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SEMIANNUAL PROGRESS REPORT

for Period Ending August 15, 1956

Alexander Hollaender, Director

Stanley F. Carson, Assistant Director

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CONTENTS

PUBLICATIONS AND LECTURES 1

Publications 1
Scientific Society Lectures and Traveling Seminars 4
Biomedical Directors Meet in Oak Ridge 8
Visiting Lecturers 9
Speakers at Professional Societies, Fall 1956 10
A. Hollaender and S. Wolff Attend European Meetings 11
Education 11

CYTOLOGY AND GENETICS 13

Cytogenetic Effects of Radiation 13

Effect of Posttreatment with Streptomycin on X-Ray-Induced Mutation in *Paramecium aurelia* 13

Chromosomal Aberration Production by X Rays and by Monochromatic 2.5- and 14-Mev Neutrons 14

Noninteraction of X-Ray- and Neutron-Induced Chromosome Breaks 16

Anaphase Bridge Cycles in Maize 17

Synergism of Two Cytoplasmically Inherited Mutants in *Neurospora crassa* (Abstract) 18

Biochemical Studies of Cytoplasmically Controlled Synergism in *Neurospora crassa* 18

Recombination Studies with *ad*-3A Mutants in *Neurospora crassa* 19

Effects of Temperature on Growth of Timothy Roots 19

Insect Cytology and Genetics 20

Ultraviolet Radiation Effects on the *Habrobracon* Egg Cytoplasm 20

Effect of Oxygen on Radiation-Induced Mitotic Inhibition in the Neuroblast of the Grasshopper Embryo 22

Drosophila Genetics 22

Nonrandom Distribution of Induced Dominant Lethals Within the Genome of *Drosophila melanogaster* 22

Meiotic Behavior of Free-X-Chromosome Heterochromatic Duplications in *Drosophila melanogaster* 23

MAMMALIAN GENETICS AND DEVELOPMENT 25

Genetic and Developmental Effects of Radiation in Mice 25

Radiation-induced Mutations in the Mouse 25

Dominant Lethals Induced in Mouse Sperm by 14-Mev Neutrons from a Deuterium-Tritium Reaction 26

Sensitivity of Spermatogonia of the Mouse to 14-Mev Neutrons 27

Further Studies on the Notch Locus of *Drosophila melanogaster* 29

MICROBIAL PROTECTION AND RECOVERY 30

Radiation Protection and Recovery in Bacteria 30

Mechanism of Protective Action of Cysteamine on *Escherichia coli* 30

Effect of Cysteamine Pretreatment on Mutant Induction of Two *Escherichia coli* Auxotrophs 32

Pre- and Postirradiation Studies with Long-Ultraviolet and Short-Visible Radiations on *Escherichia coli* B/r 33

MAMMALIAN RECOVERY 35

Modification of Radiation Injury in Mice 35

Studies on Lethally Irradiated (950 r) Mice Treated with Rat Bone Marrow 35

Isologous vs Homologous Bone Marrow in the Treatment of Irradiation Injury 36

Effect of Bone-Marrow Treatment and Chemical Protection on the Bone-Marrow Response of X-Irradiated Mice 36

Homologous Leukemoid Blood in the Treatment of Irradiation Injury 37

Recovery of Lethally Irradiated Mice by Treatment with in Vitro Cultivated Bone-Marrow Cells 37

Short-Term Preservation of Mouse Bone Marrow at 2 to 5°C and Room Temperature for Irradiation Experiments 41

PATHOLOGY AND PHYSIOLOGY 42

Pathologic and Physiologic Effects of Radiation 42

Effects of Chronic Exposure to Fast Neutrons and Gamma Rays 42

Localization of S^{35}-Methionine-Labeled Bone-Marrow Cells Injected into Irradiated Mice 42

Pathogenesis of Radiation-Induced Myeloid Leukemia 44

Chemical Studies of Blood Platelets 44

CELL PHYSIOLOGY 45

Electrophoretic Characterization of Rat Serum Proteins 45

Cytotoxicity of Rabbit Antiserum Prepared Against the Soluble Cytoplasmic Fraction of Ehrlich Ascites Tumor Cells 45

Studies on Nucleoprotein Gels 46

A Critical Period in the Acute Lethal Response of the Chick Embryo to X Radiation 46

Electroconvection-Electrophoresis of Normal Rat Serum 47

BIOCHEMISTRY 48

Studies on Nucleic Acids, Enzymes, and Radiation-Protective Compounds 48

Structure and Activity in Radiation-Protective Sulfhydryl Compounds 48

Ion-Exchange Study of the Rearrangement of AET to MEG and 2AT 50

Chromatographic Detection of Differences Between Bacteriophage-Related Deoxyribonucleic Acids 51
Ribonucleic Acid Metabolism in T2-Infected *Escherichia coli* 52
Composition of *Escherichia coli* Ribonucleic Acids 53

ENZYMOLOGY AND PHOTOSYNTHESIS 54

Enzymology 54
One-Carbon Metabolism in Bacteria: The Exchange Between Pyruvate Carboxyl and Formate or Bicarbonate 54

Studies on the Mechanism of Protein Biosynthesis 54
Enzymatic Activation of Amino Acids 54
Incorporation of Amino Acids into Protein of Various Tissues 55

Photosynthesis 56

MICROBIOLOGY 57

Tracer Studies on Intermediary Metabolism 57
A Coupled Malic-Lactic Dehydrogenase from *Veillonella gazogenes* 57
Mechanism of Electron Transport Between Reduced Pyridine Nucleotides and Flavoprotein 57
Oxidation of *l*-Arabinose by *Pseudomonas saccharophila* 58

PLANT BIOCHEMISTRY 59

Sugar Metabolism in *Sedum* 59
Sedoheptulose in the *Coleus* Plant 59
Identification of Phosphoryl Choline as an Important Constituent of Plant Saps 60
Aspartic Acid Metabolism in Barley and Wheat Leaves 60
Anomalous Behavior of Orthophosphate-P^{32} on Anion Exchangers 60

BIOPHYSICS 61

Molecular Biophysics and Radiological Physics 61
Cockcroft-Walton Acceleration Activity 61
Bone-Marrow Treatment of Mice After Fast-Neutron Irradiation 61
Combination Ion Chamber–Proportional Counter Dosimeter 61
Comparative Study of Thyroid Function in Two Species of Salamanders, *Triturus viridescens* and *Desmognathus fuscus* 63
Relation Between Several Breakage-Fusion-Bridge Cycles in One Nucleus 64
Analysis of Published Data on Induced Radiation Effects in Maize 64

BIOLOGY DIVISION SEMIANNUAL PROGRESS REPORT

PUBLICATIONS AND LECTURES

Publications. – The Symposium on Structure of Enzymes and Proteins heads the list of long publications during the period. Forty-three papers, a semiannual report, and 31 abstracts and book reviews complete the list. Seventy-two papers and 38 abstracts and book reviews are in press.

Most of the manuscripts representing talks given at the Symposium on Biocolloids in Gatlinburg in April 1956 have been received and will be compiled and published as a supplement to the *Journal of Cellular and Comparative Physiology*. This will be the ninth conference supplement.

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Anderson, N. G.	A Note on "Homogenizers" for Tissue Brei Preparation (brief note)	*J. Biophys. and Biochem. Cytol.* 2, 219–220 (1956)
———	Techniques for the Mass Isolation of Cellular Components	*Physical Techniques in Biology* (ed., G. Oster and A. W. Pollister), vol 3, p 300–352, Academic Press, New York, 1956
Atwood, K. C.	Ultraviolet Effects on *Neurospora* Heterokaryons	*Proceedings of the First International Photobiological Congress* (4th Intern. Light Congr.), p 143–145, H. Veenman & Zonen, Wageningen
Auerbach, R.	Effects of Single and Fractionated Doses of X Rays on Mouse Embryos (communication to editor)	*Nature* 174, 574 (1956)
von Borstel, R. C., and H. Moser	Differential Ultraviolet Irradiation of the *Habrobracon* Egg Nucleus and Cytoplasm	*4th International Conference on Radiobiology, Cambridge, 1955* (ed., J. S. Mitchell, B. E. Holmes, and C. L. Smith), p 211–215, Oliver and Boyd Ltd., London, 1956
Cohn, W. E.	A New Concept of Ribonucleic Acids	*Currents in Biochemical Research* (ed., D. E. Green), p 460–473, Interscience, New York, 1956
Cohn, W. E., and D. G. Doherty	The Catalytic Hydrogenation of Pyrimidine Nucleosides and Nucleotides and the Isolation of Their Riboses and Respective Ribose Phosphates	*J. Am. Chem. Soc.* 78, 2863–2866 (1956)
Congdon, C. C., T. W. McKinley, Jr., H. Sutton, and P. Urso, Jr.	The Effect of Transfusions of Blood Showing Extreme Leukocytosis on Survival of X-irradiated Mice	*Radiation Research* 4, 424–434 (1956)
Conger, A. D.	Radiation Effects on Ascites Tumor Chromosomes	*Ann. N. Y. Acad. Sci.* 63, 929–937 (1956)
Craig, D. L., J. S. Kirby-Smith, and J. N. Dent	Mitotic Inhibition and Chromosome Breakage in Salamanders of the Genus *Gyrinophilus*	*Trans. Am. Microscop. Soc.* 75, 234–241 (1956)
Delwiche, E. A., E. F. Phares, and S. F. Carson	Succinic Acid Decarboxylation System in *Propionibacterium pentosaceum* and *Veillonella gazogenes*. I. Activation, Decarboxylation, and Related Reactions	*J. Bacteriol.* 71, 598–603 (1956)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Dent, J. N., E. L. Gadsden, and J. Furth	Further Studies on Induction and Growth of Thyrotropic Pituitary Tumors in Mice	*Cancer Research* 16, 171–174 (1956)
Dolin, M. I.	Evidence for an Enzyme Substrate Complex Between Pyridine Nucleotides and DPNH Peroxidase Flavoprotein (letter to editor)	*Arch. Biochem. and Biophys.* 60, 499–501 (1956)
Gude, W. D., A. C. Upton, and T. T. Odell, Jr.	Blood Platelets of Human and Rat: A Cytochemical Study	*Lab. Invest.* 5, 348–358 (1956)
Hollaender, A.	Modification of Radiation Response	*Proceedings of the International Conference on the Peaceful Uses of Atomic Energy*, vol 16, p 106–113, United Nations, New York, 1956
Hollaender, A., and S. F. Carson	Biology Division Semiannual Progress Report for Period Ending Feb. 15, 1956	ORNL-2060
———	Modification of Radiation Response (A Revision of Geneva Conf. Lecture)	*Bull. Atomic Scientists* 12, 76–80 (1956)
———	Preface (to the Proceedings)	*Proceedings of the First International Photobiological Congress* (4th Intern. Light Congr.), H. Veenman & Zonen, Wageningen
Hollaender, A. (ed).	Symposium on Structure of Enzymes and Proteins	*J. Cellular Comp. Physiol.* 47, Suppl. 1 (1956)
Hollaender, A., and R. F. Kimball	Modification of Radiation-induced Genetic Damage	*Nature* 177, 726–730 (1956)
Hollaender, A., and G. E. Stapleton	Studies on Protection by Treatment Before and After Exposure to X and Gamma Radiation	*Proceedings of the International Conference on the Peaceful Uses of Atomic Energy*, vol 11, p 311–314, United Nations, New York, 1956
Hollaender, A., and M. R. Zelle	The Use of Action Spectra for the Evaluation of Some Basic Biological Problems	*Proceedings of the First International Photobiological Congress* (4th Intern. Light Congr.), p 128–134, H. Veenman & Zonen, Wageningen
Makinodan, T.	Serological Studies on X-irradiated Animals. II. Circulating Rat Cells in Lethally Irradiated Mice Protected with Rat Bone Marrow	*Proc. Soc. Exptl. Biol. Med.* 92, 174–179 (1956)
Naylor, A. W., and N. E. Tolbert	Glutamic Acid Metabolism in Green and Etiolated Barley Leaves	*Physiol. Plantarum* 9, 220–229 (1956)
Phares, E. F., E. A. Delwiche, and S. F. Carson	Succinic Acid Decarboxylation System in *Propionibacterium pentosaceum* and *Veillonella gazogenes*. II. Evidence for an Active "C_1" Complex	*J. Bacteriol.* 71, 604–610 (1956)
Pittenger, T. H., and K. C. Atwood	Stability of Nuclear Proportions During Growth of *Neurospora* Heterokaryons	*Genetics* 41, 227–241 (1956)
Russell, L. B.	X-ray-induced Developmental Abnormalities in the Mouse and Their Use in the Analysis of Embryological Patterns. II. Abnormalities of the Vertebral Column and Thorax	*J. Exptl. Zool.* 131, 329–395 (1956)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Russell, L. B., and W. L. Russell	Hazards to the Embryo and Fetus from Ionizing Radiation	*Proceedings of the International Conference on the Peaceful Uses of Atomic Energy*, vol 11, p 175–178, United Nations, New York, 1956
———	The Sensitivity of Different Stages in Oogenesis to the Radiation Induction of Dominant Lethals and Other Changes in the Mouse	*4th International Conference on Radiobiology, Cambridge, 1955* (ed., J. S. Mitchell, B. E. Holmes, and C. L. Smith), p 187–192, Oliver and Boyd Ltd., London, 1956
Russell, W. L.	Comparison of X-ray-induced Mutation Rates in *Drosophila* and Mice	*Am. Naturalist* 90 (Suppl.), 67–80 (1956)
———	Genetic Effects of Radiation in Mice and Their Bearing on the Estimation of Human Hazards	*Proc. Intern. Conf. on the Peaceful Uses of Atomic Energy*, vol 11, p 382–383, United Nations, New York, 1956
———	Radiation in Mice – the Genetic Effects and Their Implications for Man	*Bull. Atomic Scientists* 12, 19–20 (1956)
St. Amand, W.	Mitotic Inhibition and Chromosome Breakage Induced in Grasshopper Neuroblasts by X Irradiation at Known Mitotic Stages	*Radiation Research* 5, 65–78 (1956)
Sarvella, P. A., and L. B. Russell	Steel, a New Dominant Gene in the House Mouse, with Effects on Coat Pigment and Blood	*J. Heredity* 47, 123–128 (1956)
Shaw, E. I.	Protection by Sodium Hydrosulfite Against X-ray-induced Mitotic Inhibition in the Grasshopper Neuroblast	*Proc. Soc. Exptl. Biol. Med.* 92, 232–236 (1956)
Sheppard, C. W.	Introductory Radiological Physics	*Therapeutic Use of Artificial Radioisotopes* (ed., P. F. Hahn), p 12–29, Wiley, New York, 1956
Stapleton, G. E., and C. W. Edington	Temperature Dependence of Bacterial Inactivation by X Rays	*Radiation Research* 5, 39–45 (1956)
Tolbert, N. E., and F. B. Gailey	Carbon Dioxide Fixation by Etiolated Plants After Exposure to White Light	*Plant Physiol.* 30, 491–499 (1956)
Tolbert, N. E., and H. Wiebe	Phosphorus and Sulfur Compounds in Plant Xylem Sap	*Plant Physiol.* 30, 499–504 (1956)
Upton, A. C., F. P. Conte, G. S. Hurst, and W. A. Mills	The Relative Biological Effectiveness of Fast Neutrons, X Rays, and Gamma Rays for Acute Lethality of Mice	*Radiation Research* 4, 117–131 (1956)
Upton, A. C., J. Furth, and W. T. Burnett, Jr.	Liver Damage and Hepatomas in Mice Produced by Radioactive Colloidal Gold	*Cancer Research* 16, 211–215 (1956)
Upton, A. C., G. S. Melville, Jr., M. Slater, F. P. Conte, and J. Furth	Leukemia Induction in Mice by Fast Neutron Irradiation	*Proc. Soc. Exptl. Biol. Med.* 92, 436–438 (1956)
Volkin, E., and L. Astrachan	Phosphorus Incorporation in *Escherichia coli* Ribonucleic Acid After Infection with Bacteriophage T2[1]	*Virology* 2, 149–161 (1956)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Volkin, E.	Phosphomonoesterase of Bone	*Methods in Enzymology* (ed., S. P. Colowick and N. O. Kaplan), vol II, P 539–541, Academic Press, New York, 1955
Whiting, A. R., and W. M. Murphy	Differences in Response of Irradiated Eggs and Spermatozoa of *Habrobracon* to Anoxia	*J. Genet.* 54, 297–303 (1956)
Wolff, S., and H. E. Luippold	The Biochemical Aspects of Chromosome Rejoining	*4th International Conference on Radiobiology, Cambridge, 1955* (ed., J. S. Mitchell, B. E. Holmes, and C. L. Smith), p 217–221, Oliver and Boyd Ltd., London, 1956

Scientific Society Lectures and Traveling Seminars. – Of the 89 lectures given by members of the Biology Division during the half-year, the greater number have been presented at professional societies. Twenty-one societies are represented, but most of the presentations were for the Federation of American Societies for Experimental Biology and the Radiation Research Society.

AUTHOR(S)	TITLE	PLACE PRESENTED
Anderson, N. G.	A Device for the Electroseparation of Proteins [*Federation Proc.* 15, 4 (1956)]	Fed. Am. Soc. Exptl. Biol. (Am. Physiol. Soc.), Atlantic City
———	Discussion of the Origin of Life	15th Symp. of the Society for the Study of Growth and Development, Providence
———	New Fractionation Methods for Isolating Cellular Proteins [*Bull. Am. Phys. Soc.* 1, SII, 267 (1956)]	Am. Phys. Soc. (S. E. Sec.), Nashville
Arnold, W. A.	A Biophysical Aspect of Photosynthesis	Dept. of Botany, University of Tennessee, Knoxville
Astrachan, L.	DNA Produced by *E. coli* After T2 Bacteriophage Infection in the Presence of Proflavin [*Federation Proc.* 15, 213 (1956)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Atlantic City
Benedict, W. H.	Adenoma of Harder's Gland in Mice	Wilmer Institute Residents' Annual Meeting, Johns Hopkins Hospital, Baltimore
Billen, D.	Bone Marrow Tissue Culture in the Treatment of X-irradiated Mice	Radiation Research Soc., Chicago
von Borstel, R. C.	Effects of Radiation on the Egg Nucleus and Cytoplasm of the Wasp *Habrobracon*	Dept. of Zoology, University of Pennsylvania, Philadelphia
———	As above	Dept. of Biophysics, Yale University, New Haven
———	As above	Dept. of Biology, Amherst College, Amherst
Brumfield, R. T., and D. E. Foard	The Effects of Temperature on Cell Division and Growth in Timothy Roots [*ASB Bull.* 3, 8–9 (1956)]	Assoc. S. E. Biol., Durham
Carson, S. F., and E. F. Phares	Properties of Bacterial Succinic Acid Decarboxylase System [*Bacteriol. Proc.* (1956), p 111]	Soc. Am. Bacteriol., Houston

AUTHOR(S)	TITLE	PLACE PRESENTED
Congdon, C. C.	The Mechanism of Recovery from Irradiation Injury	Philadelphia County Med. Soc., Philadelphia
———	Radiation as a Factor in Experimental Lymphomas and Leukemias	Third Natl. Cancer Conf., Detroit
Congdon, C. C., and I. Urso	Isologous Versus Homologous Bone Marrow in the Treatment of Irradiated Mice	Radiation Research Soc., Chicago
Conger, A. D.	Cytological Consequences of Radiation and Its Significance to Plant Breeding	Southeastern Plant Breeders' Assoc., University of Tennessee, Knoxville
———	Radiobiological Studies at Atomic Bomb Detonations	N. C. High School Science Fair, Davidson College, Davidson
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 [*Federation Proc.* 15, 512 (1956)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Exptl. Pathol.), Atlantic City
Cormier, M. J.	Comparative Studies on Different Bacterial Luciferase Preparations [*Federation Proc.* 15, 40 (1956)]	Fed. Am. Soc. Exptl. Biol. (Am. Physiol. Soc.), Atlantic City
Cormier, M. J., and H. H. Rostorfer	Methemoglobin Reduction by Flavoproteins in Cell-Free Bacterial Extracts [*Bacteriol. Proc.* (1956), p 114–115]	Soc. Am. Bacteriologists, Houston
Darden, E. B., Jr.	Radiation-induced Changes in Muscle Fiber Membrane Potentials [*Bull. Am. Phys. Soc.* 1, SII, 267 (1956)]	Am. Phys. Soc. (S. E. Sec.), Nashville
Davis, J. W., J. V. Passoneau, and J. R. Totter	The Conversion of Glycine to Serine in Extracts of Acetone-dried Luminous Bacteria [*Federation Proc.* 15, 239 (1956)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Atlantic City
Dent, J. N.	Cytological Observations on the Adenohypophysis of the Intact and Thyroidectomized Newt	Am. Assoc. Anatomists, Milwaukee
Doherty, D. G.	Enzyme Substrate Relationships	Dept. of Zoology, University of Texas, Austin
———	The Hydrolysis of Carbon-Carbon Bonds by Chymotrypsin and Related Enzymes	Dept. of Biochemistry, Vanderbilt University, Nashville
———	As above	Cancer Research Lab., University of Florida, Gainesville
———	Hydrolysis by Chymotrypsin	Dept. of Biochemistry, Baylor University, College of Medicine, Houston
Edington, C. W.	The Induction of Recessive Lethals in *Drosophila melanogaster* by Radiation of Different Ion Densities	Dept. of Zoology and Entomology, University of Tennessee, Knoxville
Gaulden, M. E.	Some Investigations on Radiation-induced Mitotic Delays	Dept. of Zoology and Entomology, University of Tennessee, Knoxville
Gengozian, N., and T. Makinodan	Immune Response Studies on Lethally X-irradiated Mice Protected with Bone Marrow	Radiation Research Soc., Chicago
Hollaender, A.	The Effects of Long Ultraviolet on Biological Materials	Committee of the Photobiology Group of Great Britain, London
———	Radiation Biology	Symp. on Atomic Energy and Veterinary Medicine (sponsored by ORINS), Oak Ridge

AUTHOR(S)	TITLE	PLACE PRESENTED
Hollaender, A., and G. E. Stapleton	The Influence of Chemical Pre- and Post-treatments on Radiosensitivity of Bacteria, and Their Significance for Higher Organisms	Symp. on Influence of Ionizing Radiation on Cell Metabolism, Ciba Foundation, London
Horn, E. C., and M. E. House	The Effects of Immune Sera Prepared Against Their Various Components	N. C. Acad. Sci., Chapel Hill
Kimball, R. F.	Radiation Studies with the Protozoa	Bacteriol. Soc., University of Kentucky, Lexington
———	Radiation Genetics (three lectures)	Duke University, Durham
Kimball, R. F., N. Gaither, and S. M. Wilson	Posttreatment Modification of X-Ray Mutagenesis in *Paramecium*	Radiation Research Soc., Chicago
Makinodan, T.	Circulating Rat Cells in X-irradiated Mice Protected with Rat Bone Marrow	Radiation Research Soc., Chicago
———	The Role of Antigen in Immunity and Tolerance	Devel. Biol. Conf. Series, Bar Harbor
Melville, G. S., Jr., K. W. Christenberry, M. Slater, and A. C. Upton	A Quantitative Grading Method for the Study of Radiation-induced Lens Opacities	Radiation Research Soc., Chicago
Merwin, R. M., and C. C. Congdon	Presence of Homologous Cells in the Marrow of Mice Protected from the Effects of Irradiation by Homologous Marrow [*Federation Proc.* 15, 129 (1956)]	Fed. Am. Soc. Exptl. Biol. (Am. Physiol. Soc.), Atlantic City
Novelli, G. D.	Observations and Speculations Concerning a General Mechanism of Protein Synthesis	Bureau of Biological Research (20th Anniversary Celebration), Rutgers University, New Brunswick
Oakberg, E. F.	Gamma and X-Ray Sensitivity of Spermatogonia of the Mouse	Dept. of Zoology, University of Kentucky, Louisville
Odell, T. T., Jr.	Establishment of Transplants of Functionally Erythropoietic Elements in Rats Following Irradiation	Dept. of Medicine, Brookhaven Natl. Lab., New York
———	Successful Bone Marrow Transplantation in Fetal and Irradiated Adult Animals	Dedication Program of Jordan Hall of Biology, Indiana University, Bloomington
Odell, T. T., Jr., F. G. Tausche, and D. L. Lindsley	Transplantation of Homologous Erythropoietic Elements in the Rat After Whole Body Irradiation	Radiation Research Soc., Chicago
Randolph, M. L.	Application of Fast Neutrons to Radiobiology [*Bull. Am. Phys. Soc.* 1, SII, 267 (1956)]	Am. Phys. Soc. (S. E. Sec.), Nashville
Russell, L. B.	Radiation Hazards to the Embryo and Fetus	Berea College, Berea
Russell, W. L.	Genetic Effects of Radiation in Mice and Their Bearing on the Estimation of Human Hazards	Berea College, Berea
St. Amand, W.	Cell Division and Radiosensitivity	Dept. of Biology and Beta Kappa Chi Honorary Society, Atlanta University, Atlanta
———	As above	Dept. of Cancer Research, University of Florida, Gainesville

AUTHOR(S)	TITLE	PLACE PRESENTED
Schwartz, D.	Gene Action and Protein Specificity	Dept. of Biology, University of Mississippi, Oxford
———	On the Relation Between Nucleic Acid and Protein Specificity	Dept. of Chemistry, University of Tennessee, Memphis
———	As above	Dept. of Biochemistry, Tulane University, New Orleans
———	As above	Dept. of Biology, Spring Hill College, Mobile
Shapira, R., and D. G. Doherty	Hydrolysis of a Carbon-Carbon Bond by Trypsin [*Federation Proc.* 15, 352 (1956)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Atlantic City
Shaw, E. I.	The Effect of Hypoxia on Radiation-induced Mitotic Inhibition in the Neuroblast of the Grasshopper Embryo	Radiation Research Soc., Chicago
———	Protection by Sodium Hydrosulfite Against X-ray-induced Mitotic Inhibition in Grasshopper Neuroblasts	Dept. of Radiation Biophysics, University of Kansas, Lawrence
Slater, M., G. S. Melville, Jr., and A. C. Upton	Neutron and Gamma-Ray Dosimetry on a Po-Be Source	Radiation Research Soc., Chicago
Smith, L. H.	Leukocyte Preparations in the Treatment of Irradiation Injury	Radiation Research Soc., Chicago
Stapleton, G. E., D. H. Woodbury, and A. Hollaender	Recovery-Stimulating Activity of Bacterial Ribonucleic Acid Polynucleotide Material	Radiation Research Soc., Chicago
Tipton, S. R., C. W. Major, and J. L. Smothers	Thyroid and Adrenal Effects on Liver Protein and Enzyme Activity in Cell Fractions After Partial Hepatectomy in Rats	Intern. Physiol. Congr., Brussels, Belgium
Tolbert, N. E.	Photosynthesis	Dept. of Chemistry, Clemson College, Clemson
Tolbert, N. E., and L. M. Rohrbaugh	Effect of Carbon Dioxide Partial Pressure on Photosynthetic Products in *Sedum* Leaves [*Federation Proc.* 15, 371 (1956)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Atlantic City
Upton, A. C.	Effects of Ionizing Radiation on Mammals	Symp. on Atomic Energy and Veterinary Medicine (sponsored by ORINS), Oak Ridge
———	Acute Radiation Death	Duke University, Durham
———	Delayed Radiation Injury	Duke University, Durham
———	Radiation Studies on Mammals	Dept. of Zoology, Duke University, Durham
———	Comparative Studies of the Induction of Myeloid and Lymphoid Leukemias in RF Mice by X Radiation	VI Intern. Congr. Intern. Soc. Hematol., Boston
Upton, A. C., C. C. Congdon, and A. Hollaender	Mechanisms of Delayed Death After Total-Body Irradiation	Eighth Intern. Congr. Radiol., Mexico City
Upton, A. C., and J. Furth	Factors Influencing Induction of Myeloid Leukemia in RF Mice by Irradiation	Louisiana Cancer Conf. (sponsored by Am. Cancer Soc.), New Orleans
———	Host Factors in the Pathogenesis of Leukemia in Animals and in Man	Third Natl. Cancer Conf., Detroit

AUTHOR(S)	TITLE OF ARTICLE	PLACE PRESENTED
Upton, A. C., J. Furth, and E. S. Ledford	Inhibition of Myeloid Leukemia Induction in RF Mice by Partial Shielding During Irradiation [*Federation Proc.* 15, 512 (1956)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Exptl. Pathol.), Atlantic City
Upton, A. C., J. Furth, and F. F. Wolff	Gonadal Factors in the Induction of Myeloid Leukemia in RF Mice by X Irradiation [*Proc. Am. Assoc. Cancer Research* 2, 154 (1956)]	Am. Assoc. Cancer Research, Atlantic City
Upton, A. C., G. S. Melville, Jr., M. Slater, and F. P. Conte	Leukemia Induction in RF Mice by Fast Neutron Irradiation	Radiation Research Soc., Chicago
Urso, P.	The Bone Marrow Response of X-irradiated CAF Mice Receiving Varying Amounts of Isologous Bone Marrow Injections	Dept. of Zoology, University of Tennessee, Knoxville
Urso, P., and C. C. Congdon	Quantitative Studies on the Effect of Bone Marrow Treatment in Irradiated Mice	Radiation Research Soc., Chicago
Volkin, E.	RNA Phosphorus Turnover in T2r+-Infected *Escherichia coli* [*Federation Proc.* 15, 376 (1956)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Atlantic City
Wolff, S.	Genetics and Cytology	Chem. Eng. Graduate Seminar, University of Tennessee, Knoxville
———	The Use of Radiation in the Development of Plants and Animals	Industrial Nuclear Technol. Conf., Chicago
Wolff, S., and H. E. Luippold	Two Chemically Different Types of Radiation-Induced Chromosome Breaks	Radiation Research Soc., Chicago
Zill, L. P.	Anthrone Stability and Determination of the Heptuloses [*Federation Proc.* 15, 391 (1956)]	Fed. Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Atlantic City
———	Plant Metabolism	Dept. of Botany, University of Tennessee, Knoxville
———	As above	Dept. of Chemistry, University of Tennessee, Memphis
———	As above	All-College Seminar Program, Oklahoma A & M College, Stillwater
———	As above	Dept. of Plant Sciences, University of Oklahoma, Norman
———	As above	Depts. of Biology and Chemistry, North Texas State College, Denton

Biomedical Directors Meet in Oak Ridge. – The Biomedical Directors of AEC laboratories met in Oak Ridge for a three-day session, which ended June 2. One day was devoted to talks by ten members of the Biology Division. Discussions in this program emphasized the genetic effects of radiation and methods of protection and recovery from radiation injury.

