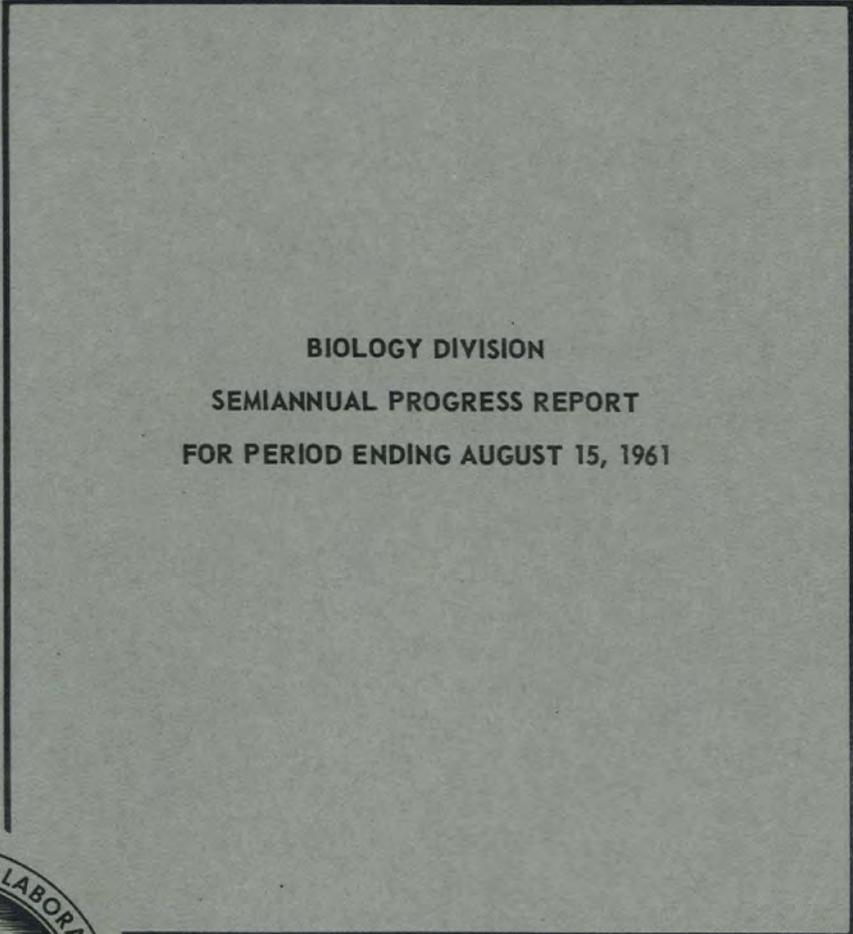


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BIOLOGY DIVISION
SEMIANNUAL PROGRESS REPORT
FOR PERIOD ENDING AUGUST 15, 1961



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BIOLOGY DIVISION
SEMIANNUAL PROGRESS REPORT
For Period Ending August 15, 1961

Alexander Hollaender, Director
Stanley F. Carson, Assistant Director

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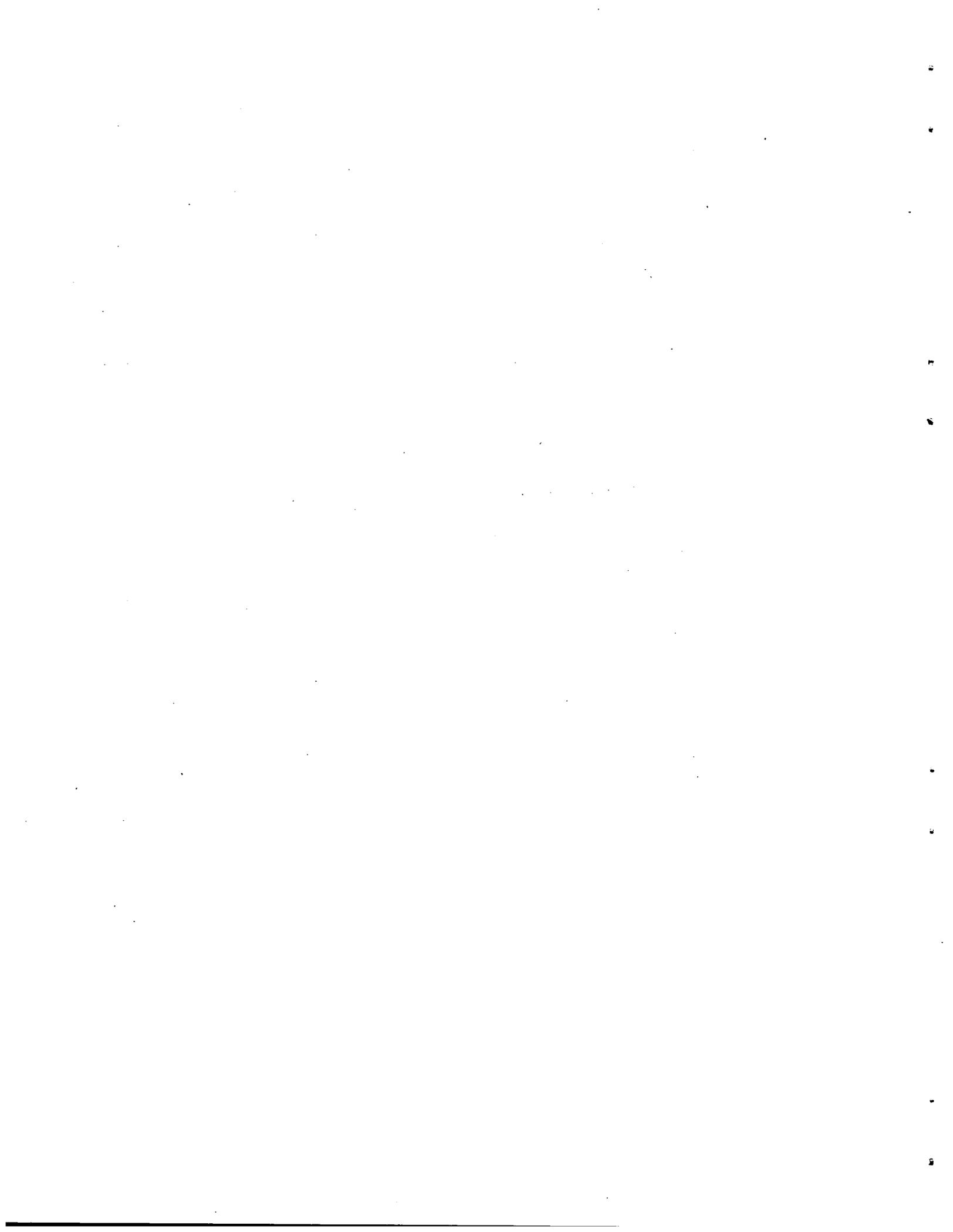
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PUBLICATIONS AND LECTURES

Publications. — During the past six months 318 papers and abstracts by members of the Biology Division have been published or have gone to press. This figure compares with 280 for the same period in 1960 and with 278 for the last reporting period, February 1961. Listed below are the 84 published papers. Not listed are 45 published abstracts and 142 papers and 47 abstracts in press.

