosome and testing such detachments for their kinetic activity by well-known methods. A genetic analysis of the detached chromosome will indicate the method of the detachment and, simultaneously, the origin of the centromere of the chromosome being tested.

**Results.** — In one set of ten detachments, the kinetic level that was measured genetically varied from the typically weak activity characteristic of ordinary X chromosomes to the strong activity of the Y chromosome; four of these detachments showed an atypical intermediate behaviour. Three of the detachments were shown by genetic tests to involve exchange with the small fourth chromosome in such a way as to leave the centromere of the X chromosome intact, yet each of the three behaved differently, in the three ways mentioned above.

A second set of seven detachments was derived in such a way that the centromere of the X chromosome might be replaced by the centromere of the small fourth chromosome. Three detachments of this set carried the pairing region of the fourth chromosome, but not its euchromatic complement, indicating that they had the centromere of chromosome four. Once again one of these detachments showed a strong activity, and another, a weak activity. Three of the others in this set exhibited an unusual intermediate activity.

**Conclusion.** — The wide range of kinetic activities exhibited by chromosomes when the centromeres are held constant and the adjacent heterochromatin is altered indicates that the variations in kinetic activity are dependent on

<sup>37</sup>E. Novitski, *Genetics*, 37, 270-287 (1952).

<sup>38</sup>E. Novitski, *J. Cellular Comp. Physiol.* 45, Suppl. 2, 151-170 (1955).

genetic changes not involving the centromere of the chromosome. Furthermore, since the unusual intermediate values derived in these experiments are not found in the simple tests of kinetic activity of the X and Y chromosomes referred to previously, it is concluded that kinetic activity is determined by a number of loci in the heterochromatic regions of the chromosome whose normal relationships have been altered by the nonspecific nature of the x-ray-induced heterochromatic exchange that has given rise to the various detachments tested.

#### Meiotic Behavior of Reversed Compound Ring-X Chromosomes in *Drosophila melanogaster*

L. M. Sandler

A genetic analysis has been made of a reversed compound ring-X chromosome. This compound is structurally like an attached-X with its free ends connected by a heterochromatic segment or, which is the same thing, it is similar to a reversed acrocentric compound-X chromosome with its free end attached to the centromere by a heterochromatic segment.

The procedure consisted in scoring the progeny of females carrying reversed rings heterozygous for recessive markers which cover, reasonably adequately, the genetic length of the X chromosome and, in addition, progeny-testing many of these  $F_1$  females in order to specify their genotypes.

The results obtained indicate the following: (1) the data from reversed rings are consistent with the usual assumptions made about the nature of crossing over (i.e., that all four nonsister chromatids participate in exchange two at a time at random with sister strands never crossing over) and are inconsistent with the assumption of sister-strand crossing over as presented, for example, by Schwartz<sup>39</sup> (however, it might be noted that the data can be made consistent if it is assumed that sister-strand exchanges always occur in pairs); (2) reversed rings and reversed acrocentrics,<sup>40</sup> which are structurally very similar to reversed rings, are found to be alike in that (a) both show a reduced frequency (or an absence) of single exchanges as compared with exchanges of ranks 0 and 2, (b) both show approximately the same frequency of no-exchange and double-exchange tetrads ( $E_0 = 0.55$  and  $E_2 = 0.45$  for the reversed acrocentric and  $E_0 = 0.63$  and  $E_2 = 0.37$  for the

reversed ring), (c) both have about the same distribution of exchanges along the length of the chromosome, and (d) both show the same reduction in crossing over (about a 50% reduction) when the parental compound-bearing females do not carry a heterochromatic homolog for the compound; and (3) there is found a sizable reduction in the recovery of reversed rings in the progeny of compound-bearing females in excess of that which can be accounted for by either reduced viability of ring-bearing individuals or by a tetrad analysis, suggesting the possibility of lethality due to unresolved interlocked complexes in reversed rings. However, there are reasons for believing that this reduction in the recovery of rings is caused by some factor other than interlocking of rings.

#### Meiotic Drive as an Evolutionary Force<sup>41</sup>

L. M. Sandler E. Novitski

Instances are known, both from studies of natural populations and laboratory experiments, in which heterozygotes produce two kinds of gametes, not with the customary equality, but with unequal frequencies. Such meiotic behavior will profoundly affect gene frequencies in a population and is referred to as "meiotic drive." Some of the consequences of meiotic drive to the genetic structure of natural populations, including those of man, and the evolutionary implications of these consequences are considered elsewhere.<sup>41</sup>

#### Are All Products of Spermatogenesis<sup>42</sup> Regularly Functional?

E. Novitski I. L. Sandler<sup>43</sup>

A genetic analysis of the behaviour of the Bar<sup>5</sup> translocation in the male of *Drosophila melanogaster* shows that the four types of gametes from such a translocation heterozygote are produced with unequal frequencies which seem to bear no relation to each other. It has been shown,<sup>42</sup> however, that the frequencies are expressible precisely in a probability formulation. This should not be the case if, as is commonly thought to be the case, all four spermatids arising from a given spermatocyte are regularly functional.

<sup>41</sup>L. Sandler and E. Novitski, *Am. Naturalist* (in press).

<sup>42</sup>E. Novitski and L. Sandler, *Proc. Natl. Acad. Sci. U.S.* (in press).

<sup>43</sup>Cytogenetic Effects of Radiation Group.

<sup>39</sup>D. Schwartz, *Genetics* 38, 251-260 (1953).

<sup>40</sup>L. Sandler, *Genetics* 39, 923-942 (1954).

## MAMMALIAN GENETICS AND DEVELOPMENT

GENETIC AND DEVELOPMENTAL EFFECTS  
OF RADIATION IN MICE

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Shortening of Life in the Offspring of Male Mice  
Exposed to Neutron Radiation from an  
Atomic BombW. L. Russell      J. S. Gower  
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**Introduction.** — Only in recent years has evidence begun to accumulate that there are slight dominant deleterious effects of mutations formerly regarded as recessive.<sup>2-5</sup> The results to be reported here, and our earlier work on mice,<sup>4-7</sup> indicate that such effects may add up to an important part, perhaps the most important part, of the genetic hazards of radiation in man. The evidence from the earlier work on mice is that appreciable deleterious effects of radiation become manifest in the first-generation offspring. This evidence is of two kinds. First, work on radiation-induced mutations at specific loci in spermatogonia has shown that among the recessive lethals, which comprise more than one-half of all

the mutations recovered, many have dominant deleterious effects which, even for individual mutations, are sometimes large enough to be detected easily. Second, over-all population damage was found in the large numbers of animals that are raised as far as three weeks of age in the specific loci studies. In all such experiments carried out, the survival to three weeks of age is significantly lower in the offspring of irradiated males than it is in the controls. (It should perhaps be pointed out that neither of the above effects, nor the effect reported in this paper, is the result of what the geneticist usually refers to as "dominant lethals," which are major chromosomal aberrations that cause early death of embryos and which, as has been pointed out elsewhere,<sup>6</sup> are probably not an important hazard.)

Our earlier work that showed a significant effect on survival to three weeks of age in the offspring of irradiated males led us to expect that there would be measurable deleterious effects later in life. The data reported here show that such is indeed the case. These data furnish a third kind of evidence of first-generation damage and perhaps the most striking one. They were obtained as a by-product of another investigation and they are not as extensive as we should like. However, they are the only data we have on this subject that were collected under the expensive and difficult conditions of a field test of a nuclear detonation. Furthermore, although small, the sample was sufficient to yield a statistically significant effect which appears to be large and, therefore, of general importance.

**Materials and Methods.** — The material used in the present longevity study was the by-product of an investigation of the relative effectiveness of neutrons from a nuclear detonation and from a cyclotron in inducing dominant lethals in the mouse.<sup>8</sup> In order to reduce the gamma component of the radiation to a proportion that would not appreciably interfere with the estimation of neutron effects, the animals were shielded with lead. The exposure chambers available were lead hemispheres of 7-in. wall thickness and 14-in. inside diameter. Young adult hybrid males, obtained by

<sup>1</sup>Consultant.<sup>2</sup>C. Stern and E. Novitski, *Science* 108, 538-539 (1948).<sup>3</sup>H. J. Muller, *J. Cellular Comp. Physiol.* 35, Suppl. 1, 205-210 (1950).<sup>4</sup>W. L. Russell, *Cold Spring Harbor Symposia Quant. Biol.* 16, 327-336 (1951).<sup>5</sup>C. Stern *et al.*, *Genetics* 37, 413-449 (1952).<sup>6</sup>W. L. Russell, Chap. 12 in *Radiation Biology* (ed. A. Hollaender), McGraw-Hill, New York, 1954.<sup>7</sup>W. L. Russell, *Proc. Intern. Conf. Peaceful Uses of Atomic Energy, Geneva, 1955* 11, 382-383, 401-402 (United Nations, New York, 1956).<sup>8</sup>W. L. Russell, L. B. Russell, and A. W. Kimball, *Am. Naturalist* 88, 269-286 (1954).

crossing inbred 101 strain females with inbred C3H strain males, were exposed inside the hemispheres placed at various distances from the detonation. Control males were placed in hemispheres two days before the detonation and for a length of time approximately the same as that required for the exposed animals. Further experimental details are described in the report of the earlier work.<sup>8</sup> One day and a half after the detonation, each male was placed with four adult untreated females of the same hybrid strain. At 18½ days after irradiation each surviving male was placed with a new group of four females. Most of the females that became pregnant were killed at a late stage of gestation for the dominant lethal study. However, since the number of pregnancies turned out to be more than adequate for the dominant lethal experiment, several of the females were allowed to come to term. It was the offspring of some of these females that were saved for the longevity study described here. All these animals came from matings made from 19 to 23 days after irradiation. A few animals died before weaning, and these were not included in the data reported here. At weaning age the sexes were separated and the animals grouped, so far as possible, six to a cage. They were kept in the same grouping throughout their life span. They were checked at least twice weekly for deaths. Only one animal died at less than one year of age, indicating that the conditions under which the animals were kept were good.

The total (neutron plus gamma radiation) dose inside each lead hemisphere was measured, as

described in the earlier publication,<sup>8</sup> by means of "tissue-equivalent" ion chambers designed for this purpose at short notice.<sup>9</sup> Subsequently, extensive testing and recalibration of these chambers<sup>10</sup> has led to a revision of the original dose estimates. The doses reported in the present publication are the revised estimates. As was reported earlier,<sup>8</sup> attempts to measure the gamma component of the radiation by means of film dosimeters left a large uncertainty as to the size of this factor. Later tests have been made in which both ionization chambers and chemical dosimeters were used to measure the gamma component inside the lead hemispheres when they were exposed to fission neutrons. According to the latest information,<sup>11</sup> these tests indicate that the gamma radiation exposure in our experiment was almost certainly less than 10% of the total dose.

**Results.** — The median and mean lengths of life, together with the number of animals, for each dose group are given in Table 4. An analysis of variance showed that neither grouping in cages nor sex had a significant effect on length of life. It seems likely that larger samples would show some effect of both these factors, but as there was no significant effect of them in the present experiment the data were pooled.

<sup>9</sup>C. W. Sheppard and E. B. Darden, appendix to J. S. Kirby-Smith and C. P. Swanson, *Science* 119, 42-45 (1954).

<sup>10</sup>C. W. Sheppard *et al.*, *Radiation Research* (in press).

<sup>11</sup>G. S. Hurst, personal communication.

Table 4. Length of Life in the Offspring of Male Mice Exposed to Neutron Radiation 19-23 Days Before Mating (Deaths Before Weaning Age Excluded)

Total Dose* to Parent (rep)	Number of Offspring	Median Length of Life of Offspring (days)	Mean Length of Life of Offspring (days)
0	103	823	792
31	50	741	754
71	5	717	699
118	22	739	723
136	8	666	688
186	2	756	756

\*Includes some gamma radiation, estimated to be less than 10% of the total dose.

To test whether there was a significant effect of radiation on the length of life of the offspring, the means were fitted to a straight line by the method of weighted least squares. This gives an intercept of 786 days and a slope of  $-0.609 \pm 0.238$ . Since the residual variance is less than the within-subclass mean square, there is no evidence of nonlinearity over the dose range tested. Even if the true shape of the curve is nonlinear, it will be conservative, in making the test of significance, to assume linearity. The larger mean square was used to compute the variance of the slope, and a two-sided t-test shows that the slope differs significantly from zero at the 1% level. If one is willing to accept a one-sided t-test as more appropriate, the significance level is 0.5%. Thus there is strong evidence of shortening of life in the offspring of the exposed males.

**Discussion.** - It is noteworthy that a significant shortening of life was detected in spite of the small sample and the considerable genetic variability that must have been present in a population that was the  $F_2$  of a cross between inbred strains. Furthermore, the weighted mean dose received by the exposed fathers was only moderate, being less than one-sixth of the 30-day median lethal dose as measured from other animals of the same strain exposed under the same conditions at distances closer to the same detonation. While it is true that certain features of the experiment, which will be discussed later, tended to maximize the shortening of life, nevertheless the result observed appears to be so large that it seems quite possible that shortening of life is an effect that might be detectable in studies of the offspring of exposed parents in human populations.

In view of the lack of information on this subject, and specifically the fact that no data

of this nature were ready for consideration prior to the writing of the 1956 Report of the National Academy of Sciences Committee on Genetic Effects,<sup>12</sup> it is desirable to consider what the present data might indicate when they are extrapolated to man. Taking the estimate obtained from the curve fitted to the mouse data, and assuming that the shortening of life in man would be proportional to this, gives, on the basis of a 70-year length of life in man, the figures shown in Table 5. It should be kept in mind that the results were obtained from neutron irradiation. The relative biological effectiveness of neutrons for this effect is not known, but it seems likely, from other data on mutations, that gamma and x radiation would be less effective than neutrons. It should also be emphasized that the effect observed here is probably a maximum one, since the offspring were obtained from matings made between 19 and 23 days after irradiation. Our data from experiments on mutations at specific loci<sup>13</sup> indicate that the sperm utilized in matings made within this time interval would have been derived from cells in a sensitive stage of gametogenesis at the time of irradiation. From approximately two to four times as many mutations are recovered from this stage as from the spermatogonial stage, which is the important one so far as radiation hazards in man are concerned.<sup>7</sup> It is also possible that the spectrum of mutations from irradiated spermatogonia would be qualitatively different and, conceivably, less effective

<sup>12</sup>The Biological Effects of Atomic Radiation, Summary Reports (Washington: National Academy of Sciences, National Research Council, 1956).

<sup>13</sup>W. L. Russell, *Biol. Semiann. Prog. Rep. Aug. 15, 1956*, ORNL-2155, p 25-29.

Table 5. Shortening of Life in the Offspring of Fathers Exposed to Neutron Radiation 19-23 Days Before Mating (Deaths Before Weaning Age Excluded)

Observed result in the mouse and extrapolation to man

	Mouse (days/rep to father)	Man (days/rep to father)
Point estimate	0.61	20
Lower 95% confidence limit	0.14	5
Upper 95% confidence limit	1.07	35

in shortening life. However, there is no direct evidence of this, whereas there is evidence from our specific loci studies that some mutations induced in spermatogonia have, even individually, a dominant effect on length of life that is detectable. To summarize this paragraph, it should be remembered that the estimates given in Table 5 are based on neutron irradiation of a postspermatogonial and sensitive stage in gametogenesis and that x or gamma irradiation of spermatogonia would almost certainly produce a smaller effect.

Another way of considering the magnitude of the observed result, so far as its human implications are concerned, is to compare the shortening of life in the offspring of irradiated fathers with that in the irradiated individuals themselves. The data on shortening of life of the males exposed to this same detonation will be presented in detail elsewhere. Briefly, the percentage shortening of life of these animals, based on 24 controls and 128 exposed animals, is 0.078% per rep. The present data, expressed in the same form, give 0.077% shortening of life in the offspring for each rep received by the father, that is, approximately as much effect as on the exposed individuals. Thus the best estimate from our present data is that, for neutron irradiation of the sensitive stages in spermatogenesis, the shortening of life in the offspring of irradiated males will be similar in magnitude to that in the exposed individuals. Again, the effect from irradiation of spermatogonial stages would probably be less. Whether the *ratio* of effect in offspring to effect in exposed individuals will be different for x and gamma rays from that observed for neutrons will, of course, depend on whether the relative biological effectiveness of neutrons is different for the effect on the offspring and the effect on the exposed individuals. Present, incomplete data on these points give no grounds for expecting that the ratio of effect in offspring to effect in exposed individuals will be less for x rays than for neutrons. Weighing the evidence reported here, and making some allowance for the many uncertainties, it seems reasonable to predict that, even under the conditions of radiation exposure in man, shortening of life in the offspring of irradiated fathers will be between 10 and 100% of the shortening of life in the exposed individuals themselves. It should be remembered that this excludes an additional effect on the offspring, namely, as measured in the mouse, death before weaning age. Also, and

more important, since the shortening of life is probably the result of mutations with slight dominant effects, the damage would not end with the first-generation offspring but would be transmitted to a certain, and probably large, degree to later generations.

**Summary.** — Length of life in the offspring of male mice exposed to moderate doses of neutron radiation from a nuclear detonation is shortened by 0.61 day for each rep received by the father over the dose range tested. This figure excludes death before weaning age. The 95% confidence limits are 0.14 and 1.07 days/rep. Extrapolating to a proportional shortening of life in man gives 20 days/rep received by the father as the point estimate and 5 and 35 days as the 95% confidence limits. The offspring were obtained from matings made from 19 to 23 days after irradiation and, therefore, represent the effect of irradiation on germ cells in a postspermatogonial and sensitive stage of gametogenesis. It is probable that irradiation of spermatogonia (the stage that is important from the point of view of human hazards) would give a somewhat smaller effect. However, since the present data show an effect on the offspring which is as large as the shortening of life in the exposed individuals themselves, it seems likely that, even when allowance is made for the conditions of human radiation exposure, shortening of life in the immediate descendants will turn out to be of a magnitude that will warrant serious consideration as a genetic hazard in man.

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#### Radiation-induced Presumed Somatic Mutations in the House Mouse

L. B. Russell                      M. H. Major

**Introduction.** — Experiments on the induction of somatic mutations can provide information in at least two main fields of interest: (1) in the comparative study of mutation induction in different types of cells; and (2) in the study of cell lineage, where the induced somatic mutations can be used as a valuable tool.

Somatic mutations involving specific loci have repeatedly been produced by radiation in *Drosophila* and in plants. In mammals, however, although several dozen spontaneous coat color mosaics have been observed, the induction of mosaics by irradiation has not been reported except in a preliminary communication.<sup>14</sup> Consequently, several points of technique had to be worked out prior to the start of the experiment. The method adopted makes it possible to distinguish the genetic effects produced from developmental effects of radiation which might otherwise obscure them.

The results indicate that somatic mutations at specific loci were induced by radiation in prospective pigment cells. The mutation rate in these cells could be calculated and compared with the rate found in mouse spermatogonia for the same loci.<sup>15</sup> Various features of the results suggest some working hypotheses and some tentative conclusions about cell lineage in mouse development.

<sup>14</sup>L. B. Russell and M. H. Major, *Genetics* 37, 621 (1952).

<sup>15</sup>W. L. Russell, *Cold Spring Harbor Symposia Quant. Biol.* 16, 327-336 (1951).

**Method.** — Embryos of a C57BL × NB cross, heterozygous for four coat (*b*, *c<sup>cb</sup>*, *d*, and *p*) genes, were irradiated with 100 or 150 r of x rays at 10<sup>1</sup>/<sub>4</sub> days postconception, and the adult fur was observed for mosaic patches. A parallel control series involved irradiation of embryos of C57BL × C57BL matings, homozygous for the wild-type alleles of the four loci. Nonirradiated offspring of both types of mating were also studied. Altogether, 701 animals were observed.

Spots of altered color in the homozygous series should represent mutations to dominants anywhere in the genome, or else nonmutational, i.e., developmental, changes (e.g., abnormal differentiation of pigment cells). When the frequency of these is subtracted from the total mosaics in the heterozygous series, only the changes due to the expression of the four coat color recessives should be left.

**Results and Conclusions.** — Mice with spots on the normally black coats were found to be of two types: (1) Animals with small white spots, always midventral, were observed only among offspring of C57BL × C57BL matings. The incidence of such individuals is significantly increased by radiation (Table 6). This is interpreted as indicating that

Table 6. Frequency of Various Spots in the Coats of Mice Irradiated 10<sup>1</sup>/<sub>4</sub> Days Postconception

	C57BL ♀ × NB ♂			C57BL ♀ × C57BL ♂		
	0 r	100 r	150 r	0 r	100 r	150 r
Number of Animals Observed						
Males	104	110	9	70	50	9
Females	98	125	12	71	32	11
Total	202	235	21	141	82	20
Per Cent with Spots of Altered Coat Color*						
Males	1.0	14.5	(0)	0	2.0	(11.1)
Females	0.0	8.0	(8.3)	1.4	0.0	(0.0)
Total	0.5	11.1	(4.8)	0.7	1.2	(5.0)
Per Cent with White Midventral Spots						
Males	0.0	0.0	0.0	2.9	22.0	33.3
Females	0.0	0.0	0.0	9.9	34.4	63.6
Total	0.0	0.0	0.0	6.4	26.8	50.0

\*Excluding white midventral spots.

radiation causes some killing of prospective pigment cells but that this becomes apparent only in a genetic background that normally provides only a barely sufficient number of such cells. (2) Animals mosaic for nonwhite spots of diffuse outline which may occur anywhere on the mouse surface were observed with different frequencies in all groups studied (Table 6). After taking account of the three groups of controls, the frequency of mosaic animals due to radiation-induced expression of the recessive at one or another of the four loci was calculated to be 10.1% for 100 r.

The following discussion applies only to spots of the second type, i.e., nonwhite spots. The proportion of the coat occupied by mutant hair ranged from 0.0145 to 0.000173 in different mosaic animals (Table 7). Assuming that the reciprocals of these figures may be taken to represent prospective pigment cells present at the time of irradiation, the extremes of the range are separated by six cell divisions. It should be noted that the distribution of mosaics shown in Table 7 does not directly indicate the distribution of  $10\frac{1}{4}$ -day embryos with respect to number of prospective pigment cells. This latter distribution, however, was derived, and it indicated that the modal number of prospective pigment cells in  $10\frac{1}{4}$ -day embryos was between 150 and 200.

It is clear from the results that most of the mosaics were due to expression of the coat color

recessives for which the C57BL × NB animals are heterozygous. At present one cannot be certain as to the mechanism by which these recessives come to express themselves. Several mechanisms were ruled out, but more than one possibility remains. In the absence of further evidence, it seems most conservative to consider the mosaics as due to somatic mutation or to small deficiencies involving the loci under study. The somatic mutation rate can be calculated as  $7.0 \times 10^{-7}/r/\text{locus}$ , and 95% confidence limits, assuming 50% error in measurement of spot size, are  $(2.51, 11.33) \times 10^{-7}$ . The germinal rate induced in spermatogonia<sup>15</sup> for the same four loci is  $2.4 \times 10^{-7}/r/\text{locus}$ .

Effect of 300 r on Mouse Testis

E. F. Oakberg      R. L. DiMinno

The experiment summarized here was undertaken in order to provide the necessary histological background for interpretation of the changes in fertility of male mice at successive weeks after exposure to 300 r of x rays.<sup>16</sup> Hybrid 101 × C<sub>3</sub>H male mice were exposed to 300 r of x rays when 12 weeks old and were killed at intervals ranging from 1 hr to 54 days after irradiation.

The high incidence of necrotic cells at 6 to 24 hr confirmed our previous observations with

<sup>16</sup>W. L. Russell, *Biol. Semiann. Prog. Rep. Aug. 15, 1956, ORNL-2155, p 25.*

Table 7. Distribution of Mosaic Animals with Respect to Proportion of Adult Coat Occupied by Hair of Altered Color\*

Area of Altered Color	C57BL ♀ × NB ♂			C57BL ♀ × C57BL ♂		
	0 r	100 r	150 r	0 r**	100 r	150 r
Total Area (= 45 cm <sup>2</sup> )						
0.000160-0.000319		4				
0.000320-0.000639		4				
0.000640-0.001279	1	2			1	1
0.001280-0.002559		7				
0.002560-0.005119		5				
0.005120-0.010239		3				
0.010240-0.020479		1	1			

\*White midventral spots (see Table 6) are not included in this table.

