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for Period Ending February 15, 1956

Alexander Hollaender, Director

Stanley F. Carson, Assistant Director

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BIOLOGY DIVISION SEMIANNUAL PROGRESS REPORT

PUBLICATIONS AND LECTURES

Publications. – A count of the publications from the Biology Division during the period of this report is as follows:

	Papers	Symposia	Abstracts and Book Reviews	Progress Reports	Total
Published	41	1	26	1	69
In press	74	1	36		111

Titles of the published papers and the symposium are listed below. Abstract references are listed with the talks that they represent.

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Amy, R. L.	A Comparative Study of the Effects of Beta Rays, Gamma Rays, and X Rays on Development in *Habrobracon*	*Radiation Research* **3**, 166–181 (1955)
Anderson, E. H., and D. Billen	The Effects of Temperature on X-Ray-Induced Mutability in *Escherichia coli*	*J. Bacteriol.* **70**, 35–43 (1955)
Anderson, N. G.	A Mechanical Device for Producing Density Gradients in Liquids	*Rev. Sci. Instr.* **26**, 891–892 (1955)
———	Studies on Isolated Cell Components. VIII. High Resolution Gradient Differential Centrifugation	*Exptl. Cell Research* **9**, 446–459 (1955)
Arnold, W., and E. S. Meek	The Polarization of Fluorescence and Energy Transfer in Grana	*Arch. Biochem. and Biophys.* **60**, 82–90 (1956)
Arnold, W., and J. T. Thompson	Delayed Light Production by Blue-Green Algae, Red Algae, and Purple Bacteria	*J. Gen. Physiol.* **39**, 311–318 (1956)
Atwood, K. C., and F. Mukai	Nuclear Distribution in Conidia of *Neurospora* Heterokaryons	*Genetics* **40**, 438–443 (1955)
Benedict, W. H., K. W. Christenberry, and A. C. Upton	Spontaneous and Radiation-induced Iris Atrophy in Mice	*Am. J. Ophthalmol.* **40**, 163–169 (1955)
von Borstel, R. C., and S. Wolff	Photoreactivation Experiments on the Nucleus and Cytoplasm of the *Habrobracon* Egg	*Proc. Natl. Acad. Sci. U.S.* **41**, 1004–1011 (1955)
Brumfield, R. T.	The Inhibition of Curvatures in Timothy Roots by Certain Chemicals	*Am. J. Botany* **42**, 958–964 (1955)
Conger, A. D.	The Effect of Oxygen on the Radiosensitivity of Mammalian Cells	*Radiology* **66**, 63–69 (1956)
Doherty, D. G.	The Hydrolysis of Carbon-Carbon Bonds by α-Chymotrypsin	*J. Am. Chem. Soc.* **77**, 4887–4892 (1955)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Furth, J.	The Concept of Conditional and Autonomous Neoplasms	In, Ciba Foundation Symposium on Leukemia Research, ed. by G. E. W. Wolstenholm and M. P. Cameron, Little, Brown, Boston, 1955, p 38–41
Furth, J., and A. C. Upton	Induction of Leukemia by Ionizing Irradiation	In, Ciba Foundation Symposium on Leukemia Research, ed. by G. E. W. Wolstenholm and M. P. Cameron, Little, Brown, Boston, 1955, p 146–158
Gaulden, M. E., and K. L. Kokomoor	Influence of Yolk on Mitotic Rate in Untreated and in X-rayed Grasshopper Neuroblasts *in vitro*	*Proc. Soc. Exptl. Biol. Med.* 90, 309–314 (1955)
Hearon, J. Z., and R. F. Kimball	Tests for a Role of H_2O_2 in X-Ray Mutagenesis. I. Estimations of the Concentration of H_2O_2 Inside the Nucleus	*Radiation Research* 3, 283–294 (1955)
Hinton, C. W.	The Behavior of an Unstable Ring Chromosome of *Drosophila melanogaster*	*Genetics* 40, 951–961 (1955)
Hollaender, A. (ed. E. J. Slaughter)	Biology Division Semiannual Progress Report for Period Ending August 15, 1955	ORNL-1953
Horn, E. C.	Ascites Tumor Development. I. An Analysis of the *in vivo* Effect of Nucleoprotein from Ehrlich Ascites Cells	*Cancer Research* 15, 663–670 (1955)
Hurst, G. S., W. A. Mills, A. C. Upton, and F. P. Conte	Principles and Techniques of Mixed Radiation Dosimetry. Application to Acute Lethality Studies of Mice with the Cyclotron	*Radiation Research* 4, 49–64 (1956)
Kimball, R. F., and N. Gaither	Behavior of Nuclei at Conjugation in *Paramecium aurelia.* I. Effect of Incomplete Chromosome Sets and Competition Between Complete and Incomplete Nuclei	*Genetics* 40, 878–889 (1955)
Kimball, R. F., J. Z. Hearon, and N. Gaither	Tests for a Role of H_2O_2 in X-Ray Mutagenesis. II. Attempts to Induce Mutations by Peroxide	*Radiation Research* 3, 435–443 (1955)
Krall, A. R.	Cytochrome Oxidase Participation in Photosynthetic Fixation of Carbon Dioxide: Specific Light Reversal of Carbon Monoxide Inhibition	*Physiol. Plantarum* 8, 869–876 (1955)
Lindsley, D. L., G. Fankhauser, and R. R. Humphrey	Mapping Centromeres in the Axolotl	*Genetics* 41, 58–64 (1956)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Lindsley, D. L., T. T. Odell, Jr., and F. G. Tausche	Implantation of Functional Erythropoietic Elements Following Total-Body Irradiation	*Proc. Soc. Exptl. Biol. Med.* **90**, 512–515 (1955)
Oakberg, E. F.	Degeneration of Spermatogonia of the Mouse Following Exposure to X Rays, and Stages in the Mitotic Cycle at Which Cell Death Occurs	*J. Morphol.* **97**, 29–54 (1955)
Odell, T. T., Jr., and A. C. Upton	Distribution of Calcium-45 in Platelets and Bone Marrow of Rats	*Acta Haematol.* **14**, 291–293 (1955)
Passonneau, J. V., and J. R. Totter	The Effects of Gamma Irradiation and of Aminopterin on the Metabolism of Formate-C^{14}, Glycine 2-C^{14}, and Adenine 8-C^{14} on Chick Embryos	*Radiation Research* **3**, 304–315 (1955)
Pittenger, T. H., A. W. Kimball, and K. C. Atwood	Control of Nuclear Ratios in *Neurospora* Heterokaryons	*Am. J. Botany* **42**, 954–958 (1955)
Randolph, M. L. (coauthor with H. G. Blosser, C. D. Goodman, and T. H. Handley)	14-Mev (n) Cross-Section Measurements	*Phys. Rev.* **100**, 429–430 (1955)
Riley, H. P.	The Protective Effect of Various Chemical Compounds Against Damage to Chromosomes by Gamma Radiation	*Am. J. Botany* **42**, 765–769 (1955)
Roberts, H. S.	The Mechanism of Cytokinesis in Neuroblasts of *Chortophaga viridifasciata* (De Geer)	*J. Exptl. Zool.* **130**, 83–105 (1955)
Rogers, R. W.	Alpha-Particle Dosimetry and the Inhibition of Mitosis in the Grasshopper Neuroblast by Low-Dosage Alpha Irradiation	*Radiation Research* **3**, 18–37 (1955)
Smith, L. H., B. Anderson, and T. T. Odell, Jr.	Binding of S^{35}-labeled Sulfate in Serum of Rats as Studied by Filter-Paper Electrophoresis	*Proc. Soc. Exptl. Biol. Med.* **90**, 360–364 (1955)
Stapleton, G. E.	Variations in the Sensitivity of *Escherichia coli* B/r to Ionizing Radiations During the Growth Cycle	*J. Bacteriol.* **70**, 357–362 (1955)
Stapleton, G. E., A. J. Sbarra, and A. Hollaender	Some Nutritional Aspects of Bacterial Recovery from Ionizing Radiations	*J. Bacteriol.* **70**, 7–14 (1955)
Totter, J. R., and C. L. Comar	Function of Molybdenum in Xanthine Oxidase	In, Inorganic Nitrogen Metabolism, ed. by W. D. McElroy and B. Glass, Johns Hopkins Press, Baltimore, 1956
Totter, J. R., and M. J. Cormier	The Relation of Bacterial Luciferase to Alternative Pathways of Dihydroflavine Mononucleotide Oxidation	*J. Biol. Chem.* **216**, 801–811 (1955)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Upton, A. C.	The Pathogenesis of the Hemorrhagic State in Radiation Sickness: A Review	*Blood* **10**, 1156–1163 (1955)
Wheeler, H. E.	Linkage Groups in *Glomerella*	*Am. J. Botany* **43**, 1–6 (1956)
Zill, L. P.	A Manometric Actinometer for the Ultraviolet Spectrum	*Arch. Biochem. and Biophys.* **59**, 473–483 (1955)

Scientific Society Lectures, Traveling Seminars, and Local Lectures. – The 76 titles listed below comprise the public oral presentations by Division members for this period.

AUTHOR(S)	TITLE	PLACE PRESENTED
Amy, R. L., and R. C. von Borstel	Inactivation of the *Habrobracon* Egg with Different Wave Lengths of Ultraviolet Radiation	Am. Assoc. Advance. Sci., Atlanta
Anderson, N. G.	Cell Function and Brei Fractions	Dept. of Zoology, Columbia Univ., New York
———	The Applications of Serological Methods to the Problem of Cell Organization	Am. Assoc. Advance. Sci., Symp., Applications of Serol. in Biol. Res., Atlanta
Arnold, W. A.	Delayed Light in Green Plants	Botanical Laboratory, University of Pennsylvania, Philadelphia
———	As above	Biology Club Seminar, The John Hopkins Univ., Baltimore
———	As above	Dept. of Biology, Princeton Univ., Princeton
Atwood, K. C.	Irreparable Mutations	Bethesda, Maryland
———	Biological Mechanisms of Radiation Damage	Depts. of Botany, Genetics, Plant Pathology, and Zoology, University of Wisconsin, Madison
———	Recessive Lethal Mutations in *Neurospora*	Dept. of Zoology, University of Chicago, Chicago
———	The Relation Between Lethal and Genetic Effects of Radiation on Microorganisms	Am. Assoc. Advance. Sci., Symp., Microbiol. in Med. Res., Atlanta
Atwood, K. C., and T. H. Pittenger	The Relation Between the X-Ray Survival Curves of *Neurospora* Microconidia and Ascospores [*Genetics* **40**, 563 (1955)]	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), East Lansing
Billen, D.	Chemical Restoration of an X-Ray-Induced Block in Deoxyribonucleic Acid Synthesis in Cysteamine-protected Bacteria	Ky.-Tenn. Branch, Soc. Am. Bacteriol., Nashville

AUTHOR(S)	TITLE	PLACE PRESENTED
von Borstel, R. C.	Effects of Radiation on the Egg Nucleus and Cytoplasm of the Wasp *Habrobracon*	Dept. of Zoology, University of Tennessee, Knoxville
———	As above	Dept. of Biology, Emory University, Emory University
———	Nucleocytoplasmic Relations in Early Embryogeny	Am. Assoc. Advance. Sci., Symp., The Formation and Early Development of the Embryo, Atlanta
———	On the Nature of Irradiation of the *Habrobracon* Egg Nucleus	Am. Assoc. Advance. Sci., Atlanta
———	Effects of Radiation on the Egg Nucleus and Cytoplasm of the Wasp *Habrobracon*	Dept. of Zoology, Florida State University, Tallahassee
———	As above	Dept. of Biology, University of Florida, Gainesville
von Borstel, R. C., and H. Moser	Nuclear and Cytoplasmic Effects of Ultraviolet Radiation on *Habrobracon* Eggs	Intern. Conf. Radiobiol., Cambridge, England
von Borstel, R. C., K. C. Atwood, and A. R. Whiting	Delayed Expression of Induced Dominant Lethals in Diploid *Habrobracon* [*Genetics* **40**, 564 (1955)]	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), East Lansing
Congdon, C. C.	(1) Experimental Treatment of Irradiation Injury (2) Development of a Research Problem (3) Comparative Pathology	Winthrop College, Rock Hill, S. C.
———	Experimental Treatment of Irradiation Injury	Science Department, Columbia College, Columbia, S. C.
Conger, A. D.	How Oxygen Causes an Increase in Chromosomal Aberration Yield	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), East Lansing
Craig, D., J. S. Kirby-Smith, and J. N. Dent	Mitotic Inhibition and Chromosome Breakage in Salamanders of the Genus *Gyrinophilus*	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), East Lansing
Darden, E. B.	Some Electrophysiologic Properties of Single Nerve and Muscle Fibers	Dept. of Zoology, University of Tennessee, Knoxville
Edington, C. W.	The Composition of Sex-linked Recessive Lethals Induced in *Drosophila* by Radiations of Different Ion Density [*Genetics* **40**, 570–571 (1955)]	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), East Lansing
———	The Nature of Recessive Lethals Induced in *Drosophila* by Radiations of Different Ion Density	Tenn. Acad. Sci., Cookeville

AUTHOR(S)	TITLE	PLACE PRESENTED
Hinton, C. W.	Analysis of an Unstable Ring-X Chromosome and Its Derivatives in *Drosophila melanogaster* [*Genetics* 40, 575 (1955)]	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), East Lansing
Hollaender, A.	Modification of Radiation Response	Intern. Conf. on the Peaceful Uses of Atomic Energy, Geneva, Switzerland
———	Studies on the Modification of Radiation Response	Biology Department, Princeton University, Princeton
Khym, J. X., L. P. Zill, and W. E. Cohn	Structural Studies on Sugars by Ion-Exchange Chromatography of Their Borate Complexes [Abstr. of Papers, 128th Natl. Meet. Am. Chem. Soc. (1955) p 8D]	Am. Chem. Soc., Minneapolis
Kimball, R. F.	Delayed Effects of Radiation on Ciliate Protozoa	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), East Lansing
Kimball, R. F., and N. Gaither	Effect of Incomplete Chromosome Sets on Behavior of Nuclei at Conjugation in *Paramecium aurelia* [*Genetics* 40, 579 (1955)]	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), East Lansing
Kirby-Smith, J. S., and D. L. Craig	Some Aspects of Ultraviolet-induced Chromosome Aberrations in *Tradescantia* Pollen [*Genetics* 40, 579 (1955)]	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), East Lansing
Krall, A. R.	Isolation of a Photosynthetic Oxidation-Reduction Coenzyme from Barley Leaves [*Plant Physiol.* (Suppl.) 30, xxiv (1955)]	Am. Inst. Biol. Sci. (Am. Soc. Plant Physiol.), East Lansing
———	Growth Substance Activity of Serine	Am. Assoc. Advance. Sci. (Am. Soc. Plant Physiol.), Atlanta
Lindsley, D. L.	Genetics of *Drosophila*	Northwestern University, Evanston
———	Implantation of Functional Erythropoietic Elements Following Total-Body Irradiation	N. Y. Acad. Sci., 2nd Conf. on Tissue Homotransplantation, New York
Luippold, H. E., and S. Wolff	Evidence of Two Types of Radiation-induced Chromosome Breaks	Tenn. Acad. Sci., Cookeville
Makinodan, T.	Methods for Producing Antibodies	Am. Assoc. Advance. Sci., Symp., Microbiol. in Med. Res., Atlanta
Naylor, A. W., and N. E. Tolbert	Glutamic Acid Metabolism in Barley Leaves [*Plant Physiol.* (Suppl.) 30, xviii (1955)]	Am. Inst. Biol. Sci. (Am. Soc. Plant Physiol.), East Lansing
Nystrom, C. W., N. E. Tolbert, and S. H. Wender	Effect of Ultraviolet Light on Photosynthesis in Anthocyanin-containing Leaves [*Plant Physiol.* (Suppl.) 30, xv (1955)]	Am. Inst. Biol. Sci. (Am. Soc. Plant Physiol.), East Lansing

AUTHOR(S)	TITLE	PLACE PRESENTED
Oakberg, E. F., and R. L. DiMinno	Gamma-Ray Sensitivity of Different Developmental Stages of Spermatogonia of the Mouse [*Genetics* 40, 588 (1955)]	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), East Lansing
Phares, E. F., and S. F. Carson	A DPNH-dependent Conversion of Pyruvate to Lactate, Requiring Catalytic Amounts of Malate [Abstr. of Papers, 128th Natl. Meet. Am. Chem. Soc. (1955) p 19D]	Am. Chem. Soc., Minneapolis
Pittenger, T. H.	Dominance Relations in *Neurospora* Heterokaryons [*Genetics* 40, 590 (1955)]	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), East Lansing
Rogers, R. W., and R. C. von Borstel	Differential Sensitivity of the Nuclear and Non-nuclear Elements of the *Habrobracon* Egg to Polonium Alpha Particles	Am. Assoc. Advance. Sci., Atlanta
Russell, W. L.	Genetic Effects of Ionizing Radiation	Department of Radiology (Symp. on Radiobiology), Columbia University, New York
Russell, L. B., and W. L. Russell	Hazards to the Embryo and Fetus from Ionizing Radiation	Intern. Conf. on the Peaceful Uses of Atomic Energy, Geneva, Switzerland
St. Amand, W.	Cell Division and Radiosensitivity	Dept. of Biology, Spring Hill College, Mobile
———	As above	Dept. of Veterinary Medicine, Tuskegee Institute, Tuskegee Institute
———	Ascites Tumors	Dept. of Zoology, University of Tennessee, Knoxville
St. Amand, W., and R. C. von Borstel	Radiosensitivity of the Unfertilized *Habrobracon* Egg During Meiosis and Early Cleavage	Tenn. Acad. Sci., Cookeville
Schwartz, D.	Mutations at the yg_2 Locus in Maize [*Genetics* 40, 594–595 (1955)]	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), East Lansing
———	Cytogenetic Studies in Maize	Botany Department, University of Tennessee, Knoxville
Shaw, E. I.	A New Medium for Grasshopper Neuroblasts	Tenn. Acad. Sci., Cookeville
Slater, J. V.	Ecological Implications of Certain Nutritional Spectra Required by Protozoans	Am. Inst. Biol. Sci. (Ecolog. Soc. Amer. and Am. Soc. Oceanog. Limnol.), East Lansing
Stapleton, G. E.	Physiological Effects of Ionizing Radiations on Bacteria	Am. Assoc. Advance. Sci., Symp., Microbiol. in Med. Res., Atlanta
Stapleton, G. E., A. J. Sbarra, and D. H. Woodbury	Synthesis of Nucleic Acids and Proteins during Recovery of *Escherichia coli* B/r from the Damaging Effects of Ionizing Radiations	Ky.-Tenn. Branch, Am. Soc. Bacteriol., Nashville

AUTHOR(S)	TITLE	PLACE PRESENTED
Tolbert, N. E.	A Compound for Phosphorus Storage and Transport [*Plant Physiol.* (Suppl.) 30, xviii (1955)]	Am. Inst. Biol. Sci. (Am. Soc. Plant Physiol.), East Lansing
_____	Photosynthesis	Am. Assoc. Advance. Sci., Symp., Atomic Energy and Agriculture, Atlanta
Totter, J. R.	Use of Bioluminescence as a Tool in Enzyme Studies	All-College Seminar Program, Oklahoma A & M College, Stillwater
_____	As above	Depts. of Plant Science and Zoology, University of Oklahoma, Norman
_____	As above	Depts. of Biology and Chemistry, North Texas State College, Denton
_____	As above	Dept. of Chemistry, University of Texas, Austin
Upton, A. C.	Carcinogenic Effects of Ionizing Radiation	University of Michigan Cancer Retreat, Baldwin
_____	As above	Dept. of Pathology, Duke Univ. School of Medicine, Durham
_____	Radiation Effects on Man	Oak Ridge High School, Chemiteers Club, Oak Ridge
_____	As above	Rocky Hill Community Club, Knoxville
_____	Hemorrhage and Related Effects	Symp. on Radiol. Physiol. (Argonne), Chicago
Upton, A. C., K. W. Christenberry, and G. S. Melville	Studies of the Relative Biological Effectiveness of Neutrons, X Rays, and Gamma Rays for Cataract Induction	Radiol. Soc. N. Amer., Chicago
Welshons, W. J.	A Comparative Study of Crossing Over [*Genetics* 40, 601 (1955)]	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), East Lansing
Whiting, A. R., and S. Caspari	Time of Death of Embryos with Dominant or Recessive Lethals Induced by X Rays in *Habrobracon* Eggs [*Genetics* 40, 602 (1955)]	Am. Inst. Biol. Sci. (Genet. Soc. Amer.), East Lansing
Wolff, S., and H. E. Luippold	The Biochemical Aspects of Chromosome Rejoining	Intern. Conf. Radiobiol., Cambridge, England
Zill, L. P., and N. E. Tolbert	The Effects of Ionizing and Nonionizing Radiation on Photosynthesis by Plants [*Plant Physiol.* (Suppl.) 30, xiv, (1955)]	Am. Inst. Biol. Sci. (Am. Soc. Plant Physiol.), East Lansing

Visiting Lecturers. – The Biology Division has been fortunate in having so impressive a group of scientists to serve on its seminar program during the past six months. Listed are the speakers, their affiliations, and the topics of their lectures.