The 1961 Gatlinburg Symposium, *Recovery of Cells from Injury*, will be published by the Wistar Institute of Anatomy and Biology as a December supplement to the *Journal of Cellular and Comparative Physiology*.

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Anderson, N. G.	Quartz flow cells for continuous spectrophotometric analysis of column effluents	<i>Anal. Chem.</i> 33 , 970-71 (1961)
Anderson, N. G., and W. D. Fisher	Studies on nuclei. IV. Evidence for the existence of DNA-protein strands in intact nuclei	<i>Exptl. Cell Research</i> 22 , 13-17 (1961).
Anderson, N. G., W. D. Fisher, and M. L. Anderson	Studies on isolated cell components. XV. The distribution of certain enzymes in the electrophoretic pattern of the soluble phase of rat liver	<i>Exptl. Cell Research</i> 23 , 318-23 (1961)
Anderson, N. G., Takashi Makinodan, and C. B. Norris	Studies on isolated cell components. XIII. The effect of heat on rat liver soluble proteins	<i>Exptl. Cell Research</i> 22 , 526-34 (1961)
Anderson, N. G., and H. D. Swanson	Studies on isolated cell components. XIV. An electrophoretic analysis of the soluble proteins of rat liver, kidney, brain, and testis	<i>Exptl. Cell Research</i> 23 , 58-68 (1961)
Arnold, William	Wave mechanics and muscular contraction	<i>J. Theoret. Biol.</i> 1 , 404-406 (1961)
Barber, A. A.	Inhibition of lipid peroxide formation by vertebrate blood serum	<i>Arch. Biochem. Biophys.</i> 92 , 38-43 (1961)
Barber, A. A., and Phillip Sheeler	Iron binding by vertebrate blood sera	<i>Comp. Biochem. Physiol.</i> 2 , 233-40 (1961)
Bender, M. A., and L. E. Mettler	Chromosome studies of primates. II. <i>Callithrix</i> , <i>Leontocebus</i> , and <i>Callimico</i>	<i>Cytologia</i> 25 (3-4), 400-404 (1960)
Bender, M. A., and Sheldon Wolff	X-ray-induced chromosome aberrations and reproductive death in mammalian cells	<i>Am. Naturalist</i> 95 , 39-52 (1961)
Brokaw, C. J.	Movement and nucleoside polyphosphatase activity of isolated flagella from <i>Polytoma uvella</i>	<i>Exptl. Cell Research</i> 22 , 151-62 (1961)
Brosseau, G. E., Benedetto Nicoletti, E. H. Grell, and D. L. Lindsley	Production of altered Y chromosomes bearing specific sections of the X chromosome in <i>Drosophila</i>	<i>Genetics</i> 46 , 339-46 (1961)