Visiting Lecturers. – The number of lectures delivered on the Biology Seminar program by visiting speakers has increased greatly over the last half-year. Thirty-six speakers, half of whom were from ten foreign countries, have accommodated the Division in this way. Speakers, their affiliations, and the subjects of their lectures are listed.

LECTURER	AFFILIATION	TOPIC
R. Speirs	Brookhaven National Laboratory, Biology Department, Upton	Relation of Eosinophils to Active Formation of Antibody
F. Bernheim	Duke University, School of Medicine, Department of Physiology and Pharmacology, Durham	Studies on the Formation and Effects of Fatty Acid Peroxides and Their Relation to Radiation
M. F. Morales	Naval Medical Research Institute, National Naval Medical Center, Bethesda	Energetics of ATP Hydrolysis
J. Oudin	Pasteur Institute, Paris, France	The Immunochemical Analysis by Diffusion in Gels – Application to the Blood Serum Components
R. E. Reeves	Louisiana State University, School of Medicine, New Orleans	Ring Shapes in Glycopyranosides
E. Juni	University of Illinois, Bacteriology Department, Urbana	A Cyclic Pathway for the Bacterial Dissimilation of 2,3-Butanediol and Related Compounds
W. J. Robbins	New York Botanical Garden, New York City	The Botanical Garden and Its Program
H. F. Falls	Department of Ophthalmic Surgery, University of Michigan Hospital, Ann Arbor	New Constitutional Syndromes
N. J. Palleroni	Universidad de Cuyo, Department of Microbiology, Mendosa, Argentina	The Carbohydrate Metabolism of *Pseudomonas saccharophiles* (D-Arabinose Oxidation)
F. Binkley	Department of Biochemistry, Emory University	Studies on Nonprotein Enzymes
T. T. Chen	Department of Zoology, Duke University, Durham	*Paramecium bursaria* I. Varieties, Mating Types and Mating Reaction. II. Killer Substances
S. W. Fox	Director, Oceanographic Institute, The Florida State University, Tallahassee	Evolution of Protein Molecules and Thermal Synthesis of Biochemical Substances
E. R. Dempster	University of California, College of Agriculture, Division of Genetics, Berkeley	Natural Selection for Segregation Ratio, with Reference to the series of Tailless Alleles
T. Alper	Medical Research Council, Experimental Radiopathology Research Unit, Hammersmith Hospital, London	Effects of Radiation on Bacteriophage
S. Elsden	Department of Bacteriology, University of Illinois, Urbana	Discussion of *Rhodospirillum rubrum*
J. W. Foster	The University of Texas, Department of Bacteriology, Austin	Morphogenesis in Bacteria: Some Aspects of Spore Formation
T. Tomiyama	Fulbright Lecturer at the University of Washington, School of Fisheries, Seattle (Chairman, Division of Fishery Chemistry, Kyushu University, Japan)	The Pathway of Uptake and Excretion of Ca^{45}, $Sr^{90}(Y^{90})$, and P^{32} in Aquatic Animals

LECTURER	AFFILIATION	TOPIC
H. T. Meryman	Yale University, New Haven	Mechanism of Biological Freezing
H. Beevers	Purdue University, Department of Biological Sciences, Lafayette	Pentose Metabolic Pathway in Plants
E. Hadorn	Zoollogisch-Vergl Anatomisches, Institut der Universität, Zürich	Patterns of Biochemical and Developmental Pleiotrophy
G. H. Beale	Institute of Animal Genetics, Edinburgh	Some Problems in the Genetics of *Paramecium*
G. Popjak	Experimental Radiopathology Research Unit, Hammersmith Hospital, London	1. Studies on the Biological Synthesis of Fatty Acids
		2. The Origin of Carbon Atoms of Chloesterol
G. Stein	The Hebrew University, Department of Physical Chemistry, Jerusalem	Radiation Chemistry of Some Organic Solutes of Biological Interest
R. J. P. Williams	Oxford University, England	Some Models for Iron-containing Enzymes
T. Caspersson	Institutet for Cellforskning och Genetik Medicinska, Nobelinstitutet Karolinska Institutet, Stockholm	Quantitative Cytochemical Methods for Cell Nuclear Studies
C. Pavan	Universidade de Sao Paulo, Sao Paulo	Structure and Function of Polytene Chromosomes of *Rynchosciara*
K. V. Thimann	Harvard University, The Biological Laboratories, Cambridge	The Formation and Destruction of Auxin in Plants
J. Huxley	British biologist and writer	Biological Aspects of Cancer
M. Errera	Universite Libre de Bruxelles, Faculte des Sciences, Bruxelles,	Alterations of Intracellular Deoxyribonucleic Acid and Their Biological Consequences
D. R. Krieg	University of Rochester, Biology Department, Rochester	Some Effects of Ultraviolet on Bacteriophage
J. Rotblat	Medical College of St. Bartholomew's Hospital, Physics Department, London	Some Experiments with the 15-Million-Volt Accelerator
E. A. Wright	Department of Pathology, St. Mary's Hospital, London	The "Oxygen Effect" in Mammals
L. H. Gray	Mt. Vernon Hospital, Northwood, Middlesex, England	New Studies on the Determination of Oxygen in Biological Materials
F. W. Went	Division of Biology, California Institute of Technology, Pasedena	Effects of Visible Radiation and Temperature on Plant Growth
Ir. M. Sangster	Antoni van Leeuwenhoek-Huis Laboratorium Bibliotheek, Amsterdam, Netherlands	An Experimental Approach in the Study of Primary Processes in the Action of Ionizing Radiation
R. E. F. Matthews	Agricultural Research Council, Plant Virus Research Unit, Moleno Institute, Cambridge, England	Studies on Plant Virus Ribonucleic Acids

Speakers at Professional Societies, Fall 1956. – At the meeting of the American Institute of Biological Sciences in Storrs, Connecticut, the Biology Division will be represented by fourteen papers. The program of the Genetics Society of America lists the following speakers: A. D. Conger (coauthors, M. L. Randolph and Helen Johnston), C. W. Edington, A. Fabergé, M. E. Gaulden, H. E. Luippold (paper by S. Wolff and H. E. Luippold), M. L. Pardue (paper by R. C. von Borstel and M. L. Pardue), T. H. Pittenger, L. B. Russell (coauthor, M. H. Major), W. L. Russell, F. J. de Serres, and W. J. Welshons. L. B. Russell will also give a paper at the American Society of Zoologists. Two papers at the American

Society of Plant Physiologists will be presented by K. C. Atwood and by A. W. Naylor (coauthors, N. E. Tolbert and L. P. Carter).

At the American Chemical Society in Atlantic City, E. F. Phares will give a paper of which M. V. Long is coauthor. L. Astrachan will participate in the Bacteriophage Conference at Cold Spring Harbor by presenting a discussion of the work done by himself, E. Volkin, and M. H. Jones. R. F. Kimball will present a paper at the Radiological Society of North America in Chicago.

R. F. Kimball and D. Schwartz will depart on August 31 for Japan to attend the International Genetics Symposia, which meets in Tokyo and Kyoto September 6–12. Both will give papers at the meeting.

Presentations from three members of this Division will be listed for the Sixth International Congress for the International Society of Hematology in Boston. Papers will be given by T. T. Odell, Jr., C. C. Congdon, and by A. C. Upton. An exhibit will also be contributed by C. C. Congdon.

C. C. Congdon will take part in the work conference on "Dynamics of Proliferating Tissues" at Brookhaven National Laboratory.

A. Hollaender and S. Wolff Attend European Meetings. – A. Hollaender attended several scientific meetings in Europe during August. In Copenhagen, he attended the First International Congress of Human Genetics. A prominent topic for discussion at this Congress was "Radiation Genetics and Its Human Implications." Dr. Hollaender also participated as special adviser to a study group of the World Health Organization whose purpose was to discuss research on the genetic effects of radiation on man. Dr. Hollaender was elected chairman of this committee.

After the Copenhagen meetings, he proceeded to Stockholm to attend the Fifth International Radiobiology Conference, where he served as chairman of the session on "Irradiation Effects on the Haematopoietic System" and also delivered a paper. The subject of the paper was "The Effects of Pre- and Posttreatment on the Radiation Sensitivity of Microorganisms."

Sheldon Wolff also attended the Radiobiology Conference in Stockholm and gave a paper entitled "Recent Studies on Chromosome Breakage and Reunion." After the Congress, Dr. Wolff attended a symposium on "Induced Mutation" at the Statens Skogsforskningsinstitut in Stockholm. Before returning to the Laboratory, he will visit a number of laboratories in the British Isles and on the Continent and discuss modification of radiation response with prominent research people.

Education. – Educational activities for this period have included teaching, study, and research. R. F. Kimball, J. S. Kirby-Smith, and A. D. Conger taught courses in Radiation Biology at Duke University. Part of the work was carried out at Durham during the regular session and part at the Marine Laboratory at Beaufort. K. C. Atwood has also done summer teaching at the University of Virginia Biological Station at Mountain Lake.

W. E. Cohn and C. W. Sheppard, who have spent the past year as research fellows, will be returning to this Laboratory by September 1; respectively, the study has been in England on Guggenheim and Fulbright Fellowships and at Harvard on a Public Health Service Fellowship.

W. St. Amand has spent the past four weeks at the University of Colorado School of Medicine in Denver, where he has been taking courses in Principles and Techniques of Cell and Tissue Culture, and Quantitative Methods of Animal Cell Culture.

W. Arnold is spending his second summer at the Institute for Muscle Research, Woods Hole, Massachusetts, where he is engaged in research with A. Szent-Gyorgyi.

CYTOLOGY AND GENETICS

CYTOGENETIC EFFECTS OF RADIATION

R. F. Kimball
K. C. Atwood
R. T. Brumfield[1]
A. D. Conger
T. H. Pittenger
D. Schwartz
F. J. de Serres[2]
S. Wolff
D. B. Asher[3]
D. E. Foard[4]
N. Gaither
H. E. Luippold
H. J. Luippold
C. B. Murray
I. L. Sandler[5]
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. – It was previously reported[6] that exposure to streptomycin after irradiation increased the detectable lethal and deleterious mutation in *Paramecium*. However, many experiments done since then have shown a decrease. In the earlier experiments it was possible to completely inhibit growth with streptomycin, but in later experiments growth was only slowed. Apparently the change in effect on mutation was associated, in some unknown way, with the change in effect on growth.

Results. – The effect on detectable mutation is almost certainly on the mutation process itself and is not a secondary result of selection of animals or nuclei with fewer mutations nor an effect of streptomycin on the expression of the mutants. There is little or no death of animals or loss of nuclei as a result of the treatment, and streptomycin is effective only if given within 1 or 2 hr after irradiation; however, the mutations are not expressed until several days afterward.

Figure 1 shows the combined results of experiments to test the relation of the effect of streptomycin to the irradiated stage in the interdivision interval. Dividing paramecia were chosen at various times, and all groups were exposed at the same time to 4.5 kr of x rays. Some of each group were exposed to streptomycin for 3 hr starting shortly after irradiation. The percentage of normal exautogamous clones (inversely related to the amount of mutation) is plotted against time after division when the paramecia were irradiated.

The insensitiveness of the latter half of the interdivision interval has been reported previously,[7] but the statistically significant increase of sensitivity during the first half of the interval is shown for the first time by the present experiments. Streptomycin has its greatest effect on animals irradiated early in the interval and appears to have less effect on animals irradiated when they are most sensitive. An experiment with higher doses failed to show any effect of streptomycin on mutation induced during the insensitive period.

Discussion. – The results with streptomycin and other[6] results show that radiation initiates a mutation process that is not completed immediately. It may be suggested that this process is completed at about the time of the change from the sensitive to the insensitive period (perhaps the stage of chromosome replication) and that it is subject to some spontaneous recovery before this time. The longer the time between irradiation and completion of mutation, the more chance there will be for recovery; streptomycin promotes recovery by increasing the time available. The increase of mutation by streptomycin in those experiments in which it completely stopped growth suggests that recovery involves some metabolic process that can be inhibited under certain circumstances. The failure of streptomycin to affect the mutation during the insensitive period probably means that these mutations are produced by a different mechanism.

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[1]Consultant.

[2]Research Associate.

[3]Temporary employee.

[4]Loanee.

[5]Arrived Aug. 1, 1956.

[6]R. F. Kimball, N. Gaither, and S. M. Wilson, *Biol. Semiann. Prog. Rep. Feb. 15, 1956*, ORNL-2060, p 13.

[7]R. F. Kimball, N. Gaither, and M. K. King, *Biol. Semiann. Prog. Rep. Aug. 15, 1954*, ORNL-1766, p 12–13.

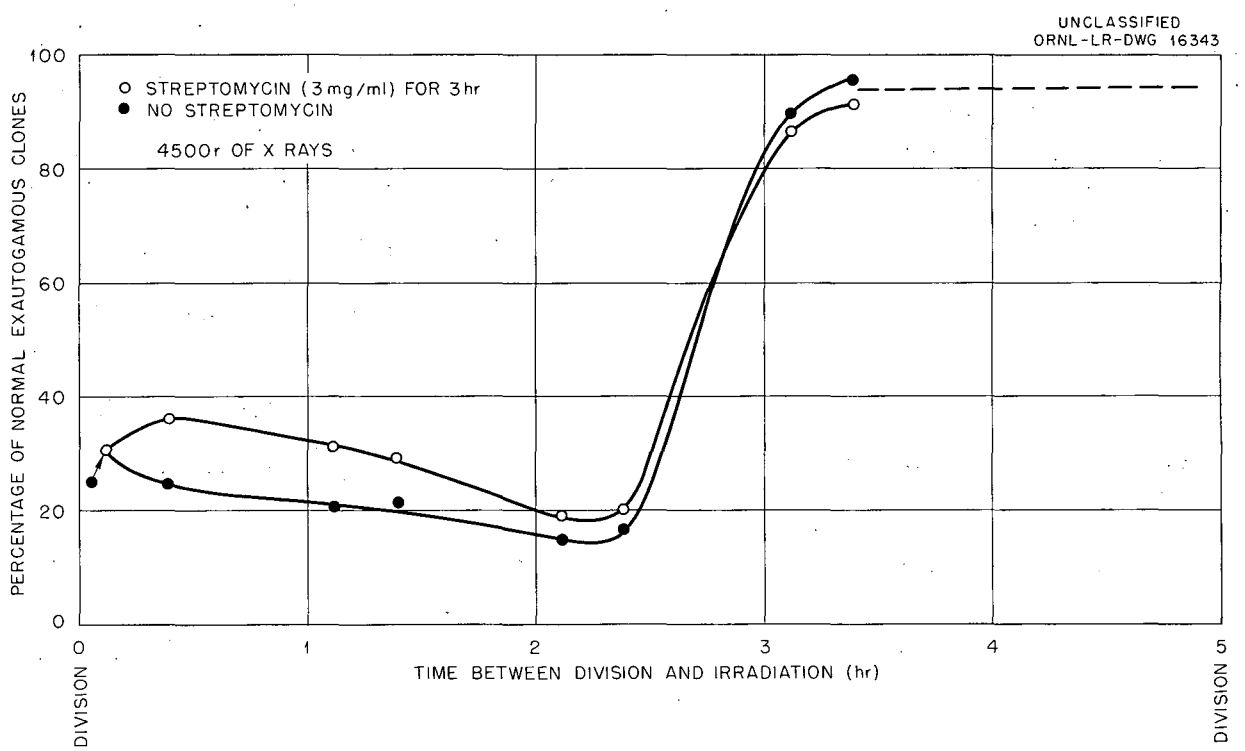

Fig. 1. Effect of Streptomycin and Time Between Division and Irradiation.

Chromosomal Aberration Production by X Rays and by Monochromatic 2.5- and 14-Mev Neutrons

A. D. Conger M. L. Randolph[8]
Helen J. Luippold

Introduction. – Considerable attention has been devoted to comparisons of the relative biological efficiency (RBE) of fast neutrons with x rays in causing biological damage. Differences in RBE are attributed to differences in the ion density or linear energy transfer (LET) (ions/micron, or kev lost per micron) of the radiation deposited in the tissue. In such neutron/x-ray comparisons, the fact has frequently been overlooked that the difference in ion density, and hence expected difference in RBE, may be as great within the fast-neutron energy range as the difference between x rays and energetic fast neutrons.

Results. – The realization of high output from the linear accelerator made it possible to measure RBE within the fast-neutron energy range. In a single large-scale experiment the effect of monoenergetic 2.5-Mev *d,d* neutrons, 14-Mev *d,t* neutrons, and 250-kvp (HVL, 1.45 mm Cu) x rays in causing *Tradescantia* chromosomal aberrations was measured. The physical situation was well known, and the tissue dose was carefully measured by two independent methods in the neutron exposures; doses are believed to be accurate to ±10%. The same comments apply to the x-ray exposures. For each type of radiation, both chromosome (four-day) and chromatid (22- to 24-hr) aberrations were observed from five or six doses. Comparisons of the effectiveness of the different types of radiation were based on coefficients of aberration production per roentgen or rep derived from weighted least-squares fits to the five- or six-point dose curves for each type of aberration.

The RBE comparisons were straightforward for the types of aberrations which increased in the same way (linearly) with dose for the three kinds of radiations. However, other types, primarily exchanges, increase linearly with dose of neutrons but as the square of the dose for x rays at high intensity. For these, comparisons were made at

[8]Biophysics Section.

that x-ray dose which caused 50% normal (aberration-free) cells, a selection considered as being approximately equivalent biologically to other kinds of systems.

The increase in RBE with an increase in LET, from this and from previous experiments, is shown in Fig. 2. Average LET was calculated from equations of Boag.[9]

The x rays of the present experiment are considered as having an RBE of 1. The additional neutron point is taken from a previous experiment[10] by a comparison of identical data (chromosome aberration production per rep) from the Y-12 cyclotron with the *d,d* and *d,t* data. The remarkable feature of the graph is the almost strict linearity of RBE with LET in the neutron energy range. By extrapolation of the line shown up to the LET of alpha particles, an RBE of about 10 is obtained, which is in quite good agreement with previous results for alpha particles. The lower three dotted points are for chromosome aberration production by Co^{60} gamma rays and 0.06 and 0.20 Å x rays, taken from data by Kirby-Smith and Daniels.[11]

Discussion. – Taken over-all, these data show that there is a consistent increase in RBE with LET, which is almost linear from the x-ray to the neutron energy range, and within the neutron energy range. It is clear that great changes in RBE are occurring in the neutron energy range, changes so large that the LET, or ion density, of neutron radiation must be considered in RBE comparisons.

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

[10] A. D. Conger and C. W. Sheppard, unpublished data.

[11] J. S. Kirby-Smith and D. S. Daniels, *Genetics* 38, 375–388 (1953).

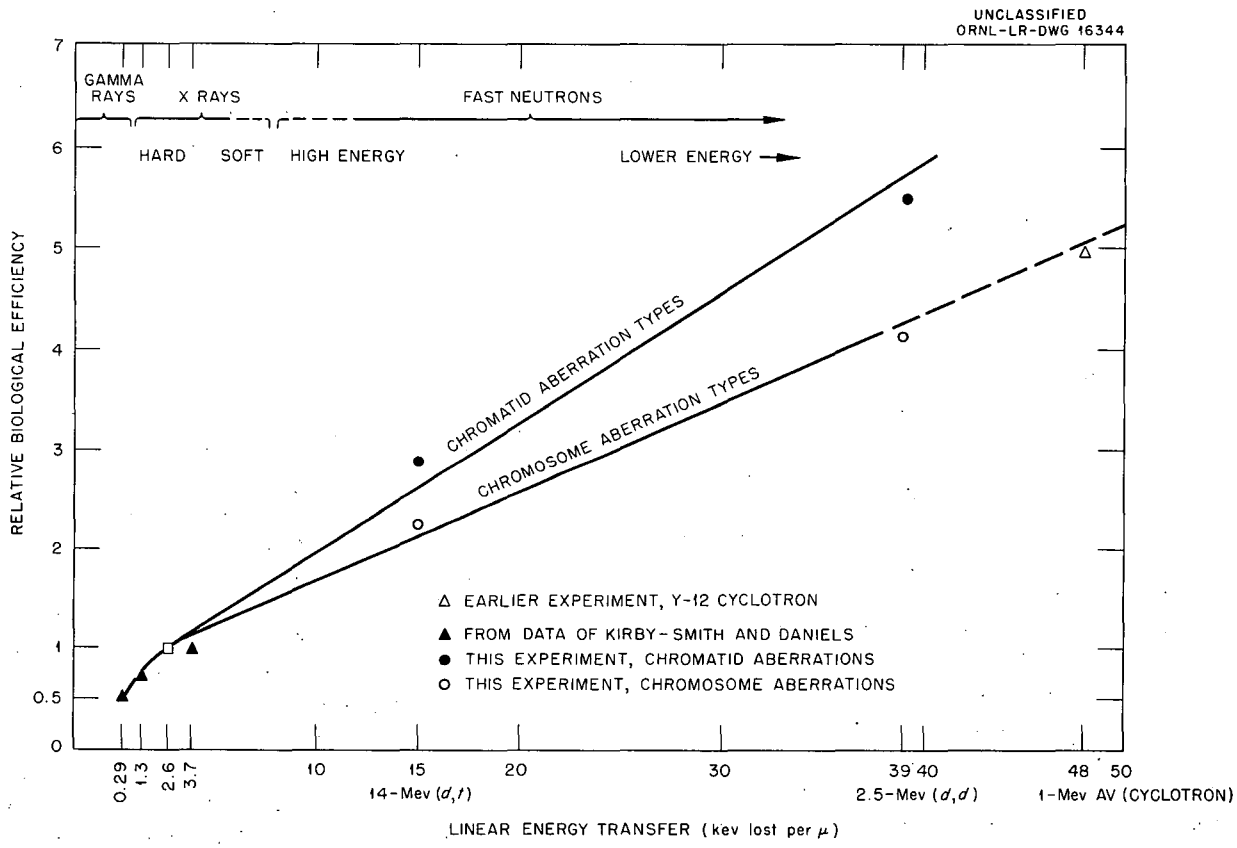

Fig. 2. Increase in RBE vs Increase in LET.

Noninteraction of X-Ray- and Neutron-Induced Chromosome Breaks

S. Wolff M. L. Randolph

Introduction. – Previous studies on the rejoining of x-ray-induced chromosome breaks in the seed of *Vicia faba* have indicated that at least one class of the breaks formed can be classified chemically as being breaks of covalent bonds. These breaks characteristically remain in the "open" condition for long periods of time before they undergo the reactions of rejoining. If the x radiation is fractionated into two separate doses, such that the second is administered before the breaks produced by the first irradiation have rejoined, then the two different groups of breaks can rejoin with one another in a manner which results in more genetic damage (two-hit chromosome aberrations) than the simple sum of the damage produced by individual doses of radiation. The present studies were designed to test whether or not x-ray-induced chromosome breaks would interact with neutron-induced breaks in the same synergistic fashion.

Results. – Seeds of *Vicia faba* which had been soaked in water for 5 hr, or in water for 4½ hr and in BAL for ½ hr, were irradiated with 600 r of 250-kvp x rays. The breaks produced remained "open" for at least 2 hr in seeds soaked in water and for ½ hr in seeds soaked in BAL. One hour later both groups of seeds were irradiated with 45 rep of 14-Mev neutrons produced by the *d,t* reaction in the Cockcroft-Walton linear accelerator. Another experiment, utilizing only a 15-min interval between fractions, was also performed on seeds soaked in water. The seeds were then allowed to germinate. As soon as the first root tip mitosis occurred, Feulgen smears were made and the cells scored for two-hit chromosome aberrations. The results are presented in Table 1.

Discussion. – In all the dose fractionation studies performed in which one dose was x radiation and

TABLE 1. EFFECT OF FRACTIONATED DOSES OF X RAYS AND NEUTRONS OF *VICIA FABA*

Experiment	First Dose	Time Breaks Remain Open (min)	Interval Between Doses (min)	Second Dose (rep)	Two-Hit Aberrations per 100 Cells	
					Observed	Expected If Two Doses Are Additive
			With BAL			
A	600 r	30			10.7	
	45 rep	?			8.7	
	600 r	30	60	45	19.3	19.4
			With H_2O			
B*	600 r	120			16.5	
	45 rep	?			10.7	
	600 r	120	60	45	28.8	27.2
C*	600 r	120			7.5	
	45 rep**	?			8.3	
	600 r	120	15	45	17.4	15.8
D	30 rep				6.1	
	45 rep**				8.3	
	60 rep				11.0	

*Experiments were performed on seeds soaked at different temperatures.