LECTURER	AFFILIATION	TOPIC
S. H. Hutner	Haskins Laboratories, New York	Some Microbiological Approaches to Problems of Stress in Mammals
J. L. Reissig	Cornell University, Ithaca	The Enzymic Interconversion of *N*-Acetylglucosamine-1-phosphate in *Neurospora*
A. S. Parkes	Medical Research Council, London, England	Low Temperature Preservation of Tissues for Transplantation
S. Zamenhof	Columbia University, New York	The Action of UV and Other Mutagens on Deoxypentose Nucleic Acids Having Biological Activity
L. M. Tocatins	Jefferson Medical College, Philadelphia	Interrelationships Between Platelets, Platelet Cofactor, and Antithromboplastin
B. Kok	Laboratory for Plant Physiological Research, Wageningen, The Netherlands	The Kinetics of Photosynthesis and Photoinhibition in Green Algae
G. E. W. Wolstenholme	Ciba Foundation, London, England	Discussion Concerning Research at the Ciba Foundation
B. Kihlman	The John Hopkins University, Baltimore	The Effect of 8-Ethoxycaffeine on Chromosome Breakage and Its Involvement in the Respiratory Cycle
D. Kanazir	Carnegie Institution of Washington, Cold Spring Harbor	Effects of UV Radiation on Metabolism of DNA and ATP
B. Ephrussi	Laboratoire de Génétique, Universite de Paris, France	Discussion of Studies Carried out in Genetics in Paris
W. J. van Wagtendonk	Indiana University, Bloomington	The Growth Requirements of *Paramecium aurelia*
N. D. Zinder	The Rockefeller Institute for Medical Research, New York	Genetic Interaction Between Bacteriophage and Its Hosts
M. Westergaard	Universitetets Genetiske Institut, Copenhagen, Denmark	Chemical Mutagenesis
F. Skoog	University of Wisconsin, Madison	The Regulation of Growth and Differentiation in Plant Tissues
L. Sandler	University of Missouri, Columbia	Some Problems Concerning Compound X Chromosomes in *Drosophila melanogaster*
G. Brecker	Hematology Service, The Clinical Center, Bethesda	The Development of Delayed Radiation Lesions
G. Fankhauser	Stanford University, Stanford University	Polyploidy in Animals and Its Significance for Experimental Biology

LECTURER	AFFILIATION	TOPIC
C. A. Price	Purdue University Agri. Expt. Station, Lafayette	The Swelling of Mitochondria
J. Furth	Children's Cancer Research Foundation, Inc., Boston	Problems in Mammalian Radiation Biology
A. Howard	Mt. Vernon Hospital and Radium Institute, Middlesex, England	Autoradiograph Studies of Dividing Cells
E. De Robertis	Instituto de Investigación de Ciencias Biológicas, Montevideo, Uraguay	Some Observations on the Submicroscopic Organization of the Nucleolus
W. H. Taliaferro	University of Chicago, Chicago	Studies on Antibody Synthesis
A. Hagberg	Swedish Seed Association, Svalov, Sweden	Cytogenetic Studies on Erectoides Mutations in Barley

Speakers at Professional Meetings, Spring 1956. – Thirty-seven papers are scheduled for presentation at professional society meetings before June 1. The societies and speakers are listed:

Ciba Foundation Symposium on "Influence of Ionizing Radiation on Cell Metabolism" – A. Hollaender (coauthor, G. E. Stapleton); *Photobiology Group of Great Britain* – A. Hollaender; *Southeastern Section, American Physical Society* – M. L. Randolph and E. B. Darden; *American Association of Anatomists* – J. N. Dent; *Louisiana Cancer Conference (American Cancer Society)* – A. C. Upton (coauthor, J. Furth); *Philadelphia County Medical Society* – C. C. Congdon; *Ninth Annual Biology Conference* – N. G. Anderson; *American Association for Cancer Research* – A. C. Upton (coauthors, J. Furth and F. F. Wolff); *Federation of American Societies for Experimental Biology* – N. G. Anderson, L. Astrachan (coauthor, E. Volkin), F. P. Conte (coauthors, G. S. Melville and A. C. Upton), M. J. Cormier, J. W. Davis (coauthors, J. V. Passonneau and J. R. Totter), R. M. Merwin (coauthor, C. C. Congdon), R. Shapira (coauthor, D. G. Doherty), N. E. Tolbert (coauthor, L. M. Rohrbaugh), A. C. Upton (coauthors, J. Furth and E. S. Ledford), E. Volkin, and L. P. Zill; *Association of Southeastern Biologists* – R. T. Brumfield (coauthor, D. E. Foard); *Society of American Bacteriologists* – S. F. Carson (coauthor, E. F. Phares), and M. J. Cormier (coauthor, H. H. Rostorfer); *Radiation Research Society* – D. Billen, C. C. Congdon (coauthor, I. Urso), W. D. Fisher (coauthor, N. G. Anderson), N. Gengozian (coauthor, T. Makinodan), R. F. Kimball (coauthors, N. Gaither and S. M. Wilson), T. Makinodan, G. S. Melville (coauthors, K. W. Christenberry, M. Slater, and A. C. Upton), T. T. Odell (coauthors, F. G. Tausche and D. L. Lindsley), E. I. Shaw, M. Slater (coauthors, G. S. Melville and A. C. Upton), L. H. Smith, G. E. Stapleton (coauthors, D. H. Woodbury and A. Hollaender), A. C. Upton (coauthors, G. S. Melville, M. Slater, and F. P. Conte), and P. Urso (coauthor, C. C. Congdon).

Ninth Annual Biology Research Conference. – Advance reservations indicate that the Biology Conference, which will be held in Gatlinburg on April 12–14, 1956, will be well attended. These conferences are sponsored by the Biology Division and supported by the Atomic Energy Commission. Dr. Norman G. Anderson was active in arranging the program for the Symposium on Biocolloids, which follows.

"Introduction" – Peter J. W. Debye, Cornell University

First Session – **RECENT ADVANCES IN THE THEORY OF COLLOIDS**

"Theoretical Aspects of Complex Coacervation" – J. T. G. Overbeek, University of Utrecht

"Formation and Pattern of Cellular Fibrils" – A. Frey-Wyssling, Institute of General Botany, Zürich

"Polyelectrolyte Gels" – I. Michaeli, Weizmann Institute, Israel

"Physical Chemistry of the Deoxyribonucleic Acid Molecule" – Paul M. Doty, Harvard University

Discussion – H. K. Schachman, University of California

Second Session – **INTERACTIONS BETWEEN BIOCOLLOIDS**

"Protein Structure and Protein Interaction" – John G. Kirkwood, Yale University

Discussion – R. F. Steiner, Naval Medical Research Institute

Third Session (Thursday Evening) – **ROUND-TABLE DISCUSSION**

"Macromolecular Fabrics and Patterns" – Paul Weiss, Rockefeller Institute for Medical Research; and F. O. Schmitt, Massachusetts Institute of Technology

Fourth Session – **INTERACTIONS BETWEEN BIOCOLLOIDS** (Continued)

"Interactions of Elongated Particles" – Gerald Oster, Polytechnic Institute of Brooklyn

Discussion – Teru Hayashi, Columbia University

"Formation of Fibrils from Globular Molecules" – David Waugh, Massachusetts Institute of Technology

"Fibrous Protein of the Nerve" – F. O. Schmitt, Massachusetts Institute of Technology

"Macromolecular Interactions" – Paul J. Flory, Cornell University

Discussion – John D. Ferry, University of Wisconsin

Fifth Session – **CELLULAR COLLOIDAL COMPLEXES**

"Introductory Discussion" – J. T. Randall, University of London King's College

"Labile Colloidal Complexes of the Cytoplasm" – N. G. Anderson, Oak Ridge National Laboratory

"Molecular Basis of Contractile Mechanisms" – K. Laki, National Institutes of Health

"Results of Experimentation on the Relation of Adenosine Triphosphate to the Contractile Properties of the Myosin Molecule" – W. J. Bowen, National Institutes of Health

Discussion – F. D. Carlson, The Johns Hopkins University

"Experimental Studies on Contractile Mechanisms" – W. F. H. M. Mommaerts, Western Reserve University

"Remarks on the Structure and Function of Proteins" and SUMMARY – Albert Szent-Gyorgyi, Institute for Muscle Research, Woods Hole, Massachusetts

The Union Carbide Nuclear Company will be host at the Smoker to be held during the evening of Thursday, April 12. An excursion to the Great Smoky Mountains will be arranged for Friday afternoon. In case of inclement weather, the program will continue, leaving Saturday afternoon open for a tour of the Biology Division laboratories. The Laboratory will be host at a banquet on Friday evening. All sessions and social functions will be held at the Mountain View Hotel.

Dr. Gaulden Visits South American Laboratories

Dr. Mary Esther Gaulden has returned from a six-week tour of biology laboratories in Peru, Chile, Uruguay, and Brazil. Dr. Gaulden gave a number of seminars in cytology, genetics, and cell physiology at the following laboratories: The Medical School of the University of São Paulo, Brazil (two seminars here); two seminars at the Laboratório de Genética, Universidade de Rio Grande do Sul, at Pôrto Alegre, Brazil; Cancer Institute of Chile, Santiago, Chile; the Laboratory of High-Altitude Physiology and the Laboratory for Investigations of Nerve Diseases, both in Lima, Peru; and the Biophysics and Embryology Departments of the Montevideo University School of Medicine, Montevideo, Uruguay. In addition, there were many informal discussions in the above fields and in biophysics in the following institutions: Institute of Biochemistry, Medical School of the University of San Marcos, Lima, Peru; research laboratories in Cuzco, Peru; the Rockefeller Foundation, the University of Chile, and Catholic University,

Santiago, Chile; Instituto de Investigación de Ciencias Biologicas, Montevideo, Uruguay; the Isotopes Laboratory, the laboratory of Genetics, and the Laboratory of Cell Physiology, University of São Paulo, Brazil; Medical School, University of São Paulo at Ribeirão Preto, Brazil; and the Institute of Biophysics, Rio de Janeiro, Brazil.

The Rockefeller Foundation gave financial aid for this very interesting and beneficial trip.

CYTOLOGY AND GENETICS

CYTOGENETIC EFFECTS OF RADIATION

R. F. Kimball
K. C. Atwood
A. D. Conger
T. H. Pittenger
D. Schwartz
F. J. de Serres[1]
S. Wolff
C. E. Bay
N. Gaither
A. H. Johnston
H. E. Luippold
S. M. Wilson

Effects of Posttreatment with Streptomycin on X-Ray-Induced Mutations in *Paramecium aurelia*

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

Several postirradiation procedures have been reported to decrease the amount of detectable mutation in *Paramecium aurelia*.[2] These treatments increased the interval between irradiation and the succeeding cell division, and it was suggested that the effects on mutation may have been causally related to this increase. More precise control of the interdivision interval has now been achieved with streptomycin. The results strengthen the evidence that mutation can be altered with posttreatments but indicate that simple lengthening of the interdivision interval is not the whole story.

Paramecia were irradiated with about 3 kr of x rays within 1 hr after division and put into streptomycin (100 ppm) either immediately, 2 hr, or 5 hr after irradiation. The last group had already undergone a division before it was put into the antibiotic. All groups were kept in streptomycin for 24 hr, and no growth or division occurred during this time. The mutation rate in the first two groups was significantly higher than in animals which were not treated with streptomycin, and there was no difference between them. The mutation rate in the last group was unchanged. No mutations were induced by streptomycin in unirradiated controls. Thus posttreatments with streptomycin *increased* the detectable mutation rate but only when given within the same interdivision interval as the radiation.

Certain hypotheses to account for the effects of posttreatments can be discarded immediately. Selective elimination of mutant or nonmutant paramecia was not involved because several of the treatments did not kill any paramecia. Ephemeral effects of the agents on the expression of mutations can be excluded because the mutations were not brought to expression for several days and many divisions after treatment. Finally, longer lasting effects of streptomycin independent of the radiation can be excluded, since there was no influence of treatment after division.

Thus it seems nearly certain that streptomycin and the other agents influence the production of mutational changes, and the results show that an appreciable fraction of the changes are not irrevocably established for at least 2 hr after irradiation. However, either chromosome reunion or the realization of potential breaks or point mutations may be involved; the data do not allow a distinction to be made between them.

The fact that streptomycin caused an increase in mutation, whereas the other agents caused a decrease, shows either that the duration of the interdivision interval is not the major factor or that the relation is more complex than one of simple temporal duration. As yet, the results do not warrant a detailed hypothesis.

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

A. D. Conger M. L. Randolph[3]
A. H. Johnston

The dependence of chromosomal aberration production on ion density in the fast-neutron range has never been adequately measured; this is particularly unfortunate, for it is in just this region that breakage efficiency is expected to change rapidly. Early work is compromised by inadequate dosimetry, and energy heterogeneity of the neutron beams employed.

The Biology Division linear accelerator provides the proper physical situation to measure this energy dependence. Monochromatic neutrons, 2.5-Mev D-D and 14-Mev D-T, are produced, and proper dosimetric measurements have been made by Randolph. Chromosomal aberration production in *Tradescantia* by x rays (hvl, 1.45 mm Cu), D-D neutrons, and D-T neutrons has been measured

[1]Research Associate.

[2]R. F. Kimball, *Ann. N.Y. Acad. Sci.* 59, 638–647 (1955); R. F. Kimball and N. Gaither, *Biol. Semiann. Prog. Rep. Aug. 15, 1955*, ORNL-1953, p 13–14.

[3]Biophysics Section.

in three experiments. The first two preliminary experiments, already completed, were for orientation; four-day chromosome aberrations from D-D neutrons and x rays (on Oct. 21) and from D-T neutrons (on Nov. 17) were observed. The last experiment, about half completed, combined in one operation all the treatments to be compared: four-day chromosome and 24-hr chromatid aberrations from x rays, D-D neutrons, and D-T neutrons were observed. Five or six doses were delivered for each of the treatments. For each kind of aberration from each type of radiation, a coefficient of aberration production per roentgen or rep was calculated from the dose curve. Comparisons of efficiency between the two neutron energies are valid because all aberrations increase linearly with dose. However, the exchange, or two-hit, aberrations increase as a power of the dose with x rays. To allow x-ray/neutron comparisons, the x-ray data were fitted as though exchanges increased linearly with dose; thus an average linear coefficient was obtained over the dose range analyzed. At present, the physical measurements are believed to be accurate to ±10%, the biological measurements, because they are incomplete, to about ±25% (Table 1).

The data clearly show that changes in aberration production efficiency are greater in the range of ion density encompassed by fast-neutron energies customarily used than are the changes in the 14-Mev neutron/x-ray energy range. As far as chromosomal breakage and aberration production in *Tradescantia* are concerned, x-ray/neutron RBE comparisons depend critically on the neutron energy, or average energy, and such comparisons are meaningless without knowledge of neutron energy distribution.

Inaccuracy of Ascertaining Radiation-induced Chromosome Damage from Anaphase Figures

S. Wolff H. E. Luippold

One of the most significant observations that led to the development of the target theory was Sax's[4] two-hit curve for x-ray-induced chromosome dicentrics and rings. There have been numerous cases in the literature,[5,6] however, of a one-hit dose curve resulting from the scoring of anaphase bridges which ostensibly result from dicentrics and rings. Even Sax[7] himself observed such a one-hit curve in *Vicia faba*. Later, Wolff[8] showed that if the metaphase configurations in *Vicia* were scored the theoretical two-hit curve was obtained. The differences in these curves were thought to be caused by such diverse phenomena as the scoring of one-hit isochromatids[8] or the differences in hydration of the material.[6] In any case, since the two-hit curve is of great theoretical importance as a basis for the target theory, it is important that the discrepancy in these reports

[4] K. Sax, *Proc. Natl. Acad. Sci. U.S.* 25, 225–233 (1939).

[5] R. S. Caldecott and L. Smith, *Cytologia* 17, 224–242 (1952).

[6] R. S. Caldecott, *Ann. N.Y. Acad. Sci* 59, 514–535 (1955).

[7] K. Sax and R. T. Brumfield, *Am. J. Botany* 30, 564–570 (1943).

[8] S. Wolff, *Nature* 173, 501 (1954).

TABLE 1. EFFICIENCY OF ABERRATION PRODUCTION BY X RAYS, 2.5-Mev AND 14-Mev NEUTRONS

(Ratios of aberrations produced per 100 cells per roentgen or rep)

Date of Experiment	Comparison	Type of Aberration		
		Chromosome Exchanges	Chromatid Plus Isochromatid Deletions	Chromatid Interchanges
Jan. 26, 1956	14-Mev neutrons/x rays	1.3	2.5	1.8
	2.5-Mev neutrons/x rays	3.0	6.8	5.7
	14-Mev neutrons/2.5-Mev neutrons	2.3	2.7	3.1
Oct. 21, 1955	2.5-Mev neutrons/x rays	2.8		
1954	Av 1-Mev neutrons*/x rays	3.8		

*Y-12 cyclotron.

be investigated. In order to test this one-hit curve for a two-hit effect, it was decided to make observations both at metaphase and at anaphase in the same material, barley, that was used by those who reported the anomolous curves.

It might be pointed out at this time that it is generally acknowledged that the scoring of anaphase figures gives less information than does that of metaphase. However, in material that is comparatively difficult to use cytologically, anaphase may be the only stage that may be scored with ease. *Hordeum vulgare*, or barley, was just such a material. The first step in these investigations, therefore, was to devise a technique that allowed the scoring of metaphase configurations in the barley root tips. The treatment devised consisted essentially in the germination of the seeds in colchicine and the subsequent application of 5% pectinase. The colchicine caused the accumulation of large numbers of cells in metaphase of the first mitotic division and destroyed the spindle so that, upon flattening, the chromosomes would spread apart from one another. The pectinase was necessary to make the cells fall apart and squash easily.

Both dry seeds and seeds that had been soaked for 8 hr in distilled water were irradiated with various doses of 250-kvp x rays (hvl, 0.55 mm Cu). One half the seeds were treated with colchicine to accumulate metaphases. The other half was not so treated so that anaphases would occur. The results are presented in Fig. 1. It may be noted that in both the dry seed and the hydrated seed that if metaphase figures are scored for dicentrics and rings a parabolic two-hit curve is obtained that increases as the square of the dose. Whereas if anaphase bridges are scored, a linear one-hit curve results.

There are many phenomena that can prevent a dicentric or a ring chromosome from yielding a bridge at anaphase. But all these phenomena should be proportional to the dose of x rays and consequently not effect a change of a two-hit

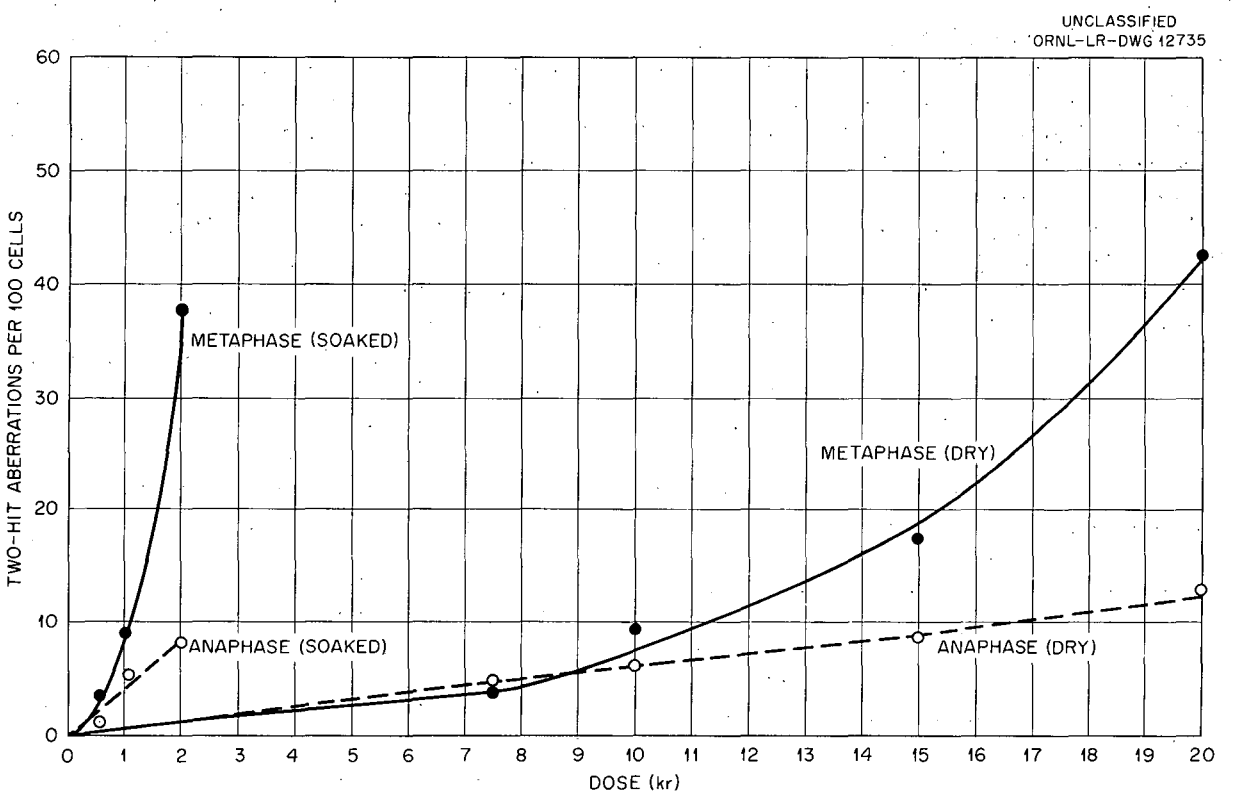

Fig. 1. Dose-Effect Curves Obtained for Two-Hit Aberrations when Metaphase and Anaphase Are Scored.

curve at metaphase into a one-hit curve at anaphase. Therefore, all that can be said at present is (1) that the theoretically important two-hit curve is obtained even in those materials where it was thought not to occur and (2) that the lack of its appearance is not caused by the formation of isochromatids or by differences in hydration but is an artifact that comes from scoring anaphases which do not yield as much information as do metaphases. Since there is no constant ratio between dicentrics and rings to anaphase bridges, it should be pointed out that much caution must be exercised about correlating other radiation effects to nuclear damage, as measured by anaphase bridges.