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AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Cameron, I. L., and D. M. Prescott	Relations between cell growth and cell division V. Cell and macronuclear volumes of <i>Tetrahymena pyriformis</i> HSM during the cell life cycle	<i>Exptl. Cell Research</i> 23 , 354-60 (1961)
Carr, D. H., M. L. Barr, E. R. Plunkett, M. M. Grumbach, A. Morishima, and E. H. Y. Chu	An XXY sex chromosome complex in Klinefelter subjects with duplicated sex chromatin	<i>J. Clin. Endocrinol. and Metabolism</i> 21 , 491-505 (1961)
Celada, Franco, and Takashi Makinodan	A new model to study hematopoietic transplanta- tion antigens	<i>J. Immunol.</i> 86 , 638-45 (1961)
Chu, E. H. Y., and M. A. Bender	Chromosome cytology and evolution in primates	<i>Science</i> 133 , 1399-1405 (1961)
Chu, E. H. Y., and Barbara A. Swomley	Chromosomes of lemurine lemurs	<i>Science</i> 133 , 1925-26 (1961)
Chu, E. H. Y., J. Warkany, and R. B. Rosenstein	Chromosome complement in a case of the "male Turner syndrome"	<i>The Lancet</i> 1 (Apr. 15), 786-88 (1961)
Clark, A. M., and V. J. Cristofalo	Some effects of oxygen on the insects <i>Anagasta kuehniella</i> and <i>Tenebrio molitor</i>	<i>Physiol. Zool.</i> 34 , 55-61 (1961)
Clayton, R. K., and William Arnold	Absorption spectra of bacterial chromatophores at temperatures from 300°K to 1°K	<i>Biochim. et Biophys. Acta</i> 48 , 319-23 (1961)
Cohn, W. E., and F. J. Bollum	Chromatography and desalting of nucleotides with ammonium bicarbonate on anion-exchange columns	<i>Biochim. et Biophys. Acta</i> 48 , 588-90 (1961)
Congdon, C. C.	Radiation and cancer	<i>Am. J. Med. Technol.</i> 27 , 165-71 (1961)
Congdon, C. C., and D. B. Duda	Prevention of bone marrow heterografting. Use of isologous thymus in lethally irradiated mice	<i>Arch. Pathol.</i> 71 , 311-23 (1961)
Congdon, C. C., Takashi Makinodan, Nazareth Gengozian, and Isabel C. Shekarchi	Early histologic changes in the lymphatic tissues of mice given foreign bone marrow	<i>Rev. Argent. de Cancerol.</i> 2 , 3-13 (1960)
de Serres, F. J.	Some aspects of the influence of environment on the radiosensitivity of microorganisms	In <i>Symposia of the Society for General Microbiology, XI, Microbial Reaction to Environment</i> (ed. by G. G. Meynell and H. Goeder), pp 196-216, University Press, Cam- bridge, 1961
Engel, M. S.	Morphology of <i>Nitrosomonas europaea</i> and classi- fication of the nitrifying bacteria	<i>J. Bacteriol.</i> 81 , 833-34 (1961)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Fisher, W. D., and C. L. Burger	Studies on nuclei. III. The effect of heat on the viscosity of nucleoprotein in 1 M NaCl	<i>Exptl. Cell Research</i> 22 , 13-17 (1961)
Foard, D. E., and A. H. Haber	Anatomic studies of gamma-irradiated wheat growing without cell division	<i>Am. J. Botany</i> 48 , 438-46 (1961)
Gengozian, Nazareth, Takashi Makinodan, and I. C. Shekarchi	Transplantation of antibody-forming cells in lethally irradiated mice	<i>J. Immunol.</i> 86 , 113-22 (1961)
Gengozian, Nazareth, I. S. Urso, R. R. Carter, and Takashi Makinodan	Immune status of irradiated mice treated with adult bone marrow and fetal hematopoietic tissue	<i>Transplant. Bull</i> 27 , 87-90 (1961)
Goodman, Joan W., and C. C. Congdon	Blood-marrow mixtures in irradiated mice. Effect of blood injection on bone marrow transplantation in lethally irradiated mice	<i>Arch. Pathol.</i> 72 , 18-26 (1961)
Goodman, Joan W., and L. H. Smith	Erythrocyte life span in normal mice and in radiation bone marrow chimeras	<i>Am. J. Physiol.</i> 200 , 764-70 (1961)
Green, D. M., and D. R. Krieg	The delayed origin of mutants induced by exposure of extracellular phage T4 to ethyl methane sulfonate	<i>Proc. Natl. Acad. Sci. U.S.</i> 47 , 64-72 (1961)
Grell, E. H.	<i>Melanogaster</i> - New mutants	<i>Drosophila Info. Serv.</i> 34 , 50 (1960)
Haber, A. H., and W. L. Carrier	Pigment formation and photosynthesis in dormant lettuce seeds	<i>Plant Physiol.</i> 36 , 351-53 (1961)
Haber, A. H., W. L. Carrier, and N. J. Enochs	Gibberellin action on growth of seedlings in the absence of photosynthesis	<i>Nature</i> 190 , 1034-35 (1961)
Haber, A. H., W. L. Carrier, and D. E. Foard	Metabolic studies of gamma-irradiated wheat growing without cell division	<i>Am. J. Botany</i> 48 , 431-38 (1961)
Hollaender, Alexander	Educational opportunities of the Biology Division, Oak Ridge National Laboratory	<i>J. Tenn. Acad. Sci.</i> 36 , 98-108 (1961)
Hollaender, Alexander, and S. F. Carson	<i>Biology Division Semiannual Progress Report for Period Ending February 15, 1961</i>	ORNL-3095
Jacobson, K. B.	Effect of substrate structure on activity of pigeon liver acetyl transferase	<i>J. Biol. Chem.</i> 236 , 343-48 (1961)
Jagger, John	A small and inexpensive ultraviolet dose-rate meter useful in biological experiments	<i>Radiation Research</i> 14 , 394-403 (1961)
Khym, J. X., and W. E. Cohn	Amine-induced cleavage of periodate-oxidized nucleotide residues	<i>J. Biol. Chem.</i> 236 , PC 9-PC 10 (1961)
Kimball, A. W.	Confidence intervals for recombination experiments with microorganisms	<i>Biometrics</i> 17 , 150-52 (1961)
Kimball, R. F., Nenita Gaither, and Stella W. Perdue	Metabolic repair of premutational damage in <i>Paramecium</i>	<i>Intern. J. Radiation Biol.</i> 3 , 133-47 (1961)

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AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Kimball, R. F., and L. Vogt-Köhne	Quantitative cytochemical studies on <i>Paramecium aurelia</i> . IV. The effect of limited food and starvation on the macronucleus	<i>Exptl. Cell Research</i> 23 , 479-87 (1961)
Kretchmar, A. L., and C. C. Congdon	Biochemical changes and increased liver weight in bone marrow chimeras	<i>Am. J. Physiol.</i> 200 , 102- 106 (1961)
Lindsley, D. L., C. W. Edington, and E. S. Von Halle	Sex-linked recessive lethals in <i>Drosophila</i> whose expression is suppressed by the Y chromosome	<i>Genetics</i> 45 , 1649-70 (1960)
Lynn, W. G., and J. N. Dent	A comparison of the responses of <i>Triturus</i> and <i>Desmognathus</i> to thyroid-stimulating hormone administration	<i>Biol. Bull.</i> 120 , 54-61 (1961)
McDonald, T. P., and Masahide Asano	Effects of nitrogen mustard on the mouse brain	<i>Am. J. Pathol.</i> 38 , 695- 709 (1961)
Maisin, Jean, and Jean Moutschen	Chemical protection of the alimentary tract of whole-body x-irradiated mice. II. Chromosome breaks and mitotic activity	<i>Exptl. Cell Research</i> 21 , 347-52 (1960)
Mans, R. J., and G. D. Novelli	In vitro amino acid incorporation into particle protein from maize seedlings	<i>Biochim. et Biophys. Acta</i> 50 , 287-300 (1961)
Mans, R. J., and G. D. Novelli	Measurement of the incorporation of radioactive amino acids into protein by a filter paper disk method	<i>Arch. Biochem. Biophys.</i> 94 , 48-53 (1961)
Mazur, Peter	Physical and temporal factors involved in the death of yeast at subzero temperatures	<i>Biophysical Journal</i> 1 , 247-64 (1961)
Moutschen, Jean	Differential sensitivity of mouse spermatogenesis to alkylating agents	<i>Genetics</i> 46 , 291-99 (1961)
Nicoletti, Benedetto	New mutants - w ^{cf} : white coffee	<i>Drosophila Info. Serv.</i> 34 , 52-53 (1960)
Nicoletti, Benedetto, and D. L. Lindsley	Cytogenetic analysis of T(X;Y)'s	<i>Drosophila Info. Serv.</i> 34 , 95-97 (1960)
Nicoletti, Benedetto, and D. L. Lindsley	Translocations between the X and the Y chromosomes of <i>Drosophila melanogaster</i>	<i>Genetics</i> 45 , 1705-22 (1960)
Parsons, D. F., M. A Bender, E. B. Darden, Jr., G. T. Pratt, and D. L. Lindsley	Electron microscopy of plasma-cell tumors of the mouse. II. Tissue cultures of the X5563 tumor	<i>J. Biophys. Biochem.</i> <i>Cytol.</i> 9 , 369-81 (1961)
Parsons, D. F., and E. B. Darden, Jr.	A technique for the simultaneous dirt-free lead staining of several electron microscope grids of thin sections	<i>J. Biophys. Biochem.</i> <i>Cytol.</i> 8 , 834-35 (1961)
Parsons, D. F., E. B. Darden, Jr., D. L. Lindsley, G. T. Pratt, and M. P. Edwards	Electron microscopy of plasma-cell tumors of the mouse. I. MPC-1 and X5563 tumors	<i>J. Biophys. Biochem.</i> <i>Cytol.</i> 9 , 353-68 (1961)
Peck, H. D.	Evidence for the reversibility of the reaction catalyzed by adenosine-5'-phosphosulfate reductase	<i>Biochim. et Biophys. Acta</i> 49 , 621-24 (1961)

AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Perkins, E. H., M. A. Robinson, and Takashi Makinodan	Agglutinin response, a function of cell number	<i>J. Immunol.</i> 86 , 533-37 (1961)
Phan The Tran, and M. A. Bender	Preservation of living cells: applications to bone marrow	<i>Rev. franç. études clin. biol.</i> 5 , 1021-29 (1960)
Popp, R. A.	Regression of grafted bone marrow in homologous irradiated mouse chimeras	<i>J. Natl. Cancer Inst.</i> 26 , 629-40 (1961)
Prescott, D. M.	The growth-duplication cycle of the cell	In <i>International Review Cytology</i> (ed. by G. H. Bourne and J. F. Danielli), vol 11, pp 255-82, Academic Press, New York, 1961
Prescott, D. M., and R. F. Kimball	Relation between RNA, DNA, and protein syntheses in the replicating nucleus of <i>Euplotes</i>	<i>Proc. Natl. Acad. Sci. U.S.</i> 47 , 686-93 (1961)
Rabson, Robert, R. J. Mans, and G. D. Novelli	Changes in cell-free amino acid incorporating activity during maturation of maize kernels	<i>Arch. Biochem. Biophys.</i> 93 , 555-62 (1961)
Randolph, M. L.	Quantitative studies of electron spin resonances produced in biologically significant materials by ionizing radiations	In <i>Free Radicals in Biological Systems</i> (ed. by M. S. Blois, Jr., R. O. Lindblom, H. W. Brown, M. Weissbluth, and R. M. Lemmon), pp 249-61, Academic Press, New York, 1961
Randolph, M. L., H. G. Jones, and D. L. Parrish	Decay of polonium-beryllium fast neutron sources	<i>Health Physics</i> 5 , 45-49 (1961)
Rogers, Palmer, and G. D. Novelli	Cell-free synthesis of ornithine transcarbamylase	<i>Biochim. et Biophys. Acta</i> 44 , 298-309 (1960)
Russell, Liane B.	Genetics of mammalian sex chromosomes	<i>Science</i> 133 , 1795-1803 (1961)
Russell, Liane B., and Jean W. Bangham	Variegated-type position effects in the mouse	<i>Genetics</i> 46 , 509-25 (1961)
Russell, Liane B., and E. H. Y. Chu	An XXY male in the mouse	<i>Proc. Natl. Acad. Sci. U.S.</i> 47 , 571-75 (1961)
Sandler, L., P. Hart, and Benedetto Nicoletti	Phenotypic changes associated with chromosome rearrangements at the <i>y</i> locus	<i>Drosophila Info. Serv.</i> 34 , 103-04 (1960)
Setlow, R. B.	The action spectrum for the reversal of the dimerization of thymine by ultraviolet light (Preliminary Note)	<i>Biochim. et Biophys. Acta</i> 49 , 237-38 (1961)
Smith, L. H., and C. C. Congdon	Modification of radiation injury by leukemoid leukocytes	<i>Lab. Invest.</i> 10 , 617-26 (1961)

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AUTHOR(S)	TITLE OF ARTICLE	PUBLICATION
Stuy, J. H.	Studies on the radiation inactivation of microorganisms. VII. Nature of the x-ray-induced breakdown of deoxyribonucleic acid in <i>Haemophilus influenzae</i>	<i>Radiation Research</i> 14, 56-65 (1961)
Upton, A. C.	The dose-response relation in radiation-induced cancer	<i>Cancer Research</i> 21, 717-29 (1961)
von Borstel, R. C.	Postmeiotic nuclear behavior in unseminated eggs of <i>Drosophila melanogaster</i>	<i>Drosophila Info. Serv.</i> 34, 110 (1960)
Vos, Otto, Joan W. Goodman, and C. C. Congdon.	Transplantation of chimeric bone marrow	<i>Intern. J. Radiation Biol.</i> 3, 29-39 (1961)
Welshons, W. J.	New mutants: <i>ln(1)pdf:pod-foot</i>	<i>Drosophila Info. Serv.</i> 34, 54 (1960)
Whiting, Anna R.	Temperature effects on lethal mutation rates of <i>Habrobracon</i> oocytes x irradiated in first meiotic metaphase	<i>Genetics</i> 46, 811-16 (1961)
Wust, C. J.	Inactivation of tryptophan synthetase from <i>Neurospora crassa</i> during dialysis	<i>Biochem. Biophys. Research Commun.</i> 5, 35-39 (1961)
(Members of the Biology Division)	<i>Summary of Reports of Bone Marrow Conferences Organized by Members of the Biology Division, ORNL</i>	Reprint No. 1813

Lectures. – Members of the Biology Division presented 168 lectures during the period February to August 1961, compared with 116 for each of the two preceding reporting periods. Of these, 96 were before professional societies at home and abroad, and 28 were presented as part of the Traveling Lecture Program. Speakers in foreign countries included Alexander Hollaender, Lazarus Astrachan, W. E. Cohn, F. J. de Serres, D. G. Doherty, F. T. Kenney, R. F. Kimball, J. S. Kirby-Smith, E. F. Oakberg, and Anna R. Whiting. Sheldon Wolff also lectured outside continental United States, giving a series of five lectures at the University of Puerto Rico at Rio Piedras and Mayaguez. Further details about the foreign speeches are given in the section "Foreign Travel."