**Same experiment.

the other neutrons, the number of two-hit aberrations was only the sum of the aberrations produced by each individual dose. This was true not only when the x-ray-induced breaks had rejoined before the neutron breaks were induced (BAL-treated seed) but also when the x-ray breaks were open at the time that the second group was formed. Thus it is seen that, unlike the situation in which all the breaks are produced by x rays, neutron-induced chromosome breaks and x-ray-induced breaks do not rejoin with one another in such a manner as to yield more two-hit aberrations than the arithmetic sum of the aberrations produced by each irradiation alone. This observation may be explained in at least two different ways. The first is that the ends of x-ray-induced breaks are qualitatively different from the ends of neutron-induced breaks, and consequently the two cannot rejoin with one another. The second is that since two-hit aberrations produced by 14-Mev neutrons, like two-hit aberrations produced by more densely ionizing neutrons, increase linearly with dose (Table 1, experiment D) rather than as the square of the dose, it is believed that both the neutron-induced breaks are produced by the same ionizing particle (proton). Thus the neutron-induced breaks, of the class which may form two-hit aberrations, already have neutron-induced partner breaks. Consequently, x-ray-induced breaks in the vicinity are superfluous for the production of two-hit aberrations. The latter explanation, which is based on differences in the spatial distribution of breaks rather than on assumed differences in their fundamental nature, is preferred at present.

Anaphase Bridge Cycles in Maize

D. Schwartz C. B. Murray

A cytological investigation was conducted on the breakage-fusion-bridge cycles in maize endosperm tissue which carries a single dose of both *Ac* and *Ds*, *Ds* being located in the standard position on the short arm of chromosome 9. The results obtained were quite unexpected in view of the *Ac-Ds* behavior as determined by analysis of variegation patterns in the mature kernel. Ten such endosperms which were scored showed 30 single bridges, 6 of which were associated with fragments, 172 double bridges, and 10 cells with two double bridges. The double bridges occurred in clusters, whereas the single bridges were distributed singly throughout the tissue. The frequency of bridges per endosperm is variable. A typical slide showed 4 singles, 25 doubles, 2 cells with two double bridges, and 74 normal anaphases. The presence of *Ac* in a nucleus causes breakage of the chromosome at the position occupied by the *Ds* locus. The most frequent result of such an event is the formation of a dicentric chromatid. This formation should initiate the chromatid breakage-fusion-bridge cycle resulting in a cluster of single bridges, each cluster indicating a *Ds* event. This appears to be inconsistent with the deficiency of single and high frequency of double bridges observed. To determine whether the results are a consequence of typical *Ac-Ds* events or a reflection of the behavior of dicentric chromatids in maize endosperm tissue, pollen irradiation experiments were carried out. Here an aberration can be introduced into the initial triploid nucleus from which the endosperm will develop. If the chromatid breakage-fusion-bridge cycle is continuous, forming a detectable single bridge in each successive division, all the anaphases in an endosperm which had received a dicentric chromatid from the pollen should show single bridges. The dose of gamma radiation used in these experiments was 1300 r. While it is known that ionizing radiation can produce many kinds of aberrations, a most frequent type is the dicentric chromatid, as determined by an analysis of variegation phenotypes in the mature kernel. From a dose of 1300 r most of the endosperms should have received at least one dicentric chromatid. Of the 109 kernels scored to date no endosperms were found which contained a single bridge in each anaphase or even in a majority of its anaphases. Eighty-six were either normal or showed a low proportion of singles. Twenty-three contained anaphases with double bridges. Of these, 8 had bridges throughout the tissue, indicating an initial radiation event which formed a dicentric chromosome. The remaining 13 were sectorial, showing clusters of double bridge anaphase figures.

From an analysis of variegation patterns of kernels which had received a dicentric chromatid, it is clear that the broken end of a chromosome does not heal in the endosperm following bridge rupture. Small spots of recessive tissue, indicating late loss of chromosomal segments, are found over the entire kernel. It is therefore suggested that fusion between broken sister chromatids is not always complete at anaphase. An incomplete

fusion would represent a weak point which would likely rupture at the early anaphase separation of the chromosomes. Thus a bridge would not be found at the mid- and late-anaphase stages where the scoring for bridges is done. The relative frequency of complete vs incomplete fusion is probably dependent upon chromosomal as well as extrachromosomal factors. The double bridge clusters are visualized as arising as a consequence of the inclusion of an unbroken dicentric chromatid into one of the daughter cells.

Synergism of Two Cytoplasmically Inherited Mutants in *Neurospora crassa* (Abstract)

T. H. Pittenger

Two different cytoplasmically inherited mutants, *mi*-1(*poky*) (ref 12) and *mi*-3 (ref 13) have been reported in *Neurospora*. These mutant strains are characterized by subnormal growth rates and defective cytochrome systems. In view of the complementarism shown by nonallelic nuclear genes in heterokaryons, it was of considerable interest to determine whether different cytoplasmic mutants could interact to produce a normal phenotype. With *poky* and *mi*-3, however, no satisfactory evidence could be obtained from mixed cultures to suggest any interaction between the two mutant particles. More recently in this laboratory a third cytoplasmically inherited mutant, designated *mi*-4, has been found.[14] In addition to other similarities, both *mi*-4 and *poky* are characterized by slow growth rates and deficiencies in their cytochrome systems that by present tests are indistinguishable. When slowly growing strains of *poky* and *mi*-4 are combined in a heterokaryon, the two defective cytoplasmic determinants coexist in the cytoplasm, a heterocytosome, and the resulting culture has a nearly normal growth rate. If the *mi*-4 or *poky* cytoplasm in these same strains is replaced by normal cytoplasm, the homokaryons and the heterokaryons all grow at the same rate. Furthermore homokaryotic strains with a mixture of *poky* and *mi*-4 cytoplasm also have essentially normal growth rates. Thus, it is clear that the synergistic effect on growth is the result of the cytoplasmic mixture of *mi*-4 and *poky* and cannot be attributed to nuclear interaction. These heterocytosomes, or those involving a normal particle and either of the mutants, are not permanently stable in certain genetic backgrounds and gradually revert to the slowly growing phenotype.

Biochemical Studies of Cytoplasmically Controlled Synergism in *Neurospora crassa*

K. C. Atwood T. H. Pittenger

Introduction. – The cytoplasmically inherited mutants of *Neurospora*, *mi*-1 (ref 12) and *mi*-4 (ref 14), are characterized by slow growth rates and defective cytochrome systems. Both are nearly devoid of cytochrome oxidase activity. It has been found that when *mi*-1 and *mi*-4 coexist in the cytoplasm the normal (fast growth) phenotype is restored. This synergistic effect on growth has been shown to be the result of the cytoplasmic mixture of *mi*-1 and *mi*-4 (a heterocytosome) and not the result of nuclear interaction. Attempts to find a biochemical basis for the synergism have given the following results.

Results. – Under spectroscopic examination the mycelial pads of both mutants and the heterocytosome appear to be mutant; that is, they show a very strong cytochrome *c* band, and the *b* and $(a + a_3)$ bands are not visible. Although no increase in cytochrome oxidase activity is found when mycelial homogenates of the mutants are mixed, homogenates of the preformed heterocytosome have slightly greater activity than either mutant but far less activity than the wild type. A soluble cytochrome peroxidase system of the mutants is about three times more active than in the wild type, but the mixed homogenates show no increase in cytochrome peroxidase activity. When reduced DPN was added to mycelial homogenates, ATP was accumulated on incubation. Both mutants accumulated ~500 times as much ATP in 1 hr as the wild type did in the same length of time. The mixture of the two mutant homogenates accumulated about half as much ATP as either mutant.

Discussion. – The defective cytochrome system of both the heterocytosome and the mutants, as revealed by spectroscopic examination and by no significant difference being evidenced at present between the mutants and the heterocytosome in regard to either of the terminal oxidase systems,

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[12] M. B. Mitchell and H. K. Mitchell, *Proc. Natl. Acad. Sci. U.S.* 38, 442–449 (1952).

[13] M. B. Mitchell, H. K. Mitchell, and A. Tissieres, *Proc. Natl. Acad. Sci. U.S.* 39, 606–613 (1953).

[14] T. H. Pittenger, *Biol. Semiann. Prog. Rep. Feb. 15, 1956*, ORNL-2060, p 16–17.

suggests that the cause of the observed synergism may lie elsewhere. These preliminary results suggest that further studies on ATP accumulation of various mixtures may be instructive in explaining the synergistic effect of the heterocytosome.

Recombination Studies with *ad*-3A Mutants in *Neurospora crassa*

F. J. de Serres

Introduction. – The *ad*-3 region of *Neurospora crassa* is occupied by at least two separate but closely linked loci, *ad*-3*A* and *ad*-3*B*. The order of these loci with reference to two closely linked markers is *hist*-2 *ad*-3*A* *ad*-3*B* *nic*-2.[15] Crossover studies on a series of 13 physiological alleles of different origin (2 spontaneous, 3 ultraviolet, 8 x ray) at the *ad*-3*A* locus have been performed to determine the linkage relationships of each of these mutants in the *hist*-2–*ad*-3*A*, *ad*-3*A*–*ad*-3*B*, and *ad*-3*A*–*nic*-2 intervals. Such data have been collected to determine the type of genetic alteration which occurred in the original wild-type strains as a prerequisite to a comparison of the reversibility of radiation-induced mutants with mutants of spontaneous origin at a single locus. In addition, various interallelic crosses of these 13 mutants have been made in order to investigate the phenomenon of "gene conversion"[16] at the *ad*-3*A* locus.

Results. – Crosses of each of the 13 strains were made with the marked strains *hist*-2 *nic*-2 and *hist*-2 *ad*-3*B*(35203) *nic*-2. The data from the first group of crosses indicate that the *ad*-3*A* locus is 1.7 to 2.1 units from the *hist*-2 locus and 2.9 to 3.4 units from the *nic*-2 locus. Data from the second group of crosses indicate that linkage values for 11 of the mutants in the *ad*-3*A*–*ad*-3*B* interval range from 0.16 to 0.83 units. Values for this same interval, from crosses of two of the x-ray-induced mutants, A3 and A9, which show marked abortion patterns in these crosses and "abnormal" linkage values in the first cross, are 1.28 and 3.55, respectively. In the interallelic crosses, adenine-independent progeny have been obtained from crosses of eight of the strains with a marked strain of mutant A2 [*hist*-2 *ad*-3*A*(2) *nic*-2]. From 9 to 108 such progeny have been recovered from individual crosses. The frequency of recovery of such progeny in these eight crosses ranges from 1.2 to 7.7 per 10^4 viable ascospores. The data from most of these crosses indicate that the progeny are distributed among all four possible classes (H++, ++N and H+N, +++) and that progeny with the parental combination of markers occur more frequently than recombinant types.

Discussion. – The linkage relationships of each of the 13 strains in the three intervals tested indicate that 11 of the mutants have resulted from localized alterations confined to the *ad*-3*A* locus. Two of the strains, however, mutants A3 and A9, are associated with chromosomal rearrangements which give marked alterations in linkage values for each interval. The data from the crosses with strain 35203 demonstrate that adenine-independent progeny with the parental combinations of markers (H++N and ++++) arise at similar frequencies from cross to cross and that the differences in linkage (0.16–0.83) result from marked variations in the frequency of the crossover progeny (H+++) recovered. Comparisons of the data from these crosses with those from the interallelic crosses suggest that when crosses are made between two closely linked mutants at separate loci the majority (if not all) of the prototrophs recovered are of one (crossover) genotype but that prototrophs recovered from crosses of mutants at the same locus are distributed among all four of the crossover and non-crossover (parental) classes.

Effects of Temperature on Growth of Timothy Roots

R. T. Brumfield D. E. Foard

Introduction. – Surface cells in the primary root of timothy (*Phleum pratense*) seedlings can be observed in the living condition during their formation by division in the meristematic region and through their elongation to maturity.[17,18] Therefore the timothy root is a suitable object for experiments concerning the effects of various environmental conditions on cell division, cell growth, and organ growth in plants. The experiments described here were undertaken to find the optimum

[15] F. J. de Serres, *Biol. Semiann. Prog. Rep. Feb. 15, 1956*, ORNL-2060, p 17.

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

[17] R. T. Brumfield, *Am. J. Botany* **29**, 533 (1942).

[18] R. H. Goodwin and W. Stepka, *Am. J. Botany* **32**, 36 (1945).

TABLE 2. TEMPERATURE COEFFICIENTS OF CELL AND ROOT GROWTH IN TIMOTHY

Temperature Range (°C)	Number of Roots	Root Growth (μ/min)	Meristematic Cell Growth (%/hr)	Elongating Cell Growth (%/hr)
10–20	4	4.22 ± 1.10	4.92 ± 2.24	4.92 ± 1.88
15–20	2	1.75 ± 0.35	2.00 ± 1.15	3.30 ± 0.14
15–25	7	2.29 ± 0.29	2.27 ± 0.62	2.48 ± 0.31
20–30	6	1.73 ± 0.19	1.15 ± 0.21	1.48 ± 0.30

temperature for growth of timothy roots and to determine temperature coefficients (Q_{10}'s) for cell division, growth of meristematic cells, elongating cells, and root growth. Since the growth rate is different for individual roots, the same root was observed for 4 hr at one temperature and for 4 hr at another. The rates of increase in cell length and root length were determined by measurement of photographs taken at successive intervals of time, temperature coefficients being calculated from these rates. The rates of cell division were estimated by counting the number of cell walls in 8-hr intervals, and the same set of roots was observed at two different temperatures.

Results. – Temperature coefficients for root and cell growth are presented in Table 2. In four roots grown at 30°C, there were 164 divisions and 94 in the same group of roots at 20°C, a Q_{10} of 1.64, which is in line with Q_{10}'s for cell and root growth rates for this temperature range.

Conclusions. – It is concluded that temperature in the range 10 to 30°C has no differential effect on root growth, cell growth, and cell division in timothy roots. The optimum temperature for growth of timothy roots is ~27.5°C.

INSECT CYTOLOGY AND GENETICS

M. E. Gaulden

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R. C. von Borstel
J. G. Carlson[20]
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Ultraviolet Radiation Effects on the *Habrobracon* Egg Cytoplasm

R. L. Amy R. C. von Borstel
M. L. Pardue

Introduction. – It has been reported previously[22] that irradiation of the concave (nonnuclear) surfaces of *Habrobracon* eggs will cause a decline in hatchability.[22] The dose-hatchability curve is sigmoid in shape, and the damage is not subject to photorestoration. The action spectrum for this nonnuclear, or cytoplasmic, damage indicates that nucleic acid or nucleoprotein is the primary chromophore.[23]

Results. – Ultraviolet irradiation of the cytoplasmic portion of the egg results in several different effects which manifest themselves during the development of the wasp. In addition to those described above, the following effects have been observed:

1. Hatchability curves have been obtained at nine wavelengths, ranging from 2220 to 3022 Å. Irradiation at wavelengths of 2537 and 2652 Å produces the most damage in the egg, but at all nine wavelengths the survival curves are sigmoid and have the same general shape and slope.
2. At wavelengths greater than 2300 Å, the dead embryos are uniform in appearance and die late in development (after tracheal elements are formed). They are characterized by atypical yolk distribution, probably resulting from herniated or incompletely developed guts.
3. At shorter wavelengths, that is, 2300 and 2220 Å, the survival time of those embryos that die before hatching is heterogeneously distributed. In general, the embryos die earlier in development than do those irradiated at longer wavelengths, and

[19] Research Participant.

[20] Research Associate.

[21] Consultant.

[22] R. C. von Borstel and S. Wolff, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 35–36.

[23] R. L. Amy and R. C. von Borstel, *Biol. Semiann. Prog. Rep. Aug. 15, 1955*, ORNL-1953, p 33–35.

it is difficult in the unstained embryo to distinguish between death from absorption in the cytoplasm and death from leakage of the radiation to the nucleus.

4. Surviving embryos which, as eggs, were irradiated on the concave surface hatch more slowly than do the controls. Delay in hatching time is roughly proportional to the dose. This phenomenon occurs at all wavelengths tested.

5. Embryos that hatched were placed on their hosts, *Ephestia* caterpillars. The adults were examined for abnormalities. A recurring abnormality observed was irregularity in the structure of the tergites, the segments on the dorsal surface of the abdomen. At the dose levels where there are few survivors, about half the wasps have irregular tergites. The data which show the percentages of adults having deformed tergites when exposed at different doses to ultraviolet light of various wavelengths are summarized in Table 3.

Discussion. – The wavelengths at which radiation is the most effective in reducing hatchability[23] are also the wavelengths at which the deaths from irradiation of the cytoplasm occur uniformly late in development. From the action spectrum data it would appear that the death late in development is brought about by absorption of ultraviolet radiation by nucleic acid or nucleoprotein. The deaths from irradiation of the cytoplasm at shorter wavelengths are unlike those from irradiation at longer wavelengths. It would seem that at shorter wavelengths some chromophore other than nucleic acid or nucleoprotein must be absorbing more efficiently. The chromophore may be a species of protein.

TABLE 3. PERCENTAGES OF ADULTS WITH ABNORMAL TERGITES FOLLOWING CYTOPLASMIC IRRADIATION OF THE EGG

Wavelength (Å)	Dose (ergs/mm^2)	Number of Adults	Number with Atypical Tergites	Percentage
2300	2500	7	1	14.3
	2700–3000	8	1	12.5
	3300–3800	19	9	47.4
2483	500–700	31	1	3.2
	900	17	4	23.5
	1000–1200	20	13	65.0
2537	300–600	298	13	4.4
	750–900	98	14	14.3
	950–1000	63	14	22.2
2652	650	45	4	8.9
	750	17	2	11.8
2753	700–800	67	4	6.0
	900	42	7	16.7
	1000–1400	17	9	52.9
2893	1100	42	2	4.8
	1200	71	9	12.7
	1400–1900	22	8	36.4

Effect of Oxygen on Radiation-Induced Mitotic Inhibition in the Neuroblast of the Grasshopper Embryo

E. I. Shaw

Introduction. – It was reported[24,25] that the deoxygenation of the culture medium by the use of 10^{-2} *M* sodium hydrosulfite reduced the effectiveness of x rays to produce mitotic inhibition in neuroblasts of embryos of the grasshopper, *Chortophaga viridifasciata*. With improved techniques more accurate data have been obtained for several different dose levels of x rays.

Results. – The period of minimum mitotic activity is first reached at about 66 min after irradiation at all the dose levels used. The average mitotic activity of the irradiated cultures at 66 min post-irradiation is divided by the average mitotic activity of the control cultures to yield the values plotted in Fig. 3. Curve A, representing cultures pretreated with sodium hydrosulfite, has the following characteristics. It is linear when a semilogarithmic scale is used for plotting the effect vs dose. There is no threshold, for the curve is linear along its entire length and extrapolates back to 100% of normal mitotic activity at zero dose. The 37% dose is approximately 21 r and is about four times as great as the 37% dose (5 r) for the unprotected cultures, curve B.

Discussion. – The protection afforded by deoxygenation of the culture medium decreases the effectiveness of x rays to produce mitotic inhibition and decreases the slope of the curve by a factor of 4. Thus the dose range in which the phenomenon can be studied has been extended. The dose reduction factor of 4 is comparable to the factor by which the production of oxidizing radicals has been decreased because of the deoxygenation of the culture medium.

In the case of the grasshopper neuroblast, mitotic inhibition caused by x rays appears to be a one-hit type of phenomenon. This is indicated by the lack of threshold (curve A in Fig. 3) and the linear relationship of the radiation effect with dose when the former is plotted on a logarithmic scale. The 37% dose of 5 r for the unprotected cultures appears to be extremely low when compared with the 37% dose for other radiation-induced phenomena.

[24] E. I. Shaw, *Biol. Semiann. Prog. Rep. Feb. 15, 1956*, ORNL-2060, p 23.

[25] E. I. Shaw, *Proc. Soc. Exptl. Biol. Med.* **92**, 232–236 (1956).

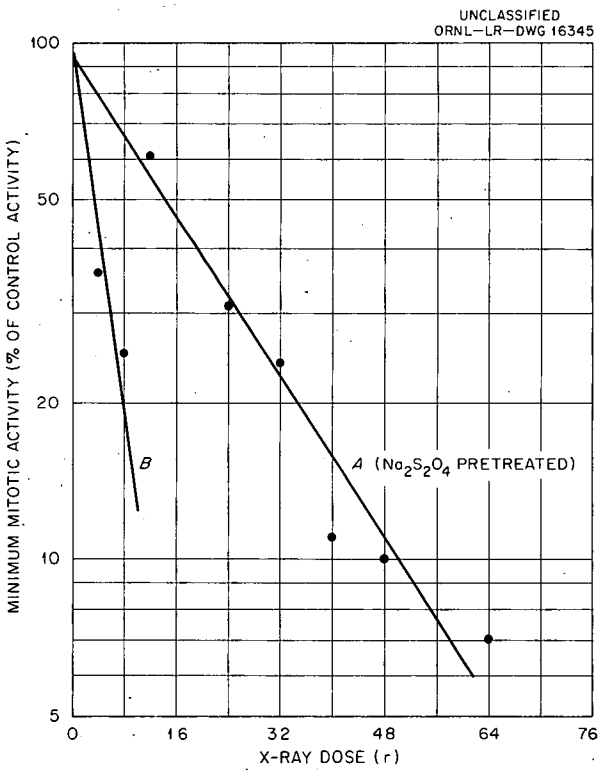

Fig. 3. Minimum Mitotic Activity vs X-Ray Dose.

DROSOPHILA GENETICS

E. Novitski

G. E. Brosseau[26] L. M. Sandler[27]
C. W. Edington E. S. Von Halle
D. L. Lindsley R. D. Wilkerson

Nonrandom Distribution of Induced Dominant Lethals Within the Genome of *Drosophila melanogaster*

D. L. Lindsley E. S. Von Halle
C. W. Edington R. D. Wilkerson

Introduction. – Normal development of zygote into an imago can be prevented in a proportion of the cases by the irradiation of one or the other of the parents prior to fertilization. In a case where every irradiated chromosome in the zygote is covered by an unirradiated homolog (i.e., irradiated ♂ × unirradiated free-X ♀), failure of the zygote to give rise to an imago is defined as an induced dominant lethal change in the irradiated genome.

[26] Temporary employee.

[27] Arrived Aug. 1, 1956.

Irradiation of mature sperm corresponds to the irradiation of a mixed population of cells, some of which are of constitution XA (where A = autosomes II, III, and IV) and others which are of constitution YA. Induction of a dominant lethal in an XA sperm results in the death of a female zygote, whereas induction of a dominant lethal in a YA sperm kills a male zygote. Any difference in the induced dominant lethal frequency in XA and YA sperm should therefore cause a shift in sex ratio which represents a measure of the difference in production of dominant lethals associated with the X and Y chromosomes.

Results. – In general, it is observed that there is either no change in sex ratio or a slight increase in males in the progeny of normal males treated with various doses of x rays. These results indicate similar frequencies of dominant lethal production in X and Y chromosomes but allow no estimate of the absolute frequency in either the X or the Y. Consequently, an experiment was designed which permitted an estimate of dominant lethal production in the X and Y chromosomes. This was accomplished by comparing the effects of given doses of radiation on the sex ratio of the progeny of males with X-bearing and Y-bearing sperm and males with X-bearing and nullo-X, nullo-Y sperm. From an experiment with doses of 1, 2, 3, and 4 kr it was estimated that X-linked dominant lethals increase at a rate of 11%/kr and that Y-linked dominant lethals increase at an average rate of 9%/kr; that is, sex-linked dominant lethals increase at an average rate of 10%/kr. Hatchability data from other sources indicate that, in the linear portion of the survival curve, total dominant lethality increases at a rate of approximately 20%/kr; consequently, 20% of the chromatin is associated with 50% of the dominant lethals.

Discussion. – A hypothesis which might be advanced to explain these results is that some portion of the sex chromosomes is particularly sensitive to irradiation. The fact that this region must be common to both the X and the Y immediately suggests the proximal heterochromatin. To test the possibility that this region contributes appreciably to the sex-linked dominant lethal frequency, tests were carried out utilizing an X chromosome [In(1)sc^{4L}, sc^{8R}] deficient for most of the proximal heterochromatin, that is, *bb*, the nucleolus organizer and Block A. Whereas data from all other X chromosomes yield estimates of X-linked dominant lethality equal to or greater than the estimated Y-linked dominant lethality, an estimate of sc^4,sc^8-linked dominant lethality was no more than one third as great as Y-linked dominant lethality.

It would appear that the sex chromosomes are involved in more than their share of dominant lethals according to length and that this disproportionality results from high sensitivity of the proximal heterochromatin. The apparent high heterochromatic sensitivity could be attributed to some physiological disturbance such as induced nucleolar disfunction, or it might be postulated that breaks induced in this region, which Muller has shown do not become involved in rearrangement, preferentially give rise to dominant lethals.[28]

Meiotic Behavior of Free-X-Chromosome Heterochromatic Duplications in *Drosophila melanogaster*

D. L. Lindsley L. M. Sandler

In an analysis of the genetic behavior of males carrying an attached-XY chromosome and a variety of free heterochromatic X-chromosome duplications,[29] it was found that certain of the duplications are associated with extremely aberrant sex ratios, particularly a reduced recovery of the attached-XY chromosome. That this reduced recovery is not a manifestation of meiotic loss of the sort demonstrated by Sandler and Braver[30] is suggested by the meiotic loss having been shown to be associated with the absence, rather than with the presence, of a homolog and is rather convincingly demonstrated by the fact that gametes from which chromosomes have been lost are not recovered in numbers nearly sufficient to account for the observed shift in sex ratio. Egg counts indicate that the aberrant sex ratios are not accompanied by any zygote mortality.

There are other instances of unequal recovery of gamete types in *Drosophila* not attributable to meiotic loss. One such case is that reported by Novitski and Sandler[31] in which certain exchange

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[28] H. J. Muller, *Genetics* 30, 15 (1945).

[29] D. L. Lindsley, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 39–40.

[30] L. Sandler and G. Braver, *Genetics* 39, 365–377 (1954).

[31] E. Novitski and L. Sandler, *Genetics* 41, 194–206 (1956).

products from compound-X chromosomes were found to be lethal if the compound-bearing female parent did not carry a homolog for the compound. Another is that reported by I. L. Sandler[32] in which an analysis of segregation in a translocation heterozygote [the translocation being between chromosomes I and IV; $T(1:4)B^S$] was made. In the latter study it was found that complementary sperm types are produced with extremely unequal frequencies. Zygote mortality has also been eliminated in this case. It may be noted that, among the apparent duplications studied here, two which show unequal sex ratios are translocations involving chromosomes one and three.

[32] I. L. Sandler, Masters thesis, University of Missouri (1956).

MAMMALIAN GENETICS AND DEVELOPMENT

GENETIC AND DEVELOPMENTAL EFFECTS OF RADIATION IN MICE

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Radiation-induced Mutations in the Mouse

W. L. Russell

J. S. Gower
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Enough data have now been obtained to warrant a preliminary report on mutation rates at specific loci in gametes irradiated in postspermatogonial stages. As in earlier experiments on mutation in spermatogonial stages, wild-type male mice were exposed to a single dose of x rays and mated to a stock homozygous for seven autosomal recessive visible genes. Control (unexposed) males were also mated to the test stock. The mating plan used was as follows: Immediately after irradiation each male was placed in a pen with two females. At the end of a week the male was removed and placed in a new pen with two new females. This procedure was repeated at weekly intervals. The offspring obtained from these matings were observed for mutations at the seven specific loci, and presumed mutants were tested genetically. The dose of x rays chosen for this experiment was 300 r. At this dose the dominant lethal incidence is low enough to permit collection of adequate numbers of viable offspring from cells irradiated in at least some of the more sensitive of the postspermatogonial stages.