Effect of High Doses of Radiation on the Growth of Maize Seedlings

D. Schwartz C. E. Bay

Further studies on the seedling height–dose curve[9] have resulted in the following observations:

1. At very high doses (500,000 r) seedlings are normal in appearance but growth stops after about five days.
2. At medium dose range (120,000 r) mitosis is only partially inhibited; root tips show reduction in number of cell divisions to about a third of the unirradiated control.
3. At over 400,000 r, cell divisions are completely and permanently inhibited.
4. The meristematic region of the root in the medium dose range shows a high degree of necrosis.
5. There is no sign of necrosis at the high doses.
6. After maximum growth is obtained, cells in the tip of the root from material irradiated at the high doses had lost their meristematic appearance, had undergone elongation and maturation, and could not be distinguished from mature cells further back along the root.

Based on these observations, the reversal in the seedling height–dose curve is explained as being the result of an interaction between chromosomal damage and the inhibition of cell division. Beginning at the low dose ranges, increased dosage results in increased chromosomal damage and decreased seedling growth. However, at approximately 120,000 r, a point is reached where the manifestation of the high chromosomal breakage becomes masked by the inhibition of cell division. Chromosome breakage gives rise to lethality as the result of aneuploidy produced in the daughter cells following division. In nondividing cells, regardless of the extent of the chromosome breakage, the genotypic constitution of the nucleus remains intact. Above 400,000 r, where cell division is completely inhibited, all signs of cell lethality disappear. These seedlings grow to their maximum height by the elongation of those cells which had already been present in the embryo at the time of irradiation. Cell division in the plumule is limited to the region just below the first node. It is to be expected that cell lethality in this critical region through which water and nutrients must pass to the plumule would have a drastic effect on the elongation viability of cells in the higher regions.

[9] D. Schwartz, *Science* 119, 3079, p 45–46 (1954).

Cytoplasmic Inheritance in *Neurospora*

T. H. Pittenger

The first instance of nonnuclear inheritance in *Neurospora* was the cytoplasmically inherited trait, *poky*,[10] which can be recognized by its slow growth and a defective cytochrome system.[11] A strain with similar characteristics has recently been found in this laboratory, and its behavior, described below, is consistent with the notion that a self-duplicating cytoplasmic particle is involved.

A lysine-requiring strain (4545) in which the cytoplasmic factor, designated *mi*-4, was first observed exhibits a very erratic pattern of growth in horizontal growth tubes. This strain has an unusually long lag period followed by a period of normal growth, after which it slows down and stops. Growth may again resume after a variable period of time, but it again eventually ceases. Conidia from this strain have a very low viability, often less than 1%, and on sorbose-containing medium conidia which do germinate grow only enough to form microcolonies and then cease growth. Such colonies usually fail to grow even when transferred to sucrose medium. Spectroscopic and biochemical analysis show that this strain has an excess of cytochrome c and a deficiency of cytochrome oxidase.

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

[11] F. A. Haskins *et al.*, *J. Biol. Chem.* 200, 819–826 (1953).

These characteristics are not transmitted by crossing when the *mi*-4 strain is used as the conidial (male) parent. The reciprocal cross has as yet been unsuccessful. The transmission of *mi*-4 is further elucidated by heterokaryon experiments. It has been shown that, in a heterokaryon between a *lys* strain with *mi*-4 cytoplasm and a *pan* strain with normal cytoplasm, the distribution of nuclei and cytoplasm in the conidia is such that *lys* and *pan* strains with normal cytoplasm are produced, as well as *lys* and *pan* strains with *mi*-4 cytoplasm and the two strains with mixed cytoplasm.

Heterokaryons with mixed cytoplasm are initially normal in their growth rate and in the ability of the conidia to germinate and form normal colonies. However, as growth proceeds, the heterokaryons gradually assume the phenotype of the *mi*-4 parent. The distance such heterokaryons grow before either slowing down or producing predominantly inviable conidia is a function of the proportion of the two mutant strains in the heterokaryon; that is, the greater the initial proportion of the *mi*-4 cytoplasm, the sooner the heterokaryons take on the *mi*-4 phenotype.

Crossing tests and transfer of *mi*-4 through heterokaryosis establish the cytoplasmic nature of *mi*-4. Since the particle-bound cytochrome oxidase system is associated with mitochondria and since the same system is defective in *mi*-4 strains, it is tentatively assumed that *mi*-4 is associated with defective or mutated mitochondria. The initial normal phenotype of heterokaryons involving a mixture of *mi*-4 and normal cytoplasm which, with continued growth, assumes the phenotype of the *mi*-4 cytoplasm indicates that the genetic basis of *mi*-4 is not the absence of a normal cytoplasmic particle, but rather the presence of an abnormal one, capable of replacing or suppressing the normal vector of cytochrome oxidase activity. Although *mi*-4 and *poky* are basically similar in phenotype, certain differences have been revealed between the two, the significance of which is not yet understood.

Structural and Functional Complexity in the *ad*-3 Region of *Neurospora crassa*

F. J. de Serres

A large number of adenine-requiring strains with a purple phenotype have been obtained to study the problem of the reversibility of x-ray-induced mutations at the *ad*-3 locus in *Neurospora crassa*. Recently, it has been shown that on the basis of heterokaryon tests these mutants fall into three distinct groups of physiological alleles: groups A and B, which are phenotypically identical and more intensely pigmented than the third group, J, the growth of which also shows a marked histidine stimulation. Preliminary genetic evidence[12] suggested that the mutants of groups A and B are members of a complex located between the closely linked *hist*-2 and *nic*-2 loci on the right arm of linkage group I (LGI) in a position corresponding to the *ad*-3 locus. The purpose of this investigation has been to determine the nature of the complexity at the *ad*-3 locus and to determine whether the group J mutants are part of this complex.

Groups A and B. – Crosses of a marked group A mutant (*hist*-2 *A*2 *nic*-2) with 21 group B mutants and of a marked group B mutant (*hist*-2 35203 *nic*-2) with three group A mutants have indicated that the origin of adenine-independent isolates in these crosses is associated with crossing over between the markers. The genotypes of the adenine-independent isolates in these two groups of crosses indicate that the group A mutants are more closely linked to the *hist*-2 locus and the group B mutants to the *nic*-2 locus. The linkage between the two groups of mutants ranges from 0.2 to 1.4 units in the various mutant combinations analyzed. Recent heterokaryon tests on the five original presumed alleles at the *ad*-3 locus[13,14] indicate that these mutants also fall into two groups. The results of these experiments have indicated that mutants 35203 and 45601 are in group B and that mutants 38701, 38709, and 68306 are in group A.

Group J. – Crosses of three group J mutants with the double mutant strain *hist*-2 *nic*-2 indicate that these three mutants are located about 1.5 to 2.5 units to the left of the *hist*-2 locus. Preliminary heterokaryon tests with a colonial strain of the "leaky" mutant 71104 suggest that group J mutants are allelic to this mutant and, thus, are located at the *ad*-5 locus on the left arm of LGI.

The results of these experiments with the deeply

[12] F. J. de Serres, unpublished Ph.D. thesis, Yale University (1955).

[13] H. K. Mitchell and M. B. Houlahan, *Fed. Proc.* 3, 370–375 (1946).

[14] R. W. Barratt *et. al.*, *Adv. Genetics* 6, 1–93 (1954).

pigmented purple adenine mutants indicate that the *ad*-3 locus is not unitary as it was once thought to be but, rather, that this region of LGI is occupied by at least two separate, but closely linked, loci. Since mutants at either of these two loci are phenotypically identical, the loci have been designated *ad*-3*A* and *ad*-3*B*. The crosses of the group J mutants indicate that these mutants are not part of the *ad*-3 complex. Since it is probable that these mutants are located at the *ad*-5 locus, for which closely linked biochemical markers are available only on the right, the 24 x-ray-induced group J mutants cannot be included in the study of the reversibility of x-ray-induced mutants at the present time.

INSECT CYTOLOGY AND GENETICS

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Acceleration of Mitosis by Agmatine

G. S. St. Amand
N. G. Anderson[17]
M. E. Gaulden

Two events essential to mitotic progress of a cell are condensation of the chromosomes and formation of the spindle. Although these processes can be readily described in terms of time of occurrence and morphology, they cannot be described in terms of the biochemical and biophysical events which are responsible for them. In view of the fact that both chromosome condensation and spindle formation can be affected by radiation, even in very small amounts, better understanding of these processes in the untreated cell is requisite to interpretation of radiation effects.

One approach to this problem can be made through the use of chemicals which alter mitotic progress. In recent years the number of substances known to inhibit mitosis has been rapidly increasing. Substances are rare, however, which accelerate mitosis and which might, therefore, be viewed as acting in a manner similar to normally occurring intracellular substances. In a search for a chemical agent to accelerate mitosis and cell division, it is feasible to select substances which, on the basis of their chemical properties, might be expected to produce a positive effect. On theoretical grounds polycations might be expected to enhance chromosome condensation and spindle formation. Decarboxylated arginine which has two positive charges and which is known to increase carbohydrate metabolism appeared promising. The effects of this substance, called agmatine, on mitosis in the grasshopper neuroblast have been studied.

Grasshopper embryos of 14 to 15 days development at 26°C were used. Control embryos were made into culture preparations in hanging drops of isotonic medium containing inorganic salts, glucose, and approximately a quarter of the yolk of an egg. Experimental embryos were mounted in hanging drops of culture medium to which had been added 1 mg of agmatine sulfate per milliliter of medium.

The first series of experiments involved the determination of the duration of mid-mitosis. Observation of a neuroblast was begun when the cell was in very late prophase. The cell was kept under observation as it progressed, and the times at which it reached certain points were recorded. The following points were picked because they could be accurately determined:

1. the breakdown of the nuclear membrane,
2. the beginning of anaphase movement,
3. the beginning of telophase change,
4. the appearance of the nucleoli (beginning of middle telophase),
5. the loss of spherical shape by the nucleoli (beginning of late telophase).

Table 2 shows the results of these timings. Some of these experiments were performed with winter embryos in which duration of mitosis varies slightly between egg cases – within cases variation is slight.[18] The durations of prometaphase-metaphase and early telophase were reduced significantly by agmatine treatment in each of the four cases. Middle telophase was significantly reduced in duration in all except one case. Anaphase was unaffected by agmatine.

Since the duration of anaphase was the same in both the control and agmatine-treated cells, it was possible to determine the number of cells going through mitosis by counting the number of anaphase cells in an embryo at 9-min (duration of

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[18]Grasshopper eggs are laid in groups (cases) of 15 to 30 eggs.

TABLE 2. DURATION OF STAGES IN CONTROL AND AGMATINE-TREATED CELLS

Concentration of agmatine: 0.005 *M*; temperature: 38°C

Case	Treatment	Duration of Stage (min)				Total	Number of Cells
		Prometaphase-Metaphase	Anaphase	Early Telophase	Middle Telophase		
1	Agmatine	14.4*	9.0	7.2*	12.6*	43.2**	10
	Control	25.0	9.2	11.6	16.3	58.1	10
2	Agmatine	10.0*	9.1	7.0*	14.6*	40.7**	5
	Control	13.0	8.8	9.0	20.2	51.0	5
3	Agmatine	11.4*	9.1	6.6*	18.1	45.2**	10
	Control	15.0	9.2	8.3	17.9	50.0	10
4	Agmatine	10.1*	8.9	6.3*	12.0*	37.3**	7
	Control	13.6	9.0	8.1	13.7	44.4	7

*Difference between controls and experimentals significant at $p < 0.05$.

**Difference between controls and experimentals significant at $p < 0.01$.

TABLE 3. EFFECT OF AGMATINE ON NUMBER OF NEUROBLASTS IN DIVISION

Difference between treatments significant at $p < 0.001$

Experiment	Number of Neuroblasts That Divided During 3 hr	
	Control	Agmatine
1	64	111
2	59	110
3	53	76
4	79	138
5	43	110
6	73	126
Mean	62	112

anaphase) intervals. The results are shown in Table 3. During the first 3 hr an average of 62 anaphase cells was observed in the control embryos. In agmatine preparations an average of 112 anaphases was observed during the same period. This difference was significant at the 0.001 level. Observations indicate that the increased yield of anaphases is sustained for at least 5 hr. These data indicate that interphase and prophase are also accelerated in their mitotic rate by agmatine. Thus it appears that the polycation agmatine enhances the changes normally occurring in all mitotic stages except anaphase.

Influence of Hypertonic Culture Medium on Radiation-induced Mitotic Inhibition

M. E. Gaulden

The only demonstrable effect of low doses of x radiation (~ 1–8 r) on the mitotic neuroblast of grasshopper embryo is retardation of middle and late prophase. This retardation in many cells is accompanied by a morphological change in the chromosomes which can best be described as "reversion" – the chromatin temporarily resumes the diffuse appearance it had at interphase.

A previous study[19] demonstrated that subjection of the neuroblast to hypertonic culture medium caused the chromatin of cells in middle prophase to assume a late prophase appearance and the chromatin of late prophase to assume a very late prophase appearance. These changes occurred within 15 to 30 sec after the cells were placed in medium hypertonic to them. This apparent advancement of the stages of mitosis was shown to be accompanied by an accelerated mitotic rate.

It was decided to set up some experiments to determine whether the "reversing" action of x rays on middle and late prophase could be prevented by subjecting the neuroblast to a hypertonic medium which advances these stages.

Grasshopper embryos were made into culture preparation in hanging drops of medium isotonic

[19] M. E. Gaulden, *Biol. Semiann. Prog. Rep. Aug. 15, 1953*, ORNL-1614, p 28.

to them. They were then given 3 r of x rays (125 kvp) in 1 min. Immediately after irradiation the isotonic medium around half the embryos was replaced with medium hypertonic (contained 1.2 × inorganic salt concentration of the isotonic medium) to the cells. Unirradiated embryos were handled in a like manner.

Twenty-one minutes after irradiation both irradiated and unirradiated embryo cultures were placed in an incubator (38°C), which enclosed the lower half of a microscope, for observation. Mitotic rate was determined by counting the number of mid-mitotic cells (prometaphase, metaphase, and anaphase) every 22 min (duration of mid-mitosis).

The results are shown in Fig. 2 as curves I and II and are expressed as mitotic ratios. It can be seen that increased tonicity of culture medium caused an accelerated mitotic rate in the unirradiated embryos for about 4 hr (curve I). The mitotic rate of irradiated neuroblasts in isotonic medium was depressed to about 25% of normal level (curve II). The mitotic rate of irradiated neuroblasts in hypertonic medium was depressed about 75% of normal level (not shown on graph). Thus, subjection to hypertonic medium immediately following irradiation resulted in less radiation damage but did not eliminate its manifestation.

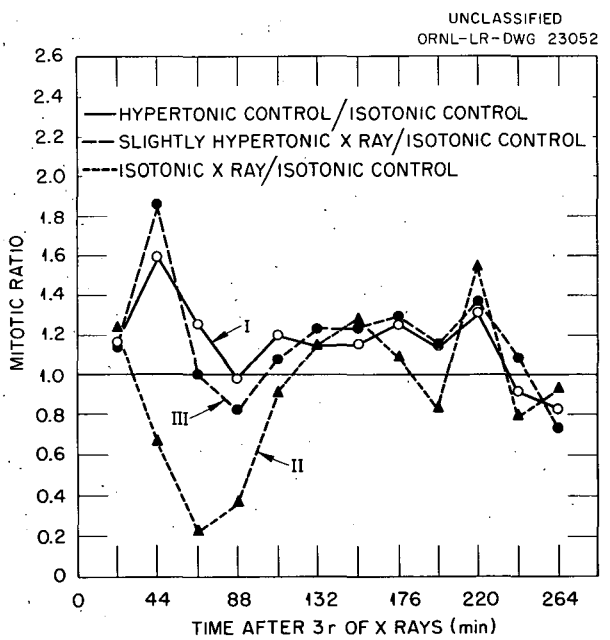

Fig. 2. Effect of Hypertonicity on Radiation-induced Mitotic Inhibition.

During the course of these experiments it was found that if the cells did not show the influence (change in appearance of chromatin) of the hypertonic medium within 1 min after irradiation they responded to the irradiation in the same manner as irradiated cells in isotonic medium (curve I). The failure to completely eliminate the manifestation of radiation damage was, therefore, thought to be due to the fact that the cells had not been subjected to hypertonic medium fast enough following irradiation. In an effort to accelerate the effects of hypertonicity, embryos were made into culture preparations in hanging drops of medium very slightly hypertonic to them. No cytological effects of this very slightly hypertonic medium can be detected. The cultures were irradiated, and immediately following irradiation the very slightly hypertonic medium was replaced with definitely hypertonic medium.

The results are given in curve III in Fig. 2. An analysis of variance of the raw data[20] revealed that this curve was not significantly different from curve I. In other words, no effect of 3 r of x radiation is detectable if cells are subjected to hypertonic medium immediately after irradiation.

These data indicate that, at least at low doses of radiation, mitotic inhibition may result from changes in physical state of the chromatin and not necessarily from alterations in strictly biochemical and physiological processes in the cell. At this time it cannot be said whether the influence of hypertonic medium is due to removal of water from the cell, to increased concentrations of certain inorganic salts, or to both.

Uptake of C^{14}-labeled Thymidine by Grasshopper Neuroblasts

M. E. Gaulden

At low doses of radiation the event primarily responsible for mitotic inhibition in the grasshopper neuroblast is "reversion" of middle and late prophase – the chromatin of nuclei in these stages "reverts" to a form comparable in every respect visually to that of interphase nuclei. The nature of this reversion is not known.

There are numerous references in the literature which suggest that interference with nucleic acid metabolism is responsible for effects of radiation

[20] We are indebted to A. W. Kimball and G. J. Atta of the Mathematics Panel for this analysis.

on the mitotic cell. Experiments have been initiated to determine at what stage or stages of mitosis synthesis of deoxyribonucleic acid (DNA) occurs in the grasshopper neuroblast, to determine whether irradiation affects this synthesis, and to determine whether inhibition of synthesis is correlated with inhibition of mitosis.

Grasshopper embryos were made into culture preparations. Neuroblasts were noted with respect to exact stage of mitosis and position in the embryo (neuroblasts lie in rows in a single layer at the ventral surface of an embryo). The medium surrounding the embryos was then replaced with a drop of medium containing thymidine labeled[21] with C^{14} (3 $\mu c/\mu$mole). When the cells reached the end of the mitotic stage being observed, the embryo was fixed immediately with the vapor of a 1% solution of osmic acid, dropped into 3:1 absolute alcohol–acetic acid, washed in water to remove unincorporated thymidine, dehydrated, and embedded in paraffin. The embryo was subsequently sectioned, stained with Feulgen reagent, and made into autoradiographs by the method of Taylor and Taylor.[22] The cells originally observed in the living condition were then examined for the presence of grains in the film above them.

Data are still being accumulated, but those acquired thus far indicate that uptake of thymidine into the DNA of chromatin begins in middle telophase and continues into very early prophase. Maximum uptake occurs during late telophase and interphase, rate of uptake in these stages being approximately the same. Thus the stages of mitosis most sensitive to radiation, namely, middle and late prophase, are not involved in the synthesis of nucleic acid (assuming that uptake of thymidine and synthesis of DNA are synonymous).

Preliminary data on irradiated cells indicate that pronounced interference with uptake of thymidine occurs only in cells which have received several hundred roentgens of x rays. Thus, it can be said, tentatively, that disturbances in the synthesis of nucleic acid are not as important in radiation damage as might be expected.

Influence of Calcium on Mitosis

G. S. St. Amand M. E. Gaulden

X-ray-induced mitotic inhibition in grasshopper neuroblasts has been shown to be influenced by hypertonic medium (see a previous section entitled, "Influence of Hypertonic Culture Medium on Radiation-induced Mitotic Inhibition"). In that study the tonicity of the medium was increased by increasing proportionately the amounts of all the inorganic constituents. In order to learn whether the cellular changes were caused by increased amounts of certain constituents, experiments have been begun to test the effects of increasing the amounts of single inorganic compounds. Some observations on the effects of calcium are now complete.

Grasshopper embryos of 14 to 15 days development at 26°C were made into hanging-drop preparations as previously described.[23] The control culture medium consisted of 6.8 g NaCl, 0.1 g $MgCl_2$, 0.2 g $CaCl_2$, 0.2 g NaH_2PO_4, 0.125 g $NaHCO_3$, and 7.7 g dextrose to 1000 ml of glass-distilled water. This medium has a calcium concentration of 0.0017 *M*. The experimental media contained varying amounts of calcium. In these the NaCl content was varied slightly to maintain isotonicity. Observation of a neuroblast cell was begun when the cell was in very late prophase. The cell was kept under observation as it progressed, and the times at which it entered prometaphase, anaphase, and early, middle, and late telophase were recorded. Only data from preparations that remained isotonic throughout the entire experiment are included in the results.

The effect of calcium concentration on the duration of the mid-mitotic stages is shown in Table 4. The omission of calcium from the medium prolonged prometaphase-metaphase significantly ($p < 0.001$), as compared with the duration of this stage when cells were exposed to 0.0017 *M* calcium. There was no significant difference in anaphase duration. The addition of a chelating agent (disodium versenate) did not appear to enhance the calcium deficiency effect. When the calcium concentration was increased above control level, prometaphase-metaphase was retarded. Sufficient numbers of cells were timed at 38°C to warrant statistical treatment only at concentrations of 0.0017 and 0.0051 *M*. The difference was significant at the 5% level for prometaphase-metaphase. The other stages did not differ significantly. The variation

[21] We are indebted to M. Friedkin of Washington University for this thymidine.