\*\*One mosaic animal occurred in this group but is omitted from the table, since its spot was not accurately measured.

20 and 600 r that depletion of spermatogonia results from killing of cells. The lowest number of spermatogonia, which was 7% of the control, was observed at five days. By seven days, an increase to 13% of the control occurred. Quantitative estimates of the regeneration of spermatogonial stages at later intervals are complicated by the depletion of postspermatogonial stages of spermatogenesis, which results in decrease in size and weight of the testis. Thus the relative proportions of cells shift drastically, and specific cells may show spuriously high incidence if proper corrections are not made. In data based on number of cells per tubule cross section, this error would result only if the tubules shrink in length. It occurred to us that a count of Sertoli cells would provide a check of this possibility, since no effect on these cells has been observed even with doses much higher than 300 r. Therefore any change in the number of Sertoli cells should result primarily from alterations in spatial distribution along the length of the tubule. A progressive increase in the number of Sertoli cells per tubule cross section was observed from 7 to 28 days, indicating that as the seminiferous epithelium is depleted, longitudinal shrinkage of the tubules occurs. Failure to allow for this possibility has led some authors<sup>17</sup> to postulate an overcompensation by spermatogonia. Correction for changes in tubule length demonstrates that no overcompensation occurs. In fact, the number of all spermatogonial types still is only 75% of control 54 days after irradiation, yet by the time these spermatogonia will have formed sperm, essentially normal levels of fertility will have been restored.

After 300 r, selection against the more drastic chromosomal damage induced in primary spermatocytes occurs as these cells undergo meiotic division. A sufficient number, however, survive to give release of significant numbers of sperm up to 28 days. Estimates of the duration of spermatogenesis indicate that sperm released from the tubules 0 to 14 days after irradiation represent cells irradiated as spermatids, maturing spermatozoa, and mature sperm; gametes maturing at days 15 to 28 represent cells irradiated during primary spermatocyte, secondary spermatocyte, and meiotic division stages. Interpretation of the

data of Sirlin and Edwards<sup>18</sup> suggests that 5 to 10 days are required for passage of sperm through the efferent ducts, epididymis, and vas deferens and should be added to the above estimates to obtain the time of appearance in the ejaculate. It must be emphasized, however, that while the estimates of the time of the release from the tubule are quite accurate, considerable variation in time taken for movement from the testis to the ejaculate may occur. The result is that even a single ejaculate may contain cells irradiated in different stages of spermatogenesis.

#### Cellular Degeneration and Repopulation in the Irradiated Testis of *Drosophila*

W. J. Welshons                      W. L. Russell

Adult *Drosophila* males were exposed to 9867, 8052, 4000, and 1000 r of x rays. At various times after irradiation the testes were removed, stained with acetoorcein, and examined histologically. Visible damage to the testis was recognized by making comparisons with unirradiated controls. A summary of the 4000-r experiment will be presented, and then the remaining experiments may be briefly described in relation to this one experiment.

Fifty-five hours after 4000 r was administered, the cells in the gonial region were greatly reduced in number, and there was an obvious reduction in the number of primary spermatocytes. Three days posttreatment there was a regeneration of cells at the tip of the testis and a further reduction in spermatocytes. At four days, the gonial region was completely normal and remained so in all further observations. Primary spermatocytes were greatly reduced or missing. From five to seven days, spermatocytes increased in number, and by day eight, spermatids were present. The spermatids were increased from days nine to eleven. From twelve days on, the testes appeared normal.

After a dose of 9867 r, the cellular elements at the tip of the testis had vanished, in most cases, by three days. There were a few sporadic attempts at regeneration over the four-to-seven-day period followed by deterioration from eight to eleven days.

In the 8052-r experiment, the results were essentially the same, except that there were a few

<sup>17</sup>J. H. D. Bryan and J. W. Gowen, *Biol. Bull.* 110, 229-242 (1956).

<sup>18</sup>J. L. Sirlin and R. G. Edwards, *Exptl. Cell Res.* 9, 596-599 (1955).

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cases of unilateral regeneration which survived up to 24 days posttreatment. Viable offspring were obtained from these males.

The results in the 1000-r experiment were very similar to those of the 4000-r experiment, except that damage was harder to assess, and the number of primary spermatocytes present was never reduced to zero.

It should be emphasized that no distinction between secondary spermatogonia and early spermatocytes was made in these preparations. However, obvious damage was recorded in the region where the synchronous divisions of secondary gonia had been observed in unirradiated material. Therefore visible damage was due to the destruction of these gonia, and it is possible that young spermatocytes were also destroyed.

These observations are in harmony with those published by Friesen,<sup>19</sup> except that damage to secondary spermatogonia is probably more extensive than Friesen believed. The claim by

Fritz-Niggli<sup>20</sup> that spermatogonia are resistant to 3000 r of x rays is erroneous. She examined the testis three days after treatment, at which time the sensitive cells have been replaced by repopulation.

The data presented here emphasize the fact that the effect of irradiation upon the testis of *Drosophila* is similar to that on the mouse.<sup>21</sup> In both animals the deletion of a portion of the cycle of spermatogenesis results in a clear-cut period of temporary sterility which can be used to separate cells irradiated as postspermatogonia from those irradiated as spermatogonia. Therefore it should be possible to obtain spermatogonial mutation rates in *Drosophila* in much the same way as is done in the mouse.<sup>22</sup> The difficult procedure of irradiating larvae to obtain spermatogonial mutation rates could thus be avoided.<sup>23</sup>

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<sup>20</sup>H. Fritz-Niggli, *Proc. Intern. Conf. Peaceful Uses of Atomic Energy, Geneva, 1955* 11, 179-183 (United Nations, New York, 1956).

<sup>21</sup>E. F. Oakberg, *Radiation Research* 2, 369-391 (1955).

<sup>22</sup>W. L. Russell, *Cold Spring Harbor Symposia Quant. Biol.* 16, 327-336 (1951).

<sup>23</sup>M. L. Alexander, *Genetics* 39, 409-428 (1954).

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<sup>19</sup>H. Friesen, *Biol. Z.* 6, 1055-1136 (1937).

## MICROBIAL PROTECTION AND RECOVERY

RADIATION PROTECTION AND RECOVERY  
IN BACTERIA

A. Hollaender

G. E. Stapleton	D. H. Woodbury
J. Jagger	N. A. Sicard <sup>2</sup>
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Role of Binding of MEA and MEG<sup>3</sup> in Protection  
of *Escherichia coli* B/r

G. E. Stapleton      D. H. Woodbury

**Introduction.** — The outstanding protection of *Escherichia coli* B/r against x- and gamma-ray inactivation by MEA (see Appendix for alphabetical list of abbreviations) suggests that the compound is protecting specific sites on or within the bacterial cell during the irradiation. Previous reports by this group<sup>4,5</sup> indicate that the concentration of MEA required for maximal protection against inactivation depends on a number of factors, including the dose of radiation used and the rate of diffusion of the compound before and during the irradiation. In anticipation of determining the possible site of action of MEA, a number of experiments were performed to determine if the MEA responsible for the protection is easily removed by washing the cells after reaction with the compound but before the irradiation.

**Methods.** — *Escherichia coli* B/r was grown in nutrient broth under constant aeration at 37°C for 20 hr. The cells were harvested by centrifugation, washed, resuspended in cold sterile distilled water, and held at ice-bath temperature. Such suspensions served as the source of cells for all experiments. All suspensions to be treated with protective agents were recentrifuged, and the pellet of cells was resuspended in the desired concentration of the compound. In part A of the

experiments, the technique consisted in incubating the cells with MEA (48  $\mu$ moles/ml) for 30 min at ice-bath temperature, centrifuging them out of the MEA, resuspending them in water, and reincubating them an additional 30 min at ice-bath temperature before irradiation. The necessary controls were cells incubated with 48  $\mu$ moles per milliliter of the compound and cells incubated in water for the same total time before irradiation. Unirradiated samples were prepared by the same technique.

Parts B and C consisted in merely diluting the MEA or MEG fivefold, after the initial 30-min incubation, and reincubation after dilution for an additional 30 min at ice-bath temperature before irradiation. The necessary controls were cells incubated with the high concentration of the compound and cells held in the lower concentration of the compound for the same total time before irradiation. All suspensions were incubated in tight-stoppered tubes during the incubation period. At the end of the 60-min total incubation period, 1-ml aliquots of the several suspensions were transferred to glass-stoppered, 2-ml volumetric tubes and irradiated at ice-bath temperature with gamma rays in a Co<sup>60</sup> source at a dose rate of 1200 r/min. After irradiation the samples were appropriately diluted and surface-plated by spreading on yeast extract agar. After incubation at 37°C for 24 hr the visible colonies were counted, and a comparison of the number from irradiated and nonirradiated samples gave the surviving fraction. None of the treatments except the irradiation had any appreciable deleterious effect on the cells.

**Results and Discussion.** — It was considered from the beginning of these experiments that if no loss of protection of the compounds occurred either by washing the cells or by reducing the concentration of the solution of the compound surrounding the cells, after an initial incubation with the compound, there would be evidence for some sort of binding of the compound required for its protective action. As is shown in Table 8, part A, there is some indication of residual protection, as indicated by the surviving fractions for those cells which were resuspended in water after reaction with MEA for 30 min. Since it was possible that there would be some carry-over of the MEA either on the cell surface or on the tube inner surfaces,

<sup>1</sup>Research Associate.<sup>2</sup>Visiting investigator from abroad.<sup>3</sup>MEG was prepared in H<sub>2</sub>O from AET·Cl by neutralization.<sup>4</sup>C. O. Doudney and A. Hollaender, *Biol. Semiann. Prog. Rep.* Feb. 15, 1955, ORNL-1863, p 46-47.<sup>5</sup>G. E. Stapleton, D. H. Woodbury, and J. H. Udinsky, *Biol. Semiann. Prog. Rep.* Aug. 15, 1956, ORNL-2155, p 30-32.

it was decided that a new procedure should be adopted, which would not involve any centrifuging and would eliminate the carry-over problem. The procedure then adopted was to react the cells with a concentration of MEA that was somewhat below the optimal protective concentration and that was therefore on the steep part of the curve relating protection and concentration, where a small dilution (fivefold) would result in a sizable decrease in ac-

tivity. This procedure was used in part B for MEA and in part C for MEG. As can be seen in Table 8, this procedure resulted in a complete loss of protection over and above that obtained by merely reacting the cells with the lower concentration for the same time. It is of interest that in the case of MEG a different result was obtained by the same procedure. The data shown in part C indicate that a good portion of the protective capacity of

Table 8. Protection and Binding of MEA and MEG

Experiment Part	Compound	Surviving Fraction at Various Gamma-Ray Doses (kr)		
		24	48	72
A <sup>a</sup>	(1) MEA (48 $\mu$ moles/ml)	$7.2 \times 10^{-1}$	$5.3 \times 10^{-1}$	$2.3 \times 10^{-1}$
	(2) MEA (48 $\mu$ moles/ml), then washed	$1.3 \times 10^{-1}$	$1.6 \times 10^{-2}$	$5.8 \times 10^{-4}$
	(3) H <sub>2</sub> O	$1.1 \times 10^{-1}$	$5.2 \times 10^{-3}$	$1.2 \times 10^{-4}$
B <sup>b</sup>	(1) MEA (30 $\mu$ moles/ml)	$7.1 \times 10^{-1}$	$1.7 \times 10^{-1}$	$9.7 \times 10^{-2}$
	(2) MEA (30 $\mu$ moles/ml), then diluted to 6 $\mu$ moles/ml	$9.1 \times 10^{-2}$	$6.0 \times 10^{-2}$	$1.1 \times 10^{-2}$
	(3) MEA (6 $\mu$ moles/ml)	$2.2 \times 10^{-1}$	$1.0 \times 10^{-1}$	$1.3 \times 10^{-2}$
	(4) H <sub>2</sub> O	$1.2 \times 10^{-1}$	$2.4 \times 10^{-3}$	$2.4 \times 10^{-5}$
C <sup>c</sup>	(1) MEG (20 $\mu$ moles/ml)	$5.9 \times 10^{-1}$	$3.2 \times 10^{-1}$	$1.6 \times 10^{-1}$
	(2) MEG (20 $\mu$ moles/ml), then diluted to 4 $\mu$ moles/ml	$2.7 \times 10^{-1}$	$1.0 \times 10^{-1}$	$2.9 \times 10^{-2}$
	(3) MEG (4 $\mu$ moles/ml)	$6.7 \times 10^{-2}$	$5.9 \times 10^{-3}$	$1.2 \times 10^{-3}$

<sup>a</sup>(1) Cells suspended in MEA at the concentration indicated and incubated for 60 min at ice-bath temperature before irradiation at ice-bath temperature.

(2) Cells suspended in MEA at the concentration indicated for 30 min at ice-bath temperature, centrifuged, and then resuspended in H<sub>2</sub>O and incubated an additional 30 min at the same temperature before irradiation.

(3) Cells suspended in H<sub>2</sub>O and incubated 60 min at ice-bath temperature before irradiation at the same temperature.

<sup>b</sup>(1) Cells suspended in MEA at the concentration indicated and incubated at ice-bath temperature for 60 min before irradiation at the same temperature.

(2) Cells suspended in MEA at the concentration indicated, incubated for 30 min at ice-bath temperature, diluted fivefold in H<sub>2</sub>O, and then incubated for an additional 30 min at the same temperature before irradiation.

(3) Cells suspended in MEA at the concentration indicated and incubated for 60 min at ice-bath temperature before irradiation.

<sup>c</sup>Procedure the same as in B except MEG was used at the indicated concentration.

the higher concentration of MEG is maintained with dilution after an initial reaction. The difference in the results obtained with MEA and MEG may indicate a different mechanism of protection, even though the site of action may be the same. It appears that the results could be made more conclusive had a larger dilution been performed. Experiments along these lines are in progress. It will finally be important to better quantitate the residual protection in terms of concentration of MEG. The experiments with MEG suggest that it may be possible, by using labeled compound, to learn something concerning the site of action of the compound, if not the site of action of radiation damage on the cell.

#### Comparative Effects of Far-Ultraviolet and Near-Ultraviolet Radiation On Survival and Potassium Retentivity in Yeast

A. K. Bruce

**Introduction.** — Previous studies<sup>6,7</sup> have demonstrated that x irradiation results in a decrease in the ability of the yeast cell to retain potassium. The response of this alteration was found to be exponential with dose, a doubling of the potassium leakage rate occurring at about 60,000 r. A similar dose-response curve is found for the survival of yeast,<sup>8</sup> although the 50% survival level is reached at about 3100 r. The site of interaction which results in cell death is taken to be nuclear. These studies were undertaken to establish the response of yeast to ultraviolet radiation and to determine the relationship between changes in cell potassium retention and viability of the cell. If a direct relation between viability and retentivity of cellular potassium exists, then the response of the cell to various radiations should produce a similar effect upon both criteria of radiation damage.

<sup>6</sup>A. K. Bruce, Ph.D. thesis, University of Rochester, 1956.

<sup>7</sup>A. K. Bruce, *Radiation Research* 5, 471 (1956).

<sup>8</sup>C. A. Beam *et al.*, *Arch. Biochem. and Biophys.* 49, 110-122 (1954).

**Experimental.** — Suspensions of fresh, starved, baker's yeast (Standard Brands) have been exposed to radiation of wavelengths of 2537 and 3400 to 4000 Å. Leakage rate of potassium and viability have been determined for both irradiated and unirradiated control suspensions. Leakage has been determined by the column elution technique<sup>6</sup> and viability by plating on potato dextrose agar (Difco) at pH 3.5 and scoring after six days of incubation at 25°C. The 2537-Å treatments were carried out in a 17-cm crystallizing dish at a distance of 18 cm from a G-E germicidal lamp at a yeast concentration of 4 mg/ml (wet weight). The treatments at 3400 to 4000 Å were at the center of four G-E H-4 lamps equally spaced about a circle of 25-cm diameter in which Corning Glass filters and copper sulfate were used to select the desired wavelength. The yeast concentration was 20 mg/ml in these experiments. All samples were bubbled with air for 10 min before and during irradiation. Samples were plated immediately after irradiation for the determination of survival. Leakage rate determinations were started approximately 30 min after the termination of the exposure.

**Results and Discussion.** — Figures 5 and 6 show the response of the yeast to radiation of wavelengths of 2537 and 3400 to 4000 Å. Both survival and potassium retentivity are decreased by these radiations. For the 2537-Å radiation a very sharp decrease in survival is found, while the retention of potassium is decreased relatively slowly. At the longer wavelength irradiation both processes are affected to approximately the same extent, potassium retentivity being slightly greater than survival. These results suggest that the interaction of 2537-Å ultraviolet radiation with the yeast cell which results in cell death and the loss of ability of the cell to retain potassium occurs at two different sites. The survival effect is generally taken to involve nuclear damage at this wavelength. At the longer wavelengths studied, the agreement between survival and the retentivity effect raises the possibility of the membrane being the determining factor. The dose relation in terms of energy absorbed by the system has not been determined as yet.

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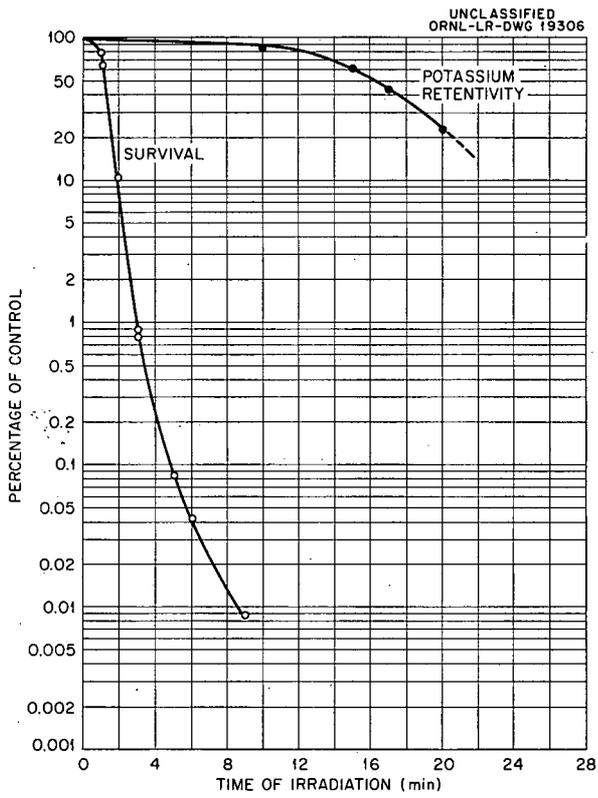


Fig. 5. Dose-Response Curve for 2537-Å Irradiation.

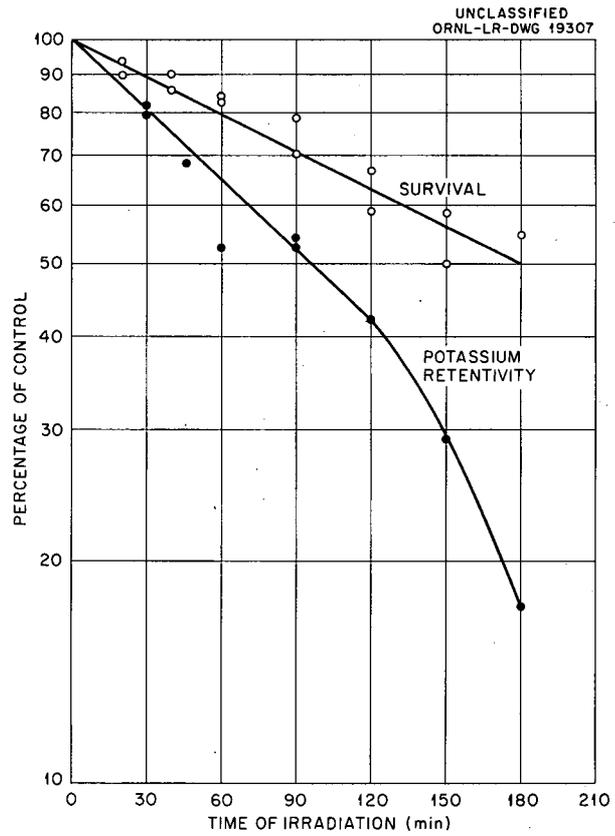


Fig. 6. Dose-Response Curve for Irradiation at 3400 to 4000 Å.

## MAMMALIAN RECOVERY

## MODIFICATION OF RADIATION INJURY IN MICE

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L. H. Smith	P. W. Rueff, Jr. <sup>2</sup>
T. W. McKinley, Jr.	A. Henley

## Cause of Death in Sublethally Irradiated Mice Given Rat Bone Marrow

C. C. Congdon T. Makinodan<sup>3</sup> N. Gengozian<sup>3</sup>

**Introduction.** — Sublethally irradiated mice that received rat bone marrow died 7 to 15 days after exposure. The cause of death was investigated in animals killed at intervals after the experimental treatment.

**Results.** — The 30-day mortality in 198 C<sub>3</sub>H × 101F<sub>1</sub> hybrid mice exposed to 710 r of x radiation was 30%. Forty-one mice similarly irradiated but given rat bone marrow intravenously afterward showed 100% mortality in 16 days. Fifty mice given 710 r and IBM (see Appendix for alphabetical list of abbreviations) had no deaths in the 30-day period. At necropsy, the mice that died after rat-bone-marrow treatment showed bone marrow aplasia as well as hemorrhage, infection, and anemia. A serial killing experiment was performed in which four groups of 20 mice each were killed and necropsied during a 30-day period. The groups were treated as follows: 710 r plus rat bone marrow, 710 r only, 710 r plus IBM, and unirradiated plus rat bone marrow. Irradiated mice that received IBM or HeBM showed recovery of bone marrow and splenic red pulp in five days. The untreated irradiated mice recovered in two weeks. The unirradiated mice injected with rat bone marrow showed no dramatic changes. Complete secondary destruction of the bone marrow and splenic red pulp was observed six to eight days after exposure in the irradiated mice given rat bone marrow. In this group, the spleen and lymph nodes showed

marked epitheloid response concomitant with the disappearance of the blood-forming tissues. The secondary destruction of the blood-forming tissues in mice given 710 r and rat bone marrow led to the death of the mice through hemorrhage, infection, and anemia. To further test this hypothesis, 20 mice were exposed to 710 r and given rat bone marrow. Six days later, each received a massive dose of IBM intravenously. Four mice died in 15 days and 16 recovered.

## Long-Term Survival of Irradiated Mice After Homologous Bone Marrow Therapy

C. C. Congdon A. C. Upton<sup>4</sup> I. S. Urso

**Introduction.** — Lethally irradiated mice treated with HBM showed many delayed (21–100 days) deaths compared with the few deaths of the mice treated with IBM.<sup>5</sup> From many HBM experiments, a considerable number of mice were accumulated that lived beyond 100 days.

**Results.** — Figure 7 shows the cumulative mortality for one year after irradiation in LAF<sub>1</sub> mice that received 900 r and IBM. The cumulative mortality for 900 r and HBM is shown under two circumstances. In one, the HBM was given on the day of irradiation as in the IBM experiment. In the other, the same dose of HBM was given on day 1, 2, or 3 after exposure to 900 r. Additional studies showed the delayed injection to be less effective in males than in females. Not every delayed-injection experiment showed good long-term survival.

**Discussion.** — Mice treated with HBM showed a peak cumulative mortality by 100 days after irradiation. After this period, the slope of the curve nearly parallels that of mice treated with IBM. Delayed injection of the HBM gave the best results obtained so far in preventing the foreign-bone-marrow reaction. This experimental modification, however, was not entirely reliable.

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results were too variable for any quantitative definition of dose-survival relationship.

The cells held at 25°C in E (ref. 9) and H media remain active for longer periods of time than do the cells held at 2 to 5°C and at 37°C. One such cell suspension in medium H was effective at 21

days (Table 9). The stained preparations of such suspensions showed much cellular debris, cell bodies that appeared to be distorted, naked nuclei, and large giant cells that were difficult to classify. It would appear that, at least functionally, these giant cells are not the same as those found in the 37°C culture.