It was first necessary to determine the maximum length of time after irradiation at which it could be safely assumed that matings would still be utilizing gametes that had been irradiated in postspermatogonial stages. With higher doses of x rays (e.g., 600 r) this interval lasts for about four or five weeks, and its end is clearly marked by the onset of a period of complete sterility. With the 300-r dose, and the mating plan used in the present experiment, it was found that the males were fertile throughout the first six weeks after irradiation, but that during the seventh week almost all matings were infertile. Thus the presterile period is somewhat longer than when higher doses are used. The details of this result, the changing pattern of fertility and dominant lethality during the six weeks of matings, are of interest in themselves and will be reported later. For the present purpose it is sufficient to note that, with the mating plan used, offspring collected from matings made up to six weeks after irradiation could safely be assumed to come from gametes irradiated in postspermatogonial stages.

The number of mutations so far obtained and tested is shown in Table 4.

The mutation rate from irradiation of postspermatogonial stages is thus 45.3×10^{-8} per roentgen, per locus. This is just twice the mutation rate (22.1×10^{-8}) obtained from earlier extensive data on irradiation of spermatogonial stages. The difference between the mutation rates in the two stages was tested by the likelihood ratio method and was found to be significant at approximately the 3% level.

[1]Consultant.

TABLE 4. X-RAY-INDUCED MUTATIONS AT SPECIFIC LOCI IN THE MOUSE

Dose to Postspermatogonial Stages	Number of Animals Examined	Number of Mutations at Locus							
		A	B	C	D	P	S	Se	Total
300 r	16,813	2	3	2	2	2	5	0	16
Control	36,351	0	0	0	0	0	0	0	0

Dominant Lethals Induced in Mouse Sperm by 14-Mev Neutrons from a Deuterium-Tritium Reaction

L. B. Russell M. H. Major
W. L. Russell C. M. Coffman

Introduction. – The determination of dominant lethal incidence in sperm provides a comparatively fast method for measuring radiation-induced chromosomal damage in mice. This method has, therefore, been used by the authors in the past to compare the effectiveness of different types of radiations[2,3] or irradiation under different conditions.[4] A source of monoenergetic neutrons, the Cockcroft-Walton linear accelerator, has recently become available to the Biology Division. In order to provide a first measure of the relative effectiveness, with regard to genetic damage in mice, of 14-Mev neutrons, an experiment was carried out to measure dominant lethals induced in sperm. In this we have had the cooperation of M. L. Randolph of the Biophysics Group.

Method. – Hybrid $(101 \times C_3H)F_1$ male mice were exposed to 14-Mev neutrons from a deuterium-tritium reaction of a Cockcroft-Walton linear accelerator. The animals were irradiated in individual Lucite tubes, arranged in a circle around the target, the target-testis distance being approximately 15 cm. Doses used were 0, 100, 200, 300, 400, and 500 rep, and there were 10 males at each dose. All exposures were made on the same day, during the course of which average dose rates varied from 176 to 255 rep/hr.

In the late afternoon of the day of irradiation, each of the 60 males was caged with four four-month-old $(101 \times C_3H)F_1$ females. Vaginal plugs were checked the following morning and daily thereafter for a total of one week, at the end of which all females were removed. Females were sacrificed 12½ to 16½ (mostly 13½ and 14½) days after mating. Corpora lutea (representing number of ovulated eggs) were counted and the uterine contents examined under a dissecting microscope.

Results. – Table 5 shows the results obtained. It may first be noted that there was little if any

[2] W. L. Russell *et al.*, *Genetics* 38, 688 (1953).

[3] W. L. Russell, L. B. Russell, and A. W. Kimball, *Am. Naturalist* 88, 269–286 (1954).

[4] W. L. Russell, J. C. Kile, Jr., and L. B. Russell, *Genetics* 36, 574 (1951).

TABLE 5. DOMINANT LETHALITY IN OFFSPRING OF MALE MICE MATING ONE TO SEVEN DAYS AFTER EXPOSURE TO 14-MEV NEUTRONS

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
			Percentage of Corpora Lutea Represented by				
Dose (rep)	Number of Pregnancies	Number of Corpora Lutea[a]	Living Embryos	Embryos Dying After Day 10	Embryos Dying Between Implantation and Day 10	Eggs or Embryos Dying Before Implantation (100 minus sum of columns 4, 5, and 6)	"Survivors" (sum of columns 4 and 5)
0	29	300	82.00	1.67	4.00	12.33	83.67
100	30	327	61.77	0.92	10.39	26.91	62.69
200	24[b]	265.2	48.64	0.75	15.46	35.14	49.40
300	28[b]	303.2	43.87	0.99	18.47	36.68	44.85
400	26	272.5	29.36	0.73	26.06	43.85	30.09
500	27[c]	279.9	26.08	0.00	24.65	49.27	26.08

[a] Doubtful corpora lutea were counted as 0.5.

[b] Includes one "pregnancy" in which all embryos had presumably died before implantation and there were no corpora lutea. The number of ovulated eggs was taken as 10.7, which is the mean number of corpora lutea per pregnancy for all pregnancies in which the corpora lutea were counted.

[c] Includes two pregnancies as described in footnote *b*.

decrease in the number of pregnancies with increasing dose, up to the highest dose used, 500 rep. In contrast, following exposure of males to a nuclear detonation or in the 86-in. cyclotron,[2,3] the number of matings decreased radically with dose, starting with doses somewhat below 300 rep, as a result of radiation sickness or death of males. There were no obvious signs of radiation sickness and no deaths following exposure to the 14-Mev neutrons.

The dominant lethal effect is summarized in the last column [col (8)] of Table 5. It is expressed in terms of survival through day 10 of gestation, since, as in earlier data,[2,3] there is no statistically significant effect of radiation on mortality between day 10 and the time of observation [col (5)].

As shown in Fig. 4, the data give a good fit to the assumption that the logarithm of survival is linearly related to dose. The slope of the regression line is -0.102 ± 0.014 (ref 5). In contrast, the slopes of lines fitted to the results of similar experiments with radiations of a nuclear detonation[3] and of the 86-in. cyclotron[2,3] are -0.257 and -0.298, respectively (Fig. 4). Exposure to the deuterium-tritium reaction in the Cockcroft-Walton, therefore, appears only about one-third as effective in producing dominant lethals in sperm as are radiations from the detonation and cyclotron. A more accurate comparison of the relative effectiveness of the various kinds of neutrons cannot be made because the cyclotron radiation and detonation radiation involved small but not accurately known gamma components.[6] There is now also evidence that the methods of dosimetry used in both these earlier sets of data may have slightly underestimated the total dose. Since these factors are known to be small and, moreover, tend to cancel each other as far as comparison with the present data is concerned, the above ratio of 1:3, for relative effectiveness of 14-Mev neutrons to cyclotron or detonation neutrons, may be assumed to be approximately correct.

[5]As calculated by the Biometrics Section, Mathematics Panel.

[6]For details, see ref cited in ref 3.

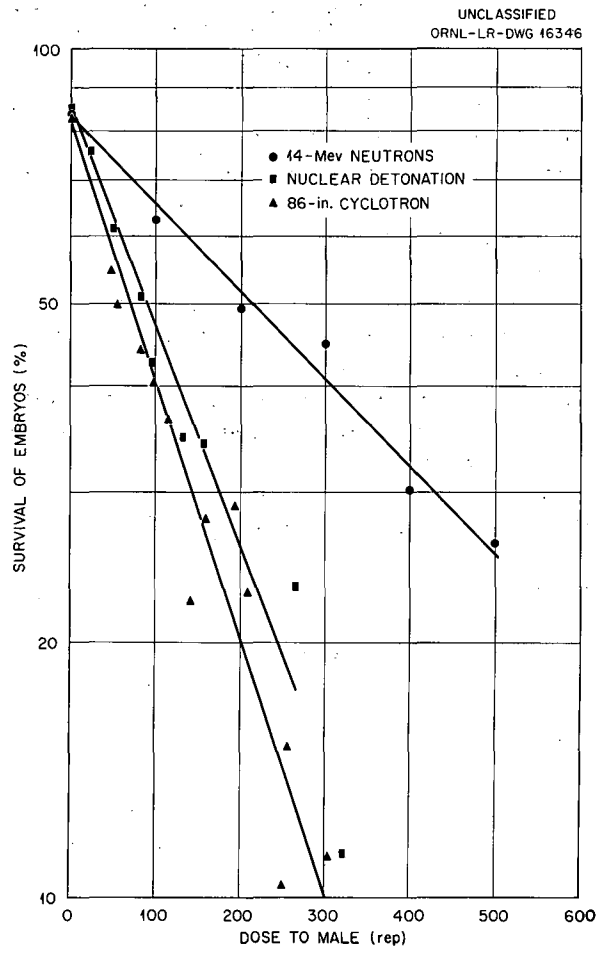

Fig. 4. Dominant Lethality in Offspring of Male Mice Mated for One Week Following Exposure to 14-Mev Neutrons. Experimental points from Table 5, column 8. Included for comparison are the results of earlier experiments using radiations of a nuclear detonation (see ref 3) and of an 86-in. cyclotron (see refs 2 and 3).

Sensitivity of Spermatogonia of the Mouse to 14-Mev Neutrons

E. F. Oakberg R. L. DiMinno

Hybrid (101 × C_3H) mice were exposed to 0, 0.5, 1.0, 1.5, 2.0, 3.0, 5.0, 7.0, 10.0, 15.0, 20.0, 25.0, 30.0, and 40.0 rep of 14-Mev neutrons from the Cockcroft-Walton accelerator.[7] All animals were

[7]We are grateful to M. L. Randolph for irradiation of the material.

killed 72 hr after exposure, and the sensitivity of the spermatogonia was determined by the quantitative procedures described elsewhere.[8]

Sensitivity of all type A spermatogonia was estimated by counts based on all tubule stages, and sensitivity of late type A and early intermediate spermatogonia by scoring number of resting primary spermatocytes in stage VII tubules. The number of both type A spermatogonia and resting primary spermatocytes was reduced at all doses, but considerable variation was observed at 0.5 to 3.0 rep (Table 6). Analysis of number of resting primary spermatocytes indicated that the apparently higher survival at 3.0 rep was not statistically significant. Analysis of data for type A spermatogonia has not been made.

[8]E. F. Oakberg, *Gamma-Ray Sensitivity of Spermatogonia of the Mouse* (in press).

Comparison of experimental/control ratios for type A spermatogonia at the eight individual exposures from 3.0 to 30.0 rep with survival after gamma-ray exposure[8] gave an average RBE of 2.6. Similar comparisons based on number of resting primary spermatocytes at stage VII for the nine exposures from 3.0 to 40.0 rep indicated an average RBE of 2.3. Thus, different developmental stages of spermatogonia of the mouse show similar relative sensitivity to neutrons. Likewise, the basic responses of spermatogonia to neutrons is the same as that for gamma rays, with a survival curve for late type A and intermediate spermatogonia (scored as resting primary spermatocytes, Table 6) similar to that obtained for gamma rays.[8] Survival of type A spermatogonia shows a flattening of the curve at 20 to 40 rep, indicating a population with a heterogeneity of sensitivities as observed previously for gamma rays.

Additional data are required for adequate evaluation of the effect of exposures in the 0.5- to 5.0-rep range.

TABLE 6. NUMBER OF TYPE A SPERMATOGONIA AND RESTING PRIMARY SPERMATOCYTES 72 hr AFTER EXPOSURE TO 14-Mev NEUTRONS

Dose (rep)	Type A Spermatogonia*		Resting Primary Spermatocytes**	
	Total Cells	Experimental/Control	Total Cells	Experimental/Control
0	1923		1877	
0.5	1806	0.939	1804	0.961
1.0	1767	0.919	1640	0.874
1.5	1530	0.800	1562	0.832
2.0	1874	0.975	1609	0.857
3.0	1716	0.892	1736	0.925
5.0	1534	0.798	1347	0.718
7.0	1344	0.699	1147	0.611
10.0	1118	0.581	919	0.490
15.0	990	0.515	433	0.231
20.0	705	0.367	288	0.153
25.0	663	0.345	167	0.089
30.0	583	0.303	91	0.048
40.0	450	0.235	10	0.005

*Five mice per dose, 100 tubules scored per mouse.

**Five mice per dose, ten stage VII tubules scored per mouse, irradiated as late type A and early intermediate spermatogonia.

Further Studies on the Notch Locus of *Drosophila melanogaster*

W. J. Welshons

It was reported earlier that exceptional wild-type offspring could be obtained from females heterozygous for the mutants *Notch* and *split*.[9] The exceptional offspring were believed to result from a crossover between the two loci. In the earlier data some *Notch* chromosomes carried the recessive mutant *yellow* so that the crosses in question were y N / + spl ♀♀ × + spl ♂♂. In these cases the exceptional offspring possessed a chromosome which, in addition to being neither *Notch* nor *split*, carried the marker *yellow*. It was concluded that if the exceptions resulted from a crossover between *Notch* and *split* the mutant *split* must be localized to the left of *Notch*.

In order to test the assumption that the exceptional chromosomes are derived from a crossover, the cross y $N^{264\text{-}47}$ + / + spl rb ♀♀ × + spl rb ♂♂ was performed. This cross yielded two y ++ exceptions out of a total of approximately 20,000 tested chromosomes. Another cross of y $N^{264\text{-}103}$ + / + spl rb ♀♀ × + spl rb ♂♂ yielded an additional 20,000 chromosomes, one of which was ++ rb and one which was not *Notch* nor *split* and carried the marker *ruby*; the presence or absence of the *yellow* marker in this chromosome could not be determined.

Several years ago the author found a spontaneous *Notch* mutation, and a salivary gland analysis of this mutant showed no visible aberration of the X chromosome. This *Notch* was utilized in the cross w^a N + / + spl dm ♀♀ × w^a spl + ♂♂. Approximately 57,000 chromosomes were tested, and the following exceptions were found: eight w^a + dm, one ++ dm, and one w^a ++.

These data indicate that in many cases the appearance of an exception is associated with a recombination between the markers on either side of *Notch* and *split*; however, in an appreciable number of cases the exceptional types are not associated with such an exchange. In this respect the *Drosophila* data are similar to those reported for *Neurospora* by Mitchell[10] and St. Lawrence.[11]

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

[10] M. B. Mitchell, *Proc. Natl. Acad. Sci. U.S.* **41**, 215–220 (1955).

[11] P. St. Lawrence, *Proc. Natl. Acad. Sci. U.S.* **42**, 189–194 (1956).

MICROBIAL PROTECTION AND RECOVERY

RADIATION PROTECTION AND RECOVERY IN BACTERIA

A. Hollaender

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Mechanism of Protective Action of Cysteamine on *Escherichia coli*

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J. H. Udinsky

Introduction. – Cysteamine, β-mercaptoethylamine (MEA), has been reported by Hollaender and Doudney[5,6] to be an outstanding protective agent. This compound has been shown to reduce the sensitivity of an aqueous suspension of *E. coli* B/r by a factor of 10 to 12 if present in optimal concentration during the time of irradiation with x or gamma rays. This remarkable desensitization of bacteria, as well as some peculiarities noted concerning the nature of the survival curves, prompted the reinvestigation of the conditions necessary for maximal protection with this compound. The following parameters were chosen for investigation: possible dependence on the cell concentration in suspension, dependence on radiation dose, and dependence on diffusion prior to and during irradiation. Cobalt-60 gamma rays were used in all experiments, at a dose rate of 1200 to 1500 r/min.

Washed cell suspensions of aerated nutrient-broth-grown *E. coli* B/r were used after growth for 16 to 20 hr at 37°C. In most cases 0.1 ml of washed cells (200×10^6 cells) was suspended in 1 ml of an aqueous solution of MEA and incubated at ice bath temperature for 30 min prior to irradiation. The cells were irradiated, immediately diluted in M/15 phosphate buffer, and then surface-plated on nutrient or yeast extract agar. After 24 hr of incubation the colonies were counted. Control suspensions with and without added MEA were held under the same conditions as were the irradiated suspensions.

Results and Discussion. – (*a*) *Independence of Cell Concentration.* – In order to determine the possible effect of cell concentration on protection, several experiments were made wherein survival was measured at a constant gamma-ray dose as a function of the concentration of MEA over the range 0.1 to 48 μmoles/ml. No significant difference could be found for suspensions containing 200×10^4, 200×10^5, or 200×10^6 cells/ml, indicating that MEA concentration was not limiting under the experimental conditions. The optimal MEA concentration was found to be about 48 μmoles/ml. When this concentration was used and survival estimated as a function of the dose of gamma rays, the surviving fraction of cells was found to be an exponential function of gamma-ray dose over the range 0 to 150 kr. The median lethal dose was found to be about 60 kr as compared with 5 kr for cells irradiated in distilled water under the same conditions.

(*b*) *Depencence on Radiation Dose.* – One possible explanation for the peculiar survival curves previously reported[5,6] could be a dependence of protection on radiation dose. The above authors attempted to test this hypothesis by testing the protective effect of irradiated solutions of MEA and found such solutions to be essentially maximally protective. Since they chose to test this effect at the optimal protective concentration (on the plateau of the concentration curve), a 50% destruction would hardly be measurable. In the present experiments it was decided to determine the relationship between surviving fractions of cells and MEA concentration at several radiation doses. The results are shown in Fig. 5. The logarithm of surviving fraction of cells is plotted as a function of the logarithm of MEA concentration for convenience. It appears from these data that the minimal and maximal protective concentrations of MEA are dependent on the radiation dose employed. Complete survival curves at limiting concentrations of MEA have not as yet been run, but

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[1]Research Associate.

[2]Temporary employee.

[3]Consultant.

[4]Loanee.

[5]C. O. Doudney and A. Hollaender, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 46–47.

[6]A. Hollaender and C. O. Doudney, p 112–115 in *Radiobiology Symposium 1954* (Z. M. Bacq and P. Alexander, eds.), Butterworths, London, 1954.

it would appear on the basis of the data on dose-dependence that nonexponential survival curves may be obtained similar to those reported previously by this group.[5,6]

(c) *Permeability and Its Effect on Protection.* – The question of cell permeability to a protective agent is always an important one in a consideration of mechanism of action. Few attempts have been made to correlate the concentration of protective compound within the cell and that required in the surrounding aqueous solution for maximal effect. While a direct measurement of the uptake of MEA by the cells would be ideal, an indirect measurement seemed less difficult and perhaps just as informative. A colorimeter sulfhydryl test[7] that has been used in this laboratory for some time[8] lent itself well to the studies. The amount of MEA removed or lost from the solution to cells introduced into it was quite easily measured by the colorimetric technique. In order to measure this loss to the cells it was found necessary to react the MEA solution with more than 10^{11} cells/ml. When fewer cells were used, no significant reduction of MEA occurred from the solution.

The cells were incubated at either ice bath temperature or at 37°C for 30 min and were removed by centrifugation at 3000 rpm for 15 min. Table 7 shows that the amount of cysteamine taken up by the cells is a small fraction of that required in the surrounding solution for maximal protection and that the diffusion of MEA into the cell has a high temperature coefficient. A few experiments were run to determine the optimal concentration of MEA for protection with incubation at ice bath temperature as compared with 37°C. As the data reported in Table 7 suggest, about 10 times less MEA was required for maximal protection with incubation at 37°C than was required with ice bath temperature. Moreover, some data have been obtained which

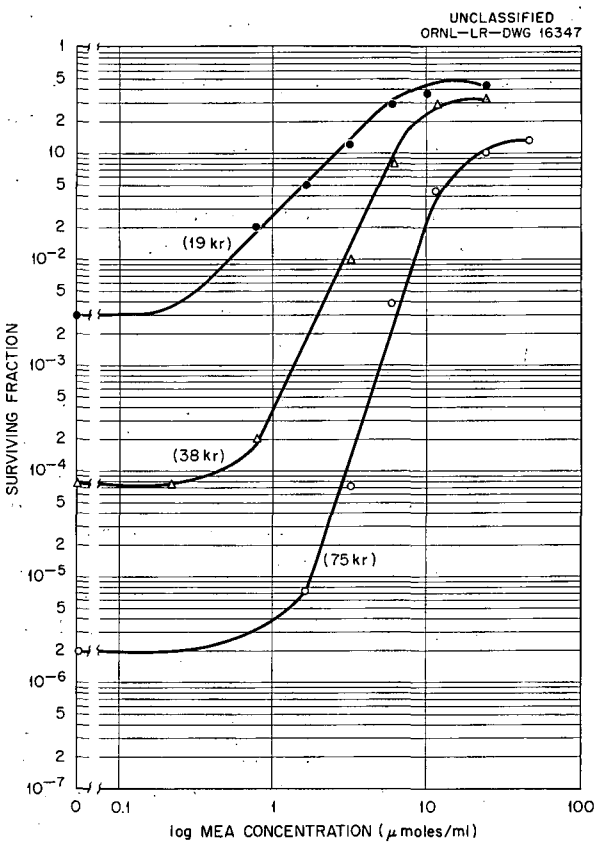

Fig. 5. Relation Between Surviving Fraction of Cells and MEA Concentration at Various Radiation Doses.

[7] R. E. Basford and F. M. Huennekins, *J. Am. Chem. Soc.* 77, 3875 (1955).

[8] J. X. Khym and A. B. Ottinger, *Biol. Semiann. Prog. Rep. Feb. 15, 1956*, ORNL-2060, p 46.

TABLE 7. UPTAKE OF MEA BY BACTERIA

Incubation Temperature (°C)	Cell Concentration (cells/ml)	MEA Concentration in Solution (μmoles/ml)	Amount Lost to Cells (μmoles)
0	6×10^{11}	0.75	0.6
0	4×10^{11}	1.50	0.5
0	6×10^{11}	48.0	0.8
37	6×10^{11}	48.0	12.0

indicate that no preincubation of cells with MEA is required for maximal protection if cells are irradiated with this compound at temperatures above 12°C. Considering the dose-dependence for MEA protection, some rough calculations would show that the number of molecules required per cell for protection and the number of ion pairs produced per cell at a particular dose of gamma rays are of the same order of magnitude. These data, as well as most of the other recent data obtained, are consistent with the idea that, at the concentrations present within the cell, MEA is readily destroyed by gamma rays and that for maximal protection it must diffuse into the cell at a rate at least as great as the rate at which the compound is destroyed by the radiation. The experiments necessary to prove this hypothesis are impractical with the technique employed in these present experiments. It is entirely possible that a dose-rate dependence could be shown for protection with a source which would make available 10 to 100 times the intensity of the present source. Some experiments with an electron generator are anticipated for the future.

No experiments to date explain why MEA is several times as efficient a protective agent as are other closely related sulfhydryl compounds. Whether or not it has a specific site of action has not been elucidated in the experiments so far performed.

Effect of Cysteamine Pretreatment on Mutant Induction of Two *Escherichia coli* Auxotrophs

R. L. Girolami A. Hollaender

Introduction. – The protective effect of cysteamine pretreatments of bacteria on survival after ionizing radiation is well known,[6] but the effect of this treatment on mutation rates has not been studied.

Methods. – Two auxotrophic strains of *E. coli* were used in this study. One, called 42-24, has the multiple requirements for serine and glycine and the other, 42-11, requires isoleucine and valine. The back mutation to independence was studied. This mutation is apparently a single step event. No mutants, of the several hundred tested by replica plating,[9] were found with a requirement for only one of the amino acid pairs.

It was necessary to carefully select the auxotrophs under study to avoid strains which showed population pressure, that is, disproportionately large mutant counts at higher dilutions. It was also found necessary to use washed agar for the mutant assay plates in order to get reproducible results.

Cells were grown in aerated nutrient broth for 18 to 20 hr at 37°C and then washed with water. The cells were divided into two portions; to one was added cysteamine to give a final concentration of 0.02 *M*, and both were held at ice bath temperature for 30 min before exposure to gamma radiation from a Co^{60} source.

Survival was determined on minimal medium plus the required amino acids, and mutation was determined on unsupplemented medium.[10] Surviving colonies were counted after two days and mutants after six days incubation at 37°C.

Results and Discussion. – An exponential survival curve was obtained for both protected and unprotected populations. The cysteamine pretreatment gave a dose reduction factor of approximately 3 for both strains.

Figure 6 shows the average regression lines (each line represents three or four separate experiments) for mutants per 10^8 survivors as related to dose on a logarithmic scale. Although the magnitude of the mutation rate differs between the two strains, a linear relationship, on a log-log plot, between the number of mutants and dose appears to characterize the data.

With strain 42-11 the rate of increase in mutants with increasing dose appears to be the same for both the protected and unprotected cultures. There is, however, a significant difference between the two treatments in the average number of mutants occurring at a given dose. The data indicate that the number of mutants is higher for unprotected than for protected populations.

With strain 42-24 the same situation appears to exist as with the previous strain. There are indications, however, that the rate of increase in mutants with increasing dose is greater in the unprotected culture.[11]

Summary. – The lethal effect of gamma irradiation on auxotrophs of *E. coli* can be altered by

[9] J. Lederberg and E. N. Lederberg, *J. Bacteriol.* **63**, 399–406 (1952).

[10] M. Demerec and E. Cahn, *J. Bacteriol.* **65**, 27–36 (1953).

[11] Statistical analysis by M. Kastenbaum, ORNL Mathematics Panel.

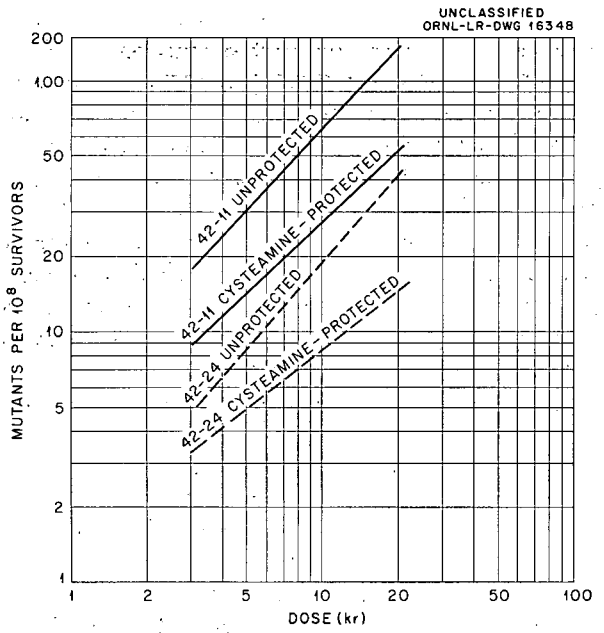

Fig. 6. Effect of Pretreatment on Mutant Induction in Two *E. coli* Auxotrophs.

pretreatment of the cells with cysteamine. The protective effect yields a dose reduction factor of about 3. Cysteamine pretreatment also results in a lower mutation rate than is observed in unprotected cultures. The magnitude of mutation protection is strain dependent.

Pre- and Postirradiation Studies with Long-Ultraviolet and Short-Visible Radiations on *Escherichia coli* B/r

R. F. Gilfillan A. Hollaender

The studies, that were begun by Hollaender[12-14] and Sbarra[13,14] on the inactivation of *Escherichia coli* B/r by long-ultraviolet and short-visible radiation (3500–4900 Å) have been continued.

Cells were cultured as described previously.[13,14] The influence of a variety of pre- and postirradiation treatments on survival has been re-examined, and the results of Hollaender and Sbarra were confirmed. These experiments have been designed to elucidate the influence of the following factors: the effect of temperature during irradiation, the temperature of incubation following irradiation, the cell concentration, the presence of inert particular material (dead cells) during irradiation, the ion composition of the suspending menstrum during and following irradiation, the removal of oxygen by nitrogen prior to irradiation, and the plating medium following irradiation. Leakage of cellular material following irradiation also has been investigated.