[22] J. H. Taylor and S. H. Taylor, *J. Heredity* **45**, 129 (1953).

[23] M. E. Gaulden and M. L. Nix, *Biol. Quar. Prog. Rep. May 15, 1950*, ORNL-727, p 41.

TABLE 4. EFFECT OF CALCIUM CONCENTRATION

Temperature (°C)	Number of Cells	Concentration of Calcium (M)	Duration of stage (min)			
			Prometaphase-metaphase	Anaphase	Early Telophase	Middle Telophase
22	13	0.000	40.3	14.9		
	13	0.0017	26.4	15.3		
38	9	0.0017	13.0	9.0	7.0	12.7
	2	0.0034	13.5	9.0	8.0	13.0
	9	0.0051	15.8	9.0	8.1	12.6
	2	0.0085	19.0	9.0		
	1	0.0170	40.0	9.0		

between the groups of cells in control medium is a result of the different temperatures at which the experiments were performed.

These data indicate that increased amounts of calcium retard the development of the spindle in the mitotic cell.

Radiation-induced Dominant Lethal Mutations in the *Habrobracon* Egg

R. C. von Borstel M. L. Pardue

It has been reported[24] that a new type of dominant lethal mutation has been discovered and demonstrated. Mutations of this type (designated as conditionally delayed dominant lethals) result in death of the haploid embryos and of the diploid larvae or pupae and are associated with eggs irradiated in the first meiotic metaphase (metaphase I) of oogenesis. Conditionally delayed dominant lethal mutations are not induced in eggs irradiated in the first meiotic prophase.[25] The restriction of induced conditionally delayed dominant lethal mutations to eggs irradiated in metaphase I can be correlated with induction of the chromosome breakage-fusion-bridge cycle. Induction of this cycle is also restricted to eggs irradiated at metaphase I.

A specific dominant lethal phenomenon induced by x rays which is manifested by death at the sixth or seventh nuclear cleavage and in which the nuclei are Feulgen-negative (apparently contain no DNA) has been described.[26] It is now known that this type of dominant lethal is induced whether eggs are irradiated in the first meiotic metaphase or prophase; they are, in fact, induced at a higher frequency in prophase I eggs killed by radiation. There is no difference in frequency between fertilized and unfertilized eggs at either meiotic stage, showing that this type of dominant lethal is not delayed in expression when an unirradiated nucleus is present. In an effort to distinguish between a hypothesis of chromosome loss and a hypothesis of a more general effect of radiation which brings about the phenomenon of Feulgen-negative nuclei production, triploid *Habrobracon* females were made, and their resulting dead embryos were examined. Triploids produce chromosome imbalance through faulty segregation at meiosis. All the embryos from triploid mothers had Feulgen-positive nuclei and all had developed past the sixth or seventh nuclei stage. This indicates that the effect of radiation which induces formation of Feulgen-negative nuclei is not brought about through chromosome loss with resulting genetic imbalance.

Examination of irradiated metaphase I eggs which died before hatching but after the sixth or seventh cleavage revealed that the age attained is profoundly influenced by ploidy. The unfertilized eggs die much earlier than those fertilized

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

[25] R. C. von Borstel, K. C. Atwood, and A. R. Whiting, *Biol. Semiann. Prog. Rep. Aug. 15, 1955*, ORNL-1953, p 29–30.

[26] R. C. von Borstel, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 34–35.

with unirradiated sperm. This again correlates well with the induction of conditionally delayed dominant lethals and the induction of the breakage-fusion-bridge cycle. When eggs in prophase I were irradiated, the low frequency of eggs getting past the sixth or seventh cleavage indicated this same tendency of haploid eggs to die earlier than diploid eggs, but the difference in number doing so was not so marked as when metaphase I eggs were irradiated. This indicates that embryo death through genetic imbalance can be induced by x rays at either meiotic stage.

Effect of Hypoxia on Radiation-induced Mitotic Inhibition in the Neuroblast of the Grasshopper Embryo

E. I. Shaw

It is well known that a reduced oxygen tension during irradiation causes a corresponding reduction in sensitivity to ionizing radiations. Gaulden, Nix, and Moshman[27] found that 64 r of x rays caused a delay of mitosis that could be shortened somewhat by irradiating the intact egg in a vacuum instead of in air. At 8 r, hypoxia had no detectable protective effect. The removal of dissolved oxygen from the fluids of the intact grasshopper egg by a vacuum is probably very incomplete. Therefore, sodium hydrosulfite, which combines readily with molecular oxygen in solution,[28] was used in the following experiments to remove oxygen.

The treatment of cultures with 10^{-2} *M* sodium hydrosulfite before irradiation prevented the occurrence of the mitotic inhibition usually caused by 8 r of x rays. Sodium hydrosulfite pretreated cultures receiving 32 r had a mitotic activity which was about 50% of normal, while the untreated cultures were totally inhibited. The recovery of normal mitotic activity occurred sooner in pretreated irradiated cultures than in the untreated irradiated cultures.

A lowering of the oxygen tension during irradiation decreased the effectiveness of x rays in producing mitotic inhibition. This indicates that the primary damage responsible for mitotic inhibition at low doses, and at least partially at high doses, must be due to the oxidation of intracellular components by HO_2 or similar oxidizing radicals whose formation is dependent upon the presence of oxygen during irradiation.

At 32 r the dose-reduction factor is about 8. At 8 r the dose-reduction factor cannot be determined accurately, for the protection is either total or statistically indistinguishable from complete protection. When dose-reduction factors for sodium hydrosulfite pretreatment at other dose levels are determined, it will be possible to resolve whether mitotic inhibition is caused by radiation damage of one or of several cell functions.

DROSOPHILA GENETICS

D. L. Lindsley, E. S. Von Halle, C. W. Edington, P. B. Turpin

Correlation of Kinetic Behavior and Distribution of Heterochromatin Within the Genome and Their Importance to the Viability of the Organism

D. L. Lindsley, E. S. Von Halle

It was reported earlier[29] that the strength of centromeres seemed to be correlated with a duplication for the heterochromatic region marked with the gene *bb*, rather than with a second arm, as had been reported previously.[30] This assertion was based on the observed kinetic behavior of chromosomes thought to be $XY^{L}\cdot$ on the basis of their origin. A cytological analysis of these chromosomes in secondary spermatocyte prophases, however, has shown that duplication for *bb* is completely correlated with the presence of a second arm.

It was further reported[31] that the recovery of $Y^{S}X(\cdot Y^{L})$ from females of constitution Y^{S} *y cv v f*$\cdot/y^{2}$ *su-w*^{a} *w*$^{a}(Y^{L}\cdot)y^{+}$ was reduced because of the dominant lethality of the particular single recombinant type. It was shown by tetrad analysis involving several different $X(Y^{L}\cdot)$ chromosomes that no-, one-, and two-exchange tetrads and the coefficient of nonrandomness, *c*, were in good agreement, whereas *x*, the relative viability of the $Y^{S}X(Y^{L}\cdot)$ recombinant, was different for different $X(Y^{L}\cdot)$ chromosomes. Similar analyses have since been performed on the progeny of females of constitution y^{2} *su-w*^{a} *w*^{a} *bb*$(Y^{L}\cdot)/Y^{S}$ *y cv v f*$\cdot y^{+}$ and y^{2} *su-w*^{a} *w*^{a} *bb*$(Y^{L}\cdot bb^{+})/Y^{S}$ *y cv v*

[27] M. E. Gaulden, M. Nix, and J. Moshman, *J. Cellular Comp. Physiol.* 41, 451–470 (1953).

[28] T. M. Lowry, *Inorganic Chemistry*, 2nd ed., p 679, Macmillan, London, 1946.

[29] D. L. Lindsley, *Biol. Semiann. Prog. Rep. Aug. 15, 1955*, ORNL-1953, p 40–43.

[30] E. Novitski, *Genetics* 37, 270–287 (1952).

[31] D. L. Lindsley and E. S. Von Halle, *Biol. Semiann. Prog. Rep. Aug. 15, 1955*, ORNL-1953, p 43–45.

$f{\cdot}y^+$, utilizing in several instances $X(Y^L{\cdot})$ chromosomes whose kinetic behavior had been previously established. The results of the analyses are shown in Table 5.

Recombinants recovered from females of constitution Y^S *y cv v f·/y v·y*$^+$ show normal viability. Recombinant chromosomes with Y^S distally but without Y^L proximally therefore are recovered with the expected frequency. The presence of Y^L proximally, however, decreases the viability of such recombinants. The extent of the inviability, given Y^L proximally, in turn seems to be correlated with centromere strength, such that recombinants with weak centromeres are less viable than the only one tested with a strong centromere. This finding supports the previous suspicion that the viability of these recombinants is in some way associated with their ability to function properly in cell division.

[32] D. G. Catcheside, *Advances Genet.* 2, 271–358 (1948).

Nonlinear Frequency-Dose Relation for Recessive Lethals Induced by X Rays in *Drosophila melanogaster*

C. W. Edington P. B. Turpin

It has been found that the frequency of recessive lethals induced by x rays in *Drosophila melanogaster* increases more rapidly with increasing dose than is expected on the basis of linearity. This finding is in contrast with previously published reports on mutation induction.[32]

The frequencies of recessive lethals induced by x rays in Oregon-R and in In(1)EN males are shown in Table 6. When the data for each of

TABLE 5. RESULTS OF TETRAD ANALYSIS ON PROGENY OF Y^S *y cv v f·y*$^+$/*y*^{2} *su-w*^{a} *w*^{a} ($Y^L{\cdot}$) ♀ × In(1) dl·49, *y w B* ♂

$X(Y^L{\cdot})$	Number Examined	E_0	E_1	E_2	c	x	Kinetic Behavior
118-2b	1260	0.106	0.602	0.292	0.695	0.400	Weak
118-12	848	0	0.676	0.324	0.632	0.685	Strong
118-13a	824	−0.010	0.762	0.248	0.616	0.677	
122-2a	95	−0.146	0.986	0.160	0.598	0	
122-13	915	−0.010	0.750	0.260	0.609	0.015	Weak
164-9	623	0.062	0.702	0.236	0.617	0.005	Weak
174-13	768	−0.002	0.814	0.188	0.599	0.078	Weak

TABLE 6. FREQUENCY OF RECESSIVE LETHALS INDUCED BY X RAYS IN OREGON-R AND IN In(1)EN MALES OF *DROSOPHILA MELANOGASTER*

Oregon-R				In(1)EN			
Dose (r)	Chromosomes Tested	Lethals	Percentage of Lethals ± 1 S.E.	Dose (r)	Chromosomes Tested	Lethals	Percentage of Lethals ± 1 S.E.
0	2647	4	0.15	0	2643	11	0.42 ± 0.13
1000	1350	28	2.07 ± 0.39	250	2461	27	1.10 ± 0.21
2000	1236	62	5.02 ± 0.62	500	1898	17	0.90 ± 0.22
2500	934	52	5.57 ± 0.75	750	2179	41	1.88 ± 0.29
3000	1454	134	9.22 ± 0.76	1000	1236	27	2.19 ± 0.42
3500	473	43	9.09 ± 1.32	2000	1113	53	4.76 ± 0.64
4000	1240	153	12.34 ± 0.93	3000	743	74	9.96 ± 1.10
				4000	654	84	12.84 ± 1.31

these experiments are fitted to a weighted linear regression, $y = a + bx$, it is found that in each case they depart significantly from linearity [Oregon-R: $\chi^2 = 11.651$, degrees of freedom (df) = 5, p = 0.02–0.05; and In(1)EN: $\chi^2 = 17.338$, df = 6, p = <0.001]. If these results are fitted to a quadratic equation, $y = a + bx + cx^2$, it is found that the χ^2 values are reduced, and the data fit the equations

$$y = 0.1494 + 1.5936x + 0.3653x^2$$

for Oregon-R ($\chi^2 = 3.866$, df = 4, $p = 0.43$) and

$$y = 0.4578 + 1.2789x + 0.4470x^2$$

for In(1)EN ($\chi^2 = 4.677$, df = 5, $p = 0.46$), where y is the percentage of recessive lethals, and x is the dose of x rays, in kiloroentgens (Fig. 3).

This nonlinear increase in the frequency of recessive lethals induced by x rays may be due to the increasing frequency at higher doses of one, two, or all of the following two-hit genetic effects: (1) two independent semilethal changes which together act as a recessive lethal, (2) gross deficiencies, and (3) "position-effect" lethals which are dependent upon gross chromosome aberrations for their expression.

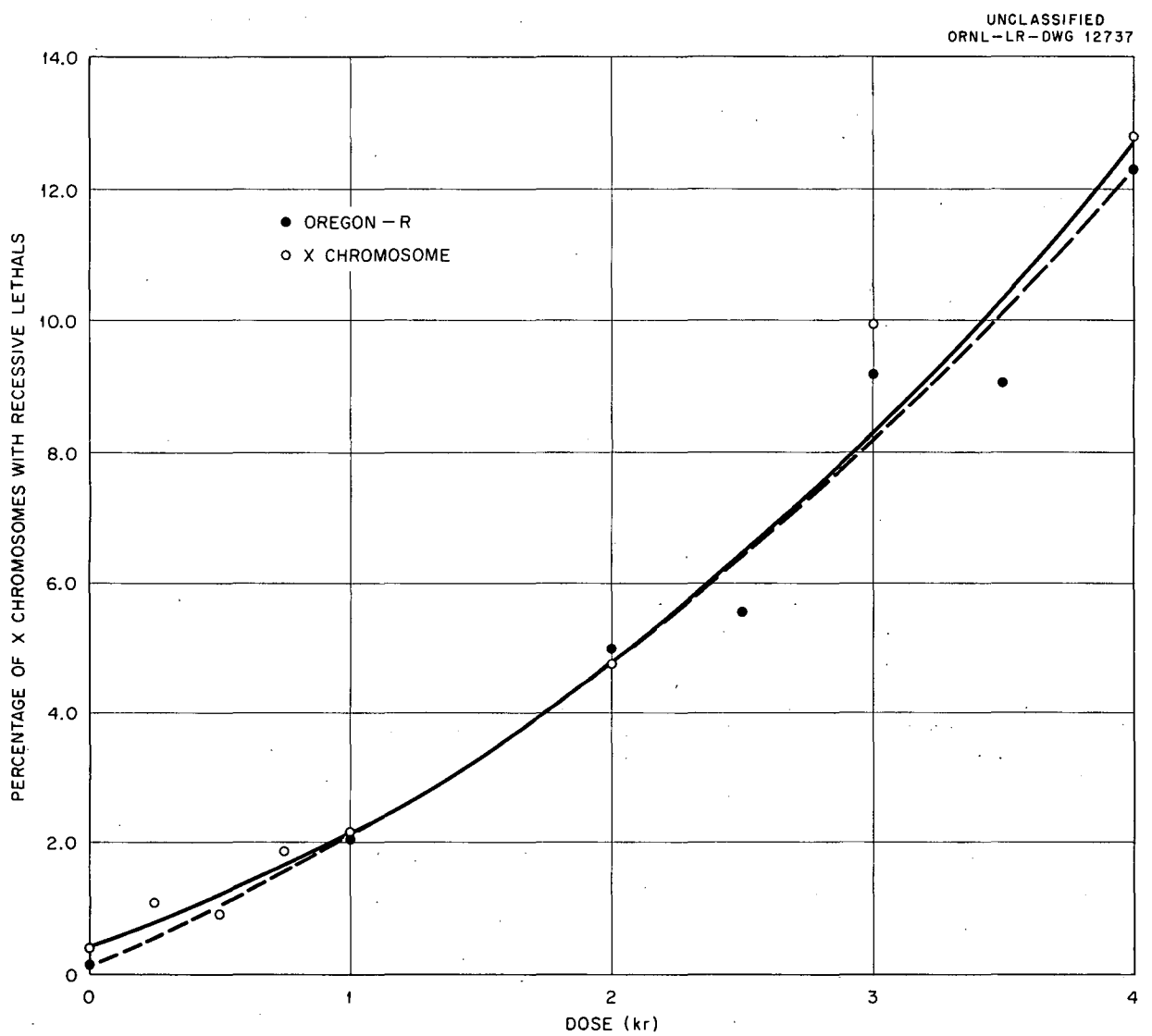

Fig. 3. Percentage of Recessive Lethals Induced by X Rays in the Oregon-R and In(1)EN X Chromosomes.

MAMMALIAN GENETICS AND DEVELOPMENT

GENETIC AND DEVELOPMENTAL EFFECTS OF RADIATION IN MICE

W. L. Russell, Section Chief

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L. B. Russell	C. M. Coffman
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Radiation-induced Mutations in the Mouse

W. L. Russell

J. S. Gower	M. B. Cupp
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The possibility for considerable expansion of the work of this section became more and more likely during the past year. The expansion was finally approved a few weeks ago. Tentative steps toward enlargement and speeding up of the major projects on mutation rate were made early in the past six-month period. Reorganization, multiplication of foundation stocks of mice, etc., are now being made as rapidly as possible. During this period of reorganization of the main projects, the space and technical assistance available for other activities change rather rapidly. It has, therefore, seemed desirable, during this period, to concentrate on experiments that can be completed in a few weeks or months. Among these, the largest one recently undertaken is the determination of mutation rates at specific loci in gametes irradiated in postspermatogonial stages. This experiment is flexible because it is run in batches, with the collection of mutants in each batch occupying only about nine weeks. Preliminary data collected some years ago indicated the possibility of a higher mutation rate in the postspermatogonial stages. Only a few of the presumed mutants collected in the new experiment have so far been tested, but the new data tend to confirm the higher mutation rate. By the time of the next semiannual report there should be enough new data to warrant a detailed account.

[1]Consultant.

[2]L. B. Russell and R. J. Spear, *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 61–63.

Fertilization and the First Cleavage Following Dominant Lethal Induction in Female Mice

L. B. Russell M. H. Major

It was shown earlier[2,3,4] that the number of living embryos from females inseminated *subsequent* to irradiation is strikingly dependent on the irradiation-to-fertilization interval. Females irradiated approximately 8.5 hr before fertilization produced only about one-sixtieth as many living embryos as those irradiated at intervals only a few hours longer. To prove that dominant lethals are induced in the germ cells of *female* mammals it is necessary to demonstrate: (1) that litter-size reduction is due to death of embryos rather than other causes and (2) that the death of embryos is due to their genetic constitution, rather than to radiation effects on the mother. For irradiation-to-fertilization intervals of ½ to 4½ days, point 1 has been very adequately proved and point 2 has been demonstrated qualitatively.[4,5] This report will attempt to determine whether point 1 applies for irradiation at the highly sensitive stage 8.5 hr before fertilization, and it will present more quantitative results on point 2 (for various irradiation-to-fertilization intervals) than had been available heretofore.

Demonstration That Litter-Size Reduction Is Due to Death of Embryos Rather Than Other Causes. – Females were irradiated (400 r) about 8.5 hr before fertilization and killed either 12 or 31 to 34 hr after fertilization. At these two killing times, controls are in the pronuclear (uncleaved) and two-cell stage, respectively. Oviducts were studied in serial sections. In irradiated females killed 12 hr after fertilization, an average number of 9.7 fertilized and 0.0 unfertilized eggs per female was found in 29 eggs studied. All eggs were of the proper stage of development. Certain nuclear abnormalities were found but none was of a nature that would raise doubts about normal fertilization having occurred, and they will be discussed at a later date. In irradiated females killed 31 to 34 hr after fertilization, 9.3 embryos per female were

[3]L. B. Russell and R. J. Spear, *Radiation Research* 3, 342–343 (1955).

[4]L. B. Russell and W. L. Russell, *Proc. 4th Internatl. Conf. on Radiobiology*, Cambridge, 1955 (in press).

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

found. Of 28 studied, 24 were in the two-cell stage and four had not cleaved; none of these four, however, showed the persisting second meiotic metaphase usually characteristic of unfertilized eggs.

It had already been shown that dominant lethals are induced by irradiating females ½ to 4½ days before fertilization. However, since sensitivity at 8.5 hr prefertilization was more than an order of magnitude higher than at the longer intervals, it was considered essential to bring a separate proof of dominant lethality for that stage, to rule out such possible radiation effects as retention of eggs, interruption of meiosis, or nonfertilizability of eggs. The results described make it clear that the first criterion for dominant lethals (i.e., death after fertilization) is satisfied. It may be recalled[2,4] that with 400 r, only 0.1 living embryo per female was found at two weeks of pregnancy and that most deaths were determined to occur before implantation. The present results show that the loss definitely occurs *after* fertilization (since 9.7 fertilized eggs per female were found); and most of it occurs after the two-cell stage (since 8.0 viable two-cell embryos per female were found).

Demonstration That Death of Embryos Is Due to Their Genetic Constitution. – Serial sections of 118 two-cell embryos were studied following irradiation given at 8.5 hr or at longer intervals before fertilization. Aberrations were usually in the form of micronuclei (i.e., small, extra nuclei) or chromatin lying free in the cytoplasm. Results are shown in Table 7. It should be mentioned that not only does the 8.5-hr group have the highest percentage of abnormal two-cell embryos and the greatest number of aberrations per embryo, but the micronuclei and fragments are, on the average, of larger size than in other groups.