Table 9. Recovery of Lethally Irradiated Mice Given Bone Marrow Cells Maintained in Vitro

Material Injected*	Number of Nucleated Cells Injected per Animal (millions)	30-Day Survival	Survival (%)
Cells incubated at 37°C			
In medium H			
10-day culture	1.8	4/4	100
10-day culture	0.9	4/7	57
13-day culture	0.8	1/10	10
15-day culture	0.5	0/10	0
15-day culture	0.4	0/10	0
16-day culture	0.4	0/12	0
Cells incubated at 25°C**			
In medium E			
16-day culture	2.4	7/10	70
18-day culture	1.7	0/12	0
20-day culture	1.7	0/9	0
In medium H			
17-day culture	4.2	6/10	60
19-day culture	1.5	6/12	50
21-day culture	3.4	8/12	67
Cells held at 2-5°C			
In medium H			
4 days	1-femur equivalent	9/10	90
7 days	1-femur equivalent	2/10	20
7 days	2-femur equivalent	5/10	50
10 days	1-femur equivalent	0/10	0
10 days	6-femur equivalent	0/10	0
Controls			
No treatment		0/21	0
Medium H only		0/20	0

\*The bone marrow cells were obtained from the femurs of 12-14 week C<sub>3</sub>H × 101F<sub>1</sub> mice. The recipient mice were 12-14 week C<sub>3</sub>H × 101F<sub>1</sub> mice that had received 900 r of x rays.

\*\*As explained in the text, most of the "bodies" counted in the 25°C culture were cell fragments. The true cell count is not known but is much less than that given.

The use of medium H as a suspending fluid for bone marrow cells to be held at 2 to 5°C was also investigated. Urso<sup>10</sup> reported that cells so treated in Tyrode's solution were effective for four to five days. The use of medium H as the suspending menstroom increased the preservation time to seven days but not beyond (Table 9).

**Discussion.** – The data obtained on the maintenance of effective bone marrow cells at the several temperatures studied indicate that preservation of active suspensions is favored at 25°C, as compared with 2 to 5°C and 37°C. The question to be answered is whether the moderate temperature favors the selective maintenance of an effective cell or whether certain necessary physiological functions of the several cell types are preserved by such treatment.

#### Mechanism of Recovery in the Leukemoid Blood Experiment

L. H. Smith                      P. W. Rueff, Jr.

**Introduction.** – Comparison of the action of IBM and leukocytes from leukemoid blood in irradiated mice was made. Hematocrits and total leukocyte counts were determined on x-irradiated BALB/c mice injected with either of these recovery agents.

**Results.** – The hematocrit and leukocyte patterns of the bone marrow and leukocyte-injected mice were essentially the same for the first 30 days after irradiation.

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<sup>10</sup>I. M. Urso, *Biol. Semiann. Prog. Rep.* Aug. 15, 1956, ORNL-2155, p 41.

**Discussion.** – These results support the hypothesis that the action of the two agents is similar.

#### Chemical Protection of Bone Marrow Cells X-Irradiated in Vitro

L. H. Smith                      P. W. Rueff, Jr.

**Introduction.** – AET·Cl and related compounds protect against lethal exposure of the whole body to radiation.<sup>11</sup> The present studies were conducted to determine whether AET·Cl protects bone marrow cells irradiated in vitro. Mouse (101 × C<sub>3</sub>H) bone marrow cells, exposed in vitro to x radiation in the presence or absence of the compound, were injected into lethally irradiated (101 × C<sub>3</sub>H) mice. The 30-day survival of these mice was recorded.

**Results.** – The in vitro exposure of bone marrow cells to 800 r destroyed their ability to promote recovery of lethally irradiated mice. This ability, however, was retained if AET·Cl was added to the bone-marrow-cell suspension (2.5 mg of AET·Cl per ml) 15 min before irradiation in vitro.

**Discussion.** – Mouse bone marrow cells exposed in vitro are sensitive to x-ray doses which also cause 100% lethality of whole-body-irradiated mice. AET protects bone marrow cells irradiated in vitro.

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<sup>11</sup>D. G. Doherty *et al.*, *Biol. Semiann. Prog. Rep.* Feb. 15, 1956, ORNL-2060, p 45-46.

## IMMUNOLOGY

T. Makinodan  
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**Antibody Response of Mice Treated with  
MEG and 950 r and Its Significance to  
the Antigen-Host Relationship**

T. Makinodan, I. C. Shekarchi,  
C. C. Congdon<sup>3</sup>

**Introduction.** — Based on the accepted definition of antigen and antibody, it is implicit that the term *foreign* is of a qualitative rather than a quantitative nature. However, results of this laboratory on the recovery of the immune mechanism following varying doses of total-body x irradiation, lethal irradiation-bone marrow treatment, and on bone marrow transplantation following x irradiation strongly suggest that the ability of the antibody-producing cell to recognize that a substance is foreign is of a quantitative nature.<sup>4-6</sup> To test the validity of this conclusion, it was felt that studies on the recovery of the immune mechanism of lethally x-irradiated mice pretreated with MEG (see Appendix for list of abbreviations) would be of importance. D. G. Doherty of the Biochemistry Section has synthesized a new protective compound, AET, which rearranges to MEG at neutral pH, and together with W. T. Burnett has demonstrated its protective effect against radiation injury in mice.<sup>7</sup>

**Results.** — At intervals of 1, 15, or 30 days post-treatment, agglutinin production to rat or sheep RBC was determined. Serum samples were obtained 5, 10, 15, 20, 30, and 45 days after antigen injection, and there were approximately 10 to 20 serum samples for each interval. No significant difference between sheep and rat agglutinin production was observed in normal and in MEG-treated

mice. However, significantly higher sheep agglutinin response was found among sublethally and lethally x-irradiated mice pretreated with MEG or posttreated with IBM. Such a difference was based on the induction period of agglutinin response, peak agglutinin titer, and on the mean total agglutinin response. The recovery of the immune mechanism of the lethally x-irradiated mice pretreated with MEG takes place at a rate comparable with that of the sublethally x-irradiated mice (475 r) when sheep RBC is employed as the test antigen, and at a rate slightly faster between 0 and 15 days and slower between 15 and 30 days posttreatment when rat RBC is employed. Hence, in terms of the rate of recovery of the immune mechanism, mice treated with MEG and 950 r are almost comparable with 475-r-treated mice. Finally, it was found that the rate of recovery of the immune mechanism of lethally irradiated mice pretreated with MEG was not significantly different from that of lethally irradiated mice posttreated with IBM.

**Discussion.** — Previous results have shown that injection of bone marrow into lethally x-irradiated mice permits transplantation of RBC, granulocyte, and platelet precursors, while definitive evidence to show transplantation of antibody-producing cells is lacking.<sup>4,6,8-11</sup> The results presented here show that the rate of recovery of the immune mechanism of lethally irradiated mice pretreated with MEG was comparable with that of lethally irradiated mice posttreated with IBM. Hence, it can be deduced that MEG protects predominantly nonantibody-producing cells.

That a residual number of antibody-producing cells is not destroyed by this dose of x radiation is evident by the positive agglutinin response of lethally irradiated mice pretreated with MEG or posttreated with IBM when antigen was injected

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<sup>2</sup>Temporary employee.

<sup>3</sup>Mammalian Recovery Group.

<sup>4</sup>T. Makinodan, *Proc. Soc. Exptl. Biol. Med.* **92**, 174-179 (1956).

<sup>5</sup>T. Makinodan, N. Gengozian, and C. C. Congdon, *J. Immunol.* **77**, 250-256 (1956).

<sup>6</sup>N. Gengozian and T. Makinodan, *J. Immunol.* **77**, 430-436 (1956).

<sup>7</sup>D. G. Doherty and W. T. Burnett, Jr., *Proc. Soc. Exptl. Biol. Med.* **89**, 312-314 (1955).

<sup>8</sup>L. H. Smith, T. Makinodan, and C. C. Congdon, *Cancer Research*, in press.

<sup>9</sup>P. C. Nowell *et al.*, *Cancer Research* **16**, 258-261 (1956).

<sup>10</sup>O. Vos *et al.*, *Acta physiol. pharmacol. neerl.* **4**, 482-486 (1956).

<sup>11</sup>C. E. Ford *et al.*, *Nature* **177**, 452-454 (1956).

only 24 hr after treatment. Furthermore, if complete destruction of the antibody-producing cells by x irradiation did take place, it would be expected that, regardless of the genetic relationship, foreign bone marrow can be transplanted. That this is not the case is clearly evident by the results presented previously.<sup>6,8-14</sup> Transplantation of HBM and closely related HeBM, such as marrow from rat and guinea pig, can take place; but transplantation of bone marrow of dog, monkey, rabbit, and chicken cannot take place. It follows then that there could exist either types of antibody-producing cells or an antibody-producing cell with the ability to recognize (a) homologous, (b) closely related heterologous, and (c) distantly related heterologous antigens; and the degree of destruction by x irradiation is in the order of  $a > b > c$ .

Although results of subsequent experiments substantiated the two basic premises of the above hypothesis, further tests are required. Such studies should contribute to the role of the *recognition* factor of the antibody-producing cell; that is, in what manner can an antibody-producing cell recognize that a substance is foreign and to what degree of foreignness?

#### Physical Properties of Circulating RBC of Lethally Irradiated Mice Treated with Rat Bone Marrow

T. Makinodan      N. G. Anderson<sup>15</sup>

**Introduction.** — Successful rat bone marrow transplants in lethally x-irradiated mice were demonstrated recently in this laboratory,<sup>4,6,8</sup> and similar findings have been reported independently by others.<sup>9-11</sup> In these treated mice, the presence of circulating rat granulocytes was demonstrated by the alkaline phosphatase test,<sup>4,8,9</sup> circulating rat platelets were demonstrated serologically,<sup>8</sup> and circulating RBC were demonstrated by a qualitative<sup>10</sup> and a quantitative immunohematologic test.<sup>14</sup> At 25 days after treatment, approximately 50% of the cells in the circulation of the mouse were rat RBC, at 65 days 100% were rat RBC.

This level has been maintained now for over 365 days in several mice. However, it was found by the double serum agar diffusion method that the serum proteins were of the mouse type.<sup>4</sup> No apparent ill effect has been observed among these treated mice following recovery from the secondary immunologic effect. Since serologically detectable normal *rat* RBC were found existing in the presence of *mouse* serum proteins in the circulation, it was felt that a comprehensive study on the physical properties of these cells would throw some light on the role of the interspecies bioenvironmental factor. Such a study is reported here.

**Results.** — Serological tests, employing specific mouse antirat RBC and rat antimouse RBC sera, showed that circulating RBC of these lethally x-irradiated mice treated with rat bone marrow were 100% of the rat type in every case studied 65 days after treatment. Since it is known that, in general, mechanically injured cells release hemoglobin, an experiment was carried out at 2°C. In spite of the large shearing force applied, only a negligible amount of hemoglobin was released by rat and rat-in-mouse RBC. In contrast, a hemoglobin concentration of approximately ten times greater was found after shearing mouse RBC. Osmotic properties of RBC were determined by exposing freshly prepared cells in distilled water at temperatures of 37, 27, and 4°C. Mouse RBC released more hemoglobin than did rat and rat-in-mouse RBC and was found to be less susceptible to temperature change. Rat RBC, on the other extreme, released the smallest amount of hemoglobin and was found to be most susceptible to temperature change.

Rat-in-mouse RBC, in relation to mouse and rat RBC, showed properties of a dual nature. Exposures at 37 and 27°C caused release of hemoglobin in concentrations only slightly more than those for rat RBC but far below that amount released by mouse RBC. On the other hand, the temperature-dependent property of the rat-in-mouse RBC is more mouse type than rat type. A very small amount of hemoglobin was released when the temperature was lowered from 27°C to 4°C. With rat RBC, however, there was approximately a twofold decrease in release of hemoglobin at a comparable temperature change. Mouse hemoglobin failed to crystallize out in all three of the methods employed. Rat and rat-in-mouse hemoglobins were readily crystallized by all three methods. The

<sup>12</sup>E. Lorenz and C. C. Congdon, *Proc. Fourth Intern. Congr. Intern. Soc. Hematol., Argentina, 1952*, p 192-211 (1953).

<sup>13</sup>C. C. Congdon, unpublished data.

<sup>14</sup>T. Makinodan, unpublished data.

<sup>15</sup>Cell Physiology Group.

denaturation property of hemoglobin was determined at 27 and at 11°C. It was found that at 27°C the rate of denaturation of hemoglobin by dilute alkali took place in an apparent first-order reaction. The velocity constants were found to be

$$K_{\text{mouse}}^{27} = 10.0 \times 10^{-3} \text{ sec}^{-1},$$

$$K_{\text{rat}}^{27} = 8.9 \times 10^{-3} \text{ sec}^{-1},$$

$$K_{\text{rat-in-mouse}}^{27} = 9.0 \times 10^{-3} \text{ sec}^{-1}.$$

At 11°C, it became obvious that more than one type of hemoglobin, in terms of the alkali denaturation property, was present in all three types studied. Regardless of the nature of rate-reaction curves, it was found that the experimental mouse hemoglobin was almost identical, if not identical, to rat hemoglobin. Paper-strip electrophoretic analysis of the hemoglobin showed that there was a marked difference in the degree of adherence of hemoglobin to the paper strip at the source of application. Relative to mouse hemoglobin, rat and rat-in-mouse hemoglobins adhered to the paper in a significantly higher concentration.

**Discussion.** — Based on these serological studies, it can be concluded that the surface molecular configuration of RBC from these experimental mice is of the rat type. The hemoglobin was found to be also very much like the rat type in its ease in crystallization, its alkali denaturation property, its electrophoretic property, and its tendency to form a paracrystalline state at low temperature. These cells possessed dualistic osmotic properties; the relative hemoglobin concentration released when cells were exposed to water was more comparable with the rat type, but its temperature-dependent property was more comparable with the mouse type.

#### Effect of Varying X-Ray Doses and Nucleated Rat Bone Marrow Cells on Mortality of Mice

N. Gengozian      T. Makinodan

**Introduction.** — It was previously reported<sup>16,17</sup> that sublethal x irradiation (710 r; LD<sub>30</sub>) of C<sub>3</sub>H × 101 mice, followed by injection of rat bone

marrow, resulted in 100% mortality for 60 mice within 16 days after treatment. The explanation offered was that this x-radiation dose would permit active transplantation of the foreign bone marrow, and the rapid recovery of the animal's immune mechanism resulted in an acute antigen-antibody reaction, causing greater mortality than 710 r alone. The objective of this study was to demonstrate active transplantation of foreign (rat) hematopoietic tissue in mice receiving low doses of x rays and to study the effect of varying x-ray doses and rat bone marrow cells on mortality.

**Results.** — Mice receiving 710 r plus rat bone marrow were tested for rat RBC (immunohematologically) and rat granulocytes (alkaline phosphatase-positive cells) over a period of 15 days. Tests for rat RBC in the peripheral blood of 23 mice were negative. However, alkaline phosphatase-positive cells were found in the peripheral blood, spleen, and bone marrow imprints of mice sacrificed daily during the course of the experiment. The greatest number of these cells occurred during the fifth, sixth, and seventh day after treatment. Normal mice receiving rat bone marrow were negative for alkaline phosphatase-positive cells three days after treatment.

Establishment of active transplantation of foreign (rat) hematopoietic tissue in mice receiving sublethal doses of x rays suggested a more critical analysis of the relationship between x-ray dose and rat-bone-marrow treatment. Table 10 shows the mortality data obtained with mice receiving x-ray doses ranging from 400 to 1300 r, followed by injection of rat bone marrow. At 500 and 600 r, the effect of the foreign bone marrow was to increase the mortality above that of the control groups receiving no treatment or isologous bone marrow; no effect was observed at 400 r. Above 710 r, the effect of the rat bone marrow was protective, decreasing the mortality below that of the control group. Deaths beyond 30 days did not occur with mice treated with 500 or 600 r plus rat bone marrow. This was attributed to the absence of rat hematopoietic tissue in the survivors of these two groups. Mice receiving x irradiation greater than 710 r plus rat bone marrow showed persistence of the foreign tissue and, as a result, delayed immunologic reaction of the host, causing additional deaths. Maximum 30-day protection was obtained with the 950- and 1150-r groups; however, from the

<sup>16</sup>N. Gengozian and T. Makinodan, *J. Immunol.* **77**, 430-436 (1956).

<sup>17</sup>T. Makinodan and N. Gengozian, *Biol. Semian. Prog. Rep.* Aug. 15, 1956, ORNL-2155, p 35.

Table 10. Per Cent Mortality of X-Irradiated (400–1300 r)  $C_3H \times 101F_1$  Mice Receiving No Treatment, Isologous Bone Marrow, or Rat Bone Marrow

X-Ray Dose (r)	Treatment					
	None, 0–30*	IBM, 0–30	Rat Bone Marrow			
			0–30	31–60	61–90	91–150
400	0	0	0	0	0	0
500	0	0	15	15	15	15
600	5	0	50	50	50	50
710	30	0	100			
800	79	0	58	73	73	77
950	100	5	22	59	63	63
1150	100	25	25	40	55	65
1300	100	70	80	85	95	95

\*Days after treatment.

30th to the 60th day, the greatest increase in mortality occurred with the former groups. This was attributed to the more rapid recovery of the host's immune mechanism among the 950-r-treated mice.

The following experiment was designed to further establish the dynamics of the in vivo antigen (transplanted foreign hematopoietic tissue)–antibody (irradiated host's immune mechanism) reaction. The status of the immune mechanism of irradiated mice at day zero may be considered to be in the order of 710 r > 800 r > 950 r, etc. It was reasoned that the injection of varying amounts of rat bone marrow into these three groups would yield dissimilar mortality data for each respective x-ray group and rat bone marrow dose. This hypothesis was based on the optimum proportions of antigen and antibody required to affect the delayed reaction observed in lethally irradiated mice treated with rat bone marrow. In this instance, the occurrence of such a reaction would be dependent upon the relative proliferation of the foreign hematopoietic tissue (antigen) and the rate of recovery of the irradiated host's immune mechanism (antibody). The 30-day-mortality data of such an experiment were as follows: injection of  $25 \times 10^6$  rat bone marrow cells resulted in 95 and 50% mortality in the 800- and 950-r groups, respectively; injection of  $45 \times 10^6$  rat bone marrow cells resulted in 70, 100, and 45% mortality for

the 710-, 800-, and 950-r groups, respectively; injection of  $140 \times 10^6$  rat bone marrow cells resulted in 100, 65, and 22% mortality for the 710-, 800-, and 950-r groups, respectively.

**Discussion.** – The results obtained in this study indicate that the injection of rat bone marrow into irradiated mice may be protective or detrimental to the host, depending upon the two variables, amount of cells injected and x-radiation dose applied to the host.

#### Effect of Bone Marrow Treatment and Chemical Protection on the Blood Leukocytes, Thymus Weight, and Spleen Weight of X-Irradiated Mice

P. Urso

**Introduction.** – The bone marrow response of irradiated mice treated with IBM, HBM, HeBM, AET, or AET plus IBM was reported earlier.<sup>18</sup> Using these same treatments, the peripheral blood leukocyte-count, thymus-weight, and spleen-weight responses were determined.

**Results.** – The leukocyte response of the irradiated (900 r) mice was parallel for the different types of bone marrow treatment. It appeared that IBM and HBM were slightly more effective than HeBM in causing recovery of spleen weight.

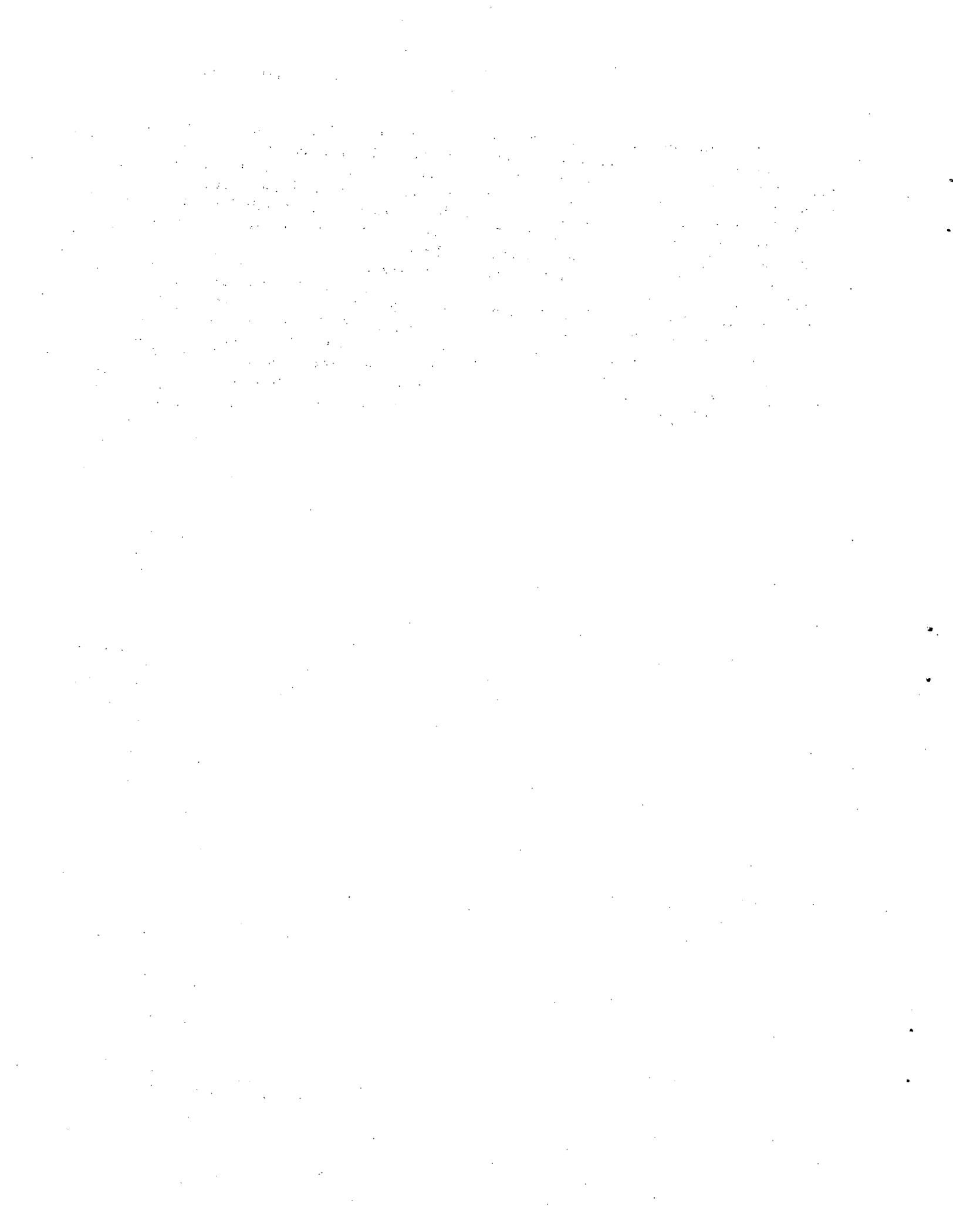
<sup>18</sup>P. Urso, *Biol. Semiann. Prog. Rep. Aug. 15, 1956*, ORNL-2155, p 36–37.

Thymus weight partially recovered and then regressed to x-ray control levels with HBM and HeBM, each event occurring quicker in the latter treatment. Recovery of thymus weight for IBM mice was subnormal and highly variable.

There was no apparent modification by AET of the initial effects of 900 r on the leukocyte count or spleen weight. With this treatment, recovery of these end points occurred after six days. However, AET appreciably modified the initial effect of 450 r on both these parameters. Thymus weight recovered temporarily, regressed, and recovered again in AET-treated mice. Recovery of the thymus was quicker with AET and 450 r than with AET and 900 r.

In irradiated (900 r) mice treated with AET and IBM, the response of leukocyte count and spleen weight was the same as the response which occurred with bone marrow alone. In contrast, the response of thymus weight was the same as that which occurred with AET alone for the first 21 days.