An increase in survival of cells with a decrease in temperature during irradiation was observed. However, the temperature coefficient varies with the electrolyte composition of the suspending menstrum; that is, a more rapid rate of killing is obtained in salt solution A, a 3.4% mixed salt solution as employed by Sbarra,[13] than in 0.85% NaCl or phosphate buffer. In contrast with the influence of temperature on the restoration of x-irradiated cells, reported by Stapleton *et al.*,[15] survival on solid media following irradiation with long-ultraviolet and short-visible light was found to be independent of the postirradiation incubation temperature over the range 0 to 37°C.

The rate of killing was observed to be dependent upon cell concentration. When 10^9, 10^7, and 10^5 cells were irradiated under a number of experimental conditions, sensitivity increased proportionately with decreasing cell concentrations. The addition of sufficient dead cells to increase the concentration 100-fold did not alter the rate of killing per unit dose.

Inactivation is dependent also on the ionic composition of the suspending fluid. Cells irradiated in salt solution A, a high concentration of mixed salts (0.517 *M* NaCl, 0.027 *M* KCl, and 0.019 *M* $CaCl_2$), are much more sensitive than those suspended in 0.14 *M* NaCl or 0.067 *M* phosphate. Continued incubation at various temperatures following irradiation in salt solution A, either in the irradiated salt mixture or in fresh solution, greatly enhances killing. Little or no enhancement of killing was observed by continued incubation for equal periods in phosphate buffer solutions.

Cell suspensions bubbled with nitrogen prior to irradiation are markedly more resistant to irradiation than those bubbled with oxygen. The oxygen

[12] A. Hollaender, *J. Bacteriol.* 46, 531–541 (1943).

[13] A. J. Sbarra and A. Hollaender, *Biol. Semiann. Prog. Rep. Aug. 15, 1955*, ORNL-1953, p 52–53.

[14] A. J. Sbarra and A. Hollaender, *Biol. Semiann. Prog. Rep. Feb. 15, 1956*, ORNL-2060, p 32–33.

[15] G. E. Stapleton, D. Billen, and A. Hollaender, *J. Cellular Comp. Physiol.* 41, 345–358 (1953).

sensitizing effect is much greater for cells irradiated in salt solution A than for those irradiated in phosphate buffer.

Following irradiation, under a variety of experimental conditions, survival can be modified by the plating medium. The incorporation of yeast extract into the glucose–basal salts–agar medium increases the number of survivors. Addition of the amino acids, purines, and pyrimidines which have been shown to replace yeast extract in restoring x-ray inactivated cells, as reported by Stapleton *et al.*,[16] was without effect on cells subjected to 3500 to 4900 Å radiation.

Studies also have been initiated to determine whether or not cellular constituents are released from irradiated cells when they are incubated at 37°C in a medium of phosphate buffer and a metabolizable substrate as Billen[17] has demonstrated for x-irradiated or ultraviolet-treated cells. Preliminary results indicate that ultraviolet-absorbing material is released under these conditions. The nature of the materials which are released has not been definitely established.

[16] G. E. Stapleton, A. J. Sbarra, and A. Hollaender, *J. Bacteriol.* 70, 7–14 (1955).

[17] D. Billen, *Arch. Biochem. and Biophys.* (in press).

MAMMALIAN RECOVERY

MODIFICATION OF RADIATION INJURY IN MICE

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Studies on Lethally Irradiated (950 r) Mice Treated with Rat Bone Marrow

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Introduction. – Previous studies[3,4] on the immune response of lethally x-irradiated mice treated with isologous bone marrow indicate that the antibody response to rat red blood cells (RBC) is subnormal when tested on the fifteenth and thirtieth day after irradiation but is normal on the sixtieth day. With sheep RBC as the antigen, animals immunized on either the thirtieth or sixtieth day after irradiation had a normal antibody response. This study was undertaken to investigate the immune response of lethally irradiated mice protected with rat bone marrow.[5,6]

Results. – The survival of lethally irradiated mice treated with rat bone marrow was influenced by caging conditions, since during the 30-day observation period fewer deaths occurred among mice housed individually than among those housed two to five per cage; and there were fewer deaths among those housed two to five per cage than among those housed eight to ten per cage. Sex differences were also evident in survival. Females showed greater 30-day survival than males. After irradiation, there was a loss in body weight during the first week; then the body weight gradually increased. The mice began to lose body weight again precipitously during the third and fourth week and continued to do so to the fifth and sixth week. In spite of the loss in weight, an abundant fecal deposit was observed, indicating normal or greater than normal food intake. The coats of these mice were ruffled and shaggy during this critical period. A period of general recovery then ensued. Surviving females appeared to be fully recovered by the eighth week and could not be differentiated from the isologous bone-marrow-treated controls.

Studies on the immune mechanism of these mice were made on the fifteenth, thirtieth, and sixtieth day after irradiation and rat bone-marrow treatment. They failed to respond to rat or mouse RBC antigens. The response to sheep RBC on the fifteenth day was also negative, but a weak antibody response was detected in animals immunized 30 and 60 days after irradiation. Circulating rat RBC in rat bone-marrow-treated mice, as demonstrated previously,[3,5] constitutes a state of "antigen excess" (tolerance), which, accordingly, explains the failure of these mice to produce antibodies to rat RBC. The weak response to sheep RBC, the poor physical condition of the animals as manifested by the secondary body weight loss and their gross appearance, and the occurrence of late deaths suggest internal stress due to a delayed immunologic response of the host to the foreign transplanted tissue (in vivo transplanted antigen-host antibody reaction). The severity and final consequence of such a phenomenon would be dependent on the rate and degree of recovery of the host's immune mechanism. This hypothesis was tested by injecting rat bone marrow into mice exposed to a sublethal x-irradiation dose (710 r) – a dose sufficient to permit active transplantation of foreign cells when injected soon after irradiation but less damaging to the hematopoietic tissue than 950 r. Preliminary studies with mice that had received 710 r showed a mortaility of 100% within 16 days postirradiation for 60 mice injected with rat bone marrow, 30% within 30 days for 200 mice receiving 710 r only, and 0% for 50 mice injected with isologous mouse bone marrow.

Discussion. – Additional studies for verification will involve lower and higher radiation doses, immunohematologic studies on the circulating RBC, histologic examination of tissues, and serum-agar diffusion studies on the hematopoietic organs.

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[1]Temporary employee.

[2]Research Participant.

[3]T. Makinodan, *Biol. Semiann. Prog. Rep. Feb. 15, 1956*, ORNL-2060, p 35–36.

[4]T. Makinodan, N. Gengozian, and C. C. Congdon, *J. Immunol.* (in press).

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

[6]N. Gengozian and T. Makinodan, *J. Immunol.* (in press).

Isologous vs Homologous Bone Marrow in the Treatment of Irradiation Injury

C. C. Congdon I. M. Urso

Introduction. – The delayed homologous bone-marrow reaction occurring in mice exposed to lethal total-body irradiation and treated with bone marrow from a foreign strain of mouse was not observed when the irradiated mice were treated with isologous bone marrow.[7]

Results. – Table 8 shows the low percentage of 100-day survivors in the homologous bone-marrow-treated mice. Histologic study of autopsied mice revealed early progressive reactive changes in the white pulp of the spleen and lymph nodes. The normal architecture of the white pulp of the spleen and the lymph nodes did not regenerate as in the case of isologous bone-marrow-treated mice.

Reticuloendothelial cell proliferation and plasma cell infiltration characterized the reactive changes. In the lymph nodes, fibrous atrophy eventually took place. The white pulp of the spleen showed hyaline degeneration of some splenic nodules. Widespread foci of simple necrosis in the liver and hemorrhagic ulceration in the pylorus and large intestine also characterized the delayed homologous bone-marrow reaction in some mice.

The reaction occurred at all doses of homologous bone marrow administered from $\frac{1}{32}$ of a femoral bone marrow to the 16-femur bone-marrow dose. It developed at exposure levels from 600 r through 1200 r but did not develop at exposures from 200 to 500 r. It was not prevented by using older (six months) irradiated recipients or by giving homologous fetal blood-forming tissues. It was not prevented by chemical protection [S,2-aminoethylisothiuronium·Br·HBr (AET)], gonadotrophin, Cortef, benadryl, estrogen, or streptomycin administration. Mixtures of isologous and homologous bone marrow did not prevent the delayed homologous reaction except when excess isologous bone marrow (3:1) was in the mixture. The most promising results in preventing the delayed homologous reaction were seen in mice given homologous bone marrow on day 1 and 2 after irradiation instead of on day 0, the usual time for injection. Fifteen out of 20 female mice treated in this manner have survived 107 days after irradiation. They resemble isologous bone-marrow treated mice and are gaining weight.

Discussion. – Further characterization of the delayed homologous bone-marrow reaction and the feasibility of preventing the reaction are the main objectives of this project.

Effect of Bone-Marrow Treatment and Chemical Protection on the Bone-Marrow Response of X-Irradiated Mice

P. Urso

Introduction. – With the aid of a quantitative technique for counting bone-marrow cells, data were obtained on the bone-marrow response of lethally x-irradiated mice treated with varying amounts of isologous bone marrow.[8] In this investigation the bone-marrow counting technique was used to further illustrate the recovery of the

[7] C. C. Congdon and I. M. Urso, *Biol. Semiann. Prog. Rep. Feb. 15, 1956*, ORNL-2060, p 37.

[8] P. Urso and C. C. Congdon, *Biol. Semiann. Prog. Rep. Feb. 15, 1956*, ORNL-2060, p 37.

TABLE 8. EFFECT OF ISOLOGOUS AND HOMOLOGOUS BONE MARROW ON SURVIVAL FOR A PERIOD OF 100 DAYS AFTER 900 r TOTAL-BODY IRRADIATION

Sex	Number of Mice	Donor*	Recipient	Number Surviving at End of Each 10-Day Period										Survival (%)
				10	20	30	40	50	60	70	80	90	100	
Male	47	$101 \times C_3HF_1$	LAF_1	44	28	15	11	9	6	4	3	2	1	2.1
Female	32	$101 \times C_3HF_1$	LAF_1	27	24	15	10	9	8	5	4	4	3	9.4
Male	35	LAF_1	LAF_1	33	33	33	33	33	33	33	33	33	33	94.3
Female	28	LAF_1	LAF_1	28	28	28	28	28	28	28	28	28	28	100.0

*Bone marrow from one femoral shaft given intravenously to each mouse.

bone marrow in x-irradiated CAF_1 mice that had been treated with homologous (101 × C_3H mouse) bone marrow or heterologous (rat) bone marrow or that received chemical protection (AET) or a combined treatment (AET plus isologous bone marrow).

Results. – Counts taken on the bone marrow used to intravenously inject mice exposed to 900 r showed that approximately eleven million cells (1-femur dose) were injected when isologous and homologous marrows were used; approximately ninety million cells were present in the heterologous-marrow injection. Several mice were injected intrapertioneally with 9 mg of AET 10 min before exposure to 900 r and either given isologous bone marrow equivalent to a 1-femur dose immediately after x ray or no additional treatment after x ray. The results on the bone-marrow response obtained by serially sacrificing the experimental mice are illustrated by Fig. 7. With bone-marrow treatment alone, the bone-marrow response of the irradiated mice was strikingly parallel for the different types of bone marrow used (Fig. 7*a*). Very few animals died within the time of recovery of the bone marrow. However, the death rate increased sharply after 21 days in mice receiving homologous or heterologous bone marrow even though the bone-marrow counts remained at an average level of ten million cells. Treatment with AET before lethal x irradiation (900 r) showed that the injurious effect of the x rays on the bone marrow is equivalent to the effect produced by a dose of 450 r (Fig. 7*b*). In addition, treatment with 9 mg of AET further modified the effect of 450 r on the bone-marrow count. Results on the bone-marrow response obtained by treating lethally irradiated (900-r) mice with AET before radiation and with isologous bone marrow after radiation show that the injected bone marrow is responsible for the quick recovery of the irradiated mouse's bone marrow and that the AET is responsible to a large degree for reducing the destructive effect of the x rays on the bone marrow (Fig. 7*c*).

Discussion. – It is clear from the results obtained that the effect of isologous, homologous, or heterologous bone marrow, AET, and AET plus isologous bone marrow on the bone-marrow response of irradiated mice varies according to the type of treatment employed.

Homologous Leukemoid Blood in the Treatment of Irradiation Injury

L. H. Smith

Introduction. – Congdon *et al.*[9] have shown that isologous leukemoid blood from tumor-bearing BALB/c mice promotes recovery in lethally irradiated BALB/c mice. It also has been shown that the leukocytes in leukemoid blood are responsible for the recovery.[10] The mode of action of leukemoid blood in the recovery process remains undetermined. Possibly, its action is similar to that of bone marrow. If so, this type of blood should be effective homologously as well as isologously.

Results. – The injection of several times the number of leukemoid blood leukocytes which is effective in BALB/c mice did not promote recovery in four genetically different types of mice (Table 9). In one experiment in which CAF_1 mice received 435 × 10^6 leukocytes, the mean death time was increased from 9.5 days for controls to 16.0 days for the treated mice.

Discussion. – It is possible that the successful use of leukemoid blood in other than the BALB/c strain depends on injecting a sufficient number of leukocytes. Bone marrow from normal BALB/c mice was effective homologously in enhancing survival of lethally irradiated LAF_1 and CAF_1 hybrid mice.

Recovery of Lethally Irradiated Mice by Treatment with in Vitro Cultivated Bone-Marrow Cells

D. Billen W. F. O'Toole

Introduction. – This investigation is a continuation of studies reported in the previous semiannual report.[11]

Results. – The results of additional studies on the use of isologous bone-marrow tissue culture in the treatment of lethally irradiated C_3H × $101F_1$ mice[11] showed that effective cultures were maintained for as long as 11 days. At an incubation

[9] C. C. Congdon *et al.*, *Radiation Research* 4, 424–434 (1956).

[10] L. H. Smith, *Biol. Semiann. Prog. Rep. Feb. 15, 1956*, ORNL-2060, p 38.

[11] D. Billen, *Biol. Semiann. Prog. Rep. Feb. 15, 1956*, ORNL-2060, p 38.

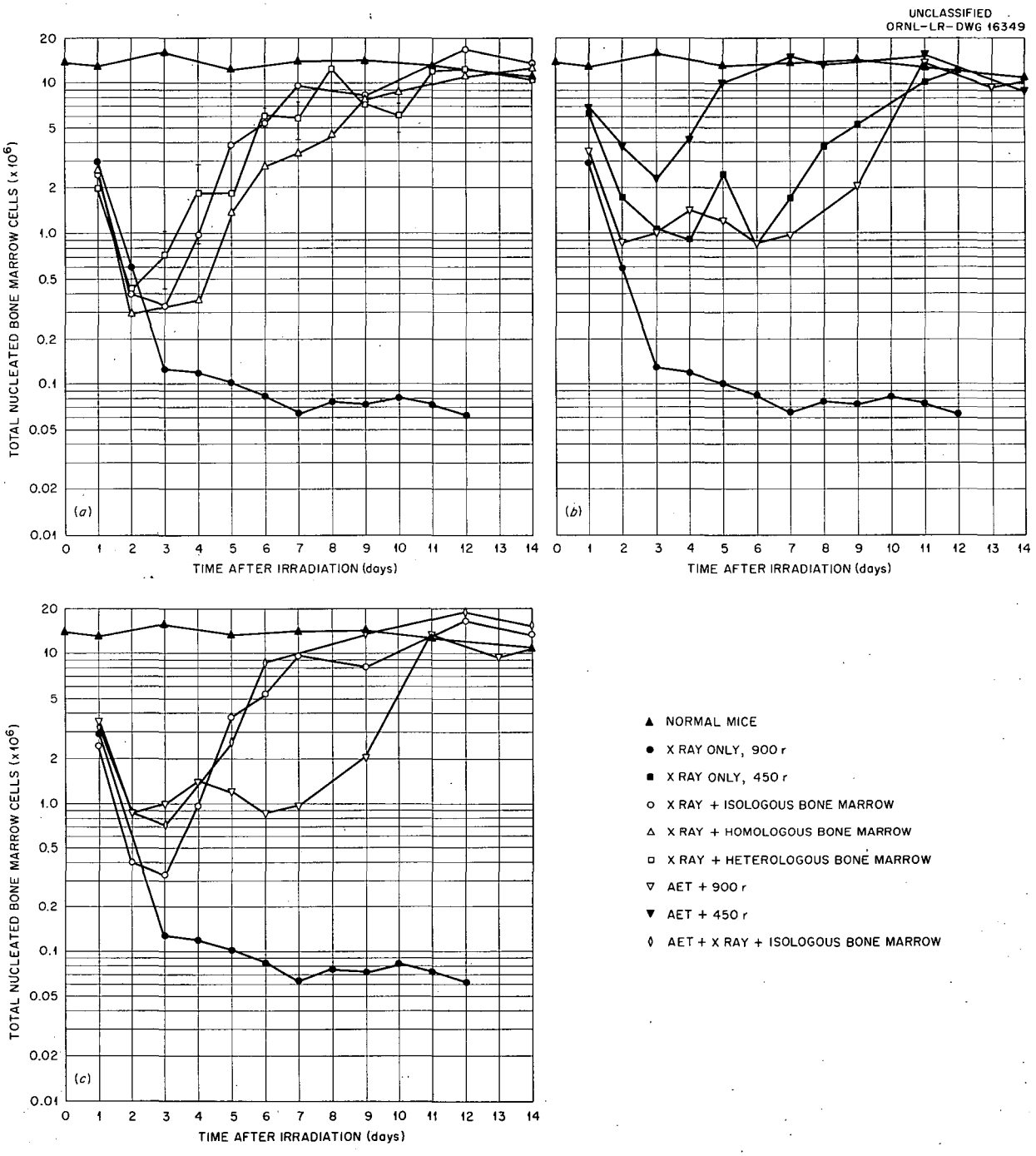

Fig. 7. Bone-Marrow Response in the Right Femur of Lethally X-Irradiated (900 r) and Sublethally X-Irradiated (450 r) CAF_1 Mice Treated with: (*a*) Isologous, Homologous (101 × C_3H Mouse), or Heterologous (Rat) Bone Marrow; (*b*) 9 mg of AET; or (*c*) 9 mg of AET Intraperitoneally Plus Isologous Bone Marrow.

TABLE 9. EFFECT OF INTRAVENOUSLY INJECTED WHOLE LEUKEMOID BLOOD FROM BALB/c MICE ON 30-DAY SURVIVAL OF CAF_1, LAF_1, RF, AND $C_3H \times 101F_1$ MICE

Postirradiation Treatment	Number of White Blood Cells Injected $\times 10^6$	Recipient		Survival (%)	X-Ray Dose (r)	Number of Mice
Single injection	132.0	CAF_1	Experimental	0	900	6
			Control	0	900	6
		$C_3H \times 101F_1$	Experimental	0	900	6
			Control	0	900	6
		RF	Experimental	0	800	6
			Control	0	800	6
		LAF_1	Experimental	0	900	6
			Control	0	900	6
		BALB/c	Experimental	100	750	6
			Control	0	750	6
Single injection	57.0	LAF_1	Experimental	0	700	7
			Control	69	700	13
		BALB/c	Experimental	50	750	2
			Control	0	750	2
Single injection	435.0	CAF_1*	Experimental	0	900	6
			Control	0	900	6
One injection each on days 0, 1, 2, and 3 postirradiation	307.0**	CAF_1	Experimental	0	900	6
			Control	0	900	6

*BALB/c controls were not used in this experiment.
**Total for four injections.

temperature of 37°C the optimal conditions included a medium with a high horse-serum content (medium E) plus a 24-hr feeding schedule (Table 10). The use of a medium composed of a lower serum concentration to which chick embryo extract had been added (medium C) decreased the length of time over which effective cells were maintained (Table 10). A surprising finding was that isologous serum (medium I) proved to be less satisfactory than heterologous horse serum (Table 10). Observations on culture preparations stained with a Giemsa-Wright stain showed that, when medium I was used, erythrocytes were present for all periods observed. Accumulation of cell debris and empty cell membranes was also seen. When bone-marrow cells were cultured in heterologous serum (mediums C and E), the cultures did not contain erythrocytes.

Incubation of the cultures at 25°C gave more effective cultures. At this temperature the cells of a 7-day culture in medium C brought about a 95% survival when injected into lethally irradiated mice as compared with 27% when the cells were obtained from similar 7-day cultures that had been incubated at 37°C (Table 10). Based on observations of stained preparations, there appeared to be a marked qualitative difference in cell population present at the two temperatures after several days of culture. Erythropoiesis seemed to be favored at the lower temperatures.

With regard to the quantity of nucleated cells injected in these experiments, the question may be raised as to the significance of the counts made. A study of stained smears made on cells obtained from the cultures showed many degenerate forms

TABLE 10. RECOVERY OF LETHALLY IRRADIATED MICE BY TISSUE-CULTURED BONE-MARROW CELLS

Material Injected	Number of Nucleated Cells Injected per Animal (millions)	30-Day Survival	Percentage Survival
	Cultures Incubated at 37°C		
Medium C			
4-day culture	0.8–3.2	17/18	92
6-day culture	1.6–3.5	6/25	24
7-day culture	3.7–7.5	4/15	27
9-day culture	1.4	0/9	0
Medium E			
7-day culture	1.4–2.8	12/13	92
9-day culture	1.3	4/5	80
10-day culture	0.7	4/4	100
Medium I			
7-day culture	20.0 (?)*	0.7	0
	Cultures Incubated at 25°C		
Medium C			
7-day culture	4.3–8.7	19/20	95
Medium E			
10-day culture	4.5	8/8	100
11-day culture	0.9–1.8	11/11	100
	Controls		
No treatment		6/334	2
Medium C only		0/8	0
Medium E only		0/8	0
Cell-free supernatant from 2-day culture – Medium C		0/4	0

*Observations on stained smears showed much cell debris; only normoblasts were recognizable.

as well as nuclear debris. In the counting procedure used it was sometimes difficult to distinguish between intact and degenerate nuclei. Where doubt existed, all forms were included in the total nucleated cell counts recorded in Table 10; thus these counts are an overestimate of the true number of intact viable cells present. To obtain additional information on this matter, the staining of dead cells by eosin dye as developed by Schrek[12] was employed. In fresh bone-marrow cell suspensions, less than 2% of all cells present stained pink. In the 11-day 37°C-incubated culture promoting recovery in irradiated mice (Table 10), 30% of the cells took up the stain, indicating the loss of a selective impermeability to the dye, and thus they were presumed to be dead.

Discussion. – It is hoped that by the tissue culture methods employed sufficient additional information will be obtained in experiments now under way to allow a more precise definition of the cell type(s) responsible for the recovery of lethally irradiated mice.

[12]R. Schrek, *J. Cellular Comp. Physiol.* **28**, 277–304 (1946).

Short-Term Preservation of Mouse Bone Marrow at 2 to 5°C and Room Temperature for Irradiation Experiments

I. M. Urso

Introduction. – Storage of normal mouse bone marrow in the laboratory for a few hours did not alter the ability of the bone-marrow cells to decrease the 30-day mortality of lethally irradiated mice. In this study CAF_1 and LAF_1 mouse bone marrow in suspension or in the excised femoral bone at room (22–25°C) or refrigerator (2–5°C) temperatures was tested to determine the optimum period of storage. The stored bone marrow was tested by its ability to cause 30-day survival of lethally irradiated mice.

Results. – At refrigerator temperature (2–5°C), storage of the bone-marrow cells suspended in Tyrode's solution or left in the excised femoral bone was possible for four to five days. At room temperature (22–25°C), storage of bone-marrow cells suspended in Tyrode's solution or left in the excised femoral bone was possible for approximately one day.

Discussion. – Long-term preservation (83 days) of blood-forming cells at –70°C in glycerol solution was demonstrated by Barnes and Loutit.[13] The data reported here were obtained primarily for their usefulness in experimental work.

[13] D. W. H. Barnes and J. F. Loutit, *J. Natl. Cancer Inst.* **15**, 901–905 (1955).

PATHOLOGY AND PHYSIOLOGY

PATHOLOGIC AND PHYSIOLOGIC EFFECTS OF RADIATION

A. C. Upton, Section Chief

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Effects of Chronic Exposure to Fast Neutrons and Gamma Rays

G. S. Melville, Jr.
M. Slater[5]
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Introduction. – From earlier studies it is evident that chronic irradiation results in an accumulation of injury in the form of delayed effects that cause shortening of the life span and that the effectiveness of a given amount of radiation decreases, in general, with reduction of the dose rate, depending on the type of injury produced. Moreover, the effectiveness of neutron irradiation has been observed to vary less markedly with changes in intensity than that of x or gamma rays. The present investigation was undertaken to obtain quantitative information about the influence of radiation intensity on the effectiveness of fast neutrons and of gamma rays in the production of delayed injuries, such as the induction of leukemia and other neoplasia, formation of lens opacities, reduction of survival, and premature aging.

Results. – Mice of the RF strain are being exposed to Co^{60} gamma rays and to fast neutrons from a Po-Be source in an irradiation facility described elsewhere.[6] Preliminary results on lethality at the higher radiation intensities are summarized in Fig. 8.

Discussion. – It is apparent from these data that, at a dose rate causing 50% mortality within 11 to 12 weeks of continuous exposure, the neutron radiation was about 5 times as effective as the gamma radiation. This ratio is substantially higher than the relative biological effectiveness (RBE) of 2.0 observed previously for the $LD_{50}/30$ days when mice of the same strain were exposed at a rate of 50 to 100 rad/min to fast neutrons from the 86-in. cyclotron.[7]

Noteworthy differences in the mechanisms of death have also been observed; in the neutron-exposed mice dying within the first 11 to 12 weeks of irradiation, inflammation of the intestines, portal tract, and liver was the most prominent histologic change, whereas in the gamma-ray-exposed mice dying in the same interval this alteration was noted infrequently but aplasia of hemopoietic elements in the bone marrow and spleen was pronounced. In contrast, in all groups of mice dying after 20 weeks of irradiation the principal cause of death thus far has been leukemia.

These observations indicate the complexity of the relationship between the mechanism of radiation death and the intensity and ion density of the radiation. The investigation is being extended to include study of mice exposed intermittently to fractionated doses of radiation and to graded intensities down to the "maximum permissible" dose level.

Localization of S^{35}-Methionine-Labeled Bone-Marrow Cells Injected into Irradiated Mice

T. T. Odell, Jr. L. H. Smith[8] W. D. Gude

Introduction. – It has been well established that the injection of isologous or homologous bone marrow promotes recovery in lethally irradiated animals. Recent evidence indicates that the injected bone marrow successfully transplants and persists in the irradiated host. To extend the knowledge of the action of bone marrow in the recovery process, isotopically tagged bone-marrow

[1] Consultant, ophthalmology.

[2] U.S. Air Force.

[3] Research Participant.

[4] Temporary employee.

[5] Biophysics Group.

[6] M. Slater, *Biol. Semiann. Prog. Rep. Feb. 15, 1956*, ORNL-2060, p 58.

[7] A. C. Upton *et al.*, *Radiation Research* 4, 117–131 (1956).

[8] Mammalian Recovery Group.