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Adler, H. I.	The bacterial cell and its environment	Tennessee Academy of Science (Collegiate Division), Tusculum College, Greeneville
Adler, H. I.	Careers in biology	Roane County High School, Kingston, Tenn.
Adler, H. I. (and M. S. Engel)	Factors influencing the survival of bacteria after exposure to ionizing radiation	Symposium on Recovery of Cells from Injury, Gatlinburg, Tennessee (ORNL Biology Division Symposium)
Anderson, N. G.	Cell fractions	University of Tennessee, Knoxville
Anderson, N. G.	New approaches to cell structure	Duke University, Durham, N.C.
Anderson, N. G.	(1) The organization of cells (2) The origin of life	South Carolina Academy of Sciences, Wofford College, Spartanburg

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Arnold, W. A.	Electrons and muscle contraction	Sigma Xi Society, University of Tennessee, Knoxville
Astrachan, Lazarus (and Thelma N. Fisher)	Resemblance of bacterial RNA and DNA [<i>Federation Proc.</i> 20, 359 (1961)]	(1) Federation Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Atlantic City, N.J. (2) University of Southern California Medical School, Los Angeles (3) Fifth International Congress of Biochemistry, Moscow, USSR
Barber, M. L. (Anderson, N. G., M. L. Barber, and Sister Francis C. Ladd)	Rapid separation of adenosine nucleotides, and nucleotides and nucleosides from RNA [<i>Federation Proc.</i> 20, 354 (1961)]	Federation Am. Soc. Exptl. Biol. (Am. Physiol. Soc.), Atlantic City, N.J.
Becker, R. R.	Properties of polypeptidyl derivatives of chymotrypsin and ribonuclease	Symposium on Poly- α -Amino-Acids, sponsored by the National Research Council and the University of Wisconsin Research Comm., Madison, Wis.
Becker, R. R. (O. A. Darling and R. G. Ona)	Enzymatic properties of poly-DL-t-leucyl- α -chymotrypsin [<i>Federation Proc.</i> 20, 380 (1961)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Atlantic City, N.J.
Bender, M. A. (and P. C. Gooch)	Somatic chromosome aberrations in normal and irradiated humans [<i>Radiation Research</i> 14, 451 (1961)]	Radiation Research Society, Washington, D.C.
Bezkorovainy, Anatoly (and D. G. Doherty)	Carbohydrate-containing proteins of bovine platelet walls [<i>Federation Proc.</i> 20, 385 (1961)]	Federation Am. Soc. Exptl. Biol. (Biochem. Sect.), Atlantic City, N.J.
Brockman, H. E.	Genetic studies at the <i>ad-3</i> region of <i>Neurospora</i>	University of North Carolina, Chapel Hill
Capalbo, E. E. (Paul Urso, and W. D. Gude)	Use of 0.1 μ diffusion chambers for primary and secondary agglutinin responses by spleen cells [<i>Federation Proc.</i> 20, 25 (1961)]	Federation Am. Soc. Exptl. Biol. (Am. Assoc. Immunol.), Atlantic City, N.J.
Carson, S. F. (Phares, E. F., and S. F. Carson)	Mechanism of methylmalonyl isomerase	Am. Soc. Microbiol., Chicago, Ill.
Celada, Franco (N. G. Anderson and Takashi Makinodan)	Solubilization and purification of homologous transplantation antigens in the mouse [<i>Radiation Research</i> 14, 455-56 (1961)]	Radiation Research Society, Washington, D.C.
Celada, Franco (and Rachel R. Carter)	Homograft-rejection capacity of isolated spleen cells [<i>Federation Proc.</i> 20, 33 (1961)]	Federation Am. Soc. Exptl. Biol. (Am. Physiol. Soc.), Atlantic City, N.J.
Celada, Franco (and W. J. Welshons)	A quantitative immunogenetic study of the male antigens in mice [<i>Radiation Research</i> 14, 456 (1961)]	Radiation Research Society, Washington, D.C.

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SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Chu, E. H. Y.	Chromosomal stabilization of cell strains	Syverton Memorial Symposium on Analytic Cell Cultures, 12th Annual Meeting of the Tissue Culture Assoc., Detroit, Mich.
Clayton, R. K.	Carotenoid-chlorophyll interactions in photosynthetic bacteria [<i>Federation Proc.</i> 20, 135 (1961)] (Read by title)	Federation Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Atlantic City, N.J.
Clayton, R. K.	Catalase in the physiology of photosynthesis	University of Florida, Gainesville
Clayton, R. K.	The first steps in photosynthesis	(1) University of Miami, Florida (2) Alabama Polytechnic Institute, Auburn
Clayton, R. K.	Light effects on plants	Duke University, Durham, N.C.
Clayton, R. K.	Phototrophy and chemo-autotrophy: Unity in the context of changing concepts	Am. Soc. Microbiol., Symposium on Autotrophy, Chicago, Ill.
Cohn, W. E. (and J. X. Khym)	Amine-catalyzed hydrolysis of periodate-oxidized nucleoside residues: application to RNA sequence analysis and 5' nucleotide cleavage [<i>Federation Proc.</i> 20, 355 (1961)]	(1) Federation Am. Soc. Exptl. Biol. (Biochem. Sect.), Atlantic City, N.J. (2) Fifth International Congress of Biochemistry, Moscow, USSR
Cole, B. T.	Separation of fatty acids and products formed by ultraviolet irradiation of linolenate by gas-liquid chromatography	South Carolina Academy of Sciences, Wofford College, Spartanburg
Cole, B. T. (and N. G. Anderson)	Fatty acids from lipids of rat liver microsomes [<i>ASB Bull.</i> 8, 28 (1961)]	Assoc. Southeastern Biologists, University of Kentucky, Lexington
Congdon, C. C.	Germinal center tissue and the immune mechanism [<i>Federation Proc.</i> 20, 23 (1961)]	Federation Am. Soc. Exptl. Biol. (Exptl. Pathol.), Atlantic City, N.J.
Congdon, C. C. (R. D. Owen, G. D. Novelli, and C. J. Wust)	Immune tissue reaction in irradiated mice [<i>Radiation Research</i> 14, 458 (1961)]	Radiation Research Society, Washington, D.C.
Cosgrove, G. E. (R. L. De Hart and A. C. Upton)	Modification of the foreign spleen reaction in irradiated mice [<i>Radiation Research</i> 14, 458-59 (1961)]	Radiation Research Society, Washington, D.C.
Cudkowicz, Gustavo	Immunological reactions by F ₁ hybrid mice against grafted parental strain tissues [<i>Federation Proc.</i> 20, 33 (1961)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Exptl. Pathol.), Atlantic City, N.J.
Cudkowicz, Gustavo	Mammary gland neoplasia in irradiated rats given the radioprotective drug AET [<i>Proc. Am. Assoc. Cancer Research</i> 3, 217 (1961)]	52nd Annual Meeting of the Am. Assoc. Cancer Research, Atlantic City, N.J.