A comparison of aberrations found in the two-cell stage (Table 7), with total death before birth,[2,4,5] indicates that 75 to 80% of all eventual deaths are already represented by gross nuclear aberrations after the first cleavage. The fact that 8.5-hr and 1½- to 2½-day irradiation-to-fertilization intervals are similar in this percentage is somewhat puzzling, in view of the higher number of aberrations per embryo in the former group. At any rate, it is clear that the second criterion for dominant lethality (i.e., that death of the embryo is due to its genetic constitution) is satisfied for all groups studied thus far.

Protective Action of AET and Hypoxia Against Radiation Damage to Spermatogonia of the Mouse

E. F. Oakberg R. L. DiMinno

The effect of *S*,*β*-aminoethylisothiuronium·Br·HBr (AET) on radiation sensitivity of spermatogonia was estimated by comparison of the number of spermatogonia and early spermatocytes in hybrid (101 × C_3H) mice given 8.8 mg of AET intraperitoneally, with controls given 0.85% NaCl. Injections were made 20 min before exposure to 0, 8, 17, 30, 47, 68, and 150 r of Co^{60} gamma rays at 5.4 r/min. Histological techniques and quantitative procedures were the same as described

TABLE 7. EFFECTS OF IRRADIATION AT VARIOUS INTERVALS BEFORE FERTILIZATION ON 31- TO 35-hr-OLD MOUSE EMBRYOS

Irradiation*-to-Fertilization	Total Number of Embryos	Percentage of Embryos			Average Number of Aberrations per Abnormal Two-Cell Embryo
		Uncleaved or Unfertilized	Two-Cell, Normal and ?	Two-Cell, Abnormal	
8.5 hr	28	14.3	21.4	64.3	1.72 + 0.28 ?
12 hr	18	0.0	44.4	55.6	1.50 + 0.60 ?
1½, 2½ days	40	7.5	72.5	20.0	1.00 + 0.00 ?
Control	32	3.1	90.6	6.2	1.00 + 0.50 ?

*400 r of x rays.

**The embryos studied in this group represent two litters, one of which resembles the 8.5-hr group, the other the 1½-, 2½-day group in incidence and type of aberration. This heterogeneity of the 12-hr group was already apparent in other data (ref. 2).

earlier.[6] Significantly higher numbers of type A spermatogonia were observed in mice given AET. Numbers of intermediate and type B spermatogonia, and early primary spermatocytes 72 hr after irradiation, were also higher in animals injected with AET; however, it was clearly demonstrated that protective action varied with dose. No significant effect was observed at 8 and 150 r. Maximum protection occurred at 30, 47, and 68 r. Protective response, therefore, depends upon interaction of radiation dose with specific spermatogonial stages.

Protective action of hypoxia was obtained by comparison of numbers of spermatogonia and early spermatocytes in hybrid (101 × C_3H) mice 72 hr after irradiation in an atmosphere of 5% O_2 and 95% He with animals exposed in 20% O_2 and 80% He. Mice were placed in a Lucite chamber and preflushed with the gas mixtures 6 min before and during exposure to 0, 17, 30, 57, and 100 r of 250-kv x rays. Return to room atmosphere was made as soon as possible after irradiation. Quantitative procedures were identical to those used in the AET experiment. The total number of type A spermatogonia was higher in mice exposed in an atmosphere of 5% O_2, but a t test gave a p value of only 0.048 for the difference. Numbers of intermediate and type B spermatogonia and early spermatocyte stages were higher for hypoxic mice at all doses, and where statistical tests could be made the difference was highly significant. Thus differences in spermatogonial response have been demonstrated, with good protection of intermediate and type B stages and only slight protection of type A cells.

Within both AET and hypoxia experiments, specific spermatogonial stages showed differences in degree of protection obtained. Furthermore, the variation between stages with AET did not parallel that observed with hypoxia.

Investigation of the *Notch* Locus of *Drosophila melanogaster*

W. J. Welshons

Intensive studies on the mutational nature of specific loci are very likely to yield information of general validity for all organisms. The *Notch* locus of *Drosophila melanogaster* was selected for such a study because a great variety of genetic tools are already available for the study of this locus.

Most *Notch* mutations are deficiencies for one or more salivary bands; some are not visibly deficient. Since salivary band 3C7 is missing in the great majority of all *Notch* deficiencies, Slizynska[7] and Demerec[8] concluded that band 3C7 is the locus of *Notch*. The wild-type alleles of the two recessive mutants *facet* and *split* also seem to be localized in band 3C7 because the phenomenon of pseudodominance is observed in the heterozygote formed from the combination of either recessive mutant with a *Notch* deficient for band 3C7. Because of a reluctance to assign more than one locus per band, Slizynska suggested that *facet* and *split* are alleles even though the combination *facet/split* has a wild phenotype.

Bauer[9] tested the mutants *split*, *facet*, *facet-notch*, and *facet-notchoid* in combination with *Notch* mutants and concluded that band 3C7 contained one locus for *split* and one locus for the *facet* alleles. Both Bauer and Slizynska noted that the pseudodominant effect was observed when the *Notches* used were not visibly deficient; these *Notches* might be deficient at a subvisible level.

Recent experiments performed here indicate that crossing over can occur between the *facet* alleles and *split*, and that the linear sequence from left to right is *facet*, *facet-notchoid*, and *split*. The mutant *facet-notch* has not yet been tested but it will probably fall within the above sequence. In addition, six nondeficient *Notches* were selected for further study, and *yellow Notch* females heterozygous for *split* were synthesized and crossed to *split* males. The next generation should consist of *split* and *Notch-split* females and *yellow-split* males. However, five of the six lines yielded at least one wild-type female or *yellow* male. It is possible to say that if the exceptional offspring resulted from crossing over, the locus of *Notch* lies to the right of *split*.

The above results indicate that a single salivary band contains the loci of four recessive mutants and, in addition, may contain the locus of the normal allele of *Notch*. Furthermore, *Notch* mutants fall into two classes which are functionally similar

[6] E. F. Oakberg, *Radiation Research* 2, 369–391 (1955).

[7] H. Slizynska, *Genetics* 38, 291–299 (1938).

[8] M. Demerec, *Carnegie Inst. Wash. Yearbook* 41, 191 (1942).

[9] H. Bauer, *Z. Abstammungs u. Vererbungslehre* 81, 374–390 (1943).

but physically distinguishable. A given *Notch* may be deficient for band 3C7 and hence physically deficient for the loci within that band, or it may possess these loci intact but remain functionally deficient because of a point mutation to the right of *split*.

The above conclusions are tentative. Additional experiments are necessary before a final conclusion can be drawn. Studies of this type must be performed on *Drosophila*, where the genetic tools are available; however, they may be of great significance when attempting to interpret the nature of mutations in the mouse, which is unfavorable material for such a study at present.

Sex Chromosomes in the House Mouse

W. St. Amand

In somatic mitoses the sex chromosomes of the male mouse are grossly unequal in size. In pachytene, a large puffy region which has been called a nucleolus or plasmosome is found at one end of the XY tetrad. According to Griffen,[10] " . . . no differential segment is otherwise included in the XY tetrad . . . any such existing area may eventually be found to lie to the right of the nucleolus." Slizynski[11] reports "two dark lines forming a half circle, one around the other" within the puffy region. The "lines" have been interpreted to represent the differential segments of the sex chromosomes.

The observations of Slizynski have been confirmed. Testis squashes stained with Sudan Black B in acid solution reveal a great deal of structure within the puffy region. The "lines" of Slizynski are found to consist of series of faintly staining chromomere-like structures. These structures show a much narrower range of variation in size, depth of staining, etc. than do the chromomeres in the pairing segments.

The structures within the puffy region superficially resemble the nucleolonema described by Estable and Sotelo[12] in nucleoli, and the possible relationship between these structures is being sought.

[10] A. B. Griffen, *J. Morphol.* 96, 123–144 (1955).

[11] B. M. Slizynski, *J. Genet.* 53, 591–596 (1955).

[12] C. Estable and J. R. Sotelo, *Stain Technol.* 27, 307–312 (1952).

MICROBIAL PROTECTION AND RECOVERY

RADIATION PROTECTION AND RECOVERY IN BACTERIA

A. Hollaender R. L. Girolami[1]
G. E. Stapleton A. S. Angel
A. J. Sbarra D. H. Woodbury
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Preparation and Activity of a Ribonucleic Acid Polynucleotide from *Escherichia coli* B/r Which Stimulates Recovery

G. E. Stapleton D. H. Woodbury

Introduction. – Previous reports[2,3] from this laboratory have shown that recovery after x or gamma irradiation of *Escherichia coli* B/r depends on the presence of guanine, uracil, and glutamic acid precursors for ribonucleic acid (RNA) and/or protein synthesis. Extracts of natural material were similarly stimulatory to recovery. If RNA synthesis is required for recovery in bacteria it should be possible to demonstrate recovery-stimulating activity of RNA polynucleotide material. The purpose of this report is to describe the preparation of RNA polynucleotide and the stimulatory activity of such material.

Results. Preparation of RNA Polynucleotide. – Mass cultures of *E. coli* B/r were grown and acetone powders of the cells prepared and dried (10 liters of 24-hr culture in inorganic salts–glucose medium[2] yielded about 4.5 g dry weight of powder). The acetone powders were finely ground in a mortar and pestle and extracted in 0.14 *M* NaCl at 75°C for 60 min (100 ml per gram of acetone powder). After extraction, the precipitate protein was removed by centrifugation (10,000 rpm, 15 min) and was discarded; the clear supernatant was adjusted to pH 5, and two volumes of cold absolute ethanol was added. After a few minutes at refrigerator temperature, a flocculent precipitate formed, which was recovered by centrifugation (3000 rpm). The precipitate was redissolved in distilled H_2O adjusted to pH 7 with sodium bicarbonate. Further deproteinization was accomplished by vigorous shaking of the solution with chloroform–amyl alcohol. A protein-chloroform gel formed, from which, after centrifugation, the aqueous supernatant was recovered. After several such treatments no more gel formed, and the aqueous layer was recovered and precipitated at pH 5 with two volumes of ethanol and then redissolved in the desired volume of water. By use of this technique it has been possible to recover 80% or more of the RNA material present in the crude extract. The polynucleotide material shows a single spot (ultraviolet absorption) by paper chromatography, and after hydrolysis (1 *N* HCl 60 min at 100°C) the hydrolytic products of RNA only were found.

Assay for Recovery Activity. – (*a*) *Bacterial Recovery.* – The assay system for bacterial recovery factors has been previously described.[2] The polynucleotide fraction, when added to a simple inorganic salts–glucose medium, gave little stimulation; however, if amino acids were simultaneously added to the medium, quantitatively similar stimulation of recovery was obtained as that brought about by extracts of natural materials. The activity per unit concentration, on a weight per unit volume basis, was about 100 times that for the crude extracts. Similar recovery stimulation was obtained in liquid or in solid media.

(*b*) *Animal Recovery.*[4] – Several batches of polynucleotide material were prepared and injected into mice (C_3H and $C_3H \times 101$ hybrid strains). The mice were injected within an hour after receiving an LD_{100} dose of x rays (800 r for C_3H mice and 900 r for the hybrid strain). Since these were designed as exploratory experiments, different amounts of the material were injected by various routes of injection. The optimal concentration and route of injection are still questionable. Some of the batches were deproteinized as described previously, while others were not so treated. Several batches of material were found to show acute toxicity to nonirradiated as well as to irradiated animals, and the irradiated animals receiving these preparations showed deaths at an earlier time than that routinely occurring in non-injected irradiated mice. Some batches were more stimulatory than others, this finding undoubtedly being related to acute toxicity. The results are shown in Table 8.

[1]Research Associate.

[2]G. E. Stapleton, D. Billen, and A. Hollaender, *J. Bacteriol.* 41, 345–358 (1953).

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

[4]These experiments were carried out in collaboration with C. C. Congdon of the Mammalian Recovery Group.

TABLE 8. SURVIVAL OF MICE INJECTED WITH BACTERIAL RNA

Strain of mice	C_3H	$C_3H \times 101$	$C_3H \times 101$
Sex	M	M	F
Dose, r	800	900	900
Noninjected, irradiated	0/16[a]	0/82	3/18[b]
Injected, irradiated	4/5	17/218[c]	11/32

[a]Fraction = $\frac{\text{number of survivors}}{\text{total number irradiated}}$.

[b]Females of this strain have a somewhat higher LD_{50} than males.

[c]This number is the total number of animals surviving, including animals in experiments where acute toxicity of injected material was shown. Corrected for those toxic batches the fraction = 15/123.

Discussion. – Previous experiments[2] indicated that new enzyme synthesis was involved in the process of recovery in bacteria. Precursors such as amino acids and purine and pyrimidine bases were shown to stimulate the recovery process after irradiation. It is logical on this basis to assume that, if RNA synthesis is involved, preformed intermediate RNA should be more stimulatory than the simple precursors, if they can pass the cell membrane. Some preliminary results indicate that this polynucleotide fraction is used as such in bacterial recovery. Experiments are planned wherein isotopically labeled polynucleotide fraction will be used to obtain more evidence on this important point.

The animal experiments, incomplete as they are, suggest that this material may stimulate cellular processes, in irradiated animals, which lead to recovery. Attempts are being made to further purify the bacterial fraction for animal use.

Pre- and Postirradiation Studies with Long-ultraviolet and Short-visible Radiation on *Escherichia coli* B/r

A. J. Sbarra A. Hollaender

A study has been initiated that is directed at the elucidation of the mechanism of inactivation of *Escherichia coli* B/r by long-ultraviolet and short-visible radiations (3500–4900 Å).

Results. – The rate of inactivation of *E. coli* B/r has been found to be dependent on:

1. temperature during irradiation,
2. presence of oxygen during irradiation,
3. growth medium before irradiation,
4. temperature and plating medium following irradiation.

Increased survival of nutrient broth-grown cells with a decrease in temperature during irradiation was observed. The temperature coefficient was about 3 when cells were irradiated at three different temperatures (10, 20, and 30°C).

Oxygen-saturated suspensions of nutrient broth-grown cells were found to be much more sensitive than oxygen-free suspensions.

Cells grown on a glucose–inorganic salts (basal) medium were found to be more resistant than cells grown on a nutrient broth medium.

The survival of organisms irradiated at 10°C and incubated for 24 hr at different temperatures on basal agar increases as the incubation temperature decreases, the survival being greatest at 6°C. The yeast-extract survival is practically linear regardless of the incubation temperature.

Discussion. – The greatest survival was obtained when organisms were irradiated at the lower temperature, the survivors decreasing as the temperature during irradiation increased. An explanation of this may be that a toxic radical of some kind is utilized by the organism at the higher temperature and is not at the lower temperature, or it may be that the radical is destroyed before the organisms have a chance to metabolize them. This finding is different from that obtained with ionizing radiation; for example, only when irradi-

ating at temperatures below 0°C is there a temperature coefficient[5] above 1.

Organisms irradiated in an oxygen-saturated system were found to be much more sensitive than those irradiated in an oxygen-free system. This finding, which also occurs with ionizing radiations,[6] suggests that the formation of toxic radicals is decreased in an oxygen-free system.

The results obtained with basal-medium-grown cells and with nutrient broth-grown cells indicate that the cells grown on basal medium are much sturdier than those grown on nutrient broth. The basal-grown cells have had to synthesize practically all the enzymes necessary for the existence of the cell. Cells cultured on nutrient broth have many important biological compounds supplied to them; thus they have had to do very little in regard to the formation of these compounds. When these cells are irradiated and plated, they must, if they are going to express themselves, synthesize many enzyme systems that may be necessary for the many synthetic reactions essential for survival. This same finding was observed with ionizing radiations;[7] that is, basal-grown cells were more resistant to x or γ rays than nutrient broth-grown organisms.

[5] G. E. Stapleton and C. W. Edington, *Radiation Research* (in press) (1956).

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

[7] G. E. Stapleton, A. J. Sbarra, and A. Hollaender, *J. Bacteriol.* 70(1), 7–14 (1955).

MODIFICATION OF RADIATION INJURY IN MICE

C. C. Congdon

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T. W. McKinley, Jr.
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I. M. Urso
P. Urso

Immune-Response Studies on Lethally X-irradiated Mice Protected with Bone Marrow

N. Gengozian T. Makinodan

The objective of this investigation was to study the immune mechanism of mice which had been lethally irradiated and protected with isologous (same strain) and heterologous (rat) bone marrow.

Preliminary studies concerning the effect of sex, age of animal, and antigen dose on the immune response of mice to a particulate antigen (rat red blood cell) have been made. The immune-response curves show that antibody production is independent of sex but dependent on antigen concentration and age of animal.

Thirty-day survival studies showed that 95% of mice receiving lethal x irradiation (950 r) can be protected if given isologous bone marrow and that 50% can be protected with heterologous bone marrow. Thirty days following irradiation and protection the immune response to particulate antigens, sheep and rat red blood cells (RBC), was studied in each group. Results with isologously protected animals show almost complete recovery of immune mechanism to sheep RBC, a distantly related antigen, and a more incomplete recovery of immune mechanism to rat RBC, a closely related antigen. Results with the heterologously protected animals show a negative response to rat RBC and a weak response to sheep RBC. The immune response of sublethally irradiated mice (710 r, LD_{30} and 600 r, LD_{10}) to sheep and rat RBC shows a greater overall response to sheep RBC, the distantly related antigen.

The results obtained with the isologously protected and sublethally irradiated animals suggest that x irradiation affects the mechanism of the recognition factor in the immune response. The results obtained with heterologously protected mice suggest a transplantation of rat hematopoietic tissue; and the immune response, death-time relationship, and gross appearance of the animal suggest a possible *in vivo* antigen-antibody reaction.

[1]Temporary employee.

Circulating Rat Cells in Lethally X-irradiated Mice

T. Makinodan

Congdon and Lorenz[2] have demonstrated that lethally x-irradiated mice can be protected by an injection of rat bone marrow. Thirty days after injection, similarly treated animals failed to respond to rat red blood cell (RBC) antigen and responded poorly to sheep RBC. Establishment of the degree of transplantation of rat hematopoietic tissue became important in an attempt to interpret the immune response of these animals.

A number of $C_3H \times 101F_1$ mice received 950 r of x radiation and rat (Sprague-Dawley) bone marrow. Control mice were of three types: non-irradiated, nonirradiated but injected with rat bone marrow, and irradiated and protected with isologous ($C_3H \times 101F_1$) bone marrow. Blood samples were taken at varying intervals, and the cells were tested immunohematologically. Rat RBC began appearing in the circulation about a week after x irradiation and injection of rat bone marrow (Fig. 4). A continuous increase in rat RBC was observed, the 50% level being attained around the twenty-fifth day. The 90 to 100% level of rat RBC was approached on about the fiftieth day and remained unchanged when tested on the sixty-fifth day. Rat granulocytes were present when tested on the forty-fifth and sixty-third days by the alkaline phosphatase staining method.[3]

It is concluded that rat erythropoietic and granulocytopoietic cells can be transplanted to lethally x-irradiated mice. Further studies are being made to determine the presence of other rat components in these animals.

[2]C. C. Congdon and E. Lorenz, *Am. J. Physiol.* 176, 297–300 (1954).

[3]P. C. Nowell *et al.*, USNRDL-TR-59 NM 006015 (Sept. 2, 1955).

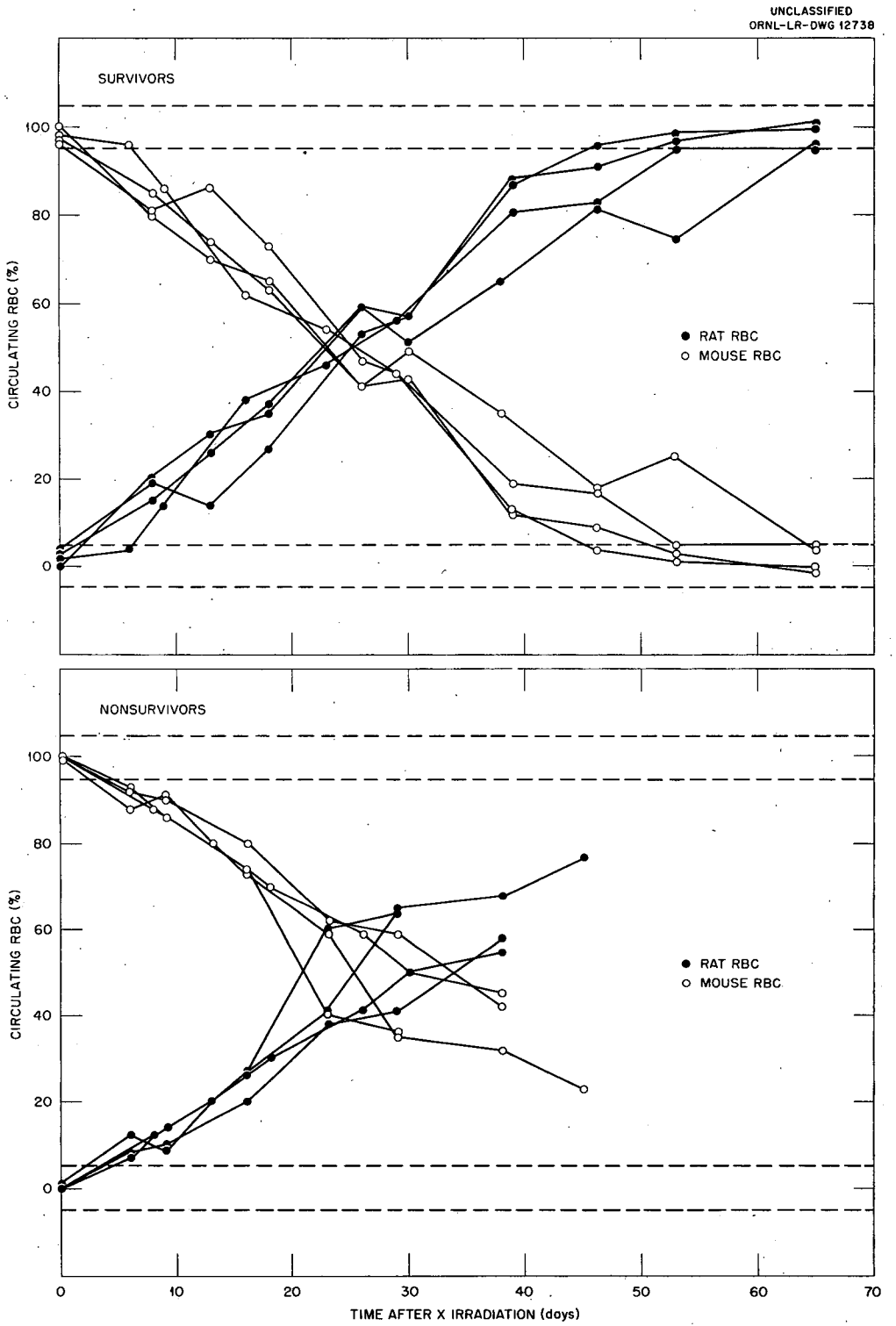

Fig. 4. Circulating Red Blood Cells in Irradiated Mice Protected with Rat Bone Marrow.