**Discussion.** - The results demonstrate that recovery of leukocyte count and spleen weight is quicker with bone marrow than with AET. On the other hand, thymus weight recovered quicker with AET than with bone marrow. AET did not have a dose-reducing effect on 900 r for these parameters, as was previously reported for the bone marrow response.<sup>18</sup>



## PATHOLOGY AND PHYSIOLOGY

### PATHOLOGIC AND PHYSIOLOGIC EFFECTS OF RADIATION

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### Effects of Chronic Exposure to Fast Neutrons and to Gamma Rays

J. A. Sproul	M. L. Randolph <sup>6</sup>
R. J. Elliott	K. W. Christenberry

**Introduction.** — This experiment, mentioned previously,<sup>7</sup> was undertaken to study the RBE (see Appendix for alphabetical list of abbreviations) of fast neutrons and of gamma rays under conditions of low-level, chronic irradiation. Although the investigation has been in progress for only about one year, because the preliminary data now available are informative, they are presented at this time.

**Results.** — Female mice of the RF strain exposed continuously throughout life (23 hr per day, 7 days per week) to fast neutrons or to gamma rays exhibited reduction of the life span in proportion to the dose rate, as has been observed with mice of other strains (Fig. 8).

If the mice were removed from the radiation field after the population began to die from leukemia and other so-called delayed effects, the mortality rate declined very little and only after an interval of several weeks, whereas if the mice were removed at a time when there was mortality from *acute radiation sickness*, a prompt drop in the

death rate occurred, owing to rapid recovery of the bone marrow and intestinal tract.

The effects on the life span and on the lens (Fig. 9) were appreciably less severe if a given amount of radiation delivered over a period of six months was administered in fractionated, biweekly or weekly treatments than if it was administered continuously, 23 hr per day, 7 days per week.

**Discussion.** — The results of the present investigation are in good agreement with those of comparable earlier experiments if allowance is made for differences in the strains of mice employed, in the energy of the radiations administered, and in the daily exposure time (i.e., mice were exposed during approximately 23 hr per day in this study, whereas in most of the earlier experiments the dose was administered each day in a matter of minutes).

The existing data indicate that the RBE of neutrons for lethality varies inversely with the intensity. At very low intensities neutrons appear to be ten times or more effective than gamma rays in shortening the life span, depending on their energy. The data also indicate that fractionation of the irradiation, even though the total period of exposure is relatively long, may significantly influence the effectiveness of a given dose as regards effects on survival and on the lens of the eye. Furthermore, it is evident from the results presented that because of the relatively long latent period that precedes *delayed*, as opposed to *acute*, radiation death, the median dose accumulated by mice under continuous daily irradiation includes appreciable wasted radiation.

### Success of Bone Marrow Homotransplantation After Graded Doses of X Radiation

T. T. Odell, Jr.      B. C. Caldwell

**Introduction.** — It has been shown that transplants of homologous bone marrow become permanently established in rats that have received 700 or 750 r of total-body irradiation.<sup>8</sup> In the experiments presented here, undertaken to study the influence of the dose of radiation on the success and extent of marrow homotransplantation, rats

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<sup>6</sup>Biophysics Section.

<sup>7</sup>G. S. Melville *et al.*, *Biol. Semiann. Prog. Rep. Aug.* 15, 1956, ORNL-2155, p 42.

<sup>8</sup>D. L. Lindsley, F. G. Tausche, and T. T. Odell, Jr., *Proc. Soc. Exptl. Biol. Med.* 90, 512 (1955).

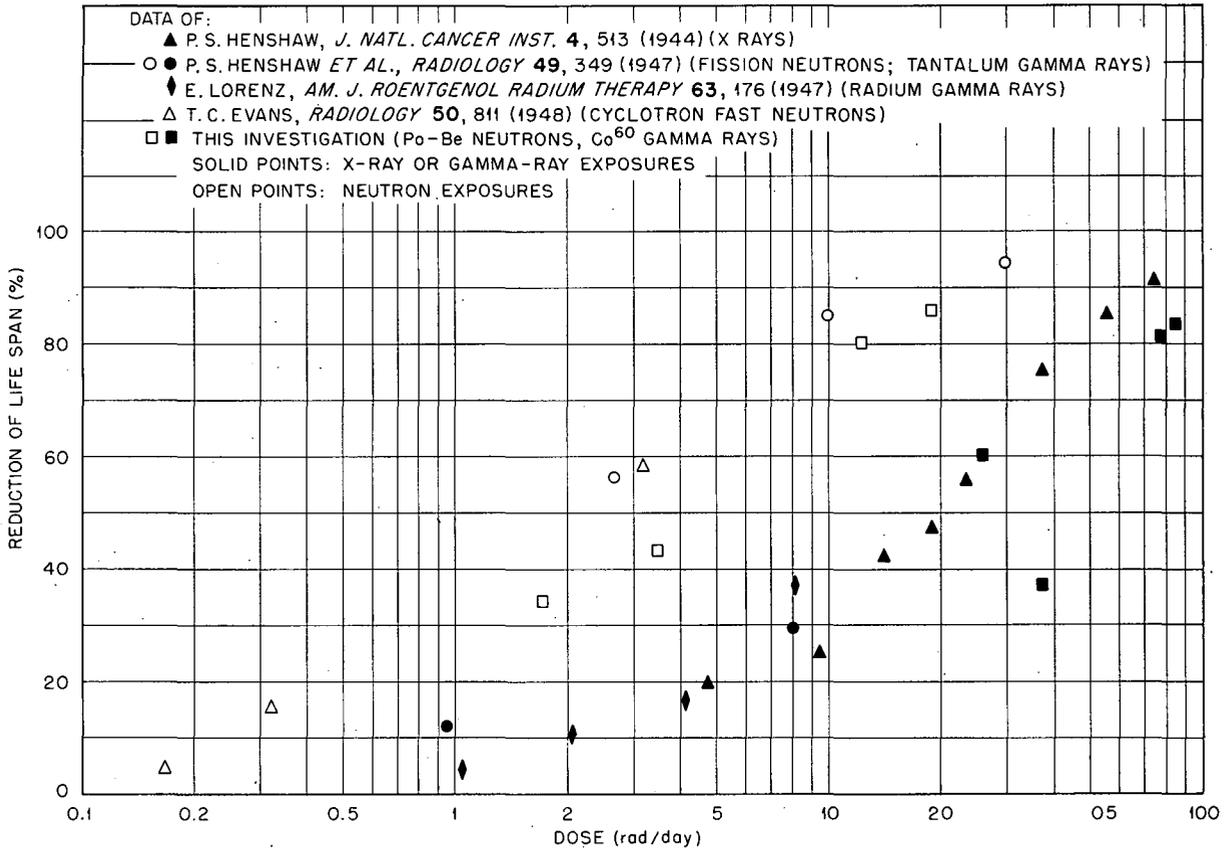


Fig. 8. Shortening of the Life Span in Mice by Daily Irradiation. Doses in neutron units or roentgens were arbitrarily converted to rad by the formulas: 1 neutron = 2.3 rad, and 1 roentgen = 0.93 rad.

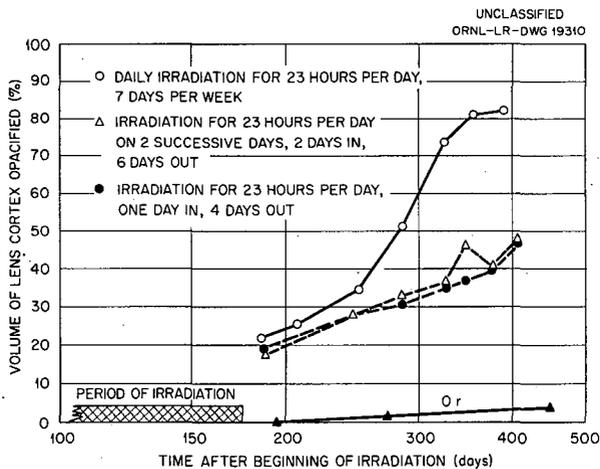


Fig. 9. The Production of Lens Opacities in Mice Chronically Exposed to Po-Be Neutrons, as Influenced by the Periodicity of Exposures. All mice were irradiated for 26 weeks and received a total accumulated dose of 673 rad.

homozygous for *D* antigen on their erythrocytes were irradiated with 0 to 500 r of 250-kvp x rays and injected intravenously 18 hr later with bone marrow from donor rats homozygous for *C* antigen on their erythrocytes.

**Results.** - A few donor (*C*) cells were present among the circulating erythrocytes of 2 of 19 non-irradiated animals and 1 of 9 rats exposed to 150 r 14 days after marrow injection, but none were detected at 43 and 56 days in the nonirradiated individuals (Table 11). Two other nonirradiated animals showed questionable positives two and three weeks after marrow injection but were negative thereafter.

After 225 r, four of eight rats had a few foreign erythrocytes in the circulation two weeks after treatment. At three weeks, about a third of the erythrocytes of one of these animals were of the foreign (*C*) type, but only a few *C* cells remained in this rat at 56 days, while the other three rats had become negative for donor cells within this

Table 11. Success and Extent of Bone Marrow Homotransplantation After Graded Doses of X Radiation

Dose (r)	Time After Marrow Injection (days)															
	14		21		28		42		56		78		105		220	
	Takes <sup>a</sup>	Donor RBC (%) <sup>b</sup>	Takes	Donor RBC (%)	Takes	Donor RBC (%)	Takes	Donor RBC (%)	Takes	Donor RBC (%)	Takes	Donor RBC (%)	Takes	Donor RBC (%)	Takes	Donor RBC (%)
0	4/19	+ <sup>c</sup>			1/19	+	0/19									
150	1/9	+	1/9	0	1/9	16	1/9	+	1/9	+	0/9					
225	4/8	+	4/7	+ to 33			3/7 <sup>d</sup>	+	1/7	+	0/6 <sup>e</sup>					
300	9/10	+			8/9	+ to 29	6/7	+ to 56	5/6	+ to 80	3/5	+ to 97	1/5	100	1/5	100
300	8/10	+	7/9	+ to 51	6/9	+ to 70			4/9	+ to 94			3/9	93 to 100		
500	4/9	+			3/5	10 to 22	3/5	12 to 58	3/5	+ to 77	2/5	69,73	1/4	83	1/1 <sup>f</sup>	80
500	4/10	+	4/10	+ to 35			3/9	+ to 71								

<sup>a</sup>The numerator indicates the number of recipients exhibiting circulating erythrocytes of donor (C) type, and the denominator the number of recipients surviving.

<sup>b</sup>The percentage of circulating red blood cells of donor type present in recipients with takes.

<sup>c</sup>+ = donor (C) cells present in small numbers not quantitatively determined.

<sup>d</sup>Actually typed at 35 days rather than 42 days.

<sup>e</sup>Regressed (one negative dead).

<sup>f</sup>Other three negative rats discarded.

interval. By 78 days, all the animals were negative.

In one group exposed to 300 r, foreign erythrocytes were present in nine of ten rats at 15 days and in five of six rats at 56 days (four animals having died from pulmonary infection). From 105 through 227 days, one of five rats exhibited 100% foreign cells, whereas the other three had completely regressed. In another group of rats given 300 r, eight of ten were positive at 14 days but only three of nine at 115 days, exhibiting 93, 100, and 100% foreign red blood cells.

In one group of ten rats that received 500 r plus C bone marrow, four of nine were positive at 15 days and three of five at 28 through 56 days (three negatives and one positive having died between 15 and 28 days). Of the three remaining positives, one regressed, one died at 93 days, and the third had 80% foreign cells at 215 days. In another group exposed to 500 r, four of ten were positive at 14 days and three of nine at 42 days, one positive having regressed and one negative having died between 14 and 42 days.

**Discussion.** - The results demonstrate that it is possible under the circumstances of these experiments to obtain a few apparently permanent takes of homologous bone marrow after relatively low doses of radiation. The results suggest that a homograft will be retained after 300 or 500 r only in those animals whose marrow becomes largely or entirely of the donor type. The threshold dose of radiation for apparently permanent homotransplants lies between 225 and 300 r of x rays.

**Long-Term Survival of Mice Protected by AET and Bone Marrow Against Supralethal Irradiation**

C. C. Congdon<sup>9</sup>  
 D. G. Doherty<sup>10</sup>      W. D. Gude  
 A. W. Kimball<sup>11</sup>      E. S. Ledford

**Introduction.** - The observation<sup>12</sup> that the combined administration of AET preirradiation and bone marrow postirradiation increased the LD<sub>50</sub>/30 days of mice by a factor of 2 to 3 made it important to observe the protected survivors throughout life to determine the extent to which these agents

would prevent the production of delayed effects of irradiation. The following are preliminary results of a large-scale investigation of this problem now in progress.

**Results.** - Small numbers of mice enabled to survive 1400 to 2600 r of whole-body x or gamma radiation because of concurrent AET or bone marrow administration have been observed for periods up to two years after irradiation. The survival data (Fig. 10) indicate that, in general, protection was afforded against the life-shortening action of radiation that was commensurate with the protection given against 30-day lethality.

**Discussion.** - Although these results are based on small numbers of animals, they demonstrate conclusively that AET and bone marrow afford marked protection against the development of delayed, as well as acute, radiation injury. Most of the animals observed to date received both AET and bone marrow; hence comparison between

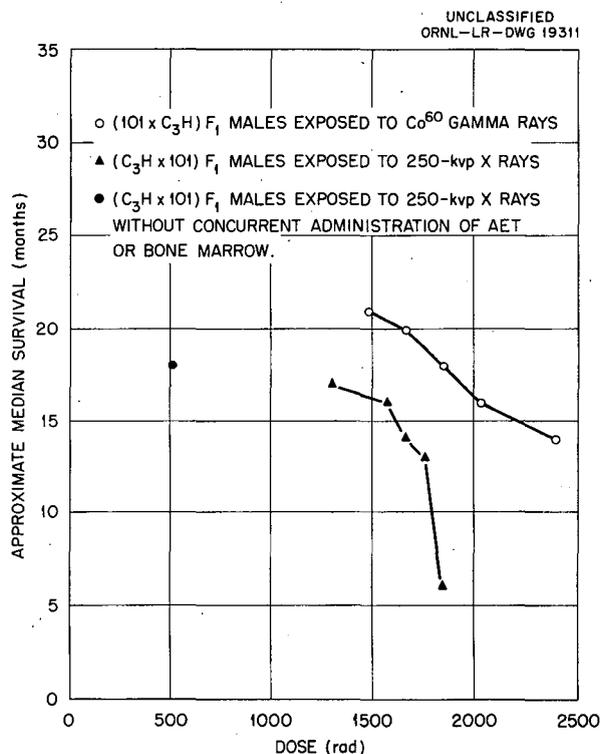


Fig. 10. The Survival of Mice Protected Against Supralethal Whole-Body Irradiation by Administration of 8.8 mg of AET Intraperitoneally 10-15 min Before Irradiation and Isologous Bone Marrow Cells Intravenously Immediately After Irradiation.

<sup>9</sup>Mammalian Recovery Group.  
<sup>10</sup>Biochemistry Group.  
<sup>11</sup>Mathematics Panel.  
<sup>12</sup>C. C. Congdon *et al.*, *Biol. Semiann. Prog. Rep.* Aug. 15, 1955, ORNL-1953, p 61-62.

the two agents must await the results of experiments now in progress.

It is noteworthy that according to the survival data of the protected mice, 250-kvp x rays were approximately 1.3 to 1.4 times as effective as  $\text{Co}^{60}$  gamma rays in shortening the life span. This ratio was also found to be the RBE of these radiations for the  $\text{LD}_{50}/30$  days in mice.<sup>13</sup>

#### The Effect of X Irradiation on the Plasma Lipide Fractions of the Rabbit

N. R. Di Luzio K. A. Simon R. J. Elliott

**Introduction.** — Close correlation has been reported between prompt elevation of blood lipides and the likelihood of death within 30 days after whole-body irradiation.<sup>14-16</sup> The purpose of this study was to investigate further the relation between the extent of radiation injury and the increase in the various lipide fractions of the plasma.

**Results.** — Marked and lasting elevation of all the plasma lipide fractions was evident within 24 hr after 1000 r of whole-body 250-kvp x radiation in rabbits destined to die within 5 days; whereas the plasma lipides of rabbits that survived beyond 30 days exhibited less pronounced and only transient elevation, if affected at all (Table 12).

**Discussion.** — These results, which are in agreement with earlier observations,<sup>14-17</sup> suggest that the degree of alteration in the plasma lipide fractions constitutes an index of the severity of radiation injury. Interpretation of the changes in the various individual lipide fractions must await further data.

#### Effects of X Radiation and Trypan Blue on the Uptake of Colloidal Gold-198 by the Reticuloendothelial System of the Rat

N. R. DiLuzio W. D. Gude  
K. A. Simon T. Mack

**Introduction.** — The importance of the reticuloendothelial cells on phagocytes and as potential

sources of hemopoietic cells makes them of fundamental importance in radiation sickness. In this experiment, attempts were made to evaluate the influence of whole-body x radiation on the phagocytic affinity and capacity of the reticuloendothelial system (RES) and on the ability of the RES to respond to sustained stimulation by repeated injections of trypan blue.

**Results.** — Successive daily intraperitoneal injections of 1 ml of a 1% solution of trypan blue for 7, 14, or 21 days caused marked hyperplasia of reticuloendothelial cells throughout the RES, with hepatomegaly and splenomegaly, in young adult rats of a subline of the Wistar strain. In addition, the administration of the trypan blue caused marked deposition of dye in the epithelium of the proximal convoluted tubules of the kidneys and anemia, with erythrophagocytosis in reticuloendothelial cells, and erythroblastosis in the spleen, bone marrow, and peripheral blood. Exposure to 400-r whole-body 250-kvp x radiation did not inhibit hyperplasia of reticuloendothelial cells in response to the subsequent daily injection of trypan blue but did depress hemopoiesis and thus prevent the erythroblastosis. Neither irradiation nor administration of trypan blue, nor both combined, markedly affected the rate of removal of intravenously injected colloidal  $\text{Au}^{198}$  from the blood stream or its concentration in liver and spleen. The total amount of gold taken up by the spleen was altered, however, in relation to the increase or decrease in the weight of the organ.

**Discussion.** — It is evident from these results that sublethal whole-body irradiation does not markedly impair the ability of the rat to remove injected colloidal  $\text{Au}^{198}$  or trypan blue from the bloodstream. Nor does it inhibit the ability of reticuloendothelial cells to proliferate in response to subsequent stimulation by repeated injections of trypan blue, despite marked depression of hemopoiesis. Hence, on the basis of these criteria, the RES cannot be considered to be radiosensitive; however, since certain other functions of the RES are reported to exhibit changes in response to

<sup>13</sup>A. C. Upton *et al.*, *Radiation Research* 4, 117 (1956).

<sup>14</sup>R. L. Rosenthal, *Science* 110, 43 (1949).

<sup>15</sup>T. L. Hayes and J. E. Hewitt, *Am. J. Physiol.* 181, 280 (1955).

<sup>16</sup>W. H. Goldwater and C. Entenman, *Radiation Research* 4, 243 (1956).

<sup>17</sup>L. T. Steadman and H. E. Thompson, *Quarterly Technical Report*, Oct. 1, 1949 thru Dec. 31, 1949, UR-103, p 20.

Table 12. Plasma Lipide Fractions<sup>a</sup> of Rabbits After 1000 r of Whole-Body X Radiation

Rabbit No.	Weight (kg)	Preirradiation (control)				24 hr After Irradiation				48 hr After Irradiation			
		Total Lipide	Neutral Fat	Phospho-lipide	Choles-terol	Total Lipide	Neutral Fat	Phospho-lipide	Choles-terol	Total Lipide	Neutral Fat	Phospho-lipide	Choles-terol
Rabbits that Survived <sup>b</sup>													
4	3.26	305	167	101	37	342	215	68	59	350	230	86	34
10	2.36	308	131	95	82	524	311	138	75	555	376	148	31
1	2.73	225	113	88	24	1320	964	210	146	675	475	164	36
3	3.97	187	110	55	22	230	171	29	29	326	234	67	25
12	2.24	262	104	103	55	720	432	168	120	514	304	131	79
6	2.46	322	151	128	43	1050	620	308	122	451	89	312	50
Mean	2.84	268	129	95	44	698	452	154	92	479	285	151	43
Rabbits that Died <sup>c</sup>													
2	3.26	246	110	103	33	1470	1170	179	121				
7	2.68	330	199	96	34	2567	2112	405	50				
8	2.97	380	266	74	40	1440	1162	247	31	1235	1059	112	64
11	2.41	320	177	90	53	3060	1683	357	1020	3174	1749	465	960
9	2.13	330	208	80	42	1210	837	213	160	1034	737	180	117
Mean	2.71	321	192	88	40	1949	1393	280	276	1814	1182	252	380

<sup>a</sup>Concentrations expressed as milligram %.

<sup>b</sup>Based on a 30-day survival period.

<sup>c</sup>Death occurred in rabbit 5 within 2 hr, in rabbits 2 and 7 on the second day, and in the remaining rabbits on the fourth day after irradiation.

smaller amounts of radiation,<sup>18,19</sup> proper assessment of the role of the RES in radiation injury requires further study.

**Studies of Acute Mortality in Mice as a Function of LET**

M. L. Randolph

J. A. Sproul      E. S. Ledford  
R. J. Elliott      D. L. Parrish<sup>20</sup>

**Introduction.** - The LD<sub>50</sub>/30 days of 14-Mev neutrons for RF male mice was determined for comparison with that of neutrons of lower energy from the 86-in. cyclotron.

**Results.** - The RBE of 14-Mev neutrons for LD<sub>50</sub>/30 days was appreciably lower than that of the cyclotron neutrons at the intensities investigated (Table 13).

**Discussion.** - The differences in RBE for a given intensity of irradiation are ascribed to the differences in the LET distributions of the radiations employed and are consistent with earlier observations.<sup>21,22</sup> The significance of RBE values, based on comparisons of LD<sub>50</sub>/30 days for neutrons vs photons, is uncertain, however, for two reasons: (1) radiations of different qualities apparently kill mice by different mechanisms, neutron-induced deaths occurring earlier than those caused by x rays or gamma rays; and (2) the values given for LD<sub>50</sub>/30 days usually denote the average soft tissue dose whereas the critical dose for x rays may be that received by the bone marrow, which probably differs significantly from the soft tissue dose.<sup>23</sup> Hence, attempts are now being made to establish RBE values in terms of the mechanism of death and the dose to the tissue predominantly affected, that is, intestine or bone marrow.

The Cockcroft-Walton accelerator produces neutron dose rates of about one-twentieth of those

<sup>18</sup>L. W. Tuttle and R. C. Baxter, *The Effect of Polonium Alpha Particle Irradiation Upon Reticuloendothelial Function as Measured with P<sup>32</sup> Labeled Particulate Chromic Phosphate*, UR-295 (Oct. 10, 1955).

<sup>19</sup>D. M. Donaldson and S. Marcus, *Aspects of the Relationship Between Irradiation Injury and Mammalian Host Defense Mechanisms*, USAF 56-50 (June 1956).

<sup>20</sup>Biophysics Section.

<sup>21</sup>A. C. Upton *et al.*, *Radiation Research* 4, 117 (1956).

<sup>22</sup>E. F. Riley *et al.*, *Radiology* 67, 673 (1956).

<sup>23</sup>H. E. Johns, p 781-792 in *Medical Physics*, Vol. II (ed. Otto Glasser), Year Book Publishers, Chicago, 1950.

Table 13. Comparison of LD<sub>50</sub>/30 Days of Fast Neutrons of Different Energies for RF Mice

Dose Rate (rad/min)	Type of Radiation	Energy (Mev)	LET: Approximate Track Average (kev/u)	LD <sub>50</sub> /30 Days (rad)	RBE
3-5	Gamma	1.1	0.27 <sup>a</sup>	920	1.0
	X	0.25 <sup>b</sup>	3.0 <sup>a</sup>	660	1.4
	Neutron	14	12 <sup>c</sup>	627 <sup>d</sup>	1.5
50-100	Gamma	1.1	0.27 <sup>a</sup>	680	1.0
	X	0.25 <sup>b</sup>	3.0	485	1.4
	Neutron	1.0 <sup>e</sup>	43 <sup>c</sup>	335 <sup>f</sup>	2.0

<sup>a</sup>From H. C. Jones, p 593 in *Radiation Dosimetry* (ed. G. J. Hine and G. L. Brownell, Academic Press, New York, 1956).