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Cudkowicz, Gustavo	Suppression of "delayed mortality" in homologous blood chimeras by preirradiation of donor mice [<i>Radiation Research</i> 14, 459-60 (1961)]	Radiation Research Society, Washington, D.C.
Davidson, Douglas	Apical initial cells and meristem reorganization in <i>Vicia</i> roots	Fifth Southeastern Developmental Biology Conference, Wakulla, Fla.
de Serres, F. J.	Some aspects of the influence of environment on the radiosensitivity of microorganisms	Eleventh Symposium of the Society of General Microbiology, London, England
Detwiler, T. C.	Age-related changes in the metabolism of RNA	Federation Am. Soc. Exptl. Biol, Atlantic City, N.J.
Doherty, D. G.	Chemical protection against ionizing radiation	University of Louisville, Kentucky
Doherty, D. G.	Chemical protection to specific systems by AET and related compounds	International Radiobiological Symposium, Radiation Effects and Milieu, sponsored by the National Swiss Fund for Research, Montreux, Switzerland
Dolin, M. I. (Elizabeth Godiniaux and M. Grunberg-Manago)	Polynucleotide phosphorylase of <i>Clostridium perfringens</i>	Am. Soc. Microbiol., Chicago, Ill.
Doria, Gino (Joan W. Goodman, Nazareth Gengozian, and C. C. Congdon)	Origin of antibody-forming cells in homologous chimeras [<i>Federation Proc.</i> 20, 36 (1961)]	Federation Am. Soc. Exptl. Biol. (Soc. Immunol.), Atlantic City, N.J.
Eisenstadt, J. M. (and G. D. Novelli)	Factors involved in the synthesis of induced β -galactosidase by <i>Escherichia coli</i>	Am. Soc. Microbiol., Chicago, Ill.
Engel, M. S. (and G. E. Stapleton)	Studies on induced radioresistance in <i>Escherichia coli</i>	Am. Soc. Microbiol., Chicago, Ill.
Finamore, F. J. (and Elliot Volkin)	The ribonucleic acids of amphibian eggs [<i>Federation Proc.</i> 20, 356 (1961)]	Federation Am. Soc. Exptl. Biol. (Nucleic acids II), Atlantic City, N.J.
Finnerty, W. R.	Mechanisms in the microbial oxidation of alkanes	Am. Soc. Microbiol., Special Symposium on Marine Microbiology, sponsored by the Office of Naval Research, Chicago, Ill.
Foard, D. E. (and A. H. Haber)	Formation of guard cells in wheat leaves during growth without cell division [<i>Am. J. Botany</i> 48, 527 (1961)]	AIBS (Botanical Soc. Am.), Lafayette, Ind.
Foreman, C. W.	Comparative aspects of tryptic peptides of several mammalian hemoglobins [<i>ASB Bull.</i> 8: 28 (1961)]	Assoc. Southeastern Biologists, University of Kentucky, Lexington
Friedberg, W. (and V. K. Jenkins)	Albumin metabolism in the lethally irradiated mouse [<i>Federation Proc.</i> 20, 401 (1961)]	Federation Am. Soc. Exptl. Biol. (Am. Physiol. Soc.), Atlantic City, N.J.

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SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Goodman, Joan W.	Transplantation of blood leukocytes [<i>Federation Proc.</i> 20, 32 (1961)]	Federation Am. Soc. Exptl. Biol. (Am. Physiol. Soc.), Atlantic City, N.J.
Goodman, Joan W.	The use of isoimmune sera for identification of lymphoid cells from blood-injected radiation chimeras	New York Academy of Sciences, Conference on Blood Groups in Infra-Human Species, New York, N.Y.
Haber, A. H. (and W. L. Carrier)	Nonessentiality of cell division for leaf morphogenesis during growth of young wheat seedlings [<i>Am. J. Botany</i> 48, 527-28 (1961)]	AIBS (Society for the Study of Development and Growth), Lafayette, Ind.
Hollaender, Alexander	(1) Basic studies on biological effects of radiation (2) Current work on radiation biology, especially mutation production and recovery (3) The development and organization of a research institute (4) The development of a center for biophysics and molecular biology (5) Effects of radiation on chromosomes and radiation protection and chromosome breakage	Tokyo University, Tokyo, Japan
Hollaender, Alexander	(1) The discussion of problems investigated by the Biology Division, ORNL (2) Modern problems in radiation research	National Institute of Radiological Sciences, Chiba, Japan
Hollaender, Alexander	(1) Bone marrow transplantation and chemical protection (2) New developments in biology	University of Nagoya, Nagoya, Japan
Hollaender, Alexander	(1) Basic problems in radiation research (2) Developments in modern biology	University of Kyoto, Kyoto, Japan
Hollaender, Alexander	Basic studies on the effects of radiation on biological materials	Osaka University, Osaka, Japan
Hollaender, Alexander	New problems in the biological effects of radiation	National Institute of Genetics, Misima, Japan
Hollaender, Alexander	Radiation biology	Atomic Energy Station, Tokai, Japan
Hollaender, Alexander	Radiation genetics and other basic biological studies at Oak Ridge National Laboratory	University of Hiroshima, Hiroshima, Japan
Hollaender, Alexander	Studies on radiation effects	National Institute of Health, Tokyo, Japan
Jagger, John	Frontiers of modern biology	Tuskegee High School, Tuskegee, Ala.
Jagger, John	Light and life	Tuskegee Institute, Tuskegee, Ala.
Jagger, John (and R. S. Stafford)	Biological and physical ranges of photoprotection in bacteria [<i>Radiation Research</i> 14, 478 (1961)]	Radiation Research Society, Washington, D.C.