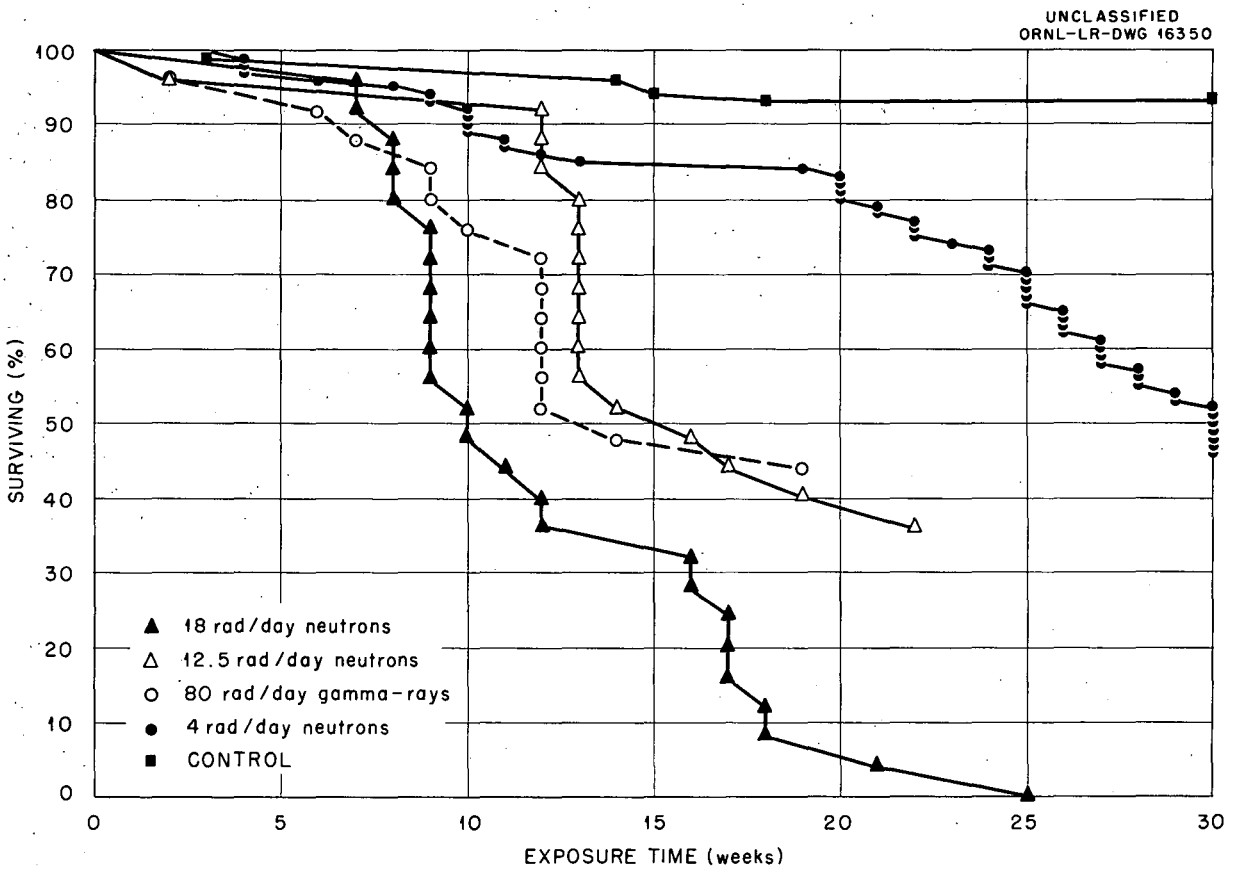

Fig. 8. Lethality of RF Mice Exposed Continuously (23 hr per day) to Fast Neutrons and to Gamma Rays.

cells have been injected into irradiated mice, and the disposition of the labeled cells has been determined autoradiographically.

Results. – Normal donor mice were injected intraperitoneally with 200 μc each of S^{35}-labeled methionine. Eighteen hours later, femoral bone marrow and humoral bone marrow were combined and injected intravenously into lethally irradiated mice (two donors per recipient, 24 hr post-irradiation). The recipients were killed 15 min, 4 hr, and 18 hr later, and bone-marrow smears and tissue sections were prepared, covered with stripping film, and exposed for 30 to 60 days.

Isotope-labeled bone-marrow cells were found in marrow smears from the irradiated recipients 15 min, 4 hr, and 18 hr after marrow injection. Labeled marrow cells were also found in sections of lung, spleen, and sternum of the 4-hr group. The majority of the tagged cells in each of the three groups were granulocytes having a ring-shaped nucleus. Other labeled cells include granulocyte precursors.

Only a small fraction of the marrow cells obtained from the recipients were labeled with the isotope. Preliminary observations indicate that the ratio of labeled to unlabeled cells is about 1:100.

Tagged bone-marrow cells were also found in normal mice injected with S^{35}-methionine-labeled marrow.

Discussion. – These results demonstrate that intravenously injected bone-marrow cells localize in the bone marrow, spleen, and lungs of irradiated animals within 15 min after injection and are still observed 18 hr later.

Pathogenesis of Radiation-Induced Myeloid Leukemia

F. F. Wolff P. Ledford W. D. Gude

Introduction. – The induction of myeloid leukemia by ionizing radiation is being investigated in mice of the RF strain.

Results. – In addition to the observations reported previously,[9] preliminary data indicate that susceptibility to the induction of this disease is minimal during the neonatal period, increasing within the first several weeks after birth (Table 11). In contrast, susceptibility to the induction of lymphoid leukemia decreases during the early months of life.

TABLE 11. INCIDENCE OF LEUKEMIA IN RF MICE AS INFLUENCED BY AGE AT IRRADIATION

Age at Irradiation* (days)	Number of Mice	Incidence of Leukemia (%)	
		Myeloid	Lymphoid, Thymic
1	70	0	24
40	95	44	19
180	43	40	14
Nonexposed control	88	1	2

*300 r x rays to whole body.

Discussion. – The age-specific differences in susceptibility to the induction of various types of leukemia by irradiation may be correlated with differences in the age distribution of spontaneous leukemias in mice, as well as in humans.

These observations are being explored further in an attempt to determine the physiologic basis for the observed age differences.

Chemical Studies of Blood Platelets

T. T. Odell, Jr. B. Anderson

Introduction. – It has been noted that the blood platelets of rats incorporate S^{35}-labeled sulfate.[10] Since inorganic sulfate introduced into rats is found almost exclusively in sulfate-containing compounds like the chondroitin sulfuric acid of cartilage and the mucopolysaccharides of mucin, this study was undertaken to determine whether the S^{35}-sulfate in platelets is present in a sulfated mucopolysaccharide.

Results. – Sulfate-labeled platelets were extracted with 2% NaOH for 7 hr in the cold, a procedure commonly used for extraction of chondroitin sulfate from cartilage.[11] The supernatant was then treated by the Sevag procedure to remove protein, dialyzed against distilled water, and then lyophilized to reduce volume and for safekeeping until needed. Around 50% of the S^{35} activity was lost during these procedures (when platelet extracts obtained by water lysis were treated by the Sevag technique, the activity was removed gradually along with the protein). The alkali extract was electrophoresed on paper strips, and the strips were then counted for radioactivity. The majority of the radioactivity (around 60%) migrated to a position immediately behind that of the dye spots corresponding to chondroitin sulfate and heparin (run simultaneously). One-dimensional chromatograms of the unknown compound revealed that it had an R_f value similar to that of chondroitin sulfate. The activity-containing spot also stained with the dye Alcian blue 8 GN, as do chondroitin sulfate and heparin; this dye is considered to be specific for mucopolysaccharides.[12]

Discussion. – The evidence thus far accumulated is consistent with the hypothesis that platelets contain a sulfated mucopolysaccharide. Since it is likely that the interendothelial cement substance also contains a mucopolysaccharide, the physiological significance of the platelet mucopolysaccharide and its possible role in the maintenance and repair of the interendothelial cement deserve investigation.

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[9] F. F. Wolff and P. Ledford, *Biol. Semiann. Prog. Rep. Feb. 15, 1956*, ORNL-2060, p 39–41.

[10] T. T. Odell, Jr., F. G. Tausche, and W. D. Gude, *Am. J. Physiol.* **180**, 491–494 (1955).

[11] E. Jorpes, *Biochem. Z.* **204**, 354 (1929).

[12] C. Rizzoli and M. A. Gliozzi, *Boll. soc. ital. biol. sper.* **31**, 426–428 (1955).

CELL PHYSIOLOGY

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Electrophoretic Characterization of Rat Serum Proteins

N. G. Anderson M. L. Anderson

Introduction. – Previous work in this laboratory indicates that rat liver soluble protein (ratlivsol) preparations contain at least five serum proteins. Characterization of tissue proteins therefore requires that means be devised for determining whether or not a given protein is also present in blood and, more specifically, which one it is. Since very little work has been done on the fractionation of rat blood proteins and since somewhat contradictory results have been obtained by previous workers, it was necessary to carry out extensive electrophoretic studies.

Results. – Pooled versenated plasma from Sprague-Dawley rats was defibrinated after the addition of calcium and then dialyzed against appropriate buffers. The buffers used were of 0.1 ionic strength, with 0.08 of the ionic strength being due to sodium chloride. Thirteen different pH's between 3 and 9 were employed. The results indicate that the presumed albumin peak observed at pH 8.5 is actually composed of at least three fractions which have the same mobility at pH 8.5 but whose mobilities differ at lower pH's. These data provide information on which a rational use of electroconvection may be based.

By a combination of ammonium sulfate fractionation and electroconvection at pH 4.6 in an acetate buffer of 0.02 ionic strength, a rat serum preparation of high purity was obtained which proved to be homogeneous both electrophoretically and serologically. The work is being continued by the use of continuous paper curtain electrophoresis.

[1]Consultant.

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[7]On leave: USPHS Fellowship.

Cytotoxicity of Rabbit Antiserum Prepared Against the Soluble Cytoplasmic Fraction of Ehrlich Ascites Tumor Cells[8]

E. C. Horn

Introduction. – The cytotoxic effect of antisera prepared against the mitochondrial-microsomal fraction of ascites tumor cells and against their nuclei has already been described.[9,10] This investigation extends those results with an antiserum prepared against the soluble fraction obtained after high-speed centrifugation (1 hr at 115,000 × *g*, average force) of ascites tumor cell homogenates.

Results. – The antiserum had a reciprocal complement fixation titer of 80 and an ascites cell agglutination titer of 80 and produced a markedly detrimental effect on the multiplication of ascites tumor cells exposed to it in vitro or in vivo. Heating the rabbit serum to 56°C for 30 min reduces only partially the cytotoxic effectiveness of the serum. Against different concentrations of tumor cells the serum showed a typical dose relationship, within the range investigated (5×10^5 to 16×10^6 cells per animal), between survival and cell numbers.

Incubation of the cells in antiserum for 30 to 60 min at room temperature prior to injection seemed to show no difference in the survival of the animals. Incubation for but 15 min, however, was not so effective in preventing tumor growth.

Discussion. – The effect of this antiserum seems to be the same as that of the antisera already described having a cytotoxic effect,[9,10]

[8]This work was supported in part by research grant [RG-4203(c)], National Institutes of Health, Public Health Service.

[9]E. C. Horn and M. E. House, *Biol. Semiann. Prog. Rep. Aug. 15, 1955*, ORNL-1953, p 123–127.

[10]E. C. Horn, *Cancer Research* 16 (1956) (in press).

namely, to inhibit cell division. Complement fixation titers obtained for the antiserum and various cellular antigens, including the homologous antigen, and cell agglutination titers seem to be unrelated to the cytotoxic effect and have no predictive value. The results which follow the destruction of complement indicate only that this system or some other heat-labile system is helpful in increasing the effectiveness of the antisera in their cytotoxic effect but apparently is not necessary for some demonstration of the effect. In a number of respects these results are remarkably similar to those of Tyler and Brookbank,[11] who showed that antisera to fertilizin extracted from sea urchin eggs are successful and perhaps specific cleavage-blocking agents.

Studies on Nucleoprotein Gels

W. D. Fisher

Introduction. – Nucleoprotein gels obtained by extraction of tissue breis with 1 *M* NaCl show appreciable viscosity changes in response to a variety of agents and appear to be much more sensitive to these agents than purified deoxyribonucleoprotein (DNP).

Results. – A suitable method for viscosity measurements has been worked out, and the response of the preparation to pH, ionic strength, extraction time, and DNP concentration has been evaluated, preliminary to a systematic investigation of the effect of chemical and physical agents. The gel is extremely sensitive to x rays, 50 r being sufficient to reduce the viscosity by one-half. There is an after-effect, and viscosity values decrease rapidly during the first 2 hr after irradiation. Centrifugation of the gel after irradiation suggests that relatively large gel islands are still present, since the DNP is sedimented in 5 min at 80,000 *g*. Irradiation of the homogenate prior to extraction also reduces the viscosity, but much larger doses of irradiation are required. Agents known to protect animals and bacteria against x irradiation, such as AET and β-mercaptoethanolamine, reduce the viscosity drop in both the extracted gel and in the homogenate, at a concentration of 10^{-3} to 10^{-2} *M*. Ultraviolet (2650 Å) also lowers the viscosity, but the energy required is about 1000 times as great as for x rays. Hydrogen peroxide (10^{-3} *M*), shearing, and freezing also lower the viscosity of the preparations.

Discussion. – Studies are now in progress on the effect of oxygen tension on x-ray effects and on the possible protective action of glycerol in freezing.

A Critical Period in the Acute Lethal Response of the Chick Embryo to X Radiation

R. A. Goff

Introduction. – As a preliminary to a study of the effects of x radiation on morphogenesis in the late stages of the chick embryo, it was necessary to determine the dose level required to initiate responses at different stages. For some time it has been known that as development proceeds in the chick embryo it becomes more sensitive to x radiation and that the chick embryo is unique in this respect. Further, Karnofsky *et al.*[12] discovered the phenomenon of acute vs delayed deaths, the former being identified by death within the first 24 hr. Acute deaths are observed when the dose rate is more than ~10 r/min, whereas the delayed deaths are related to the total dose.

In this study the dose rate was at a level which would produce acute deaths, and this factor was used as one measure of sensitivity. Emerging from this phase of the study is the suggestion that there is a hitherto unmentioned sensitive period at 9 days of incubation. Previous work[12] has shown that the sharp increase in sensitivity begins to decline at 8 days but does not reveal the critical response at 9 days.

Results. – The dose which produced ~50% acute deaths at 9 days of incubation and several days before and after is shown by the solid-line curve in Fig. 9. The mid-lethal dose at 8 and 10 days is a 75% lethal dose at 9 days, which serves to emphasize the critical nature of this period.

The dash-line curve represents the findings of the most thorough study[12] prior to this time. It shows the eighth day to be the most sensitive, but the author makes no particular point of this. The difference in the mid-lethal doses in the two curves is striking. Biological variation can make significant differences in sensitivity. For example, if mortality of embryos is high among controls or before irradiation, the surviving embryos

[11] A. Tyler and J. W. Brookbank, *Proc. Natl. Acad. Sci. U.S.* 42, 304–308; 308–313 (1956).

[12] D. A. Karnofsky, P. A. Petterson, and L. P. Ridgway, *Am. J. Roentgenol., Radium Therapy Nuclear Med.* 64, 280–288 (1950).

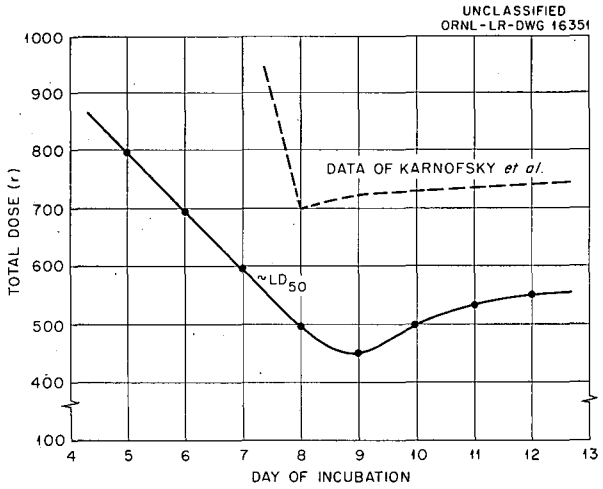

Fig. 9. Effect of Dose Rate on Incubation Period.

exhibit greater sensitivity. There may also be differences between strains of White Leghorns or the season in which the eggs are produced. The differences in radiation factors involved might also contribute somewhat to the discrepant results.

Discussion. – Deleterious stimuli from a variety of sources tend to produce their lethal effects at three fairly well-defined periods[13] in the embryogenesis of the chick, that is, 3 to 4, 12 to 14, and 19 to 20 days of incubation. The ninth day has thus not been emphasized as a critical period for other stimuli. This is not a time of major organogenesis, nor are other responsible events evident. It would be reasonable to expect the hematopoietic system to be involved in this critical period; however, at present there is no obvious reason why it should be most sensitive at 9 days. The yolk sac is probably the principal organ in the system at this time. The bone marrow is just in the process of formation in the femur. By the twelth day it produces small numbers of cells and becomes the major organ by the time of hatching. The spleen makes its appearance at 4.5 days and is most active in hematopoiesis at 14 to 18 days. The time of origin of an organ is one of its most sensitive periods to adverse conditions; but since organs can be specifically affected without lethal effect, the authors hesitate to invoke this as an explanation in the case of the bone marrow.

[13] H. L. Hamilton, *Ann. N.Y. Acad. Sci.* 55, 177–187 (1952).

Electroconvection-Electrophoresis of Normal Rat Serum

R. H. Shellhamer

Introduction. – In order to fractionate rat serum proteins into isoelectrically pure components, a starch column electroseparator was adapted for use with free solutions, making it possible to obtain three fractions in a single effort. The apparatus consists of three vertical columns supported by Lucite frames and separated by filter paper impregnated with a starch gel to prevent diffusion. Leakage of the apparatus is prevented by using filter paper gaskets impregnated with starch gel. An electrical field is applied horizontally through dialysis tubing split open into sheets.

Results. – Electroconvection of rat serum in acetate buffer pH 5.05 for 26 hr in the cold in an electrical field of 20 v and 1.8 amp yielded three fractions:

1. center column – gamma globulin contaminated with other serum proteins due to incomplete electroconvection,
2. anodic column – albumin and alpha-1,
3. cathodic column – alpha-2, beta globulins.

Fractions thus obtained, when electroconvected in turn in a two-fraction electroconvection apparatus consisting of a single vertical dialysis sac in an electrical field in which electroconvected fractions are drawn from top and bottom reservoirs at either the same pH or at other pH levels, can be further fractionated.

After concentrating to the original serum volume, electroconvected preparations were run on paper electrophoresis and compared with samples of whole serum run simultaneously. Electrophoresis was performed in veronal buffer at pH 8.6 for 16 to 20 hr, and the strips were stained with bromphenol blue, periodic acid–Schiff, Sudan black, toluidine blue, azure A, wool red, and brilliant scarlet. Paper electrophoresis of normal rat serum at pH ranges of from 8.96 to 4.25 (veronal, phosphate, and acetate buffers) followed by staining with bromphenol blue and periodic acid–Schiff revealed a polysaccharide component in the periodic acid–Schiff stained strips which was not demonstrable with the usual bromphenol blue staining. This component is associated with alpha-1 globulin and albumin at pH 8.6 and 8.96, but in the range from pH 5.2 to 7.0 it migrates ahead of both these components.

BIOCHEMISTRY

STUDIES ON NUCLEIC ACIDS, ENZYMES, AND RADIATION-PROTECTIVE COMPOUNDS

W. E. Cohn[1] R. Shapira
L. Astrachan E. Volkin
S. R. Dickman[2] D. E. Emmer
D. G. Doherty M. H. Jones
J. X. Khym A. B. Ottinger

Structure and Activity in Radiation-Protective Sulfhydryl Compounds[3]

D. G. Doherty J. X. Khym
R. Shapira T. W. McKinley, Jr.[4]
D. E. Emmer

Damage to biological material by ionizing radiation is caused primarily by free radicals. Substances added to the medium affect the degree of damage to irradiated material. Consequently it has been proposed[5] that these substances may protect the biological material by competing with it for radicals produced in the medium by ionizing radiation. This suggestion, when closely examined in the light of what is already known about free radicals and their reactions, should lead to a means of predicting reaction intermediates and products, evaluating protective agents solely on their chemical nature, and designing more effective protective agents. One limitation of this approach is in the mammalian assay for protective activity, since the distribution, metabolism, and toxicity of the protective compounds in mice may give a false evaluation of these agents.

The ability of MEA and certain related mercaptoalkyl amines to provide protection against a lethal dose of x radiation may be easily explained by the free radical hypothesis. The structure of MEA may be considered to be a resonance hybrid of two structures of similar energy (Fig. 10*a,c*) and the intermediate state (Fig. 10*b*). The reaction of MEA with a free radical results in the donation of an electron from the MEA to the radical, thus quenching it and simultaneously creating a new MEA radical (Fig. 10*d*). This radical is resonance-stabilized in a manner similar to the MEA molecule, since the unpaired electron can reside on either the S or N atom. Resonance stabilization prevents this radical from abstracting an electron from a biological molecule to create a biological radical. Instead it will react with another radical, thereby terminating both radicals. This brief, simplified explanation is supported by both chemical and biological evidence obtained in the course of the testing program. Thus MPA from purely structural considerations should be protective due to its ability to form a cyclic stabilized radical; but MBA should be much less protective, since the formation of a cyclic seven-membered ring is not favored, making this radical less stable and consequently less effective than its five- (MEA) and six- (MPA) membered counterparts (Fig. 11). The mammalian assay results support this reasoning, since it has been found that at equivalent dose levels MPA is twice as active as MEA and nine times as active as MBA.

If the radical created from the protective agent is more highly resonance-stabilized than the MEA radical without a decrease in electron donating ability of the compound, then, on solely chemical grounds, this compound should be more active than MEA. This was indeed the case when MEG was tested and found to be more protective than MEA. Correspondingly MPG was more active than MPA and twice as active as MEG (Fig. 11).

This hypothesis has been valuable in the design of protective compounds. Substances with related structures have been synthesized and found to be effective at the 2.5-μM level against a lethal dose of x radiation, that is, 15 to 20 times more effective than MEA on a molar basis. There is no reason to believe at this time that even more active compounds cannot be prepared. Thus all

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[1]On leave, Guggenheim and Fulbright Fellowships.

[2]Research Participant.

[3]Abbreviations for compounds mentioned in this section:
MEA – 2-mercaptoethylamine (formerly β-mercaptoethylamine).
MPA – 3-mercaptopropylamine (formerly γ-mercaptopropylamine).
MBA – 4-mercaptobutylamine (formerly δ-mercaptobutylamine).
MEG – 2-mercaptoethylguanidine hydrobromide.
MPG – 3-mercaptopropylguanidine hydrobromide.
2AT – 2-aminothiazoline hydrobromide.
AET – S,2-aminoethylisothiuronium bromide hydrobromide (formerly S,β-aminoethylisothiuronium bromide hydrobromide).
GED – Guanidinoethyldisulfide.

[4]Mammalian Recovery Group.

[5]D. G. Doherty *et al.*, *Biol. Semiann. Prog. Rep. Aug. 15, 1955*, ORNL-1953, p 88–91; *Biol. Semiann. Prog. Rep. Feb. 15, 1956*, ORNL-2060, p 45–46.

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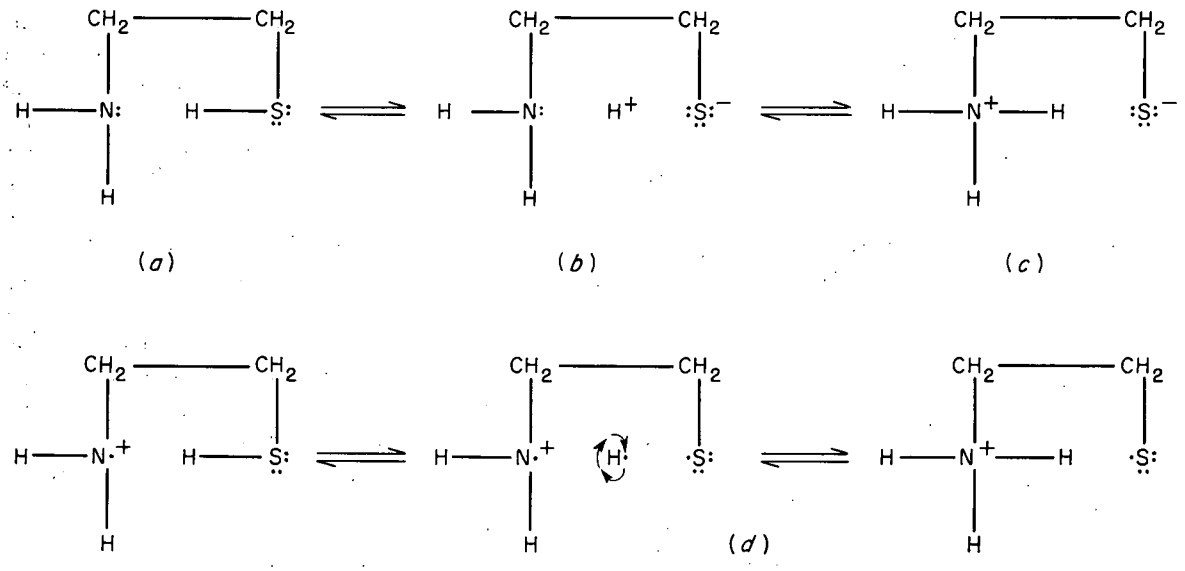

Fig. 10. Structure of MEA and MEA Radical.

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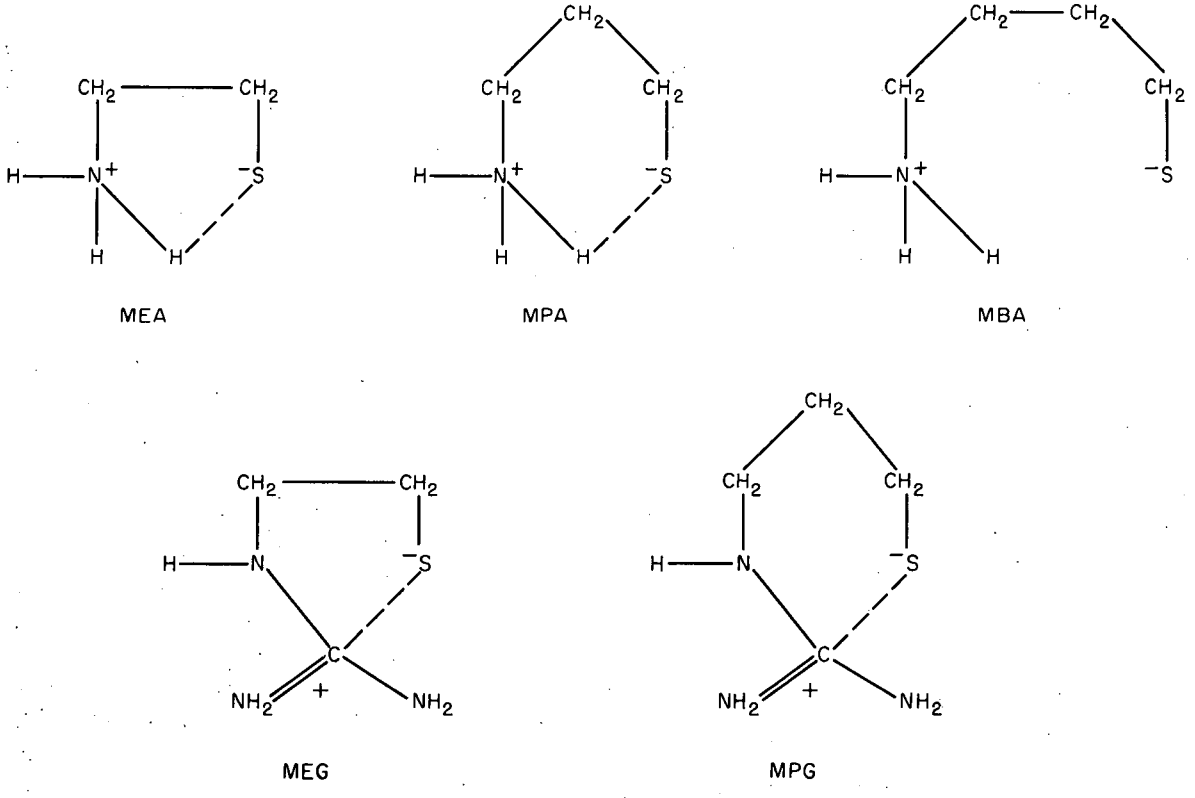

Fig. 11. Structures of Mercaptoalkylamines and Guanidines.

the evidence obtained to date seems to substantiate the proposed hypothesis and to make an explanation by an alternate mechanism less likely.