<sup>b</sup>250 kvp, hvl 0.4 mm Cu.

<sup>c</sup>Approximation by M. L. Randolph.

<sup>d</sup>Includes gamma-ray contamination of less than 5%.

<sup>e</sup>Calculation by G. S. Hurst *et al.*, *Radiation Research* 4, 49 (1956).

<sup>f</sup>Includes gamma-ray contamination of 10-15%.

used in the earlier experiments with the 86-in. cyclotron. Because the former biological facility at the cyclotron was destroyed by recent modifications to obtain an external beam, it has been impossible to repeat the earlier experiments with the cyclotron at the dose rate of the Cockcroft-Walton exposures. Hence the two neutron irradiations compared here differ in intensity as well as in energy and LET. That the difference in LD<sub>50</sub>/30 days between the neutron sources probably stems from differences in energy and not in intensity is indicated by the relative lack of intensity dependence of the LD<sub>50</sub>/30 days of neutrons observed by Clark *et al.*<sup>24</sup> Thus if the intensities of x, gamma, and neutron irradiations are diminished proportionately, the RBE of neutrons becomes higher because of the disproportionate increase in the LD<sub>50</sub>/30 days of x and gamma rays.

**Effects of Salt Concentration on Resistance of Leukemia Cells to Freezing**

N. G. Anderson<sup>25</sup>      F. F. Wolff

**Introduction.** – Studies on the stability of soluble rat liver proteins indicate that low salt concentrations produce a marked precipitation, whereas

<sup>24</sup>J. W. Clark *et al.*, *Radiation Research* 1, 128 (1954).

<sup>25</sup>Cell Physiology Group.

salt concentrations as high as 1 M have no observable deleterious effects. Similarly, high salt concentrations prevent postthawing precipitation, whereas a large amount of precipitation is seen after freezing in 0.14 M NaCl. This suggests that part of the damage occurring in frozen and thawed cells may be due to local decreases in salt concentration at sites where ice crystals thaw suddenly. Further, the superior results often obtained on slow freezing of tumor cells might possibly be due to crystallization of water outside the cells, with concomitant increase in salt concentration inside the cells. Fast freezing might not allow this change to occur. It appeared of interest, therefore, to subject tumor cells to rapid and slow freezing in the presence of several concentrations of NaCl-KCl mixtures to determine whether an increased salt concentration actually protected cells during fast freezing.

**Results.** – The effects of varying concentrations of salt on the survival of frozen and thawed rat leukemia cells are summarized in Table 14.

**Discussion.** – The results of varying concentrations of salt on the ability of leukemia cells to survive freezing are consistent with the previous observations on soluble liver protein. These experiments are being pursued further.

Table 14. Summary of Transplantation Experiments with Frozen Suspensions of Rat Lymphoid Leukemia Cells

Solution in Which Cells Were Suspended and Procedure Employed	Rate of Freezing	Experiment I		Experiment II	
		Fraction of Takes <sup>a</sup>	Average Latent Period (days)	Fraction of Takes	Average Latent Period (days)
Glucose (5%)					
Unfrozen		10/10	18.5	7/7	18.1
Frozen	Fast <sup>b</sup>	9/10	20.4	4/5	22.5
	Slow <sup>c</sup>	6/10	30.3	6/6	23.3
Solution A <sup>d</sup>					
Unfrozen		5/5	19.8		
Frozen	Fast	4/9	29	3/5	28.8
	Slow	0/13		0/5	
Solution B <sup>e</sup>					
Unfrozen		7/7	18.6		
Frozen	Fast	3/10	27.8	0/5	
	Slow	0/11		1/5	28
Solution C <sup>f</sup>					
Unfrozen		6/6	18.3		
Frozen	Fast	0/10		0/5	
	Slow	5/12	32.4	3/5	24

<sup>a</sup>Number of rats developing leukemia/number inoculated.

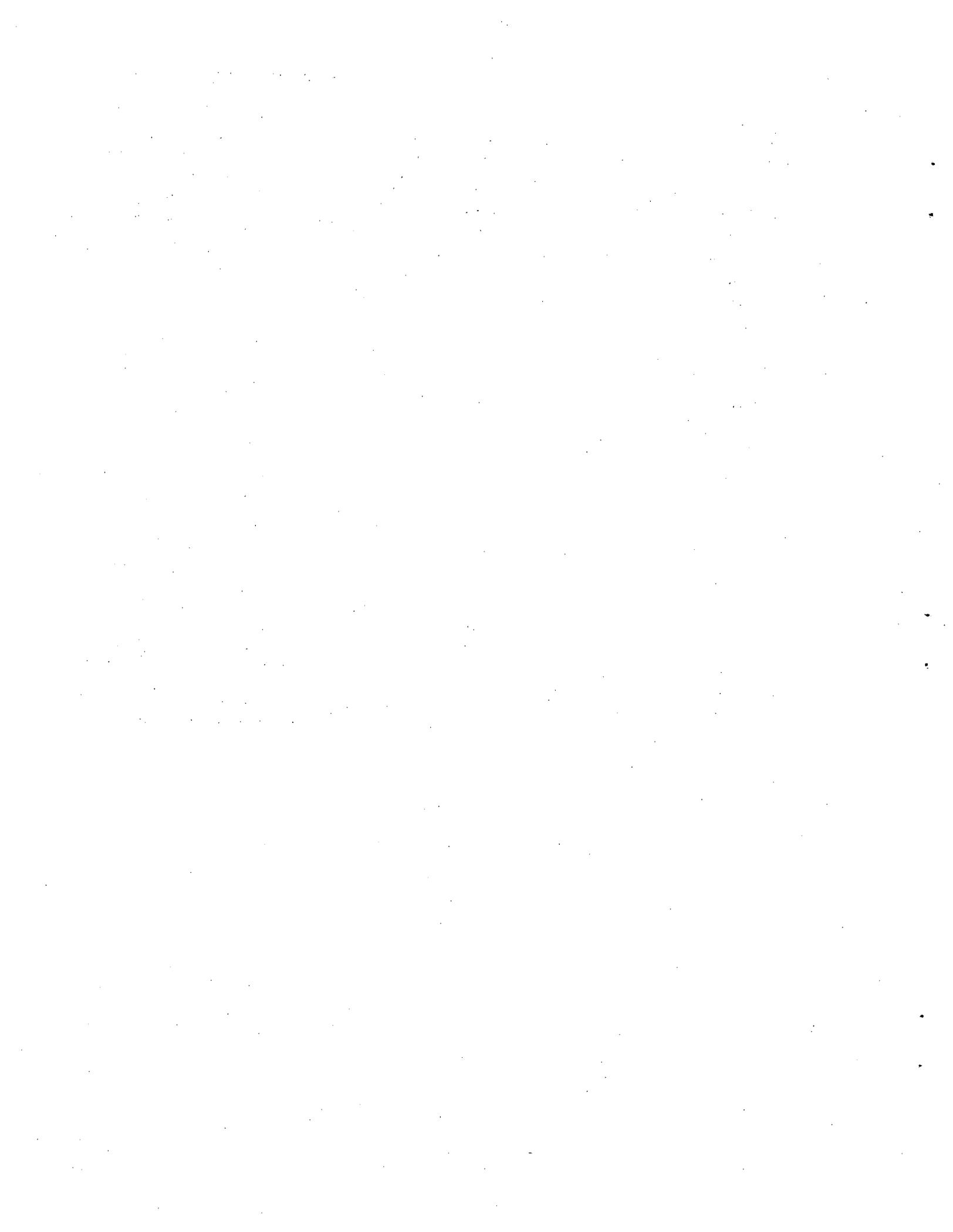
<sup>b</sup>Nearly instantaneous freezing of chilled cell suspension by immersion in acetone at dry-ice temperature (-80°C).

<sup>c</sup>Gradual freezing of chilled suspension over a period of approximately 1 hr by stepwise reduction of temperature to -80°C.

<sup>d</sup>2.7% NaCl + 0.16% KCl.

<sup>e</sup>1.8% NaCl + 0.11% KCl.

<sup>f</sup>0.9% NaCl + 0.055% KCl.



## CELL PHYSIOLOGY

### CELL PHYSIOLOGY

N. G. Anderson                      S. L. Scheinberg<sup>3</sup>  
C. W. Sheppard                      E. C. Horn<sup>4</sup>  
E. B. Darden, Jr.<sup>1</sup>                      M. L. Anderson  
W. D. Fisher<sup>2</sup>                              R. E. Canning  
J. F. Albright<sup>3</sup>                              M. T. Guess

### Fractionation of Rat Serum Proteins

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

**Introduction.** – It is difficult to prepare extracts of rat tissue proteins free of plasma proteins. It is, therefore, important to isolate and characterize rat serum proteins so that they may be distinguished from characteristically intracellular proteins. Previous work has shown that electrophoretic fractionation at two different pH's is required to separate the major serum components.

**Results.** – Orienting experiments were performed by using the continuous-flow paper-curtain electrophoresis technique. The chief difficulty encountered was with the buffer pumping system. Two sets of pumps were used and proved defective in long-continued operation in the cold. A third set of small, hard-rubber centrifugal pumps has been found satisfactory. By using pH 7.5, 0.02 ionic strength phosphate and pH 5.0 acetate buffer of the same ionic strength, rat serum albumin with no less than 1% of other serum components has been prepared. On examination of the serum albumin in the ultracentrifuge, only one sharp peak was obtained. However, when electrophoretically homogeneous  $\beta$ -lipoprotein was prepared, it showed two peaks in the ultracentrifuge. Other serum components are now being isolated and characterized.

**Discussion.** – Reproducible methods, based on continuous paper-curtain electrophoresis, have been devised for fractionating rat serum proteins.

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<sup>3</sup>Research Associate.

<sup>4</sup>Consultant.

### Separation of Bone Marrow Cells

N. G. Anderson                      T. Makinodan<sup>5</sup>  
    I. C. Shekarchi<sup>5</sup>

**Introduction.** – The protection of lethally irradiated mice by bone marrow transplants has posed the problem of which, among the many types of cells present in the marrow, is the active or transplanting cell type. While a large range of cell sizes occurs in marrow suspensions, these are not sufficiently distinct to suggest that any simple method can be evolved for segregating all the types present. Rather, it appears that a number of techniques will be required which will utilize both isopycnic-density-gradient centrifugation and differential-gradient centrifugation. In the studies now in progress, differential-density-gradient centrifugation is being explored.

**Results.** – Density gradients have been prepared by using bovine serum albumin, 15 and 25%, both dialyzed against Tyrode's solution. The gradients were prepared in 70-ml sector tubes by using the techniques devised in this laboratory. Controlled acceleration and deceleration techniques were used. The gradients were sectioned after centrifugation by using a fluorocarbon (Kel-F No. 1 oil) to raise the gradient. Four main fractions have been recovered, as shown in Fig. 11. Fraction A consists almost entirely of platelets, together with unidentified "ghosts." Fraction B contains the red blood cells and lymphocytes. Some separation of these two species is noted with the lymphocytes on top. The upper portion of C consists mostly of granulocytes, while immature cells are the predominant cell type in the lower portion of C. Megakaryocytes and cell clumps are found at D.

Preliminary studies with lethally irradiated mice suggest that the active cells are found in fraction C.

**Discussion.** – A method for fractionating bone marrow cell suspensions into four main groups has been devised. The results support the conclusion that effort should be directed toward (1) exploration of the effects of using less expensive gradient colloids, (2) the perfection of large-scale gradient

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<sup>5</sup>Radiation Immunology Group.

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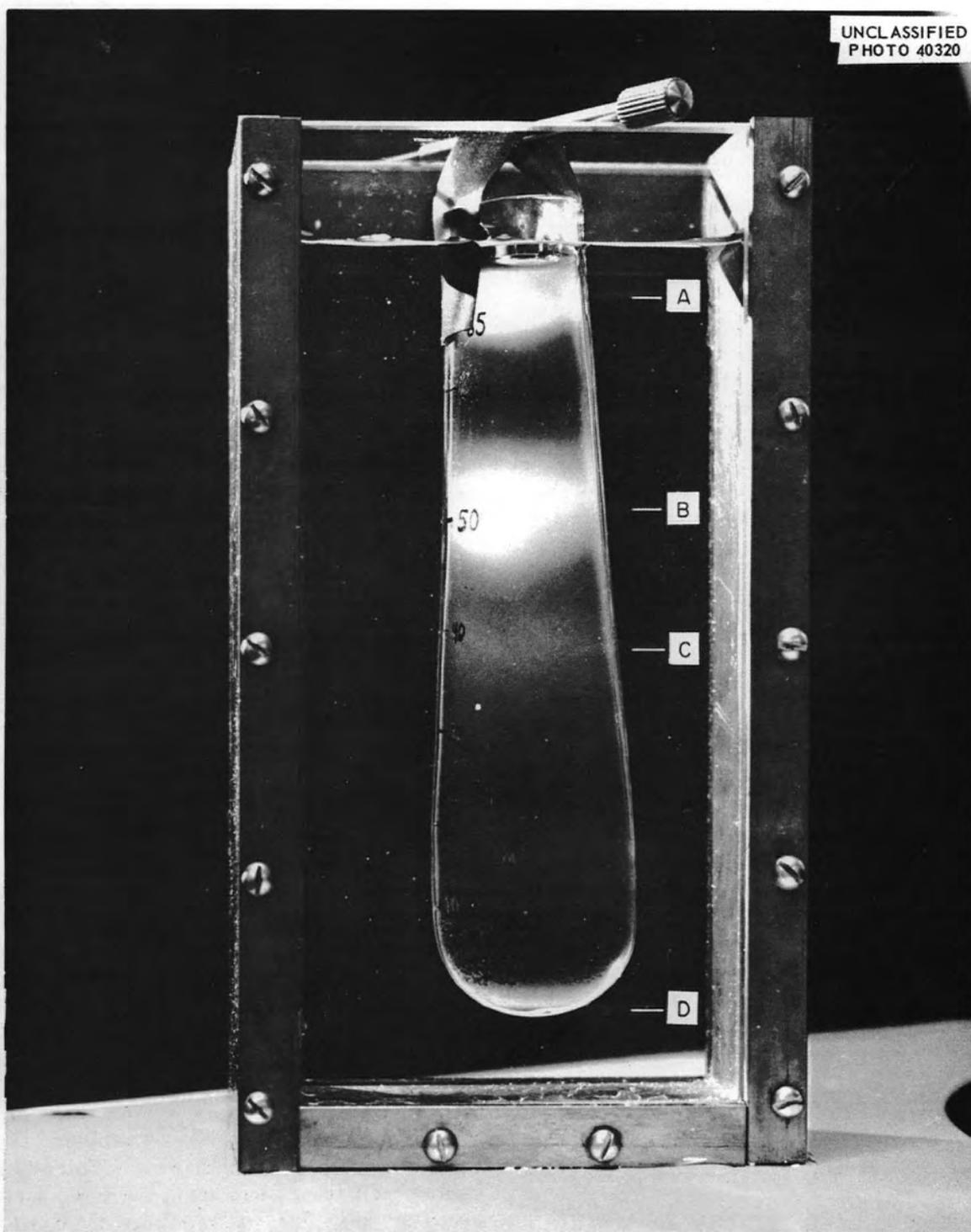


Fig. 11. Separation of Rat Bone Marrow Cells in a Gradient of Bovine Serum Albumin.

separation equipment, and (3) the further separation of the fractions obtained here by isopycnic-density-gradient centrifugation.

**Effect of X Rays on DNP from Rat Thymus**

W. D. Fisher

**Introduction.** - Although the physical properties of DNA and DNP (see Appendix for alphabetical list of abbreviations) are extensively altered by x rays *in vitro*, the exposures required to produce measurable changes are very large compared with biologically effective exposures (see review by Davison *et al.*<sup>6</sup>). Both Anderson<sup>7</sup> and Shooter (cited by Butler<sup>8</sup>) found that the viscosity of fresh crude DNP extracts made with 1 M NaCl was reduced by relatively small doses of x rays; however, they performed no systematic studies on the preparation because its viscometry was unsatisfactory.

**Results.** - Values for apparent relative viscosity agreeing to 2 to 3% can be made on crude DNP extracts about 5 hr after irradiation by measuring the flow time for the first passage of the extract through a modified Ostwald-Cannon-Fenske viscometer, size 200 (Fig. 12). Two-milliliter

aliquots of rat thymus homogenate in 0.14 M NaCl were mixed with 4.0 ml of 1.43 M NaCl and then extracted for 10 to 12 hr at 25°C. The final homogenate concentrations are shown in the graph. The dose rate was 50 r/min (250 kvp, 20 ma, 3 mm of Al). Specific viscosities were calculated by

$$\frac{\text{flow time for samples}}{\text{flow time for solvent}} - 1$$

The viscosity of the extracts was reduced by x-ray exposures of as little as 10 r (Fig. 12). The sensitivity of the extracts was increased by lowering the DNP concentration. The viscosity of the irradiated samples continued to fall for several hours. Irradiation of the homogenate decreased the viscosity of extracts prepared from irradiated samples but only at x-ray exposures about 100 times those employed on the extracts.

The sensitivity of the extracts was reduced by a variety of chemical compounds, and dose-reduction factors of 3 to 10 were obtained at a concentration of  $2 \times 10^{-3}$  M. Effective protective compounds included MEA, AED, MEG, AET, thiourea, hydroxyethylguanidine, and sodium azide. Sodium acetate, sodium cyanide, and glycine did not protect.

Freezing afforded some protection against the effects of x rays, but the exact degree of protection was hard to assess, since freezing alone caused large losses in viscosity.

Ultraviolet light and hydrogen peroxide ( $10^{-4}$  M) decreased the viscosity of the extracts.

**Discussion.** - The high sensitivity of the DNP extract studied is interesting because the exposures producing large viscosity changes are in the biological range. However, relating the *in vitro* observations to any biological effects is difficult because the high concentration of DNP in the cell and the presence of protective substances such as protein would act to reduce x-ray damage. Larger exposures are required to produce a given effect in the homogenate than in the extract, but even these doses are not enormous.

The effect of *in situ* irradiation of the thymus is being studied.

**ELECTROLYTES AND CIRCULATION**

**Radiation-induced Edema**

E. B. Darden, Jr. C. W. Sheppard

**Introduction.** - In their studies of electrolytes and water in the muscles of heavily irradiated rat

<sup>6</sup>P. F. Davison, B. E. Conway, and J. A. V. Butler in *Progress in Biophysics*, chap. 4 (ed. J. A. V. Butler and J. T. Randall), Pergamon Press, London, 1954.

<sup>7</sup>N. G. Anderson, *Federation Proc.* 13, 3 (1954).

<sup>8</sup>J. A. V. Butler, *Radiation Research* 4, 20 (1956).

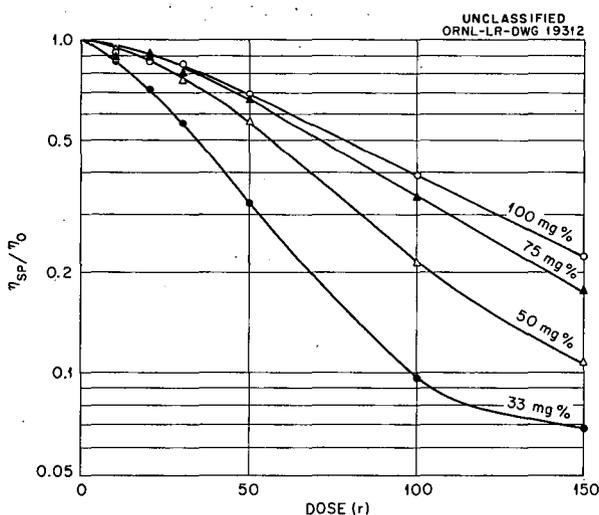


Fig. 12. Dose Curves.

BIOLOGY PROGRESS REPORT

forelimbs, Wilde and Sheppard<sup>9</sup> observed a 22% weight gain due to edema 22 hr after irradiation. This was confined, apparently, to the extracellular region as measured by inulin space determinations. Recent work on histamine production and response in irradiated skin and histamine-produced edema in rat skin has prompted a study in this laboratory to see if antihistamine substances have any inhibitory effect on the forelimb muscle edema. Results, although as yet incomplete, appear to support this thesis.

**Methods.** — Forelimbs of male rats were irradiated with a dose of 75 kr, and 22 hr afterward the muscle masses of the control and irradiated limbs were removed, cleared of connective tissue, and

the water content was determined essentially as previously described.<sup>9</sup> Two animals out of the four in each irradiated group were given diphenhydramine (Benadryl) in saline by intraperitoneal injection in single doses from 6 up to 40 mg per kilogram of body weight in schedules of from one to three doses at 6-hr intervals up to 18 hr after the end of irradiation.

**Results.** — The results are shown in Table 15. A small number of experiments not statistically sound because of anesthetic deaths during treatment etc. have not been included.

**Discussion.** — No significant effect of treatment was found at doses below 80 mg/kg. At 120 mg/kg, however, the effect is significant at the 1/2% level. In further investigations of the complex of events following tissue irradiation, the probability of release of histamine or histamine-like substances cannot be disregarded.

<sup>9</sup>W. S. Wilde and C. W. Sheppard, *Proc. Soc. Exptl. Biol. Med.* 88, 249-253 (1955).

Table 15. Edema Production in Irradiated Rat Forelimb Muscle Showing Effect of Diphenhydramine

Total Dose (mg/kg)	Number of Animals	Muscle Water (g)*				Irradiated - Control		P (%)
		Treated		Untreated		Treated	Untreated	
		Irradiated	Control	Irradiated	Control			
6	8	1.1385	0.8562	1.0805	0.8955	0.2823	0.1850	~ 13
80	8	1.1864	0.9453	1.2191	0.9528	0.2411	0.2663	> 25
120	16	1.0597	0.8632	1.1660	0.8773	0.1965	0.2887	< 0.5

\*Expressed as mean differences between wet and dry weights adjusted to a common mean dry weight.

## BIOCHEMISTRY

## NUCLEIC ACIDS, ENZYMES, AND RADIATION-PROTECTIVE COMPOUNDS

W. E. Cohn	R. Shapira
D. G. Doherty	R. H. Bradford <sup>1</sup>
J. X. Khym	A. B. Ottinger
E. Volkin	M. H. Jones
L. Astrachan	D. E. Emmer

## Minor Constituents of Ribonucleic Acids

W. E. Cohn

Ion exchange chromatograms of hydrolyzates of RNA (see Appendix for alphabetical list of abbreviations) from various sources have, from time to time, shown the presence of ultraviolet-absorbing, phosphorus-containing substances in the mononucleotide region. The propensity of nucleic acid preparations to include and tenaciously retain a variety of impurities makes it necessary to prove in each case that these new substances contain the correct components in the usual array and that they are incorporated into the usual phosphodiester linkage in the macromolecule. For at least one such constituent, apparently a modified uridylic acid, most of these criteria have been met. It appears in a wide variety of yeast nucleic acid preparations as the conventional *a* and *b* forms after alkaline hydrolysis, as the *b* form after ribonuclease hydrolysis, and as a riboside after snake-venom hydrolysis. Its position in chromatograms near uridylic acid and uridine, its pronounced bathochromic shift at a pH of about 10, and the absence of spectral shifts below pH 8 indicate that it is a uracil derivative. Its ultraviolet spectra at neutral and alkaline pH's are almost identical with those of uracil and thymine in shape and extinction coefficient, with maximums and minimums lying between or near them. Inasmuch as the substance is a nucleotide and not a base, the similarity of its spectra to those of the free pyrimidine bases is most unusual and indicates that the pyrimidine ring is able to assume a form which uracil and thymine cannot assume after they are converted to their ribosides, or deoxyribosides, or otherwise substituted on nitrogen atom 3.

The absence of the substance from commercial yeast RNA indicates that it occurs in the more

soluble and more easily fragmented yeast nucleic acids. Thus it is necessary to prepare RNA *de novo* in order to obtain it.