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Kenney, F. T.	Studies on the mechanism of adreno-cortical control of hepatic transaminase activity	Fifth International Congress of Biochemistry, Moscow, USSR
Khym, J. X.	Labilization of the phosphate bond of periodate-oxidized 5'-ribonucleotides by interaction with amines in the pH range of 6 to 8	139th Meeting of the Am. Chem. Soc., St. Louis, Mo.
Kimball, R. F.	Chromosome duplication and mutation	International Radiobiological Symposium, Radiation Effects and Milieu, sponsored by the National Swiss Fund for Research, Montreux, Switzerland
Kimball, R. F.	Postirradiation processes in the induction of recessive lethals by ionizing radiation	Symposium on Recovery of Cells from Injury, Gatlinburg, Tenn. (ORNL Biology Division Symposium)
Kimball, R. F.	Postirradiation processes in mutation induction	(1) British Empire Cancer Campaign Research Unit, Northwood, Middlesex, England (2) Institute of Genetics, University of Pavia, Italy (3) Institute of Genetics, University of Stockholm, Sweden
Kimball, R. F.	RNA, DNA, and protein synthesis in the macronucleus of <i>Euplotes</i>	Institute of Cell Research, Karolinska Institute, Stockholm, Sweden
Kirby-Smith, J. S.	Radiation biophysics	Institute of Genetics, University of Pavia, Italy
Kirby-Smith, J. S. (Benedetto Nicoletti and Mitzi L. Gwyn)	Synergistic action of x rays and ultraviolet radiation on chromosomal breakage in <i>Tradescantia</i> pollen	International Biophysics Congress, Stockholm, Sweden
Kirby-Smith, J. S. (and M. L. Randolph)	Modification of radiation-induced electron spin resonances	Symposium on Recovery of Cells from Injury, Gatlinburg, Tenn. (ORNL Biology Division Symposium)
Krieg, D. R.	A test of specificity in induced mutation	University of Southern California, Los Angeles
Kretchmar, A. L. (and C. C. Congdon)	Amino acid changes in mice with grafted homologous bone marrow [<i>Federation Proc.</i> 20, 402 (1961)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.) Atlantic City, N.J.
Lea, Charlotte R. (and G. D. Novelli)	A cell-free amino acid incorporating system from mouse liver [<i>Federation Proc.</i> 20, 391 (1961)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Atlantic City, N.J.
McDonald, T. P.	(1) Acute radiation injury in mammals (2) Delayed effects of radiation injury (3) Radiation protection and recovery	489th C. A. Co., and 310th C. A. Group, Fort Gordon, Ga.

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SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
McDonald, T. P. (Masahide Asano and G. D. Doherty)	AET protection against $\text{HN}_2\text{-HCl}$ toxicity in the mouse [<i>Federation Proc.</i> 20, 157 (1961)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Exptl. Pathol.), Atlantic City, N.J.
Makinodan, Takashi	(1) Antibody formation (2) Studies on immune reactions in in vivo cultures	University of Georgia, Athens
Makinodan, Takashi	Cellular kinetics in antibody formation	University of Pittsburgh School of Medi- cine, Pittsburgh, Pa.
Mans, R. J.	The incorporation of amino acids by cell- free preparations from maize seedlings [<i>Federation Proc.</i> 20, 390 (1961)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Atlantic City, N.J.
Mans, R. J.	A rapid method for measuring labeled proteins and other materials	Soc. Nuclear Medicine (SE Chapter), Atlanta, Ga.
Mazur, Peter	Manifestations of physical injury in yeast cells exposed to subzero temper- atures	Am. Soc. Microbiol., Chicago, Ill.
Mazur, Peter	Biological implications of the structure and properties of water	Louisiana State University, Baton Rouge
	The effects of subzero temperatures on living cells	(1) Tulane University, New Orleans, La. (2) Southwest Texas College, San Marcos
	The role of water and temperature in living organisms	(1) Incarnate Word College, San Antonio, Tex. (2) Trinity University, San Antonio, Tex. (3) Our Lady of the Lake College, San Antonio, Tex. (4) St. Mary's University, San Antonio, Tex.
Mead, Charles G.	Isolation and characterization of <i>Drosophila</i> DNA	Federation Am. Soc. Exptl. Biol., Atlantic City, N.J.
Monesi, Valerio (read by Dr. Borghese)	Ciclo cellulare negli spermatogoni del testicolò di topo studiato con timidina tritiata (in Italian)	XXXI Convegno dell 'Unione Zoologica Italiana, Turin, Italy
Monesi, Valerio (read)	Relazione della sensibilita' ai raggi X col ciclo mitotico negli spermatogoni del topo (in Italian)	VI Congresso Nucleare, Rome, Italy
Novelli, G. D.	Mechanisms involved in the control of enzyme synthesis	Fifteenth Annual Symposium on Funda- mental Cancer Research, Houston, Tex.
Novelli, G. D.	The role of DNA in the synthesis of in- duced enzymes	Gordon Conference on Cell Structure and Metabolism, Meriden, N.H.
Novelli, G. D.	The synthesis of proteins in bacteria	Wayne State University, Detroit, Mich.
Novelli, G. D.	The use of radiation and radioisotopes to study biochemical processes	Hearings of the Subcommittee on Research and Development of the Joint Committee on Atomic Energy, Congress of the United States, Washington, D.C.