Ion-Exchange Study of the Rearrangement of AET to MEG and 2AT

J. X. Khym D. G. Doherty
R. Shapira A. B. Ottinger

The proposed mechanism[6] of intratransguanylation of AET to MEG has been fully substantiated by quantitative chromatographic separation and identification of all the reaction products. In aqueous solution AET can undergo a series of chemical transformations that are primarily dependent on the pH of the solution (Fig. 12). Thus AET, stable in 0.2 M HCl, is converted quantitatively to MEG at pH 7.0 and at pH 2.5 is slowly converted to 2AT. In strong alkali at room temperature, AET is converted to a mixture of MEA, MEG, and dicyandiamide.

[6]D. G. Doherty *et al.*, *Biol. Semiann. Prog. Rep. Aug. 15, 1955*, ORNL-1953, p 88–91.

Ion-exchange chromatography was used to demonstrate the validity of the changes shown in Fig. 12; 2AT was isolated and characterized chemically, and the other compounds were synthesized for use as reference compounds. The identity of the peaks was determined by nitrogen and sulfur analyses as well as by cochromatography with reference compounds.

Separations of all the products shown in Fig. 12 were carried out on Dowex 50, a strong acid cation exchanger used in the H^+ form. The column size was 1 $cm^2 \times 5$ cm of 200- to 400-mesh resin and the flow rate was about 0.6 ml/min. In Fig. 13, a chromatogram of materials obtained from AET is given. The ordinate represents the concentration of nitrogen (solid line) and also the concentration of —SH in each cut (broken line) as determined colorimetrically by a micro-Nesslerization procedure for nitrogen and the dichlorophenol indophenol method for the determination of —SH groups, respectively. The eluting agent is shown at the top of the figure, and the amount of compound initially placed on exchanger is given in

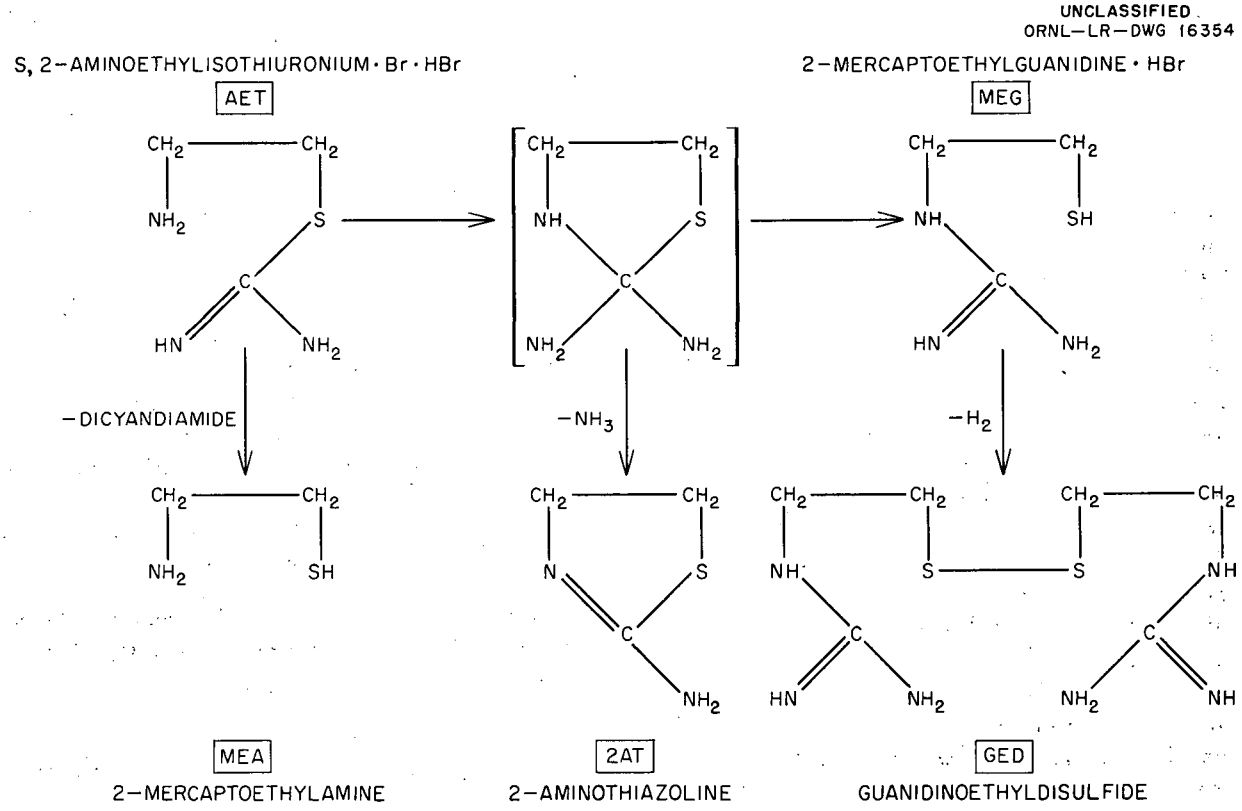

Fig. 12. Rearrangement of AET to MEG and 2AT.

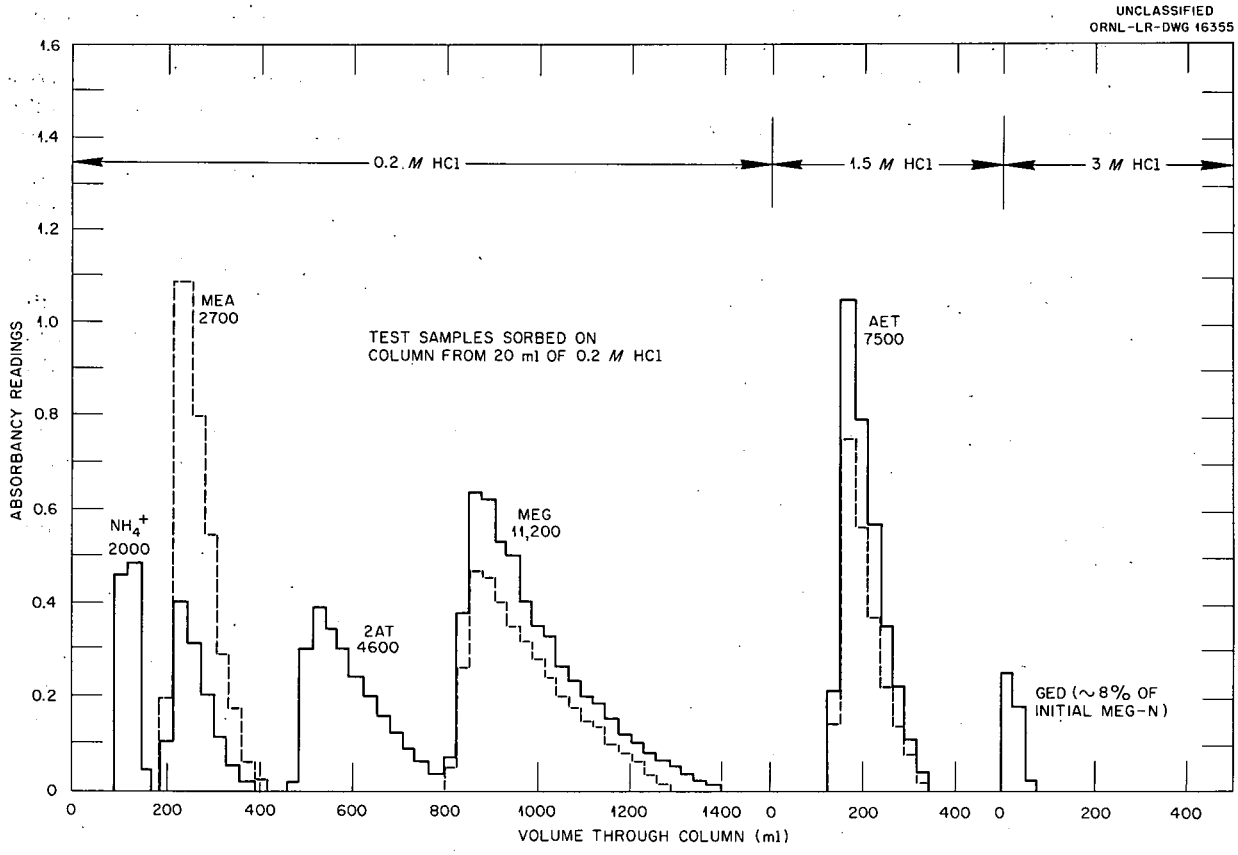

Fig. 13. Chromatogram of Materials Obtained from AET.

terms of total micrograms of nitrogen. Recoveries ranged from 92 to 97%. Only MEA, GEM, and AET give both a nitrogen and an —SH test. The ratio of N:—SH in the order given is 1:1, 3:1, and 3:1, and only NH_4^+ gives N determined inorganically. The products shown in Fig. 12 are never all present at any one pH. For instance, AET dissolved in 0.2 *M* HCl and chromatogramed gives only one peak at the position indicated, and AET that has been adjusted to pH's of the neutral range (above 6.0) gives only MEG and a small amount of GED (~8% of MEG-N, through autoxidation) at their positions. If AET is dissolved in formate buffer at pH 2.5 and allowed to stand for one day, only NH_4^+ and 2AT are formed, and these in equimolar quantities (see middle equation of Fig. 12). Similar experiments at pH 3.5 and 4.5 give NH_4^+, 2AT, and MEG – the higher the pH the more MEG. Unreacted AET is present only if the time of contact in pH's above 2 is short (20 sec to several hours depending on pH). Only in strong base (e.g., 2 *N* NaOH) does MEA appear. In this case the products are dicyandiamide, MEA, and MEG. The first two form in a ratio of 1:2 and about half the initial AET is found as MEG, indicating that even in strong base the mechanism of ring closure to form MEG is in strong competition with the mechanism that splits off the guanyl group leaving MEA and dicyandiamide (the condensation of two guanyl groups). Dicyandiamide breaks through the exchanger (not shown) and is found in the initial effluent. All products are stable to the acid cation exchanger.

Chromatographic Detection of Differences Between Bacteriophage-Related Deoxyribonucleic Acids

L. Astrachan E. Volkin M. H. Jones

In the previous report[7] a method was described for comparing two DNA species with respect to

[7] L. Astrachan, E. Volkin, and M. H. Jones, *Biol. Semiann. Prog. Rep. Feb. 15, 1956*, ORNL-2060, p 47.

their content of various polynucleotides. In brief the method consisted in labeling one species with P^{32} and then mixing it with the other species, the latter species in the mixture being twenty times the amount of the P^{32}-labeled species. Polynucleotides were obtained from the mixture, separated on ion-exchange columns, and measured for their specific activities. Differences in specific activities of the isolated polynucleotides would reflect differences between the two DNA's.

This method was used in an attempt to detect differences between bacteriophage DNA and DNA isolated from bacteriophage-infected, proflavin-inhibited *Escherichia coli*. When low molecular weight polynucleotides were obtained from the mixture by enzymatic digestion, no differences between the two DNA's were observed, since all the isolated polynucleotides had the same specific activities. However, the two DNA's were shown to be different when high molecular weight polynucleotides, produced by heating the DNA mixture, exhibited considerable variations in their specific activities. The explanation for observing differences in one case and not the other lies in the probability that, as polynucleotides increase in size, they have correspondingly more chances to be different.

Ribonucleic Acid Metabolism in T2-Infected *Escherichia coli*

E. Volkin L. Astrachan M. H. Jones

It has been found that inorganic phosphorus is incorporated into RNA of bacteriophage-infected *E. coli*[8] and that separable fractions of the RNA were able to incorporate phosphorus at widely divergent rates.[9] When mononucleotides were isolated after alkaline hydrolysis of the labeled RNA, it was found that the specific activities of adenylic and uridylic acids were 50% higher than those of cytidylic and guanylic acids. This heterogeneous incorporation suggested that infection of *E. coli* with phage induces the bacteria to synthesize a species of RNA, the base composition of which differs from normal bacterial RNA. This report deals with investigations concerning the metabolic function and the relation to bacteriophage synthesis of the new RNA.

With the possibility in mind that the new RNA might be incorporated into bacteriophage, the RNA content of bacteriophage was determined. Bacteriophage, highly labeled with P^{32}, was mixed with an excess of RNA and the mixture hydrolyzed to yield mononucleotides. Chromatography of the mononucleotide mixture resulted in no radioactivity being found which was associated with the mononucleotides. This means that RNA is absent from the T2r+ virus.

With the first possibility denied, another role for the new RNA might be to serve as precursor for bacteriophage DNA. A primary requirement of this function is that the newly synthesized RNA not only incorporate P^{32} but also release it. Turnover of RNA phosphorus was demonstrated in experiments where P^{32} was supplied to the phage-infected coli for a short time and then effectively removed by adding a great excess of unlabeled inorganic phosphate. The amount of P^{32} in RNA reached a high plateau level almost instantly, and then, when $P^{32}O_4$ was removed from the medium, P^{32} was lost by the RNA. Furthermore, the incorporation into RNA preceded P^{32} incorporation into DNA, and, while P^{32} was being released from RNA, P^{32} was incorporated into DNA.

In a similar turnover experiment, the infected cells were separated into two particulate and one supernatant fraction before isolation of RNA. The RNA of one particulate fraction, containing less than 5% of the total cell RNA, exhibited very rapid turnover of P^{32}. The other particulate fraction, containing 60% of the cell RNA, incorporated very little P^{32} and had no turnover, while the supernatant fraction incorporated an intermediate amount of P^{32} which it did not release.

The precursal relationship of RNA to DNA was indicated again in an experiment in which $P^{32}O_4$ was provided to infected cells in the presence of chloramphenicol. These cells incorporated P^{32} into the RNA but not into the DNA. When $P^{32}O_4$ was removed, the RNA did not lose any label; but when the chloramphenicol was also removed, label was lost from RNA, and DNA was synthesized. The amount of label lost from RNA was very close to that incorporated into DNA.

These experiments indicate that RNA phosphorus may be a precursor for DNA phosphorus. Further experiments with carbon labeling should deal more directly with the relationship of RNA to DNA.

[8] E. Volkin and L. Astrachan, *Virology* 2, 149 (1956).

[9] *Ibid.*, p 433.

Composition of *Escherichia coli* Ribonucleic Acids

S. R. Dickman

Introduction. – Volkin and Astrachan have studied the metabolic turnover of *E. coli* ribonucleic acid (RNA) in bacteriophage-infected cells to obtain data on the question of the precursors of bacteriophage deoxyribonucleic acid (DNA). For comparative purposes, a knowledge of the nucleotide composition of the RNA from three fractions of noninfected *E. coli* would be of considerable interest. In carrying out this objective, it was found that one RNA fraction contained a hitherto undiscovered and apparently unknown nucleotide. This substance was isolated, and experiments were performed to determine its structure.

Results and Discussion. – *Escherichia coli* was grown in broth medium and was harvested at the mid-point of the log phase of growth. It was disrupted by grinding, and the particulate matter was separated by differential centrifugation into three fractions: P_1, sedimented at 17,500 rpm for 20 min, largely composed of bacterial membranes; P_2, sedimented at 38,000 rpm for 80 min, composed of the bacterial equivalent of microsomes; and S, the supernatant fraction containing material in solution. The nucleic acids of the P_2 and S fractions were solubilized and then hydrolyzed in dilute alkali, and their nucleotide composition was determined by separation of the constituent nucleotides by ion-exchange procedures. The results of two separate preparations are presented in Table 12. The P_2 fraction contains a high percentage of guanylic acid. The S fraction differs in composition from the P_2 in that it contains less guanylic acid and slightly more uridylic acid. In addition the S fraction contains the new nucleotide derivative (termed "X"), which was not detected in the P_2 fraction.

The X substance originally appears as a contaminant of the guanylic acid fraction. It was separated from guanylic acid by further chromatography. It possesses spectral characteristics of a cytidine derivative and apparently contains 2 moles of phosphorus per mole of pyrimidine. Analysis for a pyrophosphate linkage was negative. Further attempts to locate the phosphate residues involved the utilization of specific phosphatase enzymes. It was not hydrolyzed by the barley 3′–nucleotidase or snake venom 5′–nucleotidase with the liberation of inorganic phosphate. Prostate acid phosphatase, however, cleaved both phosphates. Work is now under way to isolate and identify the hydrolytic products formed from X by this enzyme.

Since this material comprises from 16 to 20% of the total cytidine nucleotides of the S fraction, its discovery has important implications concerning the structure of this RNA. It may be present as an end group or possibly as a branch point in the RNA molecule. Further work is necessary to determine its structure and site in RNA.

TABLE 12. NUCLEOTIDE COMPOSITION OF *ESCHERICHIA COLI* RIBONUCLEIC ACIDS

Nucleotide	P_2 Fraction				S Fraction			
	Experiment I		Experiment II		Experiment I		Experiment II	
	Micromoles	Per Cent	Micromoles	Per Cent	Micromoles	Per Cent	Micromoles	Per Cent
Cytidylic acid	7.4	24.5	5.89	25.6	22.2	24.4	22.4	24.4
Adenylic acid	6.8	22.5	5.85	25.6	20.4	22.4	18.6	20.3
Uridylic acid	6.1	20.0	3.87	16.9	20.2	22.2	20.5	22.4
Guanylic acid	10.0	33.0	7.32	32.0	22.7	24.9	19.7	21.5
"X"					5.8	6.3	10.5	11.5

ENZYMOLOGY AND PHOTOSYNTHESIS

G. D. Novelli
W. A. Arnold
J. W. Davis
A. N. Best
M. J. Cormier[1]
H. K. Sherwood

ENZYMOLOGY

One-Carbon Metabolism in Bacteria: The Exchange Between Pyruvate Carboxyl and Formate or Bicarbonate

G. D. Novelli H. K. Sherwood

Introduction. – In an investigation of the phosphoroclastic split of pyruvate in extracts of *Escherichia coli*, it was observed that the exchange of C^{14}-formate with the carboxyl group of pyruvate is apparently catalyzed by a multienzyme system.[2] Subsequently it was accidentally discovered that extracts of certain anaerobes such as *Clostridium butylicum* and *Micrococcus lactilyticus* although apparently unable to metabolize formic acid are nonetheless capable of exchanging C^{14}-formate with the carboxyl group of pyruvate.[3] Since these anaerobes also carry out the exchange of CO_2 with pyruvate carboxyl, the question of a common C_1 intermediate in these series of reactions arises. The present investigation is designed to cast some light on this question.

Results. – In attempting to set up an assay system for one of the enzyme components of the system, it appeared reasonable that the rate of exchange, expressed as μmoles per hour, should be a linear function of enzyme concentration. When this property was examined for the formate-pyruvate exchange by *M. lactilyticus*, it was found that the rate of exchange increased almost exponentially as a function of enzyme concentration. This behavior suggests the presence of an autocatalytic step in the exchange process. When the rate of exchange for any given enzyme concentration is plotted as a function of time, where a constant rate would be expected to be found, actually, a rapidly increasing rate up to 30-min incubation time is found, and then the rate remains constant for the duration of the experiment. This phenomenon was observed with three different levels of extract and is as yet unexplained.

In studying the kinetics of the exchange of CO_2 with pyruvate carboxyl as catalyzed by *Veillonella gazogenes* (which extracts cannot catalyze the formate-pyruvate exchange), it was found that in contrast with formate exchange in *M. lactilyticus* the CO_2 exchange was a linear function of enzyme concentration. It was also observed that, whereas concentrated extracts of *V. gazogenes* could easily be stored in the deep freeze for extended periods of time without loss of activity, diluted extracts were completely inactivated by overnight storage in the deep freeze.

With extracts of *M. lactilyticus* it was observed that treatment with Dowex-1 resulted in a loss of ability to exchange both formate and CO_2. This property could not be restored by the addition of a heated extract. Dialysis of the extract led to a large loss of formate exchange and a large increase in CO_2 exchange. The formate exchange in both the Dowex-treated and the dialyzed extracts of *M. lactilyticus* could be restored by the addition of a small amount of *V. gazogenes* extracts (which can catalyze the CO_2 exchange but not the formate exchange).

Discussion. – The relation between CO_2-pyruvate and formate-pyruvate exchange strongly suggests some fundamental property in common in the two reactions. The most likely common property could be a common C_1 intermediate. A study of the exchange between CO_2 and formate in the presence and absence of pyruvate should allow the development of a system in which the nature of the C_1 intermediate can be studied.

STUDIES ON THE MECHANISM OF PROTEIN BIOSYNTHESIS

Enzymatic Activation of Amino Acids

G. D. Novelli A. N. Best

Introduction. – Previous studies have revealed that amino acids can be activated by enzyme systems widely distributed in animal and microbial tissues.[4] This activation appears to involve the

[1] ORINS Fellow.

[2] G. D. Novelli, *Soc. Am. Bacteriologists, Proc. 55th, New York City* 1955, 112.

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

[4] J. A. DeMoss and G. D. Novelli, *Biochim. et Biophys. Acta* 18, 592 (1955).

interaction of a specific amino acid with ATP to form an enzyme-bound amino acyl-AMP. A chemical synthesis of such compounds has been described,[5] but the method gives poor yields and is not generally applicable to all amino acids. The present investigation was designed to develop another chemical method for the synthesis of amino acyl-AMP compounds which could be applied to all amino acids with reasonably good yields. These compounds would then be used to investigate further the sequence of metabolic reactions leading to protein synthesis.

Results. – *(a) Synthesis of Amino Acyl-AMP Compounds with Dicyclohexylcarbodiimide in Pyridine.* – Specific conditions with respect to concentration of reactants, pH, and time of reaction were worked out for the successful synthesis of leucyl-AMP. The yields were 70 to 80% of theory. However, it was found that the method is not generally applicable to all amino acids; for example, all attempts to prepare glycyl-AMP failed. In addition, it proved to be very difficult to isolate the product from the reaction mixture without suffering large losses. The product, when isolated, was grossly contaminated with decomposition products of the reagent.

(b) Synthesis of Amino Acyl-AMP Compounds Through the Mixed Anhydride.[6] – By protecting the amino group with a carbobenzoxyl (CBZ) group a mixed anhydride of the amino acid could be prepared with ethylchloroformate in acetonitrile with triethylamine as catalyst. Without isolation, the mixed anhydride could be used to smoothly acylate AMP in aqueous pyridine. With CBZ leucine and CBZ phenylalanine, 90% yields were obtained and the isolated product was 90 to 100% pure. Conditions for the removal of the CBZ group have not yet been determined.

(c) Enzymatic Conversion of Amino-Acyl-AMP Compounds to ATP. – When leucyl-AMP is incubated with inorganic pyrophosphate and a soluble extract from *Escherichia coli*, there is a 10 to 20% conversion of the compound to ATP within 3 min. With a soluble extract from rat liver the conversion is of the order of 1 to 2%. The remainder of the compound is rapidly hydrolyzed. In these enzyme systems, CBZ leucyl-AMP is totally inactive, and so it is clear that the protecting group must be removed to make the compounds enzymically active.

Discussion. – It appears that the use of blocked amino acids and the employment of the mixed anhydride will give a general method for the synthesis of amino-acyl-AMP compounds, when conditions for the removal of the blocking group have been worked out. These compounds should prove useful in working out subsequent steps in the biosynthesis of protein.

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

[6] A. W. D. Avison, *J. Chem. Soc.* 1955, 732.

Incorporation of Amino Acids into Protein of Various Tissues

J. W. Davis G. D. Novelli

Introduction. – The availability of a cell-free system giving significant net protein synthesis is of paramount importance in studying the sequential enzymic reactions leading to protein biosynthesis. Experience has shown that certain integrated enzyme systems are more readily prepared in a functional state from some tissues than from others. For this reason it seemed worth while to survey many sources for a protein synthesizing system. In this survey the ability to incorporate labeled amino acids into protein has been used as a criterion of potential protein synthesizing ability. Baker's yeast, a rapidly proliferating microorganism, and tobacco- and turnip-leaf preparations, in which the energy for protein synthesis may come from radiant energy, have been investigated.

Results. – Incubation with C^{14}-leucine followed by suitable breakage of the cells and centrifugal separation of the cell constituents provided the protein for this study. Yeast cells displayed a high specific activity in a small particle fraction (13,000 × *g*, 1 hr to 30,000 × *g*, 1 hr) with a lesser amount of activity in larger particles (3,200 × *g*, 15 min to 13,000 × *g*, 1 hr). Incubation of isolated particles with C^{14} amino acid has failed to label the protein at a level considered significant for these studies. In the tobacco leaves the initial incorporation appeared in the microsomes, with chloroplasts, grana, and mitochondria gaining in specific activity with time. Macerates of the turnip leaf incorporated labeled leucine to the extent of about 25% of the labeling of the whole tobacco leaves.

Discussion. – Knowledge of the unknown steps between amino acid activation and synthesized protein awaits the demonstration of adequate

protein synthesis in an isolated cell-free system. Among the materials so far examined, plant extracts show some promise in this role.

PHOTOSYNTHESIS

W. A. Arnold H. Sherwood

During the last few months two new phenomena in chloroplasts have been under investigation. The results can be interpreted by treating the chloroplasts as semiconductors.

Chloroplasts, from any of a number of higher plants, washed in distilled water, were dried as thin films on metal plates. Such films emit red light on being heated, provided that the heating follows a period of illumination. The illumination must be at wavelengths that can be absorbed by chlorophyll.

Aside from the wavelength dependence, the light emission on heating mimics in all respects the "glow curves" made with inorganic crystals. Glow curves have for some years been interpreted on the basis of semiconduction.

The second new effect concerns the electrical resistance of the chloroplast films. The resistance, as a function of the temperature, follows the equation for a semiconductor unless the film has been illuminated. The first time that the film is heated after illumination there is superimposed on the dark conduction curve a "spike." This spike is a kind of glow curve in the conductance. It could be interpreted as the untrapping of electrons. In order to elicit the spike, the film must have been illuminated with blue light, which indicates that chlorophyll is not absorbing the light here.

Certainly the experiments made so far do not prove that chloroplasts are semiconductors. They do, however, suggest that possibly a role may eventually be found for free electrons in biology.

These experiments were made with the help of J. B. Davidson of the Instrument and Controls Division.

MICROBIOLOGY

TRACER STUDIES ON INTERMEDIARY METABOLISM

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E. F. Phares
R. E. Koeppe[1]
M. V. Long
R. M. Weimberg[2]

A Coupled Malic-Lactic Dehydrogenase from *Veillonella gazogenes*

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

Introduction. – A previous report[3] described a combination of enzymes from extracts of *Veillonella gazogenes* and *Propionibacterium pentosaceum* that converted pyruvate to lactate in the presence of reduced diphosphopyridine nucleotide (DPNH) and catalytic amounts of malate.

Results. – Experiments with a combination of purified active components from each extract demonstrated the following system: a coupled dehydrogenase from the *Veillonella* extract, which reduced pyruvate to lactate; malate, the hydrogen donor, formed oxalacetate (OAA). The OAA was reconverted to malate by DPNH and a malic dehydrogenase which was contributed by the *Propionibacterium* extract.

The coupled dehydrogenase was purified twenty-fold by treatment with streptomycin, calcium phosphate gel, and ammonium sulfate and by electrophoresis. The enzyme was assayed by following OAA formation spectrophotometrically, at 280 mμ, with malate and pyruvate as substrates. The reaction apparently involves a single enzyme and is reversible. The only hydrogen donor-acceptor systems which were found to be active were malate-pyruvate and lactate-OAA. Other hydrogen donors and acceptors which were tested included oxygen, keto acids, dyes, and pyridine nucleotides. Oxalacetate, *p*-chloromercuribenzoate, and concentrated malonate inhibited the system, but atabrine did not; iodoacetate and ammonium sulfate stimulated slightly. Metal ions did not affect the enzyme but merely complexed the OAA to increase absorption and reversed OAA inhibition slightly. Flavin content did not coincide with activity.