Isologous vs Homologous Bone Marrow in the Treatment of Irradiation Injury

C. C. Congdon I. M. Urso

The treatment of irradiated mice with homologous (interstrain) and heterologous (rat) bone marrow showed that there was a poor 30-day survival and many delayed deaths in comparison with isologous (intrastrain) bone-marrow treatment. The nature of the disorder produced with homologous bone marrow was investigated.

Fifty-two irradiated (900 r) LAF_1 mice showed 96% 30-day survival after treatment with isologous bone marrow. The survival from experiment to experiment was remarkably uniform. During the next 30 days the animals appeared normal but developed gray hair. Seventy-one similarly exposed LAF_1 mice showed an average of 58% 30-day survival after treatment with homologous ($C_3H \times 101F_1$) bone marrow. The survival in different experiments varied from 20 to 89%. Many deaths occurred during the third and fourth weeks postirradiation and continued to occur during the second month. These delayed deaths were associated with the development of a secondary disease process characterized by a ruffled appearance of the mice, a continued weight loss in the presence of approximately normal food intake, failure to gray properly, a generalized dermatitis, and loss of hair. The autopsy revealed atrophy and necrosis of lymphatic tissues, frequent hemorrhage into the wall of the intestinal tract at specific locations, and occasional bacterial infections. Bone-marrow regeneration was complete. A few homologously treated mice escaped this secondary disease. Apparently, they became desensitized or developed an acquired tolerance to the homologous bone marrow. At a lower exposure (650 r), it was observed that LAF_1 mice treated with homologous bone marrow developed the secondary disease, while the mice treated with isologous bone marrow and the surviving untreated x-ray controls did not, thus indicating that the homologous bone marrow was the initiating etiologic factor in causing the disorder.

These results indicate that a serious chronic anaphylactic type of disease develops in most homologously treated mice. Essentially the same disorder appears in heterologously treated mice. The few mice that escape the severe reaction and survive indefinitely (more than 60 days) show the feasibility of preventing these delayed deaths.

Quantitative Studies on the Effect of Bone-Marrow Treatment in Irradiated Mice

P. Urso C. C. Congdon

Results obtained by many investigators on the recovery of irradiated animals treated with bone marrow and related tissues have demonstrated a need for more detailed quantitative data on several aspects of the recovery phenomenon. In this investigation the effect of varying amounts of isologous bone marrow on the irradiated animal's bone-marrow response and on survival was studied. Correlation of the amount of bone marrow injected with other recovery parameters such as thymus weight, spleen weight, body weight change, and peripheral-blood leukocyte count was also studied.

A quantitative technique for counting the number of nucleated bone-marrow cells of the femoral bone shaft in normal and irradiated mice was developed. The variation in bone-marrow response in time and in amount was directly related to the amount of bone-marrow cells injected. It was found that a dose of 600,000 to 1,000,000 normal bone-marrow cells gave approximately 50% survival at 30 days after 900 r of x rays. With this dose of injected bone marrow, the irradiated mouse's bone marrow returned to a normal level in 14 days. A dose of 237×10^6 cells gave 100% 30-day survival, and the irradiated mouse's bone marrow returned to normal in four days. The amount of bone marrow injected also correlated with the irradiated mouse's recovery of body weight, thymus weight, spleen weight, and peripheral-blood leukocyte count. Massive doses of bone marrow gave the quickest recovery of these parameters. A dose of 237×10^6 bone-marrow cells given on the third day after irradiation caused a sevenfold increase, within 8 hr, of the bone-marrow count in the irradiated animal's femur.

Evidently, the recovery of the irradiated animal's bone marrow and the survival of the animal depend upon the amount of bone marrow received. This dose-response relationship is also evident for the other recovery parameters within certain limits. It is clear that whatever is responsible for the recovery of the irradiated animal (humoral factor, transplantation and repopulation, or both) is in a direct quantitative relationship to the amount of bone marrow made available.

Leukocyte Preparations in the Treatment of Irradiation Injury

L. H. Smith

Results of many experiments have indicated the importance of circulating granulocytes in the recovery of animals exposed to whole-body irradiation. Congdon *et al.*[4] have shown that injections of blood showing an extreme granulocytosis (leukemoid reaction to a transplantable squamous-cell carcinoma) promote recovery of lethally irradiated mice. In the present experiments, the formed elements of the leukemoid blood were tested for their effectiveness in promoting recovery from lethal irradiation. Leukocytes from other types of blood were also used.

Leukocytes were obtained from leukemoid blood by fibrinogen agglomeration of the RBC, while platelets and RBC were obtained by differential centrifugation. Mice exposed to 750 r of x rays were given a single intravenous injection of these isologous preparations following irradiation. It was found that recovery is associated with the white blood cells (WBC) rather than with the RBC or platelets. About 5.0×10^7 WBC per mouse gave the greatest survival (50–100%). Leukocytes were also effective when given intraperitoneally. Survival was not obtained if the WBC were injected prior to irradiation. The following procedures rendered the WBC ineffective: incubation at 38°C for 28 hr, standing at 3°C for 24 hr, and alternate quick freezing and thawing.

Leukocytes isolated from the blood of leukemic patients and normal rabbits were not effective. Attempts to obtain large enough numbers of WBC from normal mice were not entirely successful. However, two out of six lethally irradiated mice, each of whom received 5.0×10^7 WBC from normal mice, have survived more than 60 days.

Many forms of the myelocytic series are present in leukemoid blood. The recovery obtained by injecting leukocytes from this blood might be associated with a blast cell acting like elements of bone marrow or with mature, highly differentiated forms such as the polymorphonuclear leukocyte, which comprises a large percentage of the total leukocyte population.

[4] C. C. Congdon *et al.*, *Radiation Research* (1956) (in press).

Bone-Marrow Tissue Culture in the Treatment of X-irradiated Mice

D. Billen

Tissue culture techniques were applied to mouse bone-marrow explants, with the following objectives in mind: (1) the retention of the protective character of bone-marrow cells *in vitro* for an extended length of time and (2) the identification of cell type responsible for the recovery of lethally irradiated animals.

It has been established that isologous bone marrow may be maintained in a Gey's solution, chick embryo extract, or horse serum medium for four days and still bring about recovery when given intravenously to strain $C_3H \times 101F_1$ mice exposed to 900 r of x rays. Preliminary results have shown a six-day culture to be effective in reversing an otherwise lethal exposure. The cell suspension methods of Osgood and Krippaehne[5,6] were employed with modifications that allow for testing the ability of such cultures to influence animal response to irradiation. The presence of serum proteins (horse serum) in the culture medium and a minimum of culture disturbance during incubation were found to be essential for obtaining an effective four-day culture. Cell-free media obtained from one- or two-day cultures do not protect mice against x-ray exposure.

Attempts to single out a responsible cell type in the four-day cultures have proved difficult, since the distribution of cell types appears to be as complex as that of fresh bone-marrow samples. However, maturation of the myelocyte series continues in such cultures, as evidenced by a higher percentage of mature polymorphonuclear leukocytes than is found in fresh bone marrow. As a result of the use of heterologous serum in the medium, mature erythrocytes are not seen.

The specific medium requirements for an effective cell culture at four days and the lack of protection afforded by one- or two-day cell-free culture media indicate that living cells are necessary for animal recovery brought about by bone-marrow cells. Either the living cells are required per se for the protective action, or a humoral factor is provided by such cells which is so extremely labile as not to be detectable under these experimental conditions.

[5] E. E. Osgood, *Ann. N.Y. Acad. Sci.* 59, 806–913 (1955).

[6] E. E. Osgood and M. L. Krippaehne, *Exptl. Cell Research* 9, 116–127 (1955).

PATHOLOGY AND PHYSIOLOGY

PATHOLOGIC AND PHYSIOLOGIC EFFECTS OF RADIATION

A. C. Upton, Section Chief

T. T. Odell, Jr.
W. H. Benedict[1]
K. W. Christenberry[1]
G. S. Melville[2]
F. P. Conte[2]
W. D. Gude
F. G. Tausche
B. Anderson
E. S. Ledford
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T. Mack

Establishment of Homotransplants of Functional Erythropoietic Elements in Rats Following Total-Body Irradiation

D. L. Lindsley[3] T. T. Odell, Jr.
F. G. Tausche

Bone marrow from rats having erythrocytes of one antigenic type has been successfully transplanted into whole-body irradiated rats having erythrocytes of another antigenic type.[4] The following data have been obtained on the growth rate and fate of the transplanted homologous marrow.

It has been found that erythrocytes of the foreign type can first be detected immunologically in the peripheral circulation seven days following marrow injection (eight days after irradiation with 700 or 750 r of x rays), at which time they make up less than 1 to 2% of the total circulating erythrocytes (it is recognized that several days probably elapse between the first steps in the production of red cells and their appearance in the peripheral circulation). Their percentage then increases and by the thirty-fifth day reaches an equilibrium which appears to remain essentially constant thereafter; some mosaic animals have now been followed for over 200 days. After 700 to 750 r of x radiation the number of rats in which the marrow successfully implants is greater than 50% but less than 100%, and the final percentage of erythrocytes of the implanted type varies from 10 to 95% of the total erythrocyte population.

These experiments demonstrate that homologous transplants of normal adult tissue may become permanently established following whole-body irradiation in the median lethal dose range.

Leukemia Induction by Fast-Neutron Irradiation

G. S. Melville M. Slater[5]
R. J. Elliott

Mice of the RF strain surviving acute lethality experiments carried out in the ORNL 86-in. cyclotron[6] have been observed throughout life in an effort to obtain information about the relative biological effectiveness (RBE) of fast neutrons for leukemia induction and other delayed effects.

The observations to date are summarized in Table 9. The median survival was essentially the same in neutron- and in x-irradiated mice at corresponding dose levels.

These data indicate that, at dose levels equivalent in 30-day lethality, fast neutrons and x rays are of comparable leukemogenic potency. Since neutrons were observed to be about 1.5 times as effective as x rays in producing 30-day mortality, the RBE for leukemia induction would appear to be in the neighborhood of 1.5; however, a lower incidence of thymic lymphomas and greater frequency of myeloid leukemias occurred in neutron-exposed, as compared with x-irradiated, mice, suggesting that the RBE may vary slightly with the type of leukemia. These observations are currently being extended in a comparative investigation of the effects of chronic exposure to fast neutrons or gamma rays at graded dose rates, down to and including the "maximum permissible" exposure level (see, "Radiological Physics," this report).

Pathogenesis of Radiation-induced Myeloid Leukemia

F. F. Wolff E. S. Ledford

Factors influencing the induction of myeloid leukemia by ionizing radiation are being investigated in mice of the RF strain.

In addition to the observations reported previously,[7] preliminary data indicate that the induction of this disease is inhibited by splenectomy

[1] Consultant.

[2] U. S. Air Force.

[3] *Drosophila* Genetics Group.

[4] D. L. Lindsley, T. T. Odell, Jr., and F. G. Tausche, *Biol. Semiann. Prog. Rep. Aug. 15, 1955*, ORNL-1953, p 69–72.

[5] Biophysics Section.

[6] A. C. Upton *et al.*, *Biol. Semiann. Prog. Rep. Aug. 15, 1954*, ORNL-1766, p 55.

[7] F. F. Wolff, P. Ledford, and W. D. Gude, *Biol. Semiann. Prog. Rep. Aug. 15, 1955*, ORNL-1953, p 69.

TABLE 9. LEUKEMIA IN RF MICE EXPOSED TO FAST NEUTRONS AND TO X RAYS

Sex	Number of Exposures	Days Between Exposures	Neutron Irradiation				X Irradiation			
			Dose (rep)	Surviving 30 Days (%)	Myeloid Leukemia (%)	Thymic Lymphoma (%)	Dose (rep)	Surviving 30 Days (%)	Myeloid Leukemia (%)	Thymic Lymphoma (%)
Male	1	0	0	100	3	4				
	1	0	220–245	100	44	5	350*	100	29	17
	1	0	265–345	19–100	42	8	512**	95	11	17
Female	1	0	0	100	2	16				
	1	0	215–250	100	29	21	350*	100	18	33
	1	0	260–360	63–100	25	29	512**	95	4	35
	2	2	370–400	50–55	11	11	625–725	40–70	17	37
	2	5	345–390	85–95	19	25	525–625	85–100	19	64
	2	5	410–415	70–95	16	32	675–775	75–85	19	52
	2	7–8	380–450	65–85	11	35	625–775	65–100	24	66
			Average incidence:		25	21	Average incidence:		18	40
			Combined incidence:		46		Combined incidence:		58	

*Previous experiment [A. C. Upton and J. Furth, *Blood* 9, 686–695 (1954)].

**Previous experiment [A. C. Upton, J. Furth, and K. W. Christenberry, *Cancer Research* 14, 682–690 (1954)].

preirradiation (Table 10). Similarly, induction of both myeloid and lymphoid leukemias is markedly curtailed if the volume of tissue irradiated is reduced by partial shielding (Table 10).

It is evident that the induction of these types of leukemia is not proportional to the integral dose of radiation. Other experiments are in progress to study the possible inhibitory effects of administration of AET preirradiation or bone marrow postirradiation on the induction of myeloid leukemia.

Lamellar Cataracts Induced by Radiation in Newborn Mice

W. H. Benedict K. W. Christenberry

This study was undertaken to investigate the influence of age on the radiosensitivity of the lens.

Exposure of RF mice to 300 r of total-body x radiation on the day of birth results in the formation of lamellar cataracts within the first month of life. Perinuclear lens opacities are visible with the slit lamp when the eyes first open (at about 10–14 days of age). These progress, with extensive involvement of the lens cortex within the following four to six weeks, and are associated in certain instances with subluxation of the lens.

Opacities of this type differ markedly from those observed in animals irradiated later in life, owing to embryological differences in growth and radiosensitivity, but are similar to lamellar cataracts in humans resulting from developmental disturbances *in utero*. The radiosensitivity of the lens and pattern of opacity formation are being investigated further in relation to the age of the animal and dose of irradiation.

Effects of Hypothermia on Radiation-induced Intestinal Injury

W. D. Gude T. Mack

The cellular degeneration and inhibition of mitosis, which are detectable in the crypts of the small intestine of the rat within 30 min following whole-body exposure to doses of x radiation in excess of 100 r, constitute a highly sensitive, rapid, and reproducible measure of radiation injury. In an attempt to investigate the mechanism of these cytologic changes, the intestine was chilled immediately postirradiation, by the application of cold compresses directly to the exposed bowel.

It was observed that prompt reduction of the temperature of the tissue markedly interfered with the development of the cytologic manifestations of damage (Table 11).

It is not yet clear whether the observed effect represents inhibition or merely delay of radiation-induced damage. This question and the mechanism of the phenomenon are being explored further.

Tracer Studies on the Pharmacology of AET

F. P. Conte D. G. Doherty[8]
G. S. Melville R. Shapira[8]

To explore the mechanism of action of *S*,*β*-aminoethylisothiuronium·Br·HBr (AET) and to

[8]Biochemistry Section.

TABLE 10. INHIBITION OF MYELOID LEUKEMIA INDUCTION BY SPLENECTOMY OR PARTIAL SHIELDING

Dose of X Rays (r)	Treatment	Number of Mice	Dead of Leukemia (%)	
			Myeloid	Thymic Lymphoma
0		88	1	2
150		104	26	9
300		95	45	19
300	Splenectomy	62	18	26
450		86	23	28
450	Partial shielding*	76	8	7

*Pelvis and lower extremities shielded with lead, receiving only 1.5% of the dose administered to the remainder of the body.

TABLE 11. INHIBITION OF RADIATION-INDUCED INTESTINAL INJURY BY HYPOTHERMIA

Time Postirradiation (min)	Average Number of Mitoses* per 10 Crypts	
	Normal Temperature	Chilled
Irradiated (750 r)		
60	3	38
90	0	40
120	5	36
Nonirradiated		
60	31	48
90	25	29
120	30	30

*Each value is based on 40 jejunal crypts counted per rat, one to three rats in each treatment group. Mitosis was considered to include stages of cell division from late prophase through telophase. In general, the amount of cellular degeneration, as judged morphologically, corresponded to the degree of mitotic inhibition, being negligible in the controls and hypothermia-protected rats.

determine the optimal timing and route of administration of this drug, the absorption, distribution, and excretion of S^{35}-labeled AET have been studied.

Serial sampling of the blood, tissue, and urine at intervals after the intra-arterial injection of tagged AET into rats disclosed rapid loss of the activity from the blood stream and excretion of the activity in the urine. Considerable activity was distributed throughout all tissues. Similarly, following the intraperitoneal or oral administration of the drug, activity was absorbed into the blood stream and widely distributed throughout the body.

Although it is not certain that the activity observed in the blood, tissue, and urine samples represented unaltered AET, it is tentatively inferred that this was the case. Relatively high concentrations of activity were noted in such radiosensitive tissues as spleen, bone marrow, and lymph nodes. The gradual absorption of this compound via the gastrointestinal tract indicates the need for exploring further the feasibility of prolonged or continued protection against radiation injury by the oral administration of this material.

GENERAL PHYSIOLOGY

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N. G. Anderson
E. C. Horn[2]
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M. E. House[2]

Cell Fractionation

N. G. Anderson M. L. Anderson

The cell fractionation project has been organized with the aim of fractionating the liver cell into as many molecular species as possible. To this end several fractionation techniques have been devised, including an electroseparator for rapidly fractionating protein mixtures into smaller groups of substances and in some instances into pure fractions.

An electroseparator design has been completed and tested, using both blood and liver preparations. The device consists of a series of plastic frames supporting a thin sheet (3 mm) of protein in starch or agar against a thicker (13.5 mm) sheet of the protein. Glass screen, filter paper, and cellophane hold the supporting material in the frames. The proteins migrate through the supporting medium into a narrow space between the glass screen and the cellophane, where they are concentrated by electroconvection and removed continuously or intermittently from the bottom. Since the breakthrough time for a given protein is inversely proportional to its mobility, the emergence pattern, although showing the same peak order as free electrophoresis, will show small intervals between fast components and much longer intervals between slow components. A graphic method for relating the two patterns has been devised. With the use of only 24 v, a soluble liver nucleoprotein has been isolated from soluble liver proteins in 50 min. Gram quantities of serum albumin have been separated in a few hours. The volume of the starting layer is 68 ml in the small model described. To sharpen the starting layer, the current is reversed in order to pile up the faster components against the back cellophane sheet.

While the device described does not have sufficient resolution to resolve all the many peaks seen in free electrophoresis of liver soluble proteins, it allows rapid separation of these mixtures into much simpler fractions which may be further purified by other techniques.

Rat Liver Catalase Level and Sensitivity to X Radiation

W. Friedberg

If differences in the liver catalase level are responsible for differences in the lethal effects of x radiation, a reduction of liver catalase should result in an increased sensitivity to x radiation.

In Sprague-Dawley rats, a single injection of 3-amino-1,2,4-triazole, 1000 mg/kg, reduces the liver catalase to 4% of normal $4\frac{1}{2}$ hr after injection. Two groups of 12 rats each were irradiated with 934 r of x radiation $4\frac{1}{2}$ hr after administering aminotriazole to one group and physiological saline to the other. Three aminotriazole- and four saline-injected rats died during the 30-day observation period following irradiation.

The decrease in liver catalase did not increase the lethal effects of x radiation.

A Method for Determining the Reliability of Protein Labels

W. Friedberg

When determining the fate of an injected protein by using a radioactive label, it is important to know whether the bulk of the label persisting in a tissue represents the originally injected protein.

The extent of transfer of S^{35}-azophenylsulfonate from beef serum albumin to a normal liver protein of injected guinea pigs was determined. One week after the intravenous administration of the labeled protein the specific activity of the liver catalase protein was approximately 20% of the liver homogenate protein.

Assuming catalase to be a representative liver protein, it may be concluded that 80% of the radioactivity in the liver protein was bound to the injected protein. Control experiments indicate that even more than 80% of the radioactivity may represent injected protein.

[1]On leave; USPHS Fellowship.

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

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L. Astrachan E. Volkin
D. G. Doherty M. H. Jones
J. X. Khym A. B. Ottinger

Structure and Activity in Radiation-protective Sulfhydryl Compounds

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R. Shapira T. W. McKinley, Jr.[2]
A. B. Ottinger

The theory, previously discussed,[3] relating the structure of protective compounds with their activities in mice has been corroborated by the use of several new compounds. Unfortunately, all new compounds were found to be more toxic than *S*,β-aminoethylisothiuronium · Br · HBr (AET) in mice (Table 12).