As the result of a gift from S. A. Morell of the Pabst Laboratories of 300 g of a special yeast RNA preparation, several hundred milligrams of the new nucleotide have been isolated in order to determine the nature of the base and the point of attachment of the ribose residue.

## Structure and Activity in Radiation-Protective Sulphydryl Compounds

D. G. Doherty	T. W. McKinley, Jr. <sup>2</sup>
R. Shapira	D. E. Emmer

The screening program for radiation-protective agents has established that some 17 mercaptoalkylguanidines (prepared from the corresponding aminoalkylisothiuronium salts by neutralization), all conforming to the same general structural pattern, provided effective protection to mice, LD<sub>0-10</sub>/30 days, against an otherwise lethal single dose<sup>3</sup> of x radiation, 900 r. In order to determine the most effective of these compounds the radiation dose had to be increased to 1400 r. At that level two compounds, MEG (prepared from AET) and MPG (prepared from APT), provided consistent survival at the maximum compound dose that could be safely administered intraperitoneally. They were therefore chosen for further study as to their therapeutic ratio, route of administration, increase in radiation LD<sub>50</sub>/30 and length of time they could be administered prior to the x irradiation. Male and female mice of the 101 × C<sub>3</sub>H strain were used whose LD<sub>50</sub>/30 was determined to be 725 ± 25 r. The compound doses reported will be in terms of the thiuronium salt weighed out. When graded doses of MEG were injected intraperitoneally 20 min prior to 900-r x irradiation, the minimum effective dose LD<sub>0</sub>/30 was 160 mg/kg and the LD<sub>50</sub>/30 dose was 60 mg/kg. Similarly, MPG gave values of 120 mg/kg for LD<sub>0</sub>/30 and 60 mg/kg for LD<sub>50</sub>/30. The maximum compound dose that could be administered to animals which were to be irradiated was 360 mg/kg for MEG and 240 mg/kg for MPG. Animals that received

<sup>2</sup>Mammalian Recovery Section.

<sup>3</sup>D. G. Doherty *et al.*, *Biol. Semiann. Prog. Rep.* Aug. 15, 1956, ORNL-2155, p 48-51; *Biol. Semiann. Prog. Rep.* Feb. 15, 1956, ORNL-2060, p 45-46.

<sup>1</sup>ORINS Fellow.

no radiation could tolerate slightly higher doses with no fatalities. The increase in radiation  $LD_{50}/30$  was determined for both compounds at two compound dose levels with radiation doses from 900 to 1650 r in 75-r increments. MEG at 176 mg/kg intraperitoneally, 20 min prior to radiation, gave an  $LD_{50}/30$  of  $1200 \pm 30$  r, while 352 mg/kg gave an  $LD_{50}/30$  of  $1500 \pm 50$  r. MPG at 120 mg/kg gave  $LD_{50}/30$  values of  $1100 \pm 30$  r, and 240 mg/kg gave  $1400 \pm 50$  r.

Both compounds were found to be effective when administered in maximal doses intramuscularly, subcutaneously, and orally. Since the oral route offered an easy way to give the compounds, it was examined in a manner similar to that used in the intraperitoneal route. The mice were not fed for 5 hr, the compound was injected by stomach tube, and the mice were irradiated 30 min later. The compound  $LD_{50}/30$  dose for 900 r for MEG was 160 mg/kg and  $LD_{10}/30$  was 240 mg/kg;  $LD_0/30$  was 320, 480, and 720 mg/kg. The similar values for MPG were as follows:  $LD_{10}/30$ , 160 mg/kg;  $LD_0/30$ , 240, 320, and 480 mg/kg. The radiation  $LD_{50}/30$  for MEG at 640 mg/kg was found to be  $1450 \pm 50$  r, while the corresponding figure for MPG at 480 mg/kg was  $1250 \pm 30$  r.

Thus both compounds provide effective protection to mice whether given orally or intraperitoneally, although MEG seems to be better on the basis of its performance at high radiation doses. It is worth while to note that both compounds have a therapeutic ratio of 2 and that the elevation of the radiation  $LD_{50}$  is proportional to the amount of compound given until a dose reduction factor of 2 is obtained. Less toxic compounds are needed before tests can be made for determining where the upper limit is to radiation protection to mice.

#### Intracellular Distribution of Labeled Protective Compounds

R. Bradford                      R. Shapira  
D. G. Doherty

Determinations of the distribution of several effective  $S^{35}$ -labeled compounds established that the spleen, bone marrow, and liver were among the more highly labeled tissues. Intracellular distribution was therefore investigated to determine whether any selective absorption occurred. Nuclear, mitochondrial, microsomal, and soluble cytoplasmic fractions of the liver were prepared by

the procedure of Hogeboom and Schneider, and the activity, dry weight, and nitrogen content of aliquots of these fractions were determined. When MEG- $S^{35}$  was injected and the liver removed 45 min later, a preferential distribution of the activity was observed. Increased doses of MEG- $S^{35}$  gave large relative increases in the specific activity of the corresponding fractions, although the distribution remained unchanged. An optically active pair of compounds, the D isomer of which was effective at a level where the L afforded no survivors, that is, D- and L-1-mercaptomethyl-propylguanidine, was prepared with  $S^{35}$  label and examined by the above procedure. When each isomer was injected at the dose where the D isomer was effective and the L isomer ineffective, it was found that the activity of the microsomal fraction obtained with the D isomer was twice that of the L isomer. Doubling the L isomer dose (an effective protective level) increased the activity of the microsomal fraction to that of the D isomer. Similar activity distributions were obtained by using  $C^{14}$ -labeled compounds. The nature of the binding of this activity was studied by equilibrium dialysis experiments<sup>4</sup> with the various cell fractions in several solvent systems. A significant portion of the activity remained bound to the particulate fractions even after dialysis against water, followed by cyanide or thioglycollate. The specific activity remaining bound was proportional to the specific activity of the individual particulate fractions before dialysis as well as to the amount of labeled material administered. This interesting observation is being examined further to determine its relation to the protection afforded by these compounds.

#### Intertransguanylation Reactions of Aminoalkylisothiuronium Salts

J. X. Khym                      A. B. Ottinger

In a previous report<sup>5</sup> it was shown that aminoethylisothiourea underwent chemical change through an intratransguanylation mechanism, involving a cyclic intermediate, to yield MEG. It has now been demonstrated, in higher homologues of AET,

<sup>4</sup>D. G. Doherty and F. Vaslow, *J. Am. Chem. Soc.* 74, 931-936 (1952).

<sup>5</sup>J. X. Khym *et al.*, *Biol. Semiann. Prog. Rep. Feb. 15, 1956*, ORNL-2155, p 50-51.

that the intratransguanylation mechanism is replaced by an intertransguanylation process if the amino group of the thiuronium compounds is separated by at least four carbon atoms from the isothiuronium group. This can be demonstrated by the results of the ion exchange analysis (see Fig. 13) of solutions of ABT. The products found when ABT was dissolved in buffer solutions of pH 9.2 were MBA, MBG, and GBT. The intermolecular method of rearrangement, through which these products are formed, is considered to involve the following reactions:

- (1)  $2\text{ABT} \rightarrow \text{GBT} + \text{MBA}$
- (2)  $\text{ABT} + \text{MBA} \rightarrow \text{MBG} + \text{MBA}$
- (3)  $\text{ABT} + \text{GBT} \rightarrow \text{MBG} + \text{GBT}$
- (4)  $\text{GBT} + \text{MBA} \rightarrow 2\text{MBG}$

In order to test the feasibility of reactions 2 and 4 without having the interference due to Eqs. 1 and 3, the following reactions were separately studied at pH 7.0, where, as demonstrated in Fig. 13, curves 1 and 4, guanidinoalkylisothiuronium salts and ABT are stable:

- (5)  $\text{ABT} + \text{MEA} \rightarrow \text{MBA} + \text{MEG}$
- (6)  $\text{GET} + \text{MEA} \rightarrow 2\text{MEG}$

MEA and GET were used in place of MBA and GBT because the former two compounds were more readily available. The products of reactions 5 and 6, together with those for reactions 1 through 4, are given in Fig. 13. The requirement that an uncharged alkyl group is necessary for reactions 1 to 6 to occur is indicated by the fact that reaction 7 does not occur at pH 7.0:

- (7)  $\text{GET} + \text{ABT} \rightarrow \text{MEG} + \text{GBT}$

Thus it can be seen (Fig. 13, curve 5) that essentially all the MEA present was converted via the intertransguanylation reaction to MEG.

This evidence offers additional support for the rearrangement mechanism previously postulated for this class of compounds.<sup>6</sup> It also explains the partial protective activity of GET, which, in vivo, may transfer its guanyl group to a biological amine, forming limited amounts of MEG.

<sup>6</sup>D. G. Doherty et al., *Biol. Semiann. Prog. Rep.* Aug. 15, 1956, ORNL-2155, p 48-51; *Biol. Semiann. Prog. Rep.* Feb. 15, 1956, ORNL-2060, p 45-46.

### RNA Turnover in Phage-infected Bacteria

E. Volkin            L. Astrachan  
M. H. Jones

It has been shown that the RNA in bacteriophage-infected *Escherichia coli* can incorporate and subsequently release  $\text{P}^{32}\text{O}_4$ , although the total amount of RNA remains constant. When  $\text{P}^{32}$  is presented to the infected bacteria, RNA initially incorporates much more  $\text{P}^{32}$  than does DNA, although DNA, as the only nucleic acid end product of the infected cells' metabolism, will eventually accumulate far more isotope than the RNA.

It has now been found that addition of the protein inhibitor, chloramphenicol, to phage-infected bacteria will almost completely block DNA synthesis but will allow  $\text{P}^{32}$  to accumulate in the RNA without turnover of the latter. Upon removal of inhibitor and isotope from the medium, it was observed that  $\text{P}^{32}$  was released from the RNA while a coincident rise of isotope into DNA occurred. Thus, 20 min after removal of  $\text{P}^{32}$  and chloramphenicol, the radioactivity of the RNA had decreased by  $2.5 \times 10^4$  counts/sec, while the DNA gained  $2.4 \times 10^4$  counts/sec. After 50 min, the RNA had released  $4.1 \times 10^4$  counts/sec, while the DNA had accumulated  $5.8 \times 10^4$  counts/sec. A complication in attempting to quantitate the relation between the degree of RNA turnover and DNA synthesis arises from the fact that at all times in this experiment the level of  $\text{P}^{32}$  in the acid-soluble fraction remained quite high. It is therefore quite possible that DNA synthesis occurs exclusively by way of this latter pool and functions virtually independently of RNA turnover.

This experiment has been repeated by using  $\text{C}^{14}$ -adenosine in which the ribose contained about half the  $\text{C}^{14}$  content as the adenine. Although the results of the experiment with chloramphenicol and  $\text{P}^{32}$  were qualitatively confirmed with the isotopic riboside as precursor, certain differences were noted. First, it was observed that, after removal of inhibitor and labeled adenosine from the medium, the increase in radioactivity of DNA was less than the reduction in radioactivity of RNA after a 45-min time interval. Second, although the RNA-guanosine and the DNA-deoxyguanosine base/sugar radioactivity ratios were quite similar, and the RNA-adenosine base/sugar

## BIOLOGY PROGRESS REPORT

radioactivity ratio was similar to that of the original precursor, the radioactivity of the DNA-adenine was inordinately high at the time before removal of medium  $C^{14}$ -adenosine and chloramphenicol. It is quite possible that the adenine from the  $C^{14}$ -adenosine exchanged with preformed

DNA-adenine by a transfer mechanism apart from *de novo* DNA synthesis. As in the experiment with  $P^{32}$ , the acid-soluble-fraction  $C^{14}$  level remained quite high, obscuring any definitive conclusions concerning the relationship between RNA turnover and DNA synthesis in this system.

### DATA RELATIVE TO FIG. 13

Exchange resin:  $1.0 \text{ cm}^2 \times 5 \text{ cm}$ , Dowex 50- $H^+$ , 200 to 400 mesh

Flow rates: 0.6 to 0.8 ml/min

Ordinate: Concentration of nitrogen determined colorimetrically by Nesslerization

Abscissa: Milliliters through column as shown

Numbers above peaks: Micromoles recovered

DS: Estimate (in  $\mu\text{moles}$ ) of disulfide contamination in the guanidine alkylisothiuronium peaks

Curve 1: 89  $\mu\text{moles}$  per milliliter of ABT in 0.4 M  $\text{PO}_4^{---}$  buffer; pH, 7.0; time, 24 hr

3 ml of ABT solution was diluted to 50 ml with 0.2 M HCl and was put on exchanger

Curve 2: 89  $\mu\text{moles}$  per milliliter of ABT in 0.4 M borate buffer; pH, 9.2; time,  $5\frac{3}{4}$  hr

6 ml of ABT solution was diluted to 100 ml with 0.2 M HCl and was put on exchanger

Curve 3: Same as above, only the ABT solution remained in the borate buffer for 22 hr

Curve 4: GET at 80  $\mu\text{moles/ml}$  in 0.4 M  $\text{PO}_4^{---}$  buffer; pH, 7.0; time, 24 hr

1.5 ml of GET solution was diluted to 20 ml with 0.2 M HCl for column run

Curve 5: 50  $\mu\text{moles/ml}$  and 40  $\mu\text{moles/ml}$ , of MEA and GET, respectively, in 0.4 M  $\text{PO}_4^{---}$  buffer; pH, 7.0; time, 4 hr

6 ml of the solution was diluted to 100 ml with 0.2 M HCl for column run

Curve 6: Same as curve 5, only with 50  $\mu\text{moles/ml}$ , each of ABT and MEA, maintained at pH 7.0 for  $5\frac{1}{4}$  hr

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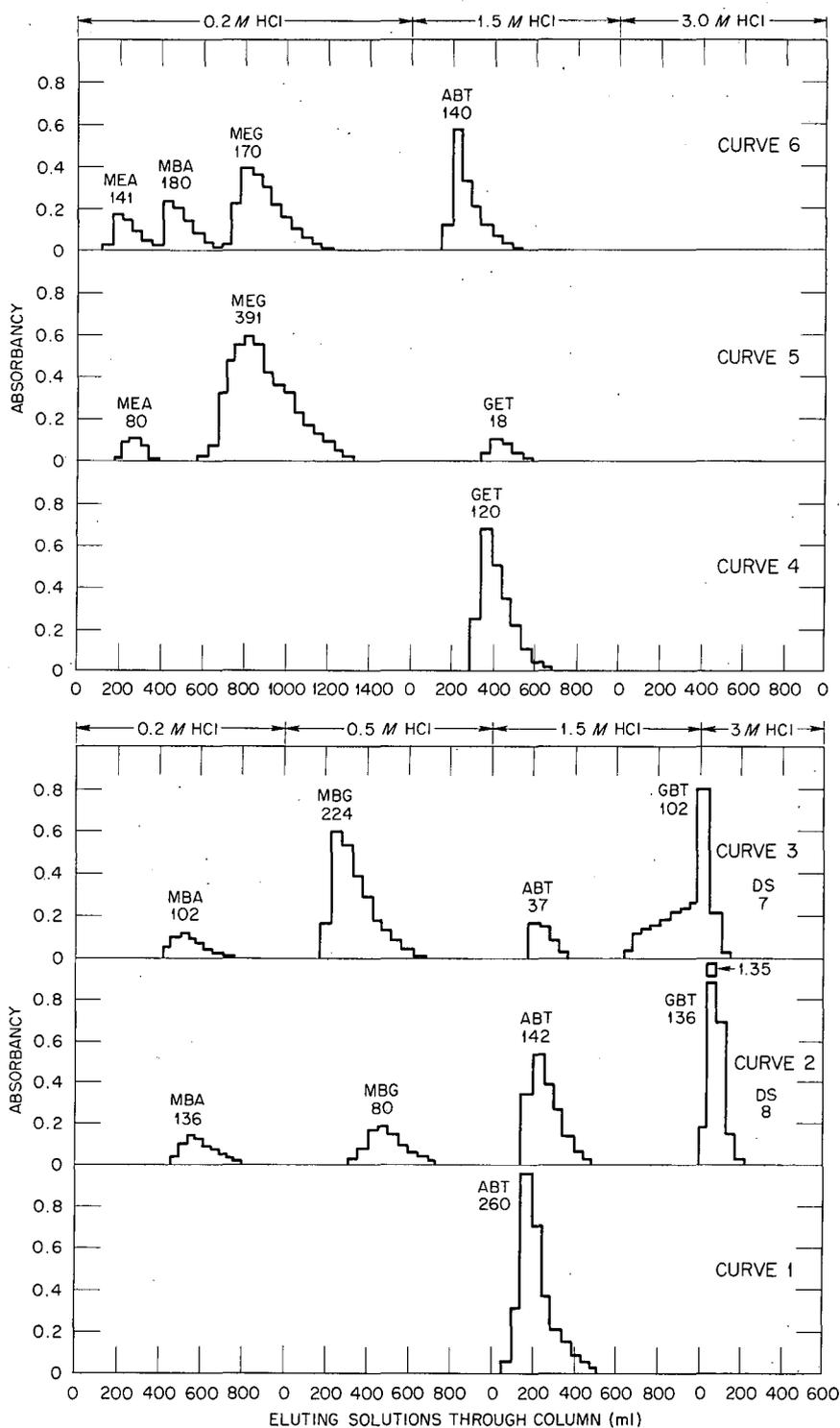
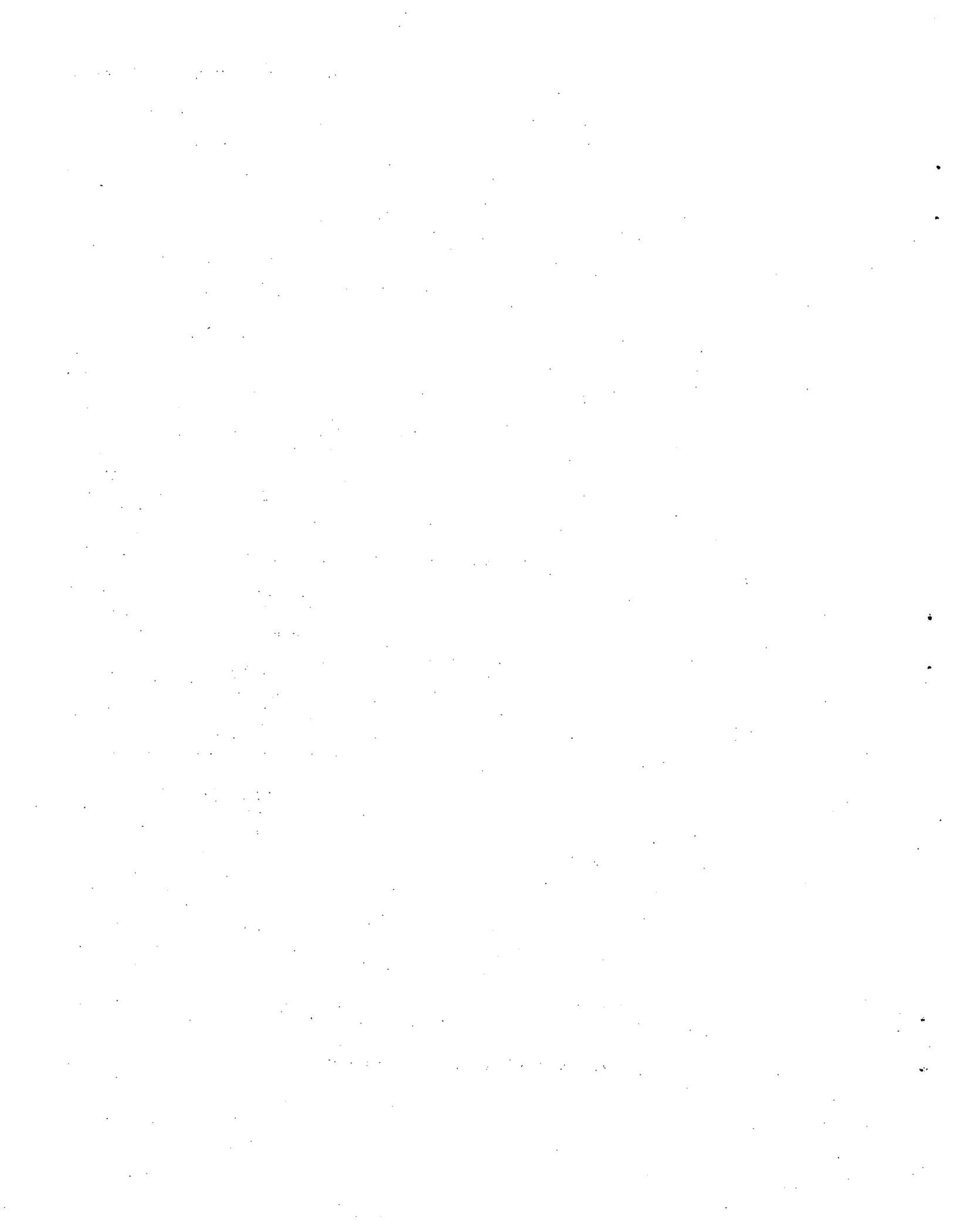


Fig. 13. Products of Intertransguanylation Reactions.



## ENZYMOLGY AND PHOTOSYNTHESIS

G. D. Novelli

J. W. Davis  
A. N. Best  
W. A. Arnold

H. K. Sherwood  
M. J. Cormier<sup>1</sup>  
J. A. DeMoss<sup>2</sup>

### STUDIES ON THE MECHANISM OF PROTEIN SYNTHESIS

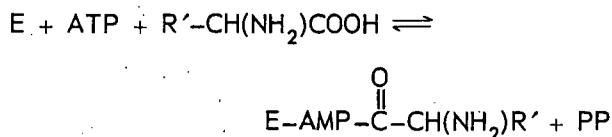
G. D. Novelli

#### Enzymatic Activation of Amino Acids

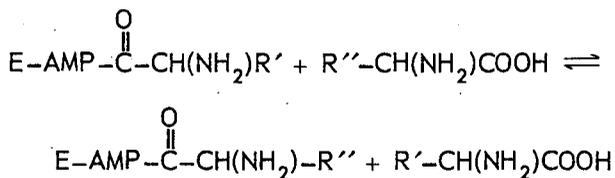
A. N. Best

**Introduction.** - From a consideration of available information,<sup>3-6</sup> a working hypothesis of the mechanism of protein synthesis can be formulated as a three-stage process as follows:

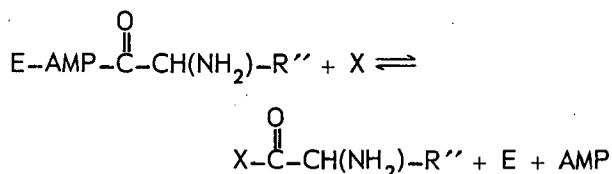
1. Primary Activation of Amino Acids -



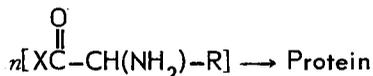
(a) Secondary Activation (anhydride exchange) -



2. Transport -



3. Polymerization -



<sup>1</sup>ORINS Fellow.

<sup>2</sup>ORINS Fellow; arrived Feb. 4, 1957.

<sup>3</sup>F. Lipmann, p 599-604 in *The Mechanism of Enzyme Action* (eds. W. D. McElroy and B. Glass), Johns Hopkins Press, Baltimore, 1954.

<sup>4</sup>H. Borsook, *J. Cellular Comp. Physiol.* **47**, Suppl. 1, 35-79 (1956).

<sup>5</sup>M. B. Hoagland, *Biochim. et Biophys. Acta* **16**, 288-289 (1955).

<sup>6</sup>J. A. DeMoss and G. D. Novelli, *Biochim. et Biophys. Acta* **22**, 49-61 (1956).

Reaction 1 indicates the activation of amino acids through the formation of aminoacyl-AMP compounds which appear to be tightly enzyme bound.<sup>7</sup> Because only about ten amino acids seem to participate in this primary activation step, reaction 1a is introduced as a possible mechanism for the activation of those amino acids which do not participate in reaction 1. Such anhydride exchanges are not uncommon in biological systems, for example, the activation of acetoacetic acid by interaction with succinyl CoA.