Discussion. – The system is distinctly different from previously reported lactic and malic dehydrogenases, which are specific for malate or lactate and which usually mediate hydrogen transfer through pyridine nucleotides. In this case the pyridine nucleotides were found to be totally inactive, and only the reduced form of the corresponding organic acid can act as the hydrogen donor for reduction. The enzyme can be used to study certain aspects of the mechanism of enzymatic hydrogen transfer.

Mechanism of Electron Transport Between Reduced Pyridine Nucleotides and Flavoprotein

M. I. Dolin

Introduction. – Reduced diphosphopyridine nucleotide (DPNH) peroxidase of *Streptococcus faecalis*[4] is being used as a model to study the general problem of pyridine nucleotide–flavoprotein interaction during electron transport. The aim is to define the mechanism of the reaction and the nature of the intermediate complex[5] formed between reduced pyridine nucleotides and flavoprotein. At present several approaches are being pursued:

1. Studies on complex formation between flavoprotein and pyridine nucleotides and pyridine nucleotide analogs are being continued.
2. Kinetic studies have been carried out by employing both one- and two-electron acceptors. In addition, the kinetics of an inactivation reaction that takes place in the presence of reduced pyridine nucleotides is under investigation.
3. Two new modification reactions of DPNH, leading to compounds with spectra similar to DPNH-X,[6] are being investigated.

Results and Discussion. – Kinetic studies show that the rate-limiting step in the oxidation is proportional to one molecule of either a one- or

[1] Research Participant.

[2] Research Associate.

[3] S. F. Carson, E. F. Phares, and M. V. Long, *Biol. Semiann. Prog. Rep. Feb. 15, 1956*, ORNL-2060, p 53.

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

[5] M. I. Dolin, *Arch. Biochem. and Biophys.* 60, 499 (1956).

[6] G. W. Rafter, S. Chaykin, and E. G. Krebs, *J. Biol. Chem.* 208, 799 (1954).

two-electron acceptor. While the results do not establish a mechanism, they are in harmony with the concept of one-electron transport via a semiquinone free-radical form of flavin[7] and indicate that the DPNH-flavoprotein complex can function efficiently as a one-electron donor. Since the enzyme contains only very small amounts of metals, the results further indicate that one-electron transfer does not require a special metalloflavoprotein. The incubation of DPNH with enzyme, in the absence of oxidant, leads to inhibition of enzyme activity. In this inactivation reaction, DPNH saturation is roughly equivalent to its saturation value in the oxidation with peroxide as oxidant. At saturation a first order decay of activity takes place. The simultaneous presence of peroxide largely prevents the inactivation.

These results may be interpreted as follows. The DPNH combines with the enzyme to form initially the active DPNH-flavoprotein complex. However, this complex is transformed at a finite rate into an inactive one. The latter may represent a reduction state from which electrons are only slowly donated or, alternatively, a complex in which a second molecule of DPNH is bound at the site normally occupied by H_2O_2. The presence of peroxide would prevent either one of these reactions from occurring. Further analysis of the system should furnish information on the still unsettled problem of the existence of ternary complexes in enzyme catalyzed reactions.

Oxidation of *l*-Arabinose by *Pseudomonas saccharophila*

R. M. Weimberg

Introduction. – Characterization of the intermediate formed from *l*-arabonate by crude cell-free preparations of *Pseudomonas saccharophila*, in the absence of DPN or any other added hydrogen acceptor, is proceeding.[8]

Results and Discussion. – The intermediate may be isolated by gradient elution on a Dowex-1 column in the formate form. Water and formic acid are removed by lyophilization from that part of the eluate containing the intermediate. The residue, a thick syrup which shows no signs of crystallizing, is believed to be a single compound, since it is eluted from the column as a single sharp peak and because paper chromatograms of the eluted material, developed in five different solvents, contain only one spot when sprayed either with an acid indicator or an α-keto acid indicator.

The isolated intermediate reacts with 2,4-dinitrophenylhydrazine, as is shown by the intense reddish-brown color produced under alkaline conditions. However, the 2,4-dinitrophenylhydrazone appears to be water soluble. An attempt was made to isolate the 2,4-dinitrophenylhydrazone, reduce it to the amino acid with hydrogen,[9] and identify the amino acid. The yield of amino acid was very poor, and seven distinct spots were detected on two dimensional chromatograms of the amino acid solution. Therefore no further effort was made to identify any of the spots.

The intermediate is oxidized by periodate with the production of one mole of formaldehyde per mole of substrate. This shows the presence of a primary alcohol adjacent to another carbon containing either a hydroxyl or carbonyl group. The compound is oxidized by hypoiodite with the formation of iodoform; however, the reaction was not quantitative. It is also oxidized by ceric sulfate and hydrogen peroxide with the evolution of one mole of carbon dioxide per mole of substrate.

Reduction of the intermediate can be achieved with hydrogen, platinum oxide being the catalyst, or with sodium borohydride. One mole of hydrogen is consumed per mole of substrate. Very likely it is the carbonyl group that is reduced, since the reaction mixture no longer gives an α-keto acid test.

Though these results give some idea as to the functional groups on the molecule, they are not sufficient for completely characterizing the compound. Further work is required in order that the intermediate may be identified.

[7] L. Michaelis, M. P. Schubert, and C. V. Smythe, *J. Biol. Chem.* 116, 587 (1936).

[8] R. Weimberg, *Biol. Semiann. Prog. Rep. Feb. 15, 1956*, ORNL-2060, p 54.

[9] G. H. N. Towers, J. F. Thompson, and F. C. Steward, *J. Am. Chem. Soc.* 76, 2392-2396 (1954).

PLANT BIOCHEMISTRY

N. E. Tolbert

L. P. Zill
A. W. Naylor[1]
G. E. Hunt[1]
L. P. Carter[1]
L. M. Rohrbaugh[1]
P. C. Kerr
D. A. Mondon
R. Rabson

Sugar Metabolism in *Sedum*

L. M. Rohrbaugh N. E. Tolbert

Introduction. – The effect of CO_2 partial pressure on the sugar reservoirs and their rate of synthesis in leaves have been examined in a *Sedum* plant to study the metabolic relationships among these sugars in vivo. Variations in these reservoirs during a day and night have also been examined.

Results. – The incorporation of $C^{14}O_2$ into sedoheptulose was 7 to 20% of the total $C^{14}O_2$ fixed in 10 min. This amount of C^{14} incorporation and the total amount of sedoheptulose present did not vary when changing the CO_2 in the air between 0.005 and 12.0%, after 3 hr in the air at high CO_2 concentration, or during a daily cycle of light and dark. The $C^{14}O_2$ incorporation into sucrose in 10 min was likewise always fast, while glucose and fructose were labeled more slowly. Large amounts of C^{14} in glucose and fructose accumulated only at high CO_2 concentrations for longer periods of time.

The sucrose pool was saturated with C^{14} before the hexose reservoirs were labeled. The data indicate that the major pathway for the formation of free glucose and fructose was from the hydrolysis of sucrose. The alternate and more direct pathway for free hexose synthesis by the action of phosphatases on the hexose phosphates of the photosynthetic carbon cycle was not implicated.

Discussion. – The amount of sedoheptulose in the leaves did not vary during the day or under the influence of various environmental factors. However, this sugar must be readily utilized, since the rate of labeling showed that it was being continually and rapidly synthesized. The sedoheptulose phosphate reservoir varied in a different fashion than did the free sugar. When the CO_2 concentration was increased, there was a marked decrease in the amount of sedoheptulose phosphate and an increase in phosphoglyceric acid. This indicates that the carbon chain of sedoheptulose phosphate may be quickly utilized through ribulose diphosphate as material for the first steps in the photosynthetic carbon cycle.

The data suggest two different roles for sedoheptulose in these cells. The free sugar seems relatively inert and is probably present chiefly in the cytoplasm. The phosphate ester is relatively active and may exist mostly in the chloroplasts.

[1]Research Participant.

Sedoheptulose in the *Coleus* Plant

N. E. Tolbert P. C. Kerr

Introduction. – Large amounts of the seven-carbon sugar, sedoheptulose, were found in the Crassulacea plants and particularly in species of *Sedum*, from whence the sugar was named many years ago. It has now been discovered that some common varieties of *Coleus* plants also contain large amounts of free sedoheptulose equivalent to that found in *Sedum* plants (2 to 6 mg per gram wet weight of leaf).

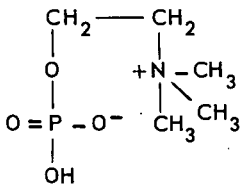
Results. – The reservoir of sedoheptulose in the *Coleus* plant, together with sucrose, appears to function in carbohydrate storage. The total amount of sedoheptulose and its rate of formation in *Coleus* plants exhibit a diurnal variation, being greatest during the first part of the day and least at night. Such diurnal variations have not been demonstrated for sedoheptulose in the *Sedum* plant (see "Sugar Metabolism in *Sedum*," this report).

Other factors also affect sedoheptulose content of the *Coleus* leaves. Leaves grown at lower light intensity contained the most free sedoheptulose. On a given plant the older leaves contained more free sedoheptulose than do the young leaves. Even dead leaves, which had dropped from the plant, contained substantial amounts of unused free sedoheptulose. Thus, as they mature, *Coleus* leaves appear to build up and maintain a sedoheptulose reservoir. Death of the leaf through aging is certainly not caused by any lack of this carbohydrate.

Identification of Phosphoryl Choline as an Important Constituent of Plant Saps[2]

N. E. Tolbert P. C. Kerr

A phosphorus-containing compound has been reported in xylem sap as a transport form of phosphorus in plants[3] and as a major compound formed from formic-C^{14} acid metabolism in plants.[4] This compound has now been identified as phosphoryl choline:



It is characterized by acid stability, weak ionic character, R_f value of 0.9 in phenol-water solvent during chromatography, and phosphatase hydrolysis to orthophosphate. It is readily labeled with $P^{32}O_4^{3-}$ or $HC^{14}OOH$. However, C^{14} was incorporated into it very slowly by $C^{14}O_2$ photosynthesis. The zwitter ion structure and organic solubility of phosphoryl choline suggest that it may act as a phosphorus carrier capable of penetrating cell membranes.

Aspartic Acid Metabolism in Barley and Wheat Leaves

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

Introduction. – In a continuing study on the metabolic pathways for metabolism of amino acid and organic acids of the tricarboxylic acid cycle, aspartic-U-C^{14} acid was fed to plants. The experimental conditions included light or darkness and aerobic or anaerobic conditions. Barley, wheat, tomato, beet, and pea plants have been used. The C^{14}-labeled products from the metabolism of this aspartic-U-C^{14} acid were determined by paper chromatographic analysis.

Results. – The metabolism of aspartic acid was severalfold faster in the light than in the dark, but this rate was from rapid conversion of the amino acid carbon chain to malic acid. On the basis of C^{14} label appearing in citric and glutamic acids, the over-all rate of cycling of the tricarboxylic acid cycle was the same in light or darkness. The C^{14} from the aspartic-U-C^{14} acid appeared only in compounds related to tricarboxylic acid cycle, which limit was not found for glutamic-U-C^{14} acid metabolism.[5]

Though the procedures would have detected 0.01% conversion of aspartic acid to β-alanine, none appeared on the chromatograms. Consequently β-alanine biosynthesis in the plants tested would appear to be by some other, as yet unknown, pathway. Likewise, only small to trace amounts of the aspartic acid carbon chain appeared in asparagine. Thus either asparagine is relatively metabolically inert or it arises primarily from some other source besides aspartic acid.

Anomalous Behavior of Orthophosphate-P^{32} on Anion Exchangers

L. P. Zill D. A. Mondon

Introduction. – During the application of the Khym and Cohn procedure to the analysis of phosphates formed by chloroplasts during photophosphorylation, a separation of radioactive orthophosphate into two peaks was observed. Such behavior would have extreme import in the interpretation of metabolic studies with P^{32} should it prove to be a true resolution of diverse molecular species. The phenomenon has been investigated with the intent of determining its real or anomalous character.

Results. – Detection of these double peaks was accomplished with a highly sensitive flow-counting technique. Isotopic identity of the two peaks was established by the "half-value absorption" technique. Possible contamination of the orthophosphate-P^{32} with phosphate ion was eliminated by rigorous chemical procedures. Addition of carrier orthophosphate to P^{32} samples and simultaneous detection by chemical and radioactive means demonstrated symmetry of the carrier phosphate with the ascending leg of the first peak and the descending leg of the second peak. At extremely low concentrations of P^{32} a single symmetrical peak of activity was obtained.

Discussion. – Although this is the first observation of anomalous double peak formation with orthophosphate, it has been observed with pyrophosphate (on anion exchangers) and with cadmium (on cation exchangers).

[2]To be published in *Plant Physiol.* **31**, September 1956, in joint authorship with J. V. Maizel and A. A. Benson, Pennsylvania State University, University Park, Pennsylvania.

[3]N. E. Tolbert and H. Wiebe, *Plant Physiol.* **30**, 499–504 (1955).

[4]N. E. Tolbert, *J. Biol. Chem.* **215**, 27–34 (1955).

[5]A. W. Naylor and N. E. Tolbert, *Physiol. Plantarum* **9**, 220–229 (1956).

BIOPHYSICS

MOLECULAR BIOPHYSICS AND RADIOLOGICAL PHYSICS

J. S. Kirby-Smith

J. N. Dent[1] M. Slater
A. C. Faberge[2] K. M. Wilbur[3]
W. G. Lynn[3] G. B. Bunyard[2]
M. L. Randolph D. L. Parrish

Cockcroft-Walton Accelerator Activity

M. L. Randolph D. L. Parrish

Introduction. – The Cockcroft-Walton accelerator has been established as a facility for the irradiation of biological materials with fast neutrons under as nearly ideal physical conditions as possible.

Results. – Current work includes two studies with the Cytology and Genetics Section, and one each with the Mammalian Genetics and Development, Pathology and Physiology, and Mammalian Recovery Sections. Irradiations for the determination of nuclear cross sections by the Electronuclear Research Division have been continued.

Discussion. – All the *d,t* 14-Mev neutrons are now being produced with the use of the rotating water-cooled target previously described.[4] Dose rates above 200 rad/hr at 15 cm from the target (total emission 3×10^{10} neutrons/sec have been obtained and a total dose of 1100 rad at 15 cm has been delivered in one working day. A study of tritium target yields and their life is being made.

Dosimetry work has been continued. The use of two independent monitors, both being simply calibrated before and after each biological exposure and more elaborately calibrated with both a Hurst fast-neutron dosimeter and a homogeneous ion chamber, makes possible absolute dose determinations to within ±5% for the *d,d* irradiations and ±10% for the *d,t* irradiations. The buildup of deuterium, after prolonged bombardment of tritium targets, has been found to produce a contaminating *d,d* dose rate of perhaps 3% of the final *d,t* dose rate. A spectral analysis of the *d,d* neutron flux is being made with photographic emulsion techniques. The gamma contamination in the *d,d* dose has been approximated, with the use of blackening of photographic emulsion and the assumption that the gamma spectrum is equivalent to that of radium, as being less than 3% of the total dose.

A superior method for mounting metallic bellows in a vacuum or pressure system has been developed.

[1] Consultant.

[2] Temporary employee.

[3] Research Participant.

[4] M. L. Randolph, *Biol. Semiann. Prog. Rep. Feb. 15, 1956*, ORNL-2060, p 57–58.

Bone-Marrow Treatment of Mice After Fast-Neutron Irradiation

M. L. Randolph C. C. Congdon[5]

Introduction. – The postirradiation treatment of mice with bone marrow as a recovery measure against fast neutrons is being investigated. Since a variety of biological effects show a dependence on the nature of the primary radiation, it might be anticipated that recovery measures would have different effectivenesses against x-ray and fast-neutron radiation damage.

Results. – The mice received total-body irradiation with 14-Mev neutrons generated by the Cockcroft-Walton accelerator. They were placed in individual thin plastic tubes at a mean distance of 15 cm from the target. No correction was made for attenuation of dose in the mice. Following irradiation at 150 to 200 rad/hr, isologous bone marrow suspended in Tyrode's solution was injected intravenously to half the irradiated mice. Survival and body weight were followed for 30 days. Table 13 and Fig. 14 give the results.

Discussion. – The preliminary results with males indicate that bone marrow increased the 14- and 30-day survival after fast-neutron irradiation. The bone marrow primarily prevented death in the second week postirradiation but did not prevent death in the first week after irradiation. The body weights of the surviving bone marrow treated mice showed a return to normal within 30 days after irradiation.

Combination Ion Chamber–Proportional Counter Dosimeter

M. Slater G. B. Bunyard
M. L. Randolph

Introduction. – One of the outstanding problems in neutron dosimetry is the accurate determination

[5] This study was undertaken in collaboration with the Mammalian Recovery Group.

TABLE 13. EFFECT OF BONE-MARROW TREATMENT ON MALE MICE IRRADIATED WITH FAST NEUTRONS

Experiment No.	Strain of Mice	Dose (rad)	Number of Femur Equivalents of Bone Marrow Injected per Test Animal	Number Surviving/Number Irradiated					
				At 7 days		At 14 days		At 30 days	
				Controls	Bone-Marrow Treated	Controls	Bone-Marrow Treated	Controls	Bone-Marrow Treated
1	RF	650	3	9/10	7/10	0/10	6/10	0/10	4/10
2	$101 \times C_3HF_1$	900	8	6/12	6/12	1/12	4/12	1/12	4/12
3	$101 \times C_3HF_1$	800	8	7/9	8/10	4/9	7/10		

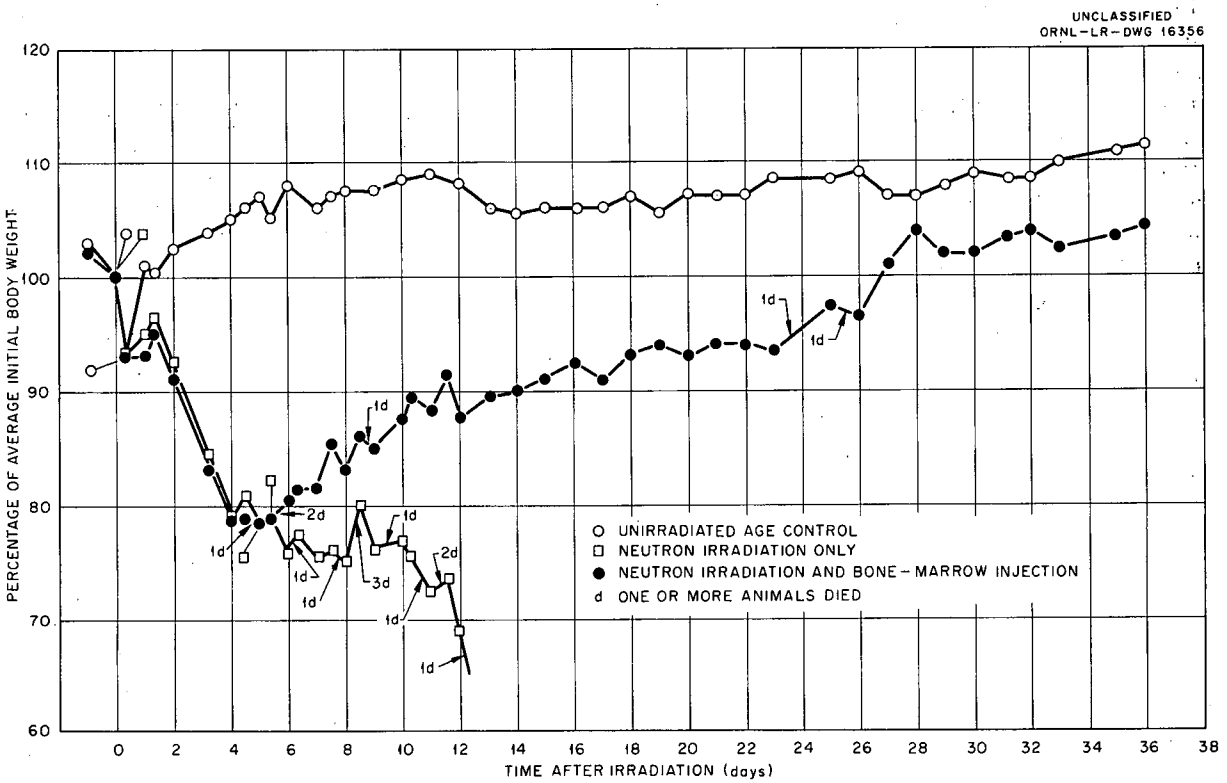


Fig. 14. Effect of Bone-Marrow Treatment on Body Weight and Survival of Mice Given Fast-Neutron Irradiation.

of the gamma-ray contamination in a relatively pure fast-neutron field. The proportional counter dosimeter[6] measures the fast-neutron component and is insensitive to the gamma-ray component. Most means of measuring the gamma-ray component depend on the difference in response between two instruments or on a detector whose response to neutrons is supposedly quite low.[7]

[6] G. S. Hurst, *Brit. J. Radiol.* 27, 353 (1954).

The instrument described here consists of a slightly modified version of Hurst's polyethylene wall, ethylene gas, absolute proportional counter.[6] These modifications permit sequential operation of the instrument as either an ionization chamber or as a proportional counter, without affecting the

[7] H. H. Rossi, p 667–691 in *Radiation Dosimetry* (G. J. Hine and G. L. Brownell, eds.) Academic Press, New York, 1956.

source-dosimeter geometry. As an ionization chamber it is operated with an electrometer to measure a current which is proportional to the total dose rate. As a proportional counter it is used with a linear amplifier and integral pulse height discriminator to measure a quantity which is proportional to the dose rate due to heavily ionizing particles. The same alpha particle source is used to calibrate the instrument both as a chamber and as a counter.

Results. – Figure 15 shows the gas multiplication curve obtained with a neutron source for operation as an ion chamber (solid curve) and as a proportional counter (dashed curve). Essentially identical curves were obtained with alpha particle and gamma ray sources. The deviation of the proportional counter curve at low multiplication is caused by collection time effects and is under further study.

Preliminary measurements on a small Po^{208}-Be neutron source have yielded a value of 0.6 for the ratio of the neutron dose to the total dose. This value is surprisingly low, although no previous measurements have been reported for a source of this type. Further investigation is being carried out on other neutron sources.

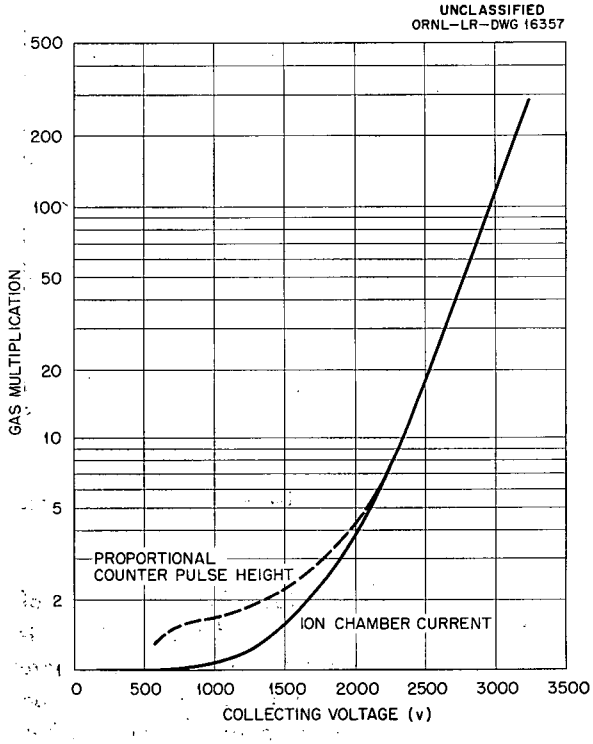


Fig. 15. Gas Multiplication Curve Obtained with a Neutron Source as an Ion Chamber and as a Proportional Counter.

Discussion. – The combination chamber-counter dosimeter described here has several advantages over other methods of separating the components in a mixed radiation field. The response is energy independent over a fairly wide range of both neutron and gamma-ray energies, and, in fact, the instrument may be used as an absolute dosimeter for either type of radiation. The measured value for the ratio of the neutron dose to the total dose is obtained as a ratio of the experimental parameters rather than as a difference between them. This value is, furthermore, completely independent of the chamber volume and gas density, source to chamber distance, alpha source energy or collimation, and of the electrometer or amplifier calibration. It is only slightly dependent on such quantities as the energy loss per ion pair, stopping power, etc. The technique used here has also yielded some useful information on the behavior of proportional counters at low gas multiplication.

Comparative Study of Thyroid Function in Two Species of Salamanders, *Triturus viridescens* and *Desmognathus fuscus*

W. G. Lynn J. N. Dent

There are indications that the thyroid gland of *Triturus viridescens* normally maintains a rather low level of secreting activity and that it fails to show any marked histological change even after prolonged administration of goitrogenic drugs. The thyroid of *Desmognathus fuscus*, on the other hand, responds to goitrogens in the usual way by hyperemia, hyperplasia, and hypertrophy. This investigation is a comparison of radioiodine uptake by the thyroid in these two animals by utilizing, for both species, several series treated for different lengths of time with $KClO_4$, which inhibits thyroid function by interference with the iodide-concentrating mechanism of the gland; several series treated for different lengths of time with thiourea, which permits iodide concentration but interferes with the formation of iodinated proteins; and suitable series of untreated controls. Preliminary experiments have demonstrated that the expected differences in radioiodine uptake do exist, and the experiments with long-term treatment, which are now in progress, should reveal this more strikingly.

The thyroid and pituitary glands of all animals will be sectioned and subjected to histological examination in an attempt to ascertain whether the difference in response in these two species is correlated with any indicated difference in production of TSH.

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

A. C. Faberge'

Introduction. – Breakage-fusion-bridge cycles are definitely established only in maize, but there is reason to think that they are of universal occurrence in other organisms, in the form of dominant lethals, and that they play an important part in somatic radiation damage. Both on general grounds, and also for the interpretation of other experiments using maize endosperm, it was important to know what happens when two independent chromatid-type breakage-fusion-bridge cycles are present simultaneously in the same nucleus. Two possibilities are open: they may remain independent of one another or they may coalesce, to form a single, chromosome-type, breakage-fusion-bridge cycle.

Results. – An experiment to distinguish between these two possibilities by a genetic technique was made, about 60 ears from the critical cross:

I Sh Bz Wx Ds ac ac ♀ × Ds C Sh Bz Wx Ac/ac ♂

being obtained. Preliminary examination shows that, in general, coalescence does not occur, the two chromatid-type cycles remaining independent. Detailed examination of this material will be necessary to ascertain whether coalescence of cycles can sometimes occur as an unusual event, and if so, with what frequency.

Discussion. – This result makes the interpretation of other data, particularly radiation data, with maize much more straightforward. The underlying chromosome mechanics are probably that those free chromosome ends which are closest in the nucleus are most likely to join.

Analysis of Published Data on Induced Radiation Effects in Maize

A. C. Faberge'

Rates of induced loss for single genetic markers have been established in several organisms, but the data for maize are numerically by far the most important. An analysis of these data has been made, which shows that the relative sensitivity of different markers depends on their position in a chromosome arm and the length of that arm. The fact that breaks are induced approximately in proportion to pachytene length and the application of simple chromosome mechanics allow a rough forecast of relative loss rate when only the cytological positions of the markers are known. Although such single marker losses are called mutations, the induced mutation rates probably have no connection with any properties of the locus as such but only with its position.