The results of the new compounds tested agree well with the hypothesis[3] that formation of a cyclic intermediate is necessary for protective activity. The activity of the one exception, *N*-formyl AET (*N*-acetyl AET was ineffective), may be readily explained by the known chemical lability of formamino groups under the test conditions. The manner in which guanidinoethyl disulfide is protective is similar to that of cystamine.

Several active and inactive isothiuronium compounds were hydrolyzed with strong base to the corresponding mercaptans, which had protective activity. In particular, *S*,Δ-aminobutylisothiuronium · Br · HBr (ABT), inactive itself, and incapable of forming a cyclic intermediate, yields an active aminobutyl mercaptan, offering further evidence that the guanyl group transfer is essential in the active thiuronium series. The AET was found to afford effective protection when administered orally, subcutaneously, intraperitoneally, and intramuscularly.

[1]On leave – Guggenheim and Fulbright Fellowships.
[2]Mammalian Recovery Section.
[3]D. G. Doherty *et al.*, *Biol. Semiann. Prog. Rep. Aug. 15, 1955*, ORNL-1953, p 88–91.

TABLE 12. PRELIMINARY RESULTS WITH NEW COMPOUNDS, AET, AND APT

[C_3H male mice given an LD_{100} dose (800 r)]

Compound	Toxicity[a] LD_{50} (mg/mouse)	Dose[b] (mg/mouse)	30-day Survival[c] (800 r)	Nitroprusside –SH Test[d]
S, β-Amino-β-methylpropylisothiuronium · Br · HBr	5	3	10/15	+
S, β-Aminobutylisothiuronium · Br · HBr	2	1.5	7/7	+
S, β-Formaminoethylisothiuronium · Cl	7	5	14/14	+
Guanidinoethyl disulfide · 2HBr	3	2	13/18	+
S, β-Phenylaminoethylisothiuronium · Br · HBr	1.5	1.0	(In progress)	+
S, β-*n*-heptyl-γ-aminopropylisothiuronium · Br · HBr	2.0	1.5	(In progress)	+
S, β-Cyclohexyl-γ-aminopropylisothiuronium · Br · HBr	2.0	1.5	(In progress)	+
S, β-Aminoethylisothiuronium · Br · HBr (AET)	12.0	4.4–8.8	143/150	+
		6.6	53/55	+
S,γ-Aminopropylisothiuronium · Br · HBr (APT)	7.0	2.3–6.9	24/25	+
		5.3	23/25	+

[a]Determined by procedure given in *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 52.
[b]Intraperitoneal dose 10 min prior to x irradiation.
[c]Irradiation procedure given in *Biol. Semiann. Prog. Rep. Feb. 15, 1955*, ORNL-1863, p 48–49. Fraction indicates survivors over total irradiated; no survivors in approximately 300 control animals.
[d]Tested at pH 7.0.

No protection was observed after the small dose of 2.0 mg was administered intravenously.

In a comparison study at equimolar amounts, cystamine, cysteamine, and AET were found to be completely effective in protecting mice against 900 r. British anti-Lewisite was 50% effective, and cysteine, glutathione, and mercaptoethanol were ineffective. The AET administered just after irradiation was ineffective. Large amounts of succinate and ascorbate provided no protection.

Colorimetric Determinations of S,β-Aminoethylisothiuronium · Br · HBr (AET) and Related Compounds

J. X. Khym A. B. Ottinger

The necessity for quantitative methods to detect the presence of AET in biological fluids and to determine its stability in solution over a wide range of conditions prompted an investigation of the various colorimetric techniques for the determination of sulfhydryl groups. A procedure involving the reduction of 2,6-dichlorophenol indophenol[4] at neutral pH was selected as the most suitable for obtaining information that could be correlated with biological protective activity and could be used in paper and column chromatography.

The stability of AET (100 μmoles/ml) in aqueous solution at various pH's at room temperature was determined over a period of two weeks. The results are presented in Fig. 5. In addition, the sulfhydryl activity relative to AET of a series of compounds capable of forming active cyclic structures[4] and of a few biologically inactive compounds was determined by this procedure. The results are summarized in Table 13.

It was found that dilute aqueous solutions of AET (1 μmole/ml) were stable only under nitrogen at pH 7.0. In the presence of air or at other pH's the ability to reduce the dye was rapidly lost. The data presented in Fig. 5 lend support to the multiple[3] equilibrium forms of AET possible in aqueous solution; AET dissolved in water readily hydrolyzes, and, when briefly boiled, 2-aminothiazoline is formed (inactive in this color test). In strong acid solution both the alkylamino and guanylamino groups retain their protons, explaining the stability of AET in 0.2 *M* HCl. As the pH is increased, the alkylamino group releases

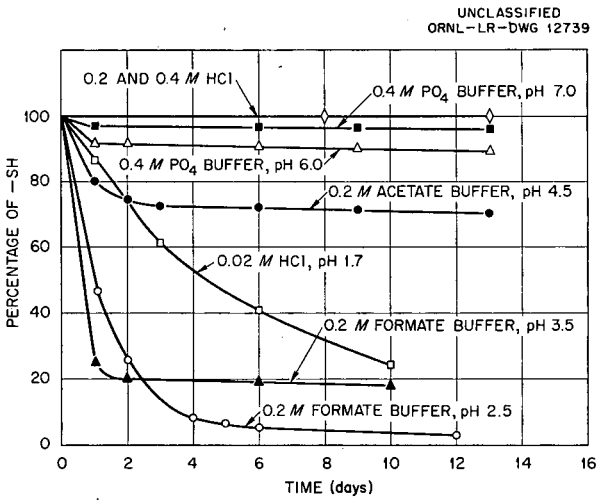

Fig. 5. Percentage of –SH Remaining vs Time of Standing of AET Solutions at Various pH's.

its proton, allowing the formation of the cyclic intermediate, which at pH's of 2 to 4 loses ammonium ion slowly to form 2-aminothiazoline. Nearer to neutrality, the transformation to the cyclic form is very rapid, and mercaptoethylguanidine forms. Under alkaline conditions the carbon-sulfur bond of AET is broken, with the formation of mercaptoethylamine and dicyandiamide. The correlation between potential sulfhydryl activity and protection is also rather striking (Table 13), if the protective activity is considered on a molar basis. Those compounds with the highest sulfhydryl activity provided the best protection, while those with no activity were completely ineffective.

Ribonucleic Acid Phosphorus Turnover in T2r+-infected *Escherichia coli*

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M. H. Jones

Infection of *E. coli* with bacteriophage T2r+ produces a vigorous synthesis of phage deoxyribonucleic acid (DNA) and protein but no significant change in the host's ribonucleic acid (RNA) content. There is no general agreement, however, concerning turnover of RNA after phage infection. This problem has been reinvestigated under conditions thought to be more definitive than those previously employed. Thus the RNA contribution from uninfected bacteria was maintained at an insignificant level, and, furthermore, RNA phosphorus was identified unambiguously with RNA

[4] R. E. Basford and F. M. Huennekins, *J. Am. Chem. Soc.* 77, 3873 (1955).

TABLE 13. THIOL GROUP ASSAY OF ISOTHIURONIUM COMPOUNDS COMPARED WITH AET AS A STANDARD

Name of Compound	Concentration Tested (μmoles/ml)	ΔO.D./ΔO.D. of AET*	Protective Activity
S, β-Aminoethylisothiuronium • Br • HBr	1.0	1.00	+
S, γ-Aminopropylisothiuronium • Br • HBr	1.0	1.11	+
S, β-Aminoethyl-1′,3′-diisopropylisothiuronium • Br • HBr	1.0	0.90	+
S, β-Phenylaminoethylisothiuronium • Br • HBr		0.88	+
S, β-Aminoethyl-1′,3′-dibenzylisothiuronium • Br • HBr	1.0	0.90	+
S, β-Aminoethyl-1′-allylisothiuronium • Br • HBr	1.0	0.87	+
S, β-Aminoethyl-1′,3′-diethylisothiuronium • Br • HBr	1.0	0.77	+
S, β-Aminoethyl-1′,3′-diphenylisothiuronium • Br • HBr		0.60	+
S, β-Methylaminoethylisothiuronium • Br • HBr		0.60	+
β-Mercaptoethylgluconamide		1.02	+
S, β-Aminoethylaminoethylisothiuronium • Br • HBr	20.0	0.03	–
S, Δ-Aminobutylisothiuronium • Br • HBr	100.0	0	–
β-Mercaptothiazoline	100.0	0	+ –
S, β-Dimethylaminoethylisothiuronium • Cl • HCl	100	0	–
S-Benzylmercaptoethylamine • HBr	100	0	–
S, γ-Hydroxypropylisothiuronium • Br • HBr	100	0	–

*ΔO.D. = difference in optical density of compound tested to that of AET.

mononucleotide phosphorus by ion-exchange chromatography.

The results reveal that medium inorganic phosphate was incorporated in RNA after phage infection to an extent some 500- to 1000-fold more than could be accounted for on the basis of the few remaining uninfected cells. A marked difference was observed in the kinetics of incorporation in peptone broth, as compared with glucose-synthetic medium. In peptone broth, a rapid incorporation of P^{32} into RNA phosphorus quickly ceased, whereas such incorporation in synthetic medium continued almost linearly for at least 60 min. In both cases, it was observed at all sample times that adenylic and uridylic acid phosphorus had specific activities, some 50% as great as the other two mononucleotides, indicating that the incorporation of isotope was heterogeneous with respect to the total RNA phosphorus. These results indicate that the host cell may contain RNA's of varying metabolic activity after phage infection. Partial separation of the infected cells' RNA by differential centrifugation revealed that supernatant RNA had P^{32} specific activities about 5 times as high as a particulate fraction RNA, although the latter contained some 60% of the total cell RNA. Moreover, the RNA of another particulate fraction, containing less than 5% of the total cell RNA, had specific activities some 10 times as great as the supernatant RNA.

The functional nature of the metabolically active RNA in this system remains to be elaborated.

DNA Produced by *Escherichia coli* After T2r+ Bacteriophage Infection in the Presence of Proflavin

L. Astrachan E. Volkin
M. H. Jones

It has been reported[5] that *E. coli* infected with bacteriophage in the presence of proflavin does

[5]R. I. De Mars, *Virology* 1, 83 (1955).

not form viable phage but does synthesize the major chemical constituents of phage; that is, phage-like DNA (hydroxymethylcytosine DNA) and phage-like protein (morphological and immunological identity). To test the possibility that DNA formed in the proflavin system may differ in nucleotide sequential arrangement from normal phage DNA, the polynucleotides produced by enzymic degradations were compared.

The proflavin system DNA was uniformly labeled with P^{32} and then mixed with 15 to 20 times its amount of authentic phage DNA. The total DNA was then isolated and hydrolyzed with either deoxyribonuclease or whole snake venom (diesterase plus monoesterase). Both enzyme systems produced a high yield of polynucleotide fragments (dinucleotide or greater). The hydrolyzates were chromatographed on Dowex 1 or cellulose-triethanolamine columns. In each chromatographed fraction, the chemically determined phosphate was predominantly a measure of material from authentic phage DNA, whereas radioactivity measured only the contribution of proflavin system DNA. Any differences in specific activities of the products would reflect sequential differences in the intact DNA's. Comparisons of specific activities showed the two DNA materials to be the same with respect to content of various polynucleotides produced by deoxyribonuclease. The polynucleotides produced by the snake venom enzymes also had the same specific activities, with the exception of one fraction which had a lower specific activity, indicating greater contribution from authentic phage DNA.

ENZYMOLOGY AND PHOTOSYNTHESIS

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ENZYMOLOGY

Comparative Studies on Different Bacterial Luciferase Preparations

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Differences in the properties of bacterial luciferase, dependent upon the method of extraction and purification of the enzyme, have been found. These differences have been exploited in the study of other reactions. Inhibitors of this luminescent system have been found, and the nature of these inhibitions has been elucidated.

Crude luciferase preparations, obtained by lysing the cells of *Achromobacter fischeri* with water or by extracting acetone-dried powders of the organism, contained a strong reduced diphosphopyridine nucleotide (DPNH) oxidase. The two enzymes cannot be separated by acid precipitation or by $(NH_4)_2SO_4$ fractionation, regardless of the type of crude extracts used. However, when either extract is subjected to starch-column electrophoresis[2] at pH 6.5 (henceforth termed purified), luciferase preparations are obtained which produce light in the presence of DPNH, flavin mononucleotide (FMN), and a long-chain aldehyde only when DPNH oxidase obtained from *Escherichia coli* is added.

In order to be sure, however, that luciferase does not have the property of oxidizing DPNH, different luciferase preparations (containing the same amount of luciferase) were compared for their ability to oxidize DPNH. The results presented in Table 14 leave little doubt that bacterial luciferase is not a DPNH oxidase. The observations of McElroy and Green[3] that luciferase preparations contained DPNH oxidase activity may be explained by assuming that their preparations were either contaminated with DPNH oxidase and could not be separated by their methods of purification, or that luciferase and DPNH oxidase occur as a single particle, the oxidase being selectively destroyed during electrophoresis procedures.

Purified luciferase obtained from crude extracts of lysed cells showed an absolute requirement for albumin before light was emitted in the presence of reduced flavin mononucleotide ($FMNH_2$) and aldehyde, whereas purified preparations obtained from extracts of acetone-dried powders gave light in the absence of albumin. Cysteine, glutathione, Versene, and BAL (2,3-dimercaptopropanol) were quite effective in replacing albumin. Apparently, the nature of the activation of purified luciferase by these compounds lies in the removal of heavy metals.

A number of inhibitors of luminescent bacterial extracts were studied. Cytochrome c, methemo-

[1] On leave to AEC, Washington.

[2] J. R. Totter and M. J. Cormier, *J. Biol. Chem.* 216, 801–812 (1955).

[3] W. D. McElroy and A. A. Green, *Arch. Biochem. and Biophys.* 56, 240–255 (1955).

TABLE 14. A COMPARISON OF THE DPNH OXIDASE ACTIVITY OF DIFFERENT PREPARATIONS OF BACTERIAL LUCIFERASE

Organism Used	Light Production (counts per 5 sec)		DPNH Oxidation Rate ($-\Delta E_{340}$ per 100 sec)			
			Crude Extract		Purified Extract	
	Crude Extract	Purified Extract	Menadione	$K_3Fe(CN)_6$	Menadione	$K_3Fe(CN)_6$
A. fischeri (acetone-dried powders)	188,000	188,000	1.500	0.345	0.000	0.000
A. fischeri (lysed cells)	450,000	500,000	0.338	0.110	0.255	0.027
Coccobacillus (lysed cells)	175,000	175,000	0.010	0.010	0.040	0.010

globin, ferricyanide, and menadione inhibit luminescence by competing with luciferase for the hydrogen of $FMNH_2$. The reduction of the above compounds by the extracts was found to be FMN dependent and to proceed nonenzymically. Spontaneous reactions of the type described here may be of general importance in biological systems.

The luminescence by purified luciferase preparations in the presence of $FMNH_2$ and aldehyde was strongly inhibited by a *p*-chloromecuribenzoate, a well-known sulfhydryl inhibitor. This was interpreted to mean that luciferase is a sulfhydryl enzyme.

Ferric and ferrous iron inhibit luminescence, but the effect is only 1% of that observed when the corresponding 8-hydroxyquinoline chelates are used. The evidence indicates that ferrous iron and its chelate inhibit by combining with luciferase, whereas ferric iron and its chelate act as oxidants for $FMNH_2$.

Hemin has been found to combine very effectively with luciferase, 0.0002 μmole per milliliter of hemin giving a 91% inhibition of luminescence.

Conversion of Glycine to Serine in Extracts of Luminous Bacteria[4]

J. W. Davis J. R. Totter
A. N. Best

An understanding of the interconversions between various nucleotides and deoxynucleotides rests largely on the knowledge of C_1 transfer mechanisms. The water extracts of an acetone-dried luminous bacterium have been found to possess a very active enzyme system for the transfer of a hydroxymethyl group to C^{14}-glycine, yielding C^{14}-serine. The transfer of a hydroxymethyl group from serine has been postulated as occurring in the formation of hydroxymethylcytosine in phage.[5]

Dowex-1-treated water extracts of a coccobacillus provided the enzyme. The β-carbon of either serine or formaldehyde could serve as the source of the hydroxymethyl group, but the fixation of either required both pyridoxal phosphate and tetrahydrofolic acid (THFA) as cofactors. Folic acid, with or without reduced diphosphopyridine nucleotide (DPN), and citrovorum factor failed to replace THFA in the system. Formate, even in the presence of reduced DPN and adenosine triphosphate (ATP), could not furnish the hydroxymethyl group, and, likewise, methanol was found to be inactive.

Aging of the water extracts impaired the activity of the enzyme. However, these extracts could be restored to full activity by the addition of 2,3-dimercaptopropanol (BAL).

While THFA is a cofactor, it is required in substrate amounts. A 1:1 molar relation between the concentration of THFA and that of serine, added as a source of the hydroxymethyl group, seems to be optimum.

The present system displays no requirement for DPN, ATP, and Mg^{++}, previously found necessary for the operation of other glycine-serine transfer systems.[6] This fact, coupled with the inability of formic acid to serve as a precursor of serine, leads to the belief that this preparation may be relatively free of enzymes which could detract from a study of C_1 transfers. Reactivation by BAL suggests that the enzyme activity depends upon the presence of free sulfhydryl groups on the enzyme surface.

PHOTOSYNTHESIS

Delayed Light in Photosynthesis

W. A. Arnold H. K. Sherwood

Chlorophyll absorbs the energy that drives the process of photosynthesis. This is shown by the detailed agreement between the action spectrum of photosynthesis and the absorption spectrum of the chlorophyll-protein complex. The fact that chlorophyll in the plant is fluorescent shows that the absorption of light leads to an excited state of chlorophyll. The next step in photosynthesis, the transfer of this excitation energy on toward the reduction of carbon dioxide, has until lately been immune to direct experimental study. The finding, made in Oak Ridge, that photosynthetic plants emit light for some time after they have been illuminated, together with the hypothesis that the delayed light represents the reversal of the early steps in photosynthesis, gives a direct method for studying the initial reactions. For such a study it is necessary to have information on how the delayed light decays with time in the dark.

The intensity of the delayed light as a function of the time in the dark has been measured from 10^{-4} to 10^4 sec. In general, not only the intensity

[4]See also, J. V. Passonneau and J. R. Totter, *Biol. Semiann. Prog. Rep. Aug. 15, 1955*, ORNL-1953, p 97–98.

[5]S. S. Cohen, *Cold Spring Harbor Symp. Quant. Biol.* 18, 221–235 (1953).

[6]R. L. Kisliuk and W. Sakami, *J. Am. Chem. Soc.* 76, 1456–7 (1954).

of the delayed light but also the shape of the decay curve depends upon the history of illumination. *Chlorella* plants, in the dark for 20 to 30 min, exposed to one short flash of light give a second-order decay curve. The results are being published in the *Proceedings of the 1955 Gatlinburg Conference on Photosynthesis.*

Since these experiments are but a part of the study of the first reactions in photosynthesis, their interpretation must wait.

TRACER STUDIES ON INTERMEDIARY METABOLISM

S. F. Carson

E. F. Phares R. Weimberg[1]
M. I. Dolin M. V. Long

The One-Carbon Fragment Produced by Propionic Acid Bacteria

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

A previous report[2] described an enzyme system, from combined cell extracts of *Propionibacterium pentosaceum* and *Veillonella gazogenes*, which decarboxylated succinyl-CoA to propionate and CO_2. However, in the presence of pyruvate and reduced diphosphopyridine nucleotide (DPNH), the one-carbon (C_1) precursor of CO_2 was "fixed" into the malate carboxyl group, apparently through oxalacetate (OAA) and the malic dehydrogenase (MDH) system. When catalytic amounts of malate replaced the substrate (succinyl-CoA), pyruvate was converted to lactate. The latter transformation did not occur directly by means of lactic dehydrogenase but probably through the agency of a "catalytic cycle" (Fig. 6) involving malate, lactate, C_1 fragment, pyruvate, and OAA.

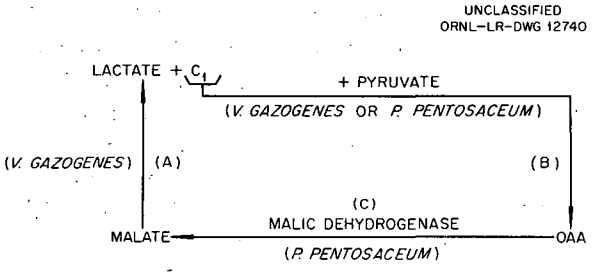

Fig. 6. Catalytic Cycle.

Direct evidence was obtained for participation of the MDH system in the conversion of lactate to pyruvate. Starch electrophoresis of each of the two cell extracts yielded an active enzyme fraction (from each extract), which oxidized DPNH in the presence of malate and pyruvate when it was added to the *other* cell extract. The MDH "activity fraction," tested by the oxidation of DPNH in the presence of OAA, corresponded almost exactly with the active enzyme fraction (discussed above) from the *P. pentosaceum* extract. A second electrophoresis purification of an active enzyme fraction yielded similar results. For the conversion of lactate to pyruvate, authentic MDH preparations replaced the requirement for *P. pentosaceum* extract, as well as for the enzyme fraction derived therefrom. Either bacterial extract exchanged C^{14} from pyruvate-2-C^{14} into OAA. With the *V. gazogenes* extract, with or without DPNH, spectrophotometric data indicated that substrate amounts of malate and pyruvate caused an accumulation of OAA. The *P. pentosaceum* extract, on the other hand, caused a rapid decrease in OAA, with a concomitant DPNH oxidation; this phenomenon was due to the large amount of MDH present in the *P. pentosaceum* extract.