Step 2 follows from the fact that the products of reaction 1, the aminoacyl-AMP compounds, remain tightly enzyme bound and, if they are to participate in protein synthesis, must be transported to specific areas in the cell.

Stage 3, which describes the polymerization of the activated amino acids to form a specific protein, is undoubtedly a complex process which will involve a template mechanism for the predetermination of the amino acid sequence in a given specific protein.

The work of the Enzymology Group is directed toward obtaining information and evidence for all three stages in the over-all process of protein synthesis.

**Results.** - (a) *Synthesis of Aminoacyl-AMP Compounds with Dicyclohexylcarbodiimide in Pyridine.* - A careful study of the conditions for the use of DCC in the synthesis of aminoacyl-AMP compounds has been completed. In general the method can be applied successfully to this synthesis with a limited number of amino acids such as leucine, phenylalanine, and serine, but not with glycine, tryptophan, histidine, and glutamic acid. When successful, the method suffers from the fact that a variety of complex products results from which purified preparations of the aminoacyl-AMP compound are difficult to prepare. Because of these findings the use of this method has been abandoned here except for the preparation of leucyl-AMP.

<sup>7</sup>J. A. DeMoss, S. M. Genuth, and G. D. Novelli, *Proc. Natl. Acad. Sci. U. S. A.* **42**, 325-332 (1956).

(b) *Synthesis of Aminoacyl-AMP Compounds Through the Mixed Anhydride.* — This method, which involves protecting the amino group with a carbobenzoxy group, has been worked out to give excellent yields. Unfortunately, it was discovered that AMP is a potent inhibitor of catalytic hydrogenation, thus effectively preventing removal of the carbobenzoxy group. Many attempts to relieve this inhibition by increasing the amount of catalyst or by preliminarily treating the AMP with Raney nickel have been unsuccessful. Attempts to solve the problem by using different catalysts are now in progress.

(c) *Activation of Amino Acids by Various Mammalian Systems.* — The activation of amino acids measured by the amino-acid-dependent exchange of PP<sup>32</sup> with ATP has been studied in rat liver, rabbit liver, mouse and rat bone marrow, and chicken erythrocytes. All tissues studied carry out this reaction. The catalysis of the PP-ATP exchange by 18 individual amino acids has been studied in rabbit liver. Only nine amino acids, phenylalanine, histidine, tryptophan, valine, isoleucine, cysteine, methionine, tyrosine, and leucine, were active. This is the same group that had been found to be active with several microbial extracts, and this finding adds further evidence that these amino acids are the ones that undergo primary activation. The remaining amino acids may be activated in another manner, for instance, by anhydride exchange (reaction 1a).

There appears to be a rather strong amino-acid-dependent PP-ATP exchange in chicken erythrocytes. The activity seems to be confined to cellular elements, since no activity could be detected in the cell-free plasma. In general, however, the mammalian systems so far tested are from 1/10 to 1/100 as active as microbial extracts.

**Discussion.** — The rather general distribution of the amino-acid-dependent exchange of PP with ATP suggests that it may indeed be involved in protein synthesis. Present and future plans involve attempts to resolve several questions posed by the hypothesis describing the mechanism of protein synthesis discussed in the introduction. Experimental evidence for the following points is being sought: (1) the direct participation of the activating system in protein synthesis, (2) the secondary activation of the "inactive" amino acids by anhydride exchange, and (3) the molecular species which transports the activated amino acids.

## Activation and Incorporation of Amino Acids by Pea Seedlings

J. W. Davis                      G. D. Novelli

**Introduction.** — Although the widespread distribution of the system for the activation of amino acids<sup>8</sup> suggests that the system may play a fundamental role in protein synthesis, direct proof of the connection between activation and protein synthesis is lacking. In an effort to correlate these two processes, measurements of the amino-acid-activating enzyme activity and of protein synthesis, as reflected in C<sup>14</sup>-leucine incorporation, were made in greening, etiolated pea plants. De Deken-Grenson has shown previously<sup>9</sup> that in such a system there is a large increase in protein nitrogen as the greening progresses.

**Results.** — Undialyzed extracts of the epicotyl portion of pea seedlings was the system chosen for these studies. The pea seedlings were grown to a height of 3 to 7 cm in the dark in sterile, moist chambers, and one-half the total crop was removed to the light at time 0. Extracts made from epicotyls of the greening or etiolated peas at corresponding times were tested for activity of the amino-acid-activating enzyme. In addition, epicotyls from the greening peas were tested for C<sup>14</sup>-leucine incorporation into protein at intervals during the greening process. The activity of the amino-acid-activating system, as evinced by the exchange of P<sup>32</sup>-pyrophosphate with ATP, and the rate of amino acid incorporation into protein were found to be maximally present in both the etiolated seedlings and in all stages of the greening plant. The same ten amino acids which are activated by the mammalian and bacterial-activating enzymes<sup>8</sup> have been found active also in the pea seedling extracts.

**Discussion.** — The increased synthesis of protein induced by the greening of etiolated pea plants does not elicit an increased activity of amino acid activation or an increased rate of amino acid incorporation. This suggests that the activation of amino acids is not the rate-limiting step in protein synthesis. The fact that the same group of ten amino acids which catalyze the PP-ATP exchange in microbial and mammalian tissues is also the

<sup>8</sup>J. A. DeMoss and G. D. Novelli, *Biochim. et Biophys. Acta* 22, 49-61 (1956).

<sup>9</sup>M. de Deken-Grenson, *Biochim. et Biophys. Acta* 14, 203-211 (1954).

active group in plant tissues is further evidence for the suggestion<sup>8</sup> that these amino acids are involved in the primary activation stage.

#### Quantum Efficiency Determinations on Components of the Bacterial Luminescence System

M. J. Cormier      G. D. Novelli

**Introduction.** — The components required for luminescence in cell-free extracts of the luminous bacterium, *Achromobacter fischeri*, are now well known [i.e., DPNH, FMN, a long-straight-chain fatty aldehyde (RCHO), and oxygen]. Furthermore, the two enzymes involved in this process (DPNH oxidase and luciferase) have been separated, and the role of each has been identified. However, the nature of the energy-yielding reaction and the fate of FMN during luminescence are among the problems that remain to be solved. In an effort to solve these problems, quantum efficiency measurements were made on components of the luminescence system.

**Results.** — The quantum efficiency determinations (expressed as molecules utilized per quantum emitted) for dodecyl aldehyde, FMN, and DPNH were made by using enzyme preparations from *A. fischeri*. For the aldehyde, the quantum efficiency values varied from 20 to 40, depending upon the enzyme preparation used. The evidence indicated that the aldehyde was being oxidized by luciferase, and not by other enzymes in the preparation. For two separate experiments the efficiency values of FMN were each about 0.3 at a point where the luminescence reaction was still proceeding at 60% of the initial rate. In one enzyme preparation it was found that approximately 0.036% of the DPNH was utilized for light production, whereas the bulk of the compound was oxidized via other pathways.

**Discussion.** — The efficiency values obtained for dodecyl aldehyde indicate that the aldehyde disappears during luminescence. McElroy and Green<sup>10</sup> have previously pointed out that a peroxidation of aldehyde to the corresponding acid would furnish sufficient energy for the excitation process in-

involved in this luminescent reaction. In view of the efficiency values obtained for aldehyde, it seems plausible that such a peroxidation might indeed furnish the necessary excitation energy.

The FMN experiments indicate that FMN was not destroyed during luminescence, but rather recycled at least three times during the time interval of the experiment. This result does not allow for any significant luminescence pathway which does not result in a regeneration of FMN.

If the value obtained for the DPNH efficiency approximates that occurring within the cell, luciferase is not very significant as part of an over-all, energy-yielding, oxidative pathway.

#### PHOTOSYNTHESIS

##### Energy Storage in Photosynthetic Systems

W. A. Arnold      H. K. Sherwood

**Introduction.** — An energy storage in green plants and in suspensions of chloroplasts was shown several years ago, inasmuch as they emit light for some time after they have been illuminated. In the last semiannual report<sup>11</sup> it was shown that chloroplasts, dried to thin films on metal plates, emit light on being heated, provided that the heating follows a period of illumination.

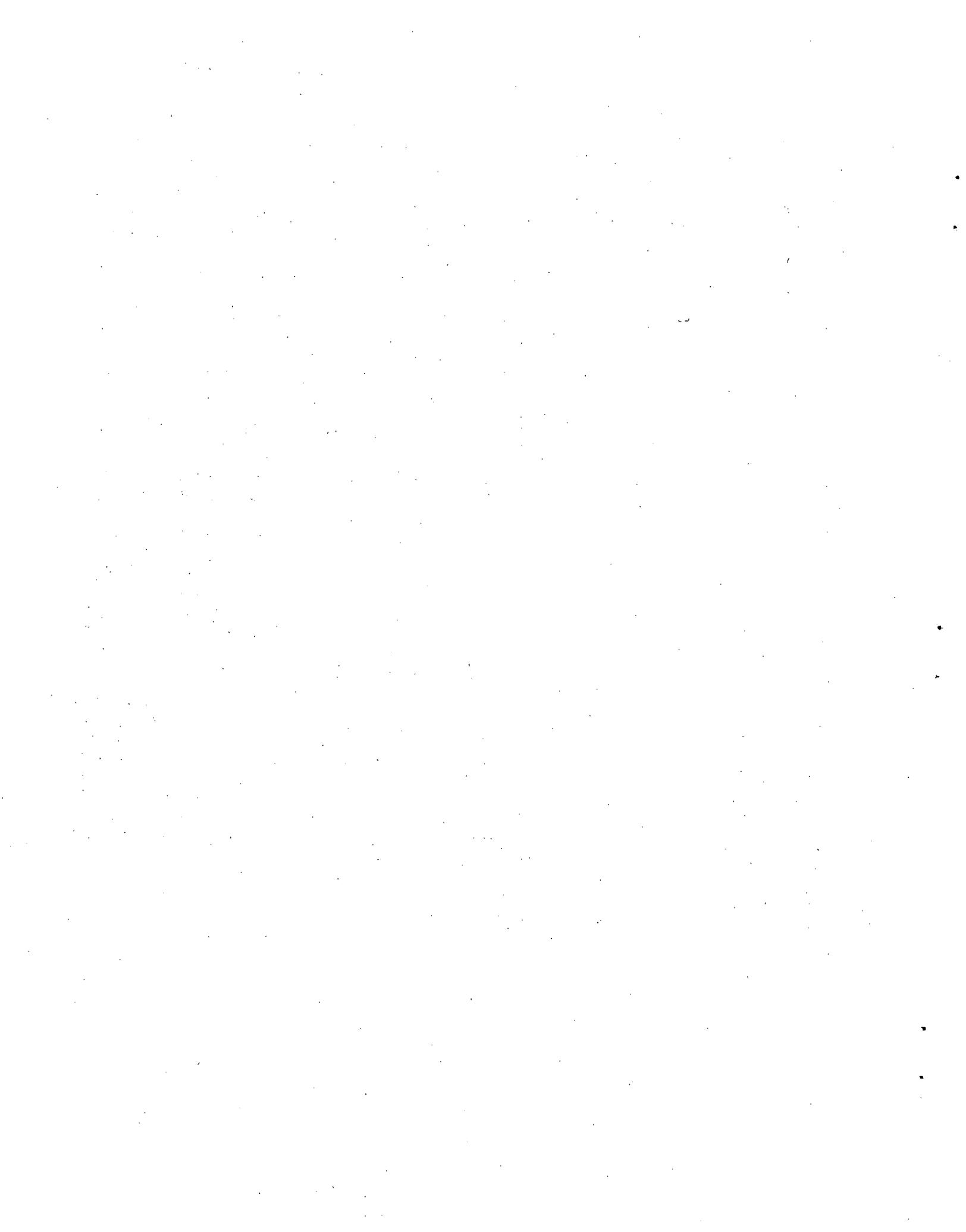
**Results.** — With the help of A. J. Fairbanks and J. S. Kirby-Smith, it has been shown that films of chloroplasts, after being dried and illuminated, give a strong signal in the paramagnetic resonance apparatus at the correct frequency and magnetic field for unpaired electrons. The signal is reduced to zero by heating the sample to 140°C in the dark. Curves recently published<sup>12</sup> show that a temperature of 140°C will free essentially all the stored light.

**Discussion.** — The finding that heating to 140°C frees the stored light and eliminates the paramagnetic signal suggests that the stored energy may be in the form of a free radical, an excited triplet, or a trapped electron.

<sup>11</sup>W. A. Arnold and H. Sherwood, *Biol. Semiann. Prog. Rep. Aug. 15, 1956*, ORNL-2155, p 56.

<sup>12</sup>W. Arnold and H. K. Sherwood, *Proc. Natl. Acad. Sci. U. S. 43*, 105-114 (1957).

<sup>10</sup>W. D. McElroy and A. A. Green, *Arch. Biochem. and Biophys.* **56**, 240-255 (1955).



## MICROBIOLOGY

### TRACER STUDIES ON INTERMEDIARY METABOLISM

S. F. Carson            E. F. Phares  
M. I. Dolin            R. E. Koeppe<sup>1</sup>  
M. V. Long

#### DPNH Oxidation by *Clostridium perfringens*

M. I. Dolin

**Introduction.** — The problem of anaerobiosis in bacteria is still unsettled. None of the explanations proposed to account for the inability of strict anaerobes to grow aerobically have been adequate.<sup>2</sup> It appeared that a reasonable approach to at least one aspect of the problem might be to characterize as completely as possible the electron transport reactions of those facultative and strict anaerobes that are capable of respiring in oxygen; and to investigate the mechanisms controlling the peroxide level. One such mechanism in the facultative anaerobe *Streptococcus faecalis* involves the function of two separate enzymes, a flavoprotein DPNH oxidase and a flavoprotein DPNH peroxidase (see Appendix for alphabetical list of abbreviations). This work has been summarized previously.<sup>3,4</sup> The present report deals with a DPNH oxidase system in the strict anaerobe *Clostridium perfringens*.

**Results.** — Soluble, nonparticulate extracts of *Clostridium perfringens* catalyze a rapid, cyanide-insensitive, oxidation of DPNH to DPN. The oxidation rate is the same in oxygen-saturated solution as it is in a solution that is in equilibrium with atmospheric oxygen. Peroxide is not formed in this oxidation nor is added peroxide decomposed in the presence or absence of DPNH. Peroxide, at a level of  $10^{-5}$  M, causes a 65% inhibition of the rate of oxidation. The absence of peroxide as a product of the oxidation is not attributable to a dialyzable component in the system, since peroxide is not formed even after the following treatments of the enzyme: dialysis, ammonium sulfate fractionation, or partial purification by zone electrophoresis. However, in the presence of FAD

(which does not affect the rate of DPNH oxidation), peroxide does accumulate during oxidation, and the amount formed depends on the FAD level. At a concentration of  $10^{-5}$  M FAD, 50% of the theoretical amount of peroxide is formed. FMN and riboflavin do not replace FAD in this reaction. With partially purified extracts, menadione, at a level of  $3 \times 10^{-4}$  M, causes a twofold stimulation of the rate of DPNH oxidation, and two-thirds of a mole of peroxide is formed per mole of DPNH oxidized.

In the presence of DPNH, enzyme activity decays with first-order kinetics. Neither oxidized DPN nor the primary acid modification product of DPNH is inhibitory. With the crude enzyme, cytochrome c also functions as an electron acceptor. The cytochrome c reductase appears to be a different enzyme from the DPNH oxidase.

**Discussion.** — The results above indicate that *C. perfringens* contains a soluble enzyme system that catalyzes a four-electron oxidation of two molecules of DPNH to 2DPN and 2H<sub>2</sub>O. Free peroxide is not formed unless the reaction is coupled to autoxidizable compounds such as menadione or FAD. With the former, the peroxide formed can be accounted for on the assumption that the increased oxidation noted has all passed through menadione and may perhaps be attributed to an enzyme other than the DPNH oxidase. Present evidence suggests that FAD does not function with a separate enzyme, but with the oxidase itself. These results may explain reports in the literature that the peroxide concentration formed in oxidations catalyzed by various anaerobes is highly variable, sometimes even with different batches of cells of the same organism.<sup>5</sup> It is not clear at present whether the prosthetic group of the oxidase is a flavin or whether one or two enzymes are involved in the oxidation. With one enzyme, a mechanism involving two molecules of bound DPNH might be pictured, while a one- or two-enzyme reaction might involve bound peroxide as an intermediate.

The inactivation of the oxidase by its substrate, DPNH, may offer an explanation for the fact that oxidative metabolism is not important in the physiology of *C. perfringens*.

<sup>1</sup>Research Participant.

<sup>2</sup>M. Stephenson, *Bacterial Metabolism*, Longmans, Green, New York, 1949.

<sup>3</sup>M. I. Dolin, *Biol. Semian. Prog. Rep. Aug. 15, 1956*, ORNL-2155, p 57-58.

<sup>4</sup>M. I. Dolin, *J. Biol. Chem.*, in press.

<sup>5</sup>I. Lieberman, *J. Bacteriol.* 68, 61-62 (1954).



## PLANT BIOCHEMISTRY

N. E. Tolbert  
L. P. Zill  
R. Rabson<sup>1</sup>

P. C. Kerr  
D. A. Mondon  
A. H. Haber<sup>2</sup>

A. W. Naylor<sup>3</sup>

## Phosphorylcholine in Plants

N. E. Tolbert                      P. C. Kerr

**Introduction.** — In a continuing study of phosphorus compounds in plants, one phosphate ester has been shown to have the unique properties of being transported in the xylem sap from the root to the leaves.<sup>4</sup> Recently<sup>5</sup> it has been identified as P-choline (see Appendix for alphabetical list of abbreviations), and at present its physiological distribution and properties are being studied to ascertain possible functions for it in plants.

**Results.**<sup>6</sup> — P-choline reservoirs in the plant amount to 5 to 30% of the total soluble phosphorus, including inorganic phosphate. P-choline is the only major ester in the barium-soluble-alcohol-soluble phosphorus fraction from plants. Changes in amounts of P-choline in the roots and leaves are similar to those for glutamine and asparagine. P-choline accumulates in roots of green or young etiolated plants and moves in the xylem sap to the leaf. As etiolated plants age, P-choline moves out of the roots and accumulates in large amounts in the dying leaves. There is almost complete  $P^{32}$  exchange within a few minutes to an hour after either labeled inorganic phosphate or P-choline is fed to a plant. Equally rapid labeling of P-choline with  $H_2C^{14}O$  and  $HC^{14}OOH$  and relatively slow labeling over one to three days with glycine- $C^{14}$  or  $C^{14}O_2$  photosynthesis suggest that the methyl groups also turn over at a rapid rate. Phospholipids are labeled substantially by  $P^{32}$  only after one or more days, suggesting that P-choline has other functions besides phospholipid synthesis. The similarities between the amides and the

P-choline reservoirs suggest that P-choline functions in phosphorus and methyl-group storage and transport. Rapid exchange of phosphate and methyl groups suggests that P-choline participates in transphosphorylation and transmethylation and that these reactions may be involved in phosphate transport.

## Ultraviolet Inhibition of Sucrose Synthesis in Plants

L. P. Zill                                      D. A. Mondon

It has been found that the mechanism of sucrose synthesis in the wheat plant is more sensitive to photochemical inhibition by ultraviolet light than is the initial fixation of carbon dioxide.<sup>7</sup> On the basis of Sinsheimer's<sup>8</sup> demonstration of photochemical instability of the uridylic acids and the demonstrated involvement of UDPG in sucrose synthesis by plants,<sup>9</sup> the effect of ultraviolet light on UDPG has been investigated. At a dose of ultraviolet light of 35 ergs/mm<sup>2</sup>/sec, predominantly at 2537 Å, an exponential destruction of the absorption at 260 mμ was observed with a half life of approximately 5 min. This half life is of the same order of magnitude as the inhibition of sucrose synthesis in the plant upon exposure to ultraviolet light of the same intensity. This evidence supports, but does not prove, the destruction of UDPG by ultraviolet light as the basis for inhibition of sucrose synthesis in the green plant. Uridine diphosphate and triphosphate were found to exhibit an almost identical sensitivity to ultraviolet light as did UDPG. The loss of absorption at 260 mμ could, in all compounds studied, be reversed by addition of acid until the pH was 1, similar to Sinsheimer's observations with the uridylic acids.

<sup>1</sup>Temporary employee.

<sup>2</sup>Research Associate.

<sup>3</sup>Research Participant.

<sup>4</sup>N. E. Tolbert and H. Wiebe, *Plant Physiol.* 31, 499-504 (1955).

<sup>5</sup>J. V. Maizel, A. A. Benson, and N. E. Tolbert, *Plant Physiol.* 31, 407-408 (1956).

<sup>6</sup>Abstract to be published in *Federation Proc.*, 1957.

<sup>7</sup>L. P. Zill and N. E. Tolbert, *Biol. Semian. Prog. Rep. Aug. 15, 1955*, ORNL-1953, p 101-103.

<sup>8</sup>R. L. Sinsheimer, *Radiation Research* 1, 505-513 (1954).

<sup>9</sup>C. E. Cardini, L. F. Leloir, and J. Chiriboga, *J. Biol. Chem.* 214, 149-155 (1955).

No inhibition of the formation of sucrose by a purified preparation of UDPG fructose transglycosylase could be demonstrated when ultraviolet-irradiated UDPG was used as substrate. It therefore becomes probable that the inhibited reaction is that in which UTP is regenerated from UDP enzymatically in the presence of adenosine triphosphate. Such an inhibition should lead to an accumulation of UDP and ATP, a phenomenon which has been reported for ultraviolet-irradiated *E. coli*.<sup>10</sup>

#### Photosynthesis in Gibberellin-treated Leaves

A. H. Haber

N. E. Tolbert

**Introduction.** — Major effects of the newly discovered class of plant growth hormones, gibberellins, are increased rates of growth in size and weight. Such growth could be the result of either an increased rate of photosynthesis or a more efficient or altered utilization of the photosynthetic products. To explore these possibilities, the rate of  $C^{14}O_2$  fixation and the distribution of  $C^{14}$  among the products from short-time photosynthesis in gibberellin-treated plants have been studied.

**Results.** — Experiments were run with a number of species from a few hours to nine days. Gibberellin treatment caused about 50 to 70% increase in average height of pea plants a few days after treatment. It did not enhance the rate of  $CO_2$  fixation per unit weight of leaf tissue and did not alter the general pathways of short-time metabolism of the newly fixed  $C^{14}O_2$  in the sugar, organic acid, and amino acid products.

<sup>10</sup>D. Kanazir, *Bull. Inst. Nuclear Sci. (Boris Kidrich)* 5, 119-122 (1955); cf. *Chem. Abstracts*, 10853i (1956).

A brief paper on the details of this investigation has been accepted for publication in *Plant Physiology*.

#### Aspartic Acid Metabolism in Plants

R. Rabson

A. W. Naylor

N. E. Tolbert

P. C. Kerr

**Introduction.** — In a previous investigation<sup>11</sup> on the metabolism of aspartic acid, the products from aspartic-U- $C^{14}$  acid in leaves were examined under a wide variety of physiological conditions. Although aspartic acid was readily converted in the leaf into other compounds of the citric acid cycle (e.g., malic and citric acids), asparagine was only infrequently formed in small amounts when leaves were in the dark, and  $\beta$ -alanine was never labeled. Because of the latter results, this project has been extended to a wider variety of plant tissues including roots and stems.

**Results.** — Aspartic-2,3- $C^{14}$  acid was converted to citric acid cycle products in roots, stems, and leaves of all plants tested. Its rate of conversion to malic acid was often exceedingly rapid. Only in the roots was asparagine- $C^{14}$  readily synthesized from aspartic acid during experiments lasting up to 6 hr. In no case were detectable amounts of  $C^{14}$ -labeled  $\beta$ -alanine formed. The amounts of glutamine and asparagine found labeled in the various plants differed widely.  $\gamma$ -Aminobutyric acid was found to contain a large proportion of the  $C^{14}$  from aspartate in stems of the plants examined, indicating an active glutamic decarboxylase system. Detail tables of these results are being prepared for publication. Other precursors for  $\beta$ -alanine synthesis are being sought.