Proceeding clockwise from malate (on Fig. 6), the addition of *V. gazogenes* extract causes accumulation of OAA (through reactions A and B) if pyruvate is present to act as an acceptor for the C_1 fragment. The OAA carboxylase (reaction B) is present in both bacterial extracts; this "OAA carboxylase" is markedly different from the known OAA carboxylases, as it neither fixes "free CO_2" into OAA nor requires the usual "activated pyruvate" cofactor system. Addition of *P. pentosaceum* extract, which contains MDH, converts the OAA to malate (reaction C) to complete the cycle.

The Enzyme-Substrate Complex of DPNH Peroxidase

M. I. Dolin

The enzyme-substrate complex formed between DPNH peroxidase flavoprotein and pyridine nucleotides[3,4] offers a means of studying the factors involved in substrate binding to an enzyme surface and may offer an insight into the mechanism of electron transport and associated reactions at the pyridine nucleotide–flavoprotein level. Nicotinamide mononucleotide (NMN), nicotinamide riboside, and *N'*-methylnicotinamide were prepared,

[1]Research Associate.

[2]S. F. Carson and E. F. Phares, *Biol. Semiann. Prog. Rep. Aug. 15, 1955*, ORNL-1953, p 79–82.

[3]*Arch. Biochem.* (in press).

[4]M. I. Dolin, *Biol. Semiann. Prog. Rep. Aug. 15, 1955*, ORNL-1953, p 82–83.

and their ability to cause the formation of the spectrophotometrically visible complex with the enzyme was compared with that of diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN).

The characteristics of the DPNH-flavoprotein complex have been described previously.[3,4] Using chemically reduced enzyme, similar spectral changes at 450 mμ are produced with TPN and NMN; however, with a 100-fold excess of these nucleotides, the change in extinction in the region 520 to 600 mμ is only about one-fifth that produced by DPN. Reduced triphosphopyridine nucleotide (TPNH) gives similar results with the oxidized enzyme. None of the changes described above occur in control experiments in which the enzyme is replaced with a solution of flavine adenine dinucleotide (FAD). By adding graded levels of oxidized nucleotide to chemically reduced enzyme and plotting the delta 450-mμ values against nucleotide concentration, typical saturation curves are obtained. The following apparent association constants can be calculated for the formation of the complex [presumably reduced nucleotide·FPH_2 (reduced flavoprotein), since hydrosulfite is present in excess]: DPN or DPNH, 3×10^4 *M*; TPN, 3×10^3 *M*; NMN, 2.5×10^3 *M*.

With the chemically reduced enzyme, addition of excess nicotinamide riboside yields only a small increase in absorption at 450 mμ and no detectable absorption above 520 mμ, whereas *N'*-methylnicotinamide in 300-fold excess causes no detectable change in absorption either at 450 mμ or above 520 mμ. Reduced *N'*-methylnicotinamide bleaches the oxidized enzyme. Reduced NMN and reduced nicotinamide riboside have not been tested as substrates. Enzymatically, TPNH undergoes oxidation at the same rate as that for DPNH, but there is only a 50% increase in rate upon the addition of peroxide; the very slow removal of reduced *N'*-methylnicotinamide, as shown by absorption measurements, is not increased by peroxide.

A comparison of the characteristic reactions of the pyridinium compounds described above suggests that the spectrophotometrically visible DPNH·flavoprotein complex may be kinetically active. The adenylic acid moiety and the 5′ phosphate on nicotinamide riboside are implicated in the binding of substrate to the flavoprotein.

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

R. Weimberg

In a study of the intermediary metabolism of *l*-arabinose by *Pseudomonas saccharophila*,[5] it was shown that *l*-arabinose is oxidized to *l*-arabono-γ-lactone. The lactone is enzymatically hydrolyzed to *l*-arabonic acid, which, in turn, is oxidized to α-ketoglutaric acid. The α-ketoglutaric acid does not arise via the tricarboxylic acid (TCA) cycle. The only cofactor required for these reactions is DPN. The studies to be reported here are concerned with the characterization of the intermediates occurring between *l*-arabonic acid and α-ketoglutaric acid.

The addition of *l*-arabonic acid to a reaction mixture containing crude enzyme from *P. saccharophila* at pH 9.0 in the absence of DPN results in the accumulation of at least one intermediate, which has been demonstrated to be an α-keto acid. Analysis of the reaction mixture by paper chromatography has shown that there is only one other acid present in these reaction mixtures besides the substrate, *l*-arabonate, and that this compound, although an α-keto acid, is not α-ketoglutaric acid. This new intermediate is a suitable substrate for the reduction of DPN in the presence of the *P. saccharophila* enzyme. The compound may be separated from *l*-arabonate by gradient elution on a Dowex 1 column in the formate form with 0.2 *M* ammonium formate, pH 6.0.

Large amounts of the new intermediate are being prepared and purified in order that its chemical structure may be determined.

[5] R. Weimberg and M. Doudoroff, *J. Biol. Chem.* **217**, 607 (1955).

PLANT BIOCHEMISTRY

N. E. Tolbert
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A Bicarbonate-Glycolate Shift in Algae

N. E. Tolbert L. P. Zill
P. C. Kerr

A bicarbonate-chloride shift in blood by a Donnan equilibrium exchange accelerates the transfer of CO_2 or bicarbonate between the plasma and the red cells. During photosynthesis a rapid movement of CO_2 into plant cells must also occur. By analyzing the composition of the suspending medium for algae before and after rapid CO_2 photosynthetic fixation, a specific bicarbonate shift with the glycolate anion has been found.

During 2- to 30-min steady-state photosynthesis experiments with *Chlorella*, 3 to 10% of the total $C^{14}O_2$ fixed is excreted into the algal medium as glycolate. This excretion is dependent upon the conditions for active uptake of bicarbonate, namely, light for photosynthesis and the presence of oxygen and bicarbonate. When any of these conditions are not met, glycolate moves from the medium into the cell, where it accumulates in relatively large amounts for a short period of time. In continuous photosynthesis, however, glycolate did not accumulate in the medium beyond 3 to 8 mg/liter. The excretion of glycolate is independent of the pH of the medium as long as the pH is above 5.5. At lower pH values, around 3.5 to 2.5, glycolate excretion decreases, but the loss of many other cell constituents then occurs.

By alternate periods of photosynthesis with $C^{14}O_2$ and $C^{12}O_2$, the glycolate was shown to arise directly from the sugar phosphate esters of the photosynthetic carbon cycle. It then moved out of the cell in exchange for the bicarbonate ion or was metabolized to glycine and serine.

Since bicarbonate uptake during photosynthesis and its excretion during respiration represent a major ionic movement, a balance of it with the diffusible glycolate anion would lessen undesirable loss or absorption of cations by the cell. If a Donnan equilibrium existed across the cell membrane, a change in bicarbonate ion concentration should result in an opposite movement of glycolate to help restore the following equality:

$$\frac{(\text{glycolate}^-)_{cell}}{(\text{glycolate}^-)_{medium}} = \frac{(HCO_3^-)_{cell}}{(HCO_3^-)_{medium}}$$

That glycolate functions in this manner rather than some other organic anion may be because of several factors. Glycolic acid, a two-carbon α-hydroxy acid, is one of the strongest, yet smallest, organic acids in the cell. It is a unique acid in that it is being directly synthesized from the photosynthetic carbon cycle, with the result that the reservoir of it in the cell is closely associated with photosynthesis and the obligated CO_2 movement.

This bicarbonate-glycolate shift may be involved in acid bursts, pH shifts, CO_2 bursts, and quantum efficiency, as calculated from short-time or flashing-light photosynthesis experiments. Algae also acquire rather long photosynthetic induction phases after having been kept in the dark. During dark respiration *Chlorella* are known to metabolize glycolate to glycine and serine. Thus glycolate reabsorption and accumulation inside the cell cannot persist for a long period in the dark. When the algae are placed in the light, a photosynthetic induction period would ensue while the glycolate pool was replenished.

Anthrone Reagent and Its Application to the Determination of the Heptuloses

L. P. Zill D. A. Mondon

The use of the anthrone reagent for the quantitative determination of the hexoses has been routine in this laboratory for several years. The nonspecificity of this reagent has been a unique advantage in the analysis of samples containing any single sugar. The method is extremely sensitive and simple in procedure. However, the widely recognized instability of the reagent has been a constant source of difficulty. An investigation of this instability has been made, and it has been found that the instability can be circumvented by the control of several factors. In addition, it has been established that the reagent may be used for the quantitative determination of another class of carbohydrates, the heptuloses.

The oxidation of anthrone by atmospheric oxygen produces dianthrone and anthraquinone. Neither of these two compounds has the spectral properties exhibited by aged anthrone samples. However, nitration of these compounds, owing to the presence

of nitrate ion in the sulfuric acid solvent, has been shown to be the basis for the reagent instability. The absorption spectrum of aged anthrone samples has a single peak with a maximum at about 430 mμ, which is in the characteristic range for nitrated aromatic compounds. Addition of nitrate ion to fresh anthrone reagent produces, immediately, an absorption spectrum identical with that mentioned above. The oxidation of anthrone by oxygen can be partially prevented by addition of the antioxidant, thiourea, to the reagent, as proposed by Roe.[1] Complete inhibition of the nitration reaction does not appear to be possible. However, the following conditions provide a reagent with very good stability: (1) sulfuric acid having the lowest nitrate concentration available should be used; (2) low acid concentration (66%) in the reagent will prevent an optimum condition for nitration; (3) storage in a refrigerator at 4°C strongly retards the reaction rate of nitration.

Previous experiments in this laboratory demonstrated that furfural derivatives were formed by the action of acid on heptuloses. Since the formation of furfural or a furfural derivative is a primary requisite for the anthrone reaction, it appeared that this reaction could be used for the determination of the heptuloses. Indeed, a linear relationship of absorbancy to concentration has now been demonstrated for the reaction of anthrone with the heptuloses.

[1] J. H. Roe, *J. Biol. Chem.* **212**, 335–43 (1955).

BIOPHYSICS

J. S. Kirby-Smith[1]
M. L. Randolph
M. Slater
A. Faberge[2]

Cockcroft-Walton Accelerator Activity

M. L. Randolph

The accelerator program is intended to establish and operate a well-characterized fast-neutron facility for irradiation studies with small, sensitive biological materials.

The following technical advances have been effected: a low-scatter, demountable water-cooled target assembly; introduction of zirconium tritide targets; a precision sphere for locating biological materials; and a rearrangement of the electronics for detectors.

The scope of potential biological studies with this facility is suggested by the accompanying list (Table 15) of irradiations, mostly preliminary, already performed. Only in the *Habrobracon* experiment was the dose given too low to observe the anticipated biological effect. The *Tradescantia* studies are the first conclusive biological work done and are discussed elsewhere.[3] The *Drosophila* experiment indicated that the effectiveness of 14-Mev neutrons at these doses lay between that of the ORNL 86-in. cyclotron neutrons (average energy 1 Mev) and 250-kvp x rays. Detailed results are not yet available from the mouse testicular damage experiment.

In collaboration with personnel of the Electronuclear Research Division, the 14-Mev neutron flux has been used in determining (n,α) cross

[1]On leave until June 1956.
[2]Temporary employee, arrived Jan. 24, 1956.

[3]A. D. Conger, M. L. Randolph, and H. Johnston, Cytology and Genetics Section of this report.

TABLE 15. USES OF THE COCKCROFT-WALTON ACCELERATOR

Biological Material	Number of Points Irradiated and Maximum Dose (rep) at 5 cm	Energy (Mev)	Biological Effect Investigated	Investigator	Date
Tradescantia inflorescences	4; 12	3	Chromatid aberration	A. D. Conger	July 1954
	6; 50	3	Chromosome aberration	A. D. Conger	Oct. 1955
	5; 100	14	Chromosome aberration	A. D. Conger	Nov. 1955
	12; 120	3	Chromosome and chromatid aberration	A. D. Conger	Jan. 1956
	11; 370	14	Chromosome and chromatid aberration	A. D. Conger	Jan. 1956
Pollen	2; 56	3	Chromosome aberration	J. S. Kirby-Smith	May 1955
Grasshopper neuroblast	1; 4	3	Mitotic inhibition	M. E. Gaulden	Oct. 1955
	1; 16	14	Mitotic inhibition	M. E. Gaulden	Dec. 1955
Habrobracon	1; 13	3	Hatchability	R. C. von Borstel	Oct. 1955
Drosophila	5; 800	14	Recessive lethality	C. W. Edington	Dec. 1955
Mice testes	10; 40 (at 15 cm)	14	Testicular damage	E. F. Oakberg	Jan. 1956

sections for a number of nuclei.[4] This mutually beneficial cooperative venture is expected to be continued.

Yield studies have also been continued. With a 150-μa focused beam at 150 kv, the initial yield from tritide targets[5] is about 8 rep/min at 5.3 cm from the target but decreases so rapidly that 5 hr was required to deliver 800 rep. A somewhat higher, much more lasting yield has recently been obtained from an old target at a higher voltage and current. With drive-in of deuterium on a zirconium foil by an 800-μa focused beam at 250 kv, the *d-d* neutron yield after an hour approaches the equilibrium value of 0.6 rep/min at 5 cm. A low-scatter, water-cooled rotating target has just been installed and was found to give a *d-t* yield of 1200 rep in its first hour of operation at 200 kv.

Intercomparisons between measurements of total ionization produced in hydrogeneous chambers and neutron flux determine the absolute dosimetry. Little 14-Mev neutron contamination in the *d-d* flux is found by silver activation, and little *d-d* neutron contamination in the *d-t* flux is found by measurement of the protons, with discrimination against alphas from the *d-t* reaction. Examination of 15 recoil-particle tracks in photographic emulsion exposed to the *d-t* flux indicated the neutron energy to be 14 ± ½ Mev in every case.[6] An angular distribution study showed the 14-Mev flux to be very nearly isotropic, as expected.

The dose-to-flux ratio for 14-Mev neutrons has been calculated for several materials, taking into account both elastic and inelastic reactions. It was found that the dose per atom is roughly constant for H, C, O, and N; that using the total cross sections and assuming only elastic scattering leads to values 12% low for wet tissue; and that of the dose in wet tissue 67½% arises from elastic scattering by hydrogen, 9½% from elastic scattering by other elements, and 23% from inelastic reactions.

A routine for monitoring biological irradiations has been developed, with pre- and postcalibration and electronic checks of two independent monitors and with cross checks and a chart record during the irradiation.

The accelerator facility has begun to be utilized for quantitative investigations of a variety of biological effects. Although the present dose rates may be increased, they are adequate for a number of studies. The *d-d* dosimetry is at a final stage, and the *d-t* dosimetry is at an intermediate stage.

[4]H. G. Blosser, C. D. Goodman, T. H. Handley, and M. L. Randolph, *Phys. Rev.* 100, 429–430 (1955).

[5]ORNL Radioisotopes Catalog, p 165 (1956).

[6]Courtesy of H. G. Blosser, Electronuclear Research Division.

Radiological Physics

M. Slater

Work on problems of dosimetry and exposure facilities, in cooperation with other groups in the Biology Division, has accounted for most of the activities during the report period.

The chronic irradiation facility, designed in cooperation with the Pathology-Physiology Section, has been in operation since October 1955. A photograph of a part of the facility is shown in Fig. 7. An extensive series of measurements with several dosimetry systems was made on the 117-curie polonium-beryllium source (initial emission of 3×10^8 neutrons/sec) used in the neutron facility and the 150-mc Co^{60} source used in the gamma-ray facility. The inverse-square method previously described[7] was used with thimble ion chambers[8] having walls of polyethylene, polystyrene, or lead and containing various gases (ethylene, acetylene, carbon dioxide, or air); with tissue-equivalent and Victoreen chambers; as well as with several polyethylene wall–ethylene gas proportional counters.[9] Data were also obtained with chemical dosimeters,[10] gamma-ray film dosimeters, and a calibrated long counter. The neutron spectrum was determined with a proton recoil spectrometer.[11] A preliminary analysis of the data indicates reasonable agreement among the methods and a 5 to 10% gamma dose contamination in the neutron source. A further analysis is being carried out.

Designs have been established for a chronic gamma-irradiation facility for the Mammalian Genetics and Development Section and for a 250-kv x-ray irradiation facility for the Mammalian Recovery

[7]M. Slater, *Biol. Semiann. Prog. Rep. Aug. 15, 1955*, ORNL-1953, p 131.

[8]E. B. Darden, Jr., and C. W. Sheppard, topical report, ORNL-1002 (1951).

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

[10]S. C. Sigoloff, USAF School of Aviation Medicine, Randolph Field, Texas (private communication). The author is indebted to Lt. Sigoloff for cooperation in performing this measurement.

[11]R. G. Cochrane and K. M. Henry, *Rev. Sci. Instr.* 26, 757 (1955). The author is indebted to Mr. Henry for cooperation in performing this measurement.

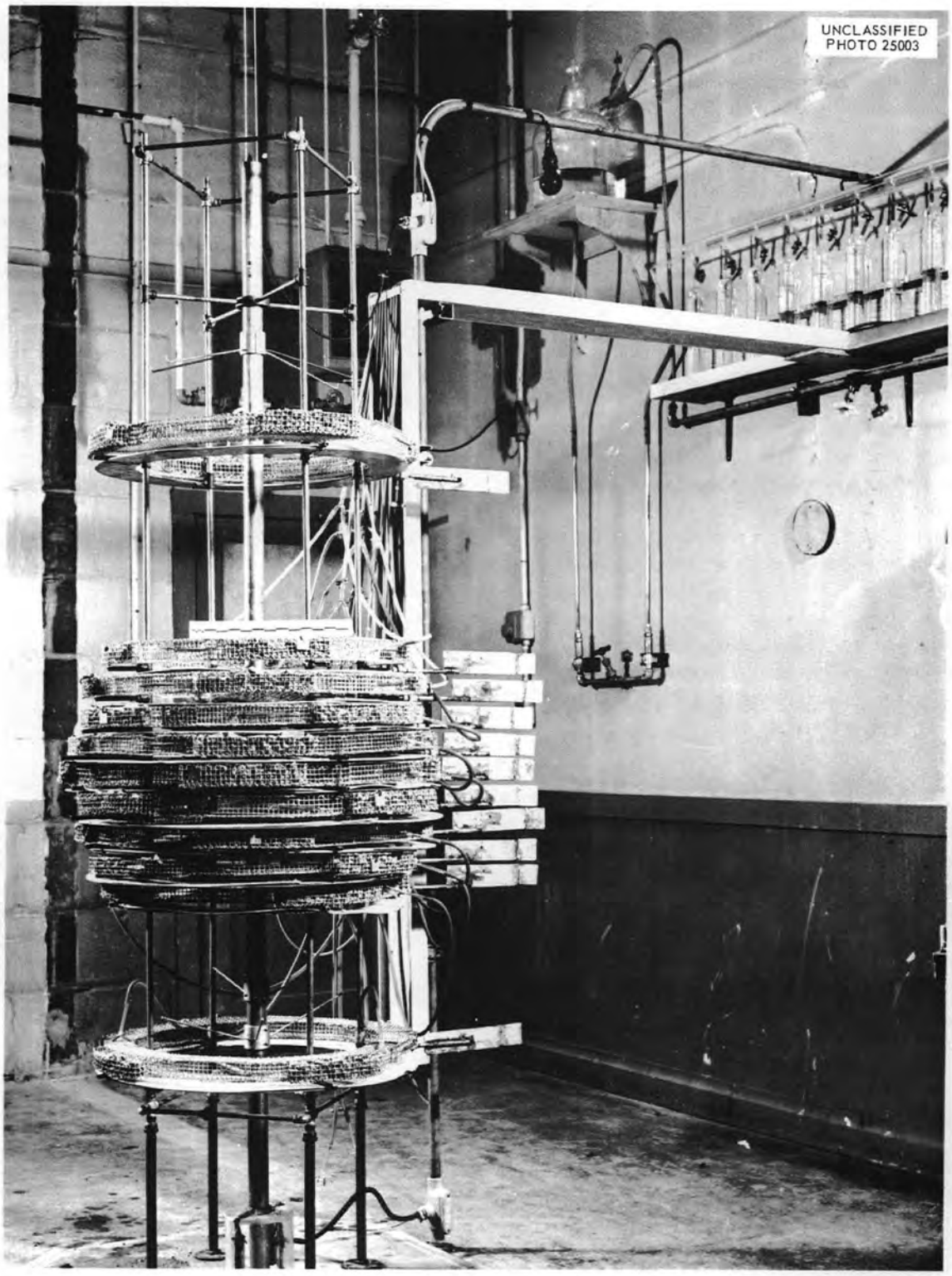

Fig. 7. Portion of Chronic Irradiation Facility.

Section and have been submitted to the Engineering Department for construction.

The operating conditions of the G-E Maxitron x-ray machine are being investigated in an attempt to ensure more stable and reliable exposures and dosimetry. A mass recalibration of all x-ray dosimeters is being carried out.

A combination ion chamber–proportional counter dosimeter for the accurate determination of the gamma-ray contamination in a neutron field has been designed and is being fabricated.

Through these cooperative ventures, one large exposure facility for biological materials is now operating and two others are in the advanced design stage. The availability of a large neutron source served as an opportunity for a precise intercomparison of neutron dosimetry systems. In general, an attempt has been made to put all exposure and dosimetry systems on a stable and reliable foundation.