<sup>11</sup>N. E. Tolbert, A. W. Naylor, and L. P. Carter, *Plant Physiol.* 31, Suppl., X (1956).

## BIOPHYSICS

MOLECULAR BIOPHYSICS AND RADIOLOGICAL  
PHYSICS

J. S. Kirby-Smith

J. N. Dent <sup>1</sup>	M. L. Randolph
A. C. Fabergé <sup>2</sup>	D. L. Parrish
W. G. Lynn <sup>3</sup>	A. J. Fairbanks <sup>4</sup>

## Average LET Values for Fast Neutrons

M. L. Randolph

**Introduction.** — Many radiobiological studies involve the concept that the RBE (see Appendix for alphabetical list of abbreviations) of various radiations for specific biological effects is primarily determined by the LET produced in the irradiated tissue. Except in cases of monoenergetic charged-particle irradiations of materials that are thin compared with the range of the initial particles, a wide distribution of LET values applies throughout the irradiated material. Hence in most irradiation circumstances, no single number can completely characterize the LET distribution. However, a well-defined average LET value may be a sufficient single-number characterization for many problems in radiobiology. Two such possible averages are the track average,

$$\bar{L}_x = \frac{\int L\psi(L) dL}{\int \psi(L) dL},$$

and the energy average,

$$\bar{L}_E = \frac{\int L\phi(L) dL}{\int \phi(L) dL},$$

where  $\psi(L) dL$  and  $\phi(L) dL$  represent the fractions of total track length and total energy of ionizing particles passing through the LET range  $dL$ . This investigation has been directed primarily to the accurate determination of these averages for first-collision doses of fast neutrons.

**Results.** — Starting from the distribution functions of Boag,<sup>5</sup> methods of calculation of  $\bar{L}_x$  and

$\bar{L}_E$  have been developed in detail to include all the heavy products from elastic and inelastic neutron interactions with all the important elements of tissue. The methods include the results of studies on the energy dissipation of nuclei with energies less than the energy at the Bragg peak of ionization.<sup>6</sup> Average values for eight monoenergetic fast-neutron energies and for two wide spectra (former biology facilities at the ORNL 86-in. cyclotron, using Sheppard's estimate of the neutron energy distribution<sup>7</sup>) have been calculated but are erroneously high, particularly for  $\bar{L}_E$  at 14 Mev, because of the false assumption that all elastic scattering of neutrons is isotropic in center-of-mass coordinates.

**Discussion.** — Precise calculations of LET values for neutron irradiations are complicated by (1) the multiplicity of ionizing products produced, (2) the uncertainty in energy dissipation rates for slow-moving ions, and (3) the uncertainties in the energies of heavy recoils. These problems become increasingly significant at neutron energies above 5 Mev. However, nuclear data presently available seem to make possible significant calculations at neutron energies of 14 Mev and below 5 Mev. The first complication mentioned is handled by taking into account all elastic and energetically possible inelastic products. The importance of inelastic nuclear products has been demonstrated previously.<sup>8</sup> The second complication is reduced by the use of Snyder and Neufeld's results<sup>6</sup> for low-energy protons and all heavy recoils. The third complication arises primarily from the uncertainty in angular distribution of elastically scattered neutrons. The sparse data on these differential cross sections, which are being assembled, will form the basis for the last correction to the present values. Preliminary calculations indicate that this will markedly reduce our present values of  $\bar{L}_E$  at 14 Mev.

<sup>1</sup>Consultant.<sup>2</sup>Temporary employee.<sup>3</sup>Research Participant.<sup>4</sup>Research Associate.<sup>5</sup>J. W. Boag, *Radiation Research* 1, 323-341 (1954).<sup>6</sup>W. S. Snyder and J. Neufeld, *Radiation Research* 6, 67-78 (1957).<sup>7</sup>C. W. Sheppard et al., *Radiation Research* 6, 173-187 (1957).<sup>8</sup>M. L. Randolph, *Biol. Semiann. Prog. Rep.* Feb. 15, 1956, ORNL-2060, p 58.

Although probably more workers in radiobiology<sup>9,10</sup> have used the concept of the track-average LET, some workers<sup>11</sup> have used the concept of the average energy. In particular cases, as Boag<sup>5</sup> has mentioned, arguments can be made that the more significant value is the track average, whereas in other cases it is the energy average. Whenever there is a distribution of LET values the energy average is greater than the track average.

### Calibration of the Chronic Facility Cs<sup>137</sup> Gamma-Ray Source

D. L. Parrish      M. L. Randolph

**Introduction.** — A 5-curie Cs<sup>137</sup> gamma-ray source with a 33-year half life and an energy<sup>12</sup> of 0.663 Mev has been installed and calibrated for the Mammalian Genetics and Development Section. This installation was designed to accommodate mice in their normal laboratory environment while being irradiated with gamma rays.

**Results.** — Average dose rates were found for various positions of mouse cages. A spatial distribution of the gamma-ray emission of the source was determined. The total emission of the source was also determined.

**Discussion.** — The polyethylene ion chamber with flowing filtered air, and described by Darden and Sheppard,<sup>13</sup> was used for primary measurements. The absolute calibration of this chamber by use of the Bragg-Gray principle, measured volume and calibrated capacitor, agreed to within 1% of the value obtained by comparison with a standard radium source calibrated by the National Bureau of Standards. Inverse-square measurements vs the Cs<sup>137</sup> source were performed along two axes, one vertical and one on an angle of 26 deg with the horizontal, to a distance of about 100 cm.

Relative distance measurements were made to at least  $\pm 0.1$  cm, and the data were analyzed by the method developed by Slater *et al.*<sup>14</sup> Ion-chamber measurements were made at various standard mouse-cage positions in the source room. These measurements were adequate to a distance of about 3 m from the source.

Du Pont type 502 film packs were used for the determination of the spatial distribution of the source and served as another means in arriving at the dose rate in various mouse-cage positions. The films were processed and densities were read through the courtesy of the Film Section of the Applied Health Physics Group. Inverse-square calibrations were made for the film along the same axes as those used for the ion chamber. Density calibrations of the film were also made with the radium standard as well as with the Cs<sup>137</sup> source itself.

The mouse cages were located in groups at four different distances, namely,

1. the "umbrella" — a semispherical rack supported at 182 cm from the source,
2. first-row floor racks at an average distance of 270 cm,
3. second-row floor racks at an average distance of 380 cm,
4. third-row floor racks at an average distance of 595 cm.

Average dose rates (from about 60 to 440 r/hr) were determined for these four distances. Several locations were noted to have decreased dose rates: in particular, the lower mouse-cage level of each of the three floor racks.

Further studies are being conducted with an ion chamber of larger volume to establish better the dose rate at the greater distances and to recheck the spatial distribution of the source.

### Cockcroft-Walton Accelerator

D. L. Parrish      M. L. Randolph

**Introduction.** — The Cockcroft-Walton accelerator serves the Biology Division as a well-characterized facility for low-level irradiations by monoenergetic fast neutrons with minimum gamma-ray contamination.

**Results.** — Forty irradiations of biological materials have been performed in the last six months without the loss of a single run because of failure

<sup>9</sup>R. E. Zirkle, p 315-350 in *Radiation Biology* (ed. A. Hollaender), McGraw-Hill, New York, 1954.

<sup>10</sup>J. W. Boag, *The Relative Biological Effectiveness of Different Ionizing Radiations*, NBS Report 2946, (1953), p 20-21.

<sup>11</sup>G. S. Hurst, *et al.*, *Radiation Research* 4, 49-64 (1956).

<sup>12</sup>*Nuclear Data*, NBS Circular 449, Suppl. 2, July 1950 to January 1951.

<sup>13</sup>E. B. Darden, Jr., and C. W. Sheppard, *A Thimble Type Gamma-Ray Dosimeter and the Measurement of the Radiation from Lumped and Distributed Type Sources*, ORNL-1002 (July 9, 1950).

<sup>14</sup>M. Slater *et al.*, *Radiation Research* 5, 604 (1956).

of the accelerator or dose monitors. Neutron studies carried on in cooperation with various other groups in the Division have included cataract formation in mice, acute mortality in mice, recovery of mice from acute radiation injury, chromosome restitution in *Vicia faba*, dominant and recessive lethality in *Drosophila*, effects on leukemia in mice, free radical formation in cysteine, and effect on root growth in timothy seedlings. Several of these studies are discussed elsewhere in this report.

**Discussion.** — The Cockcroft-Walton accelerator has been found to be a satisfactory facility for a wide variety of low-level fast-neutron irradiation studies. It is possible to do acute lethality studies on mice with this machine (doses up to 1200 rads) with reliable dosimetry, or to achieve detectable free radical formation (doses up to 20,000 rads) in one working day. The dependability of the accelerator is the result of the attention given to ion source design and assembly (largely due to the generous cooperation of C. D. Moak of the Physics Division), high-voltage stability, target cooling design, and zirconium tritide target production (by B. J. Massey of the Radioisotope Department of the Operations Division). The dependability of the monitoring system is the result of a routine of intercomparison throughout each run between two stable independent monitors, each checked vs separate standard sources before and after each run. With this system plus a complete spare electronic counting system it has been possible to quickly resolve such discrepancies between monitors as have occasionally arisen.

The gamma-ray contamination accompanying the 14-Mev neutron flux has been measured by using the counter-chamber described previously,<sup>15</sup> the proper operation of which is now understood, and by using inverse-square measurements with photographic emulsion. At 15 and 5 cm from the target, where biological irradiations are performed, the gamma-ray dose is not more than 5% of the total dose.

#### Paramagnetic Resonance Studies of Radiation-induced Free Radicals in Biochemical and Biological Systems

J. S. Kirby-Smith

A. J. Fairbanks      M. L. Randolph

**Introduction.** — The development of paramagnetic resonance spectroscopy has now reached such a

point that the applicability and usefulness of this technique to many problems in radiation biology are unquestionably assured.<sup>16,17</sup> In this laboratory, following the acquisition from the Stable Isotopes Division of a magnet and power supply of excellent field uniformity and stability and the essential completion of the major microwave instrumental phases of the project, the application of these methods to a number of specific problems is well under way.

The exploratory work completed and the studies currently in progress may be broken down into two major categories. The first of these deals primarily with what may be called the radiological physics aspects of free-radical production, that is, radical production as a function of dose, intensity, and LET of applied radiation, as well as a determination of the minimum detectable number of radicals. The second class of investigations is concerned primarily with the role of radiation-induced radicals in several specific biochemical or biological systems.

**Physical Characterization of Radical Production.** — The effect of LET on radical production has been studied by using Co<sup>60</sup> gamma rays, 14-Mev D-T neutrons, and the 5.3-Mev alpha particles from polonium. The relative effects of these radiations per unit energy absorbed are shown in Table 16, taking the effectiveness of gamma rays to be unity. The compound irradiated was glycine. Additional studies of glycine and other compounds exposed to gamma rays have

<sup>15</sup>M. Slater, G. B. Bunyard, and M. L. Randolph, *Biol. Semiann. Prog. Rep.* Aug. 15, 1956, ORNL-2155, p 61-63.

<sup>16</sup>W. Gordy, W. B. Ard, and H. Shields, *Proc. Natl. Acad. Sci. U.S.* 41, 983-996 (1955).

<sup>17</sup>*Ibid.*, p 996-1004.

Table 16. Dependence of Radical Production in Glycine on LET

Radiation	Relative Effect	LET (track av in kev/ $\mu$ )
Co <sup>60</sup> gamma rays	1.0	0.27
14-Mev D-T neutrons	0.7	12
5.3-Mev polonium alpha particles	0.1	130

shown that the radicals produced by 5000 r are readily detectable.

**Paramagnetic Resonance in Irradiated DNA and *Tradescantia* Pollen.** — Dry films of purified, highly polymerized calf thymus DNA obtained from the chemistry and general physiology groups in the laboratory have been irradiated with gamma rays, alpha particles, and ultraviolet radiation in the range 2400 to 3600 Å. No resonances indicating free-radical formation were detected for gamma-ray or alpha-particle radiation at dose levels up to  $10^7$  rad. Heavy doses of ultraviolet rays, approximately  $10^8$  ergs/cm<sup>2</sup>, did result in a small but definite free-radical production of long half life. No fine structure has yet been observed in this resonance. Commercial DNA from herring sperm exposed to ionizing radiation does show a non-specific resonance typical of most proteins which is presumed to be due to the protein impurities known to be present in these samples.

*Tradescantia* pollen treated with alpha particles, x rays, and gamma rays shows a very definite but weak resonance with some structure. To date it has not been possible to correlate this fine structure with the known patterns of pure compounds. A search for such correlation is in progress. Contrary to the usual very long half-life times (up to months in some cases) of the radicals produced in many pure compounds and dry biological materials, the patterns or radicals produced in the *Tradescantia* pollen appear to have a half life of the order of 30 min.

**Detection of Free Radicals in Irradiated Bacteria and Protective Compounds.** — Bacterial cells were protected against the lethal damage of ionizing radiation by prior incubation with the protective compound AET.Cl. These cells, along with the proper controls, were frozen in liquid nitrogen, dried under vacuum overnight, and examined for free-radical content. Evidence of differences in the resonance patterns of protected and non-protected cells was found on several occasions, although some recent results appear to be negative. Limitations of the method such as techniques of preparation of the material for analysis, necessity for using a small sample, and sensitivity of the apparatus have made reproducibility of the results quite difficult. Efforts are now being directed toward overcoming these obstacles.

Resonance studies of the pure protective compound show that no resonance is detectable in the

unirradiated material. After irradiation a readily detectable, reproducible, and long-lived radical can be shown. The nature of the particular salt influences the details of the pattern obtained.

**Discussion.** — The results of the LET studies show qualitatively what would be expected for direct chemical effects, namely, that there is considerable wastage of ionization so far as radical production is concerned for the higher ion density radiation such as neutrons and alpha particles. The figure of 5000 r required for a detectable resonance to be achieved with the present apparatus is very promising and indicates very clearly that this method is sufficiently sensitive to be applied directly to studies of bacterial killing and inactivation as well as to the more usual studies of radiation chemistry.

The preliminary measurements made on irradiated bacteria and protective compounds, as well as for DNA and *Tradescantia* pollen, show quite definitely that the magnetic-resonance approach can be expected to be of considerable value in radiobiological studies of these systems. In particular, in the case of comparative studies of the action of ultraviolet and ionizing radiation in chromosome breakage, these exploratory studies give support to the simple hypothesis that ionizing radiation acts directly on the protein in the chromosome, while ultraviolet radiation acts directly on the DNA fraction. The primary activation of the DNA component by ultraviolet radiation is clearly shown by the observation of radicals. More information concerning the energy transfer from DNA to protein molecules is necessary for formulation of a more specific hypothesis. Studies of radical production in DNA solution and in gels are being planned.

#### Nature of Broken Chromosome Ends Produced by Alpha Particles

A. C. Fabergé

**Introduction.** — Among methods of studying induced chromosome breaks and aberrations, the analysis of maize endosperm mosaics has the unique property of distinguishing between stable and unstable chromosome ends.<sup>18</sup> In genetic experiments with most organisms, newly created stable chromosome ends are not recovered, which

<sup>18</sup>A. C. Fabergé, *Z. indukt. Abstammungs- u. Vererbungslehre* 87, 392-420 (1956).

long ago led Muller<sup>19</sup> to postulate that a natural chromosome end is a special terminating particle or telomere. Under special conditions, however, mechanically generated chromosome breaks in maize zygotes can be stabilized, by a mechanism which is not understood. In maize endosperm there is no indication that stable chromosome ends are ever produced, either by x rays, by ultraviolet rays, or by ethylene oxide.<sup>18-20</sup> In *Tradescantia*, alpha particles preponderantly give aberrations of a type suggesting that broken ends are unrejoinable. For these reasons an effort was made to treat pollen of the critical maize material with plutonium alpha particles, even though their penetration barely exceeds one-third the diameter of a pollen grain.

**Results.** — A sufficient number of ears from this treatment have been produced but have not yet been analyzed. Preliminary examination shows, however, that at least a great many unstable broken chromosome ends are produced by alpha particles.

#### Relation Between Several Breakage-Fusion-Bridge Cycles in One Nucleus

A. C. Fabergé

Material obtained from the experiments already described<sup>21</sup> has been examined. No instances of fusion of two chromatid-type breakage-fusion-bridge cycles into a single chromosome type breakage-fusion-bridge cycle have been found. The rule is, then, that the broken chromosome ends generated by the breaking of anaphase bridges do not interact with one another.

#### Comparison of the Effects of Goitrogens on Thyroid Activity in *Triturus viridescens* and *Desmognathus fuscus*

J. N. Dent

W. G. Lynn

**Introduction.** — Salamanders of the genus *Triturus* have been widely used in studies of the histology, cytology, seasonal variation, and responses of the thyroid gland. Relatively few investigations of these matters have been made for members of other urodele genera. It is questionable whether findings based upon study of an exclusively aquatic newt are entirely applicable to terrestrial urodeles.

There is, in fact, some indication in the literature that the thyroid of *Triturus* may differ significantly in at least one respect from that of the terrestrial salamander, *Desmognathus*, namely, in the histological changes elicited by treatment with the goitrogenic drug thiourea. This investigation is a comparison of the responses of these animals to administration of two goitrogens, thiourea and potassium perchlorate. The comparison is based upon the thyroid histology and the uptake of radioiodine by the gland.

**Results.** — Examination of the sectioned thyroids of control animals reveals that, at the same season of the year and under the same temperature conditions, these two urodeles have characteristic differences in the level of thyroid activity — that of *Triturus* being lower than that of *Desmognathus*. Treatment with thiourea for 30 days or for 46 days results in marked hypertrophy and hyperplasia of the thyroid in *Desmognathus* but causes nothing more than a slight hyperemia in *Triturus*. Potassium perchlorate is even more effective than thiourea in *Desmognathus*, causing a great increase in epithelial height and complete loss of intra-follicular colloid. The thyroids of *Triturus* given perchlorate treatment are not significantly different from those of controls.

The uptake of I<sup>131</sup> by the thyroids of control animals follows a similar pattern in the two species. The maximum uptake occurs 6 to 8 hr after injection of a tracer dose of I<sup>131</sup>, and more than 50% of the peak quantity is still retained at 60 hr. The level of uptake is, however, consistently higher in *Desmognathus* than in *Triturus*. Perchlorate treatment results in complete inhibition of the thyroid's ability to concentrate radioiodine in both species. Thiourea treatment causes an increase in the uptake of I<sup>131</sup> in the thyroid of *Desmognathus* but produces no effect in *Triturus*.

**Discussion.** — Both on the basis of histological appearance and ability to concentrate radioiodine, the thyroid of *Triturus* has a lower level of activity than that of *Desmognathus*. In *Desmognathus* the effects of treatment with thiourea and with perchlorate ion upon radioiodine uptake and thyroid histology follow the pattern which would be expected on the basis of work on mammals. *Triturus* appears to be refractory to thiourea, and its response to perchlorate seems anomalous, in that, although radioiodine uptake is completely inhibited, no histological changes are discernible. This may

<sup>19</sup>H. J. Muller, *Collecting Net* 13, 181-195 (1938).

<sup>20</sup>A. C. Fabergé, *Genetics* 40, 571 (1955).

<sup>21</sup>A. C. Fabergé, *Biol. Semiann. Prog. Rep. Aug. 15, 1956*, ORNL-2155, p 64.

indicate that thyrotropin production is unusually low in this salamander. The low thyroid activity and the lack of histological response in *Triturus* may be of importance in connection with the animal's ability to stand a wide temperature range, a feature which is of some significance in the shallow, exposed ponds which form its natural habitat.

**Phenylthiourea Treatment and Binding of Radioactive Iodine in the Tadpole**

W. G. Lynn

J. N. Dent

**Introduction.** — In a study of the distribution of  $I^{131}$  after administration to tadpoles, Dent and Hunt<sup>22</sup> demonstrated not only the expected concentration of this substance in the thyroid gland but also significant accumulations in the thymus, the horny teeth, the melanophores of the skin, and the pigmented layer of the retina. Phenylthiourea effectively inhibits melanogenesis, and its administration to young anuran larvae results in completely unpigmented tadpoles (Millott and Lynn<sup>23</sup>). Its use serves, therefore, as a means of studying the basis for the accumulation of radioiodine by pigmented tissues.

**Results.** — Tadpoles raised in either 0.005 or 0.01% solutions of phenylthiourea from the time of hatching lose all pigment in both skin and retina within two days. Larvae put in these solutions at later stages (4 and 15 days after hatching) lose the skin pigment much more slowly, and the retinal pigment may be retained indefinitely. Autoradiographic analysis of the binding of  $I^{131}$  by such larvae was made at three different intervals

after the beginning of treatment. It was found that continuous administration of phenylthiourea at either of the concentrations tested completely inhibits  $I^{131}$  binding in the skin and tapetum nigrum, regardless of whether formed melanin is still present in these tissues. Cessation of phenylthiourea treatment is followed by recovery of some ability to concentrate  $I^{131}$  within 24 hr, although the uptake at that time is far lower than that in untreated controls.

Since phenylthiourea also has a goitrogenic activity, the familiar histological changes in the thyroid resulting from its administration can be seen in the experimental tadpoles. The autoradiograms indicate an extremely low concentration of bound  $I^{131}$  in the thyroids of continuously treated animals. Cessation of treatment is followed by prompt recovery of the ability to bind iodine, so that within 24 hr the level in the thyroid reaches that found in controls. Radioiodine binding by the thymus follows precisely the pattern just described for the thyroid.

**Discussion.** — The evidence indicates that the binding of radioiodine by pigmented tissues is not associated with formed melanin. It occurs during melanogenesis and is doubtless associated with tyrosinase activity in these tissues. Tyrosinase is involved in melanogenesis and may also be the enzyme concerned in the formation of iodinated proteins in pigmented regions. Phenylthiourea is known to inhibit tyrosinase activity in vitro. Whether the effect of phenylthiourea upon thyroid activity rests upon this same basis is doubtful. The presence of tyrosinase in the thyroid has never been demonstrated, and the much more rapid recovery of iodine-binding ability by the thyroid (and thymus) as compared with that by pigmented tissues may indicate that the mechanism of action of phenylthiourea differs in these regions.

<sup>22</sup>J. N. Dent and E. L. Hunt, *J. Exptl. Biol.* 121, 79-97 (1952).

<sup>23</sup>N. Millott and W. G. Lynn, *Quart. J. Microscop. Sc.* 95, 17-25 (1954).

## APPENDIX

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

ABT - *S*,4-aminobutylisothiourea  
 AED - 2-aminoethyldisulfide  
 AET - *S*,2-aminoethylisothiuronium bromide hydrobromide  
 AET·Cl - *S*,2-aminoethylisothiuronium chloride hydrochloride  
 AMP - adenosine monophosphate  
 APT - *S*,3-aminopropylisothiuronium bromide hydrobromide  
 ATP - adenosine triphosphate  
 CoA - coenzyme A  
 DCC - dicyclohexylcarbodiimide  
 DNA - deoxyribonucleic acid  
 DNAse - deoxyribonuclease  
 DNP - deoxyribonucleoprotein  
 DPN - diphosphopyridine nucleotide  
 DPNH - reduced diphosphopyridine nucleotide  
 FAD - flavinadenine dinucleotide  
 FMN - flavin mononucleotide  
 GBT - 4-guanidinobutylisothiourea  
 GET - 2-guanidinoethylisothiourea  
 HBM - homologous bone marrow  
 HeBM - heterologous bone marrow  
 IBM - isologous bone marrow  
 LET - linear energy transfer  
 MBA - 4-mercaptobutylamine  
 MBG - 4-mercaptobutylguanidine  
 MEA - 2-mercaptoethylamine  
 MEG - 2-mercaptoethylguanidine  
 MPG - 3-mercaptoethylguanidine  
 P-choline - phosphorylcholine  
 PP - pyrophosphate  
 RBC - red blood cells  
 RBE - relative biological effectiveness  
 RES - reticuloendothelial system  
 RNA - ribonucleic acid  
 RNAse - ribonuclease  
 UDP - uridinediphosphate  
 UDPG - uridinediphosphate glucose  
 UTP - uridinetriphosphate