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Novelli, G. D. (J. M. Eisenstadt and Tadanori Kameyama)	A requirement for genetically specific DNA in the cell-free synthesis of the enzyme β -galactosidase	98th Annual Meeting of the National Acad- emy of Sciences, Washington, D.C.
Novelli, G. D. (Tadanori Kameyama and J. M. Eisenstadt)	The effect of ultraviolet light and x rays on an enzyme-forming system	Symposium on Recovery of Cells from In- jury, Gatlinburg, Tenn. (ORNL Biology Division Symposium)
Novelli, G. D. (Tadanori Kameyama and J. M. Eisenstadt)	The nature of the system catalyzing the synthesis of induced β -galactosidase	Cold Spring Harbor Symposium on Cellular Regulatory Mechanisms, Cold Spring Harbor, Long Island, N.Y.
Oakberg, E. F.	Population dynamics of irradiated spermatogonia	Assoc. for Radiation Research, Symposium on Dynamics of Irradiated Cell Popula- tions, Oslo, Norway
Oakberg, E. F.	Radiation response and sensitivity of mammalian cells	International Radiobiological Symposium, Radiation Effects and Milieu, sponsored by the National Swiss Fund for Research, Montreux, Switzerland
Oakberg, E. F. (and Evelyn Clark)	Effect of dose and dose rate on radiation damage to mouse spermatogonia and oocytes as measured by cell survival	Symposium on Recovery of Cells from In- jury, Gatlinburg, Tenn. (ORNL Biology Division Symposium)
Odell, T. T. (and T. P. McDonald)	Stimulation of platelet production by serum of bled rats [<i>Federation Proc.</i> 20, 51 (1961)]	Federation Am. Soc. Exptl. Biol. (Am. Physiol. Soc.), Atlantic City, N.J.
Page, Sara L. (and C. J. Wust)	Primary immune response of mice to an enzyme, triose phosphate dehydrogenase [<i>ASB Bull.</i> 9, 34 (1961)]	(1) South Carolina Academy of Sciences, Wofford College, Spartanburg (2) Assoc. SE Biologists, University of Kentucky, Lexington
Parsons, D. F.	Cytologic changes produced by low doses (7r) of x rays on primary oocytes of the 4 to 6 day old mouse	Electron Microscope Soc. Am., Pittsburgh, Pa.
Peck, H. D., Jr.	The formation of adenosine-5'-phospho- sulfate from AMP and sulfite by ex- tracts of <i>Thiobacillus thioparus</i>	Am. Soc. Microbiol., Chicago, Ill.
Peck, H. D., Jr. (and Earl Fisher, Jr.)	The oxidation of thiosulfate by extracts of <i>Thiobacillus thioparus</i> [<i>Federation Proc.</i> 20, 219 (1961)]	Federation Am. Soc. Exptl. Biol. (Am. Soc. Biol. Chem.), Atlantic City, N.J.
Perkins, E. H. (and Martha A. Robinson)	Effect of time of antigen injection on the antibody response by transplanted isol- ogous spleen cells [<i>Federation Proc.</i> 20, 25 (1961)]	Federation Am. Soc. Exptl. Biol. (Am. Assoc. Immunol.), Atlantic City, N.J.
Popp, R. A.	Competence of retransplanted homolo- gous marrow in relation to time after original transplantation [<i>Radiation Research</i> 14, 491 (1961)]	Radiation Research Society, Washington, D.C.

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SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Popp, R. A.	Inheritance of hemoglobin differences in mice: its use in studying the differentiation of hematopoietic cells	University of Virginia, Charlottesville
Prescott, D. M.	Nuclear function and nuclear cytoplasmic interaction	Rutgers Institute, New Brunswick, N.J.
Prescott, D. M.	Synthetic processes in cell nuclei	Symposium of the Histochemical Society, Atlantic City, N.J.
Russell, Liane B.	Factors influencing the frequency of chromosomal loss and non-disjunction	Teratology Society, Cincinnati, Ohio
Russell, W. L.	Effect of radiation dose rate on mutation in mice	Symposium on Recovery of Cells from Injury, Gatlinburg, Tenn. (ORNL Biology Division Symposium)
Russell, W. L.	The genetic effects of ionizing radiation on mammals	Temple University, Philadelphia, Pa.
Russell, W. L.	Genetic effects of radiation	Symposium on Dental Genetics, National Institute of Dental Research, Washington, D.C.
Setlow, Jane K.	Ultraviolet inactivation of transformation principle [<i>Radiation Research</i> 14, 500 (1961).]	Radiation Research Society, Washington, D.C.
Setlow, Richard	Macromolecular sensitivity	Brookhaven Symposium on Fundamental Aspects of Radiosensitivity, Upton, Long Island, N.Y.
Shaw, M. W. (and E. H. Y. Chu)	Chromosome analysis of a mother of two mongols	Am. Soc. Human Genetics, Atlantic City, N.J.
Taylor, Mary B. (and G. D. Novelli)	Analysis of a bacterial sulfated polysaccharide	Am. Soc. Microbiol., Chicago, Ill.
Taylor, W. H. (and G. D. Novelli)	Studies on the localization and properties of dihydroorotic dehydrogenase in <i>Escherichia coli</i>	Am. Soc. Microbiol., Chicago, Ill.
Thompson, P. E.	Genetic consequences of homologous chromosome pairing	Iowa State University, Ames
Upton, A. C.	Effects of radiation on life span: relation to aging	Health Physics Seminar, ORNL, Oak Ridge
Upton, A. C.	Late effects of atom-bomb radiation in mice	Radiation Research Society Symposium on Late Effects of Ionizing Radiation, Washington, D.C.
Upton, A. C.	Recent developments in the field of cancer	Oak Ridge Health Department, Municipal Building, Oak Ridge
Upton, A. C.	Recent developments in cancer research	Oak Ridge Rotary Club
Upton, A. C. (J. W. Conklin and M. L. Randolph)	Influence of dose rate on oncogenic effectiveness of gamma rays in RF mice	52nd Annual Meeting of the Am. Assoc. Cancer Research, Atlantic City, N.J.

SPEAKER [AND COAUTHOR(S)]	TITLE	PLACE PRESENTED
Urso, Paul (and Takashi Makinodan)	Significance of mitosis and maturation in secondary precipitin response [<i>Federation Proc.</i> 20, 25 (1961)]	Federation Am. Soc. Exptl. Biol. (Am. Assoc. Immunol.), Atlantic City, N.J.
Volkin, Elliot	Synthesis and function of DNA-like RNA	Federation Am. Soc. Exptl. Biol. Sympo- sium on Topics of Current Interest in Biochemistry, Atlantic City, N.J.
von Borstel, R. C.	The genetic basis for x-radiation-in- duced mitotic inhibition [<i>Radiation Re- search</i> 14, 453 (1961)]	Radiation Research Society, Washington, D.C.
von Borstel, R. C.	Genetic methods for insect control	Gordon Conference on Biochemistry and Agriculture, Tilton, N.H.
von Borstel, R. C.	The nature of radiation-induced death in <i>Habrobracon</i> and <i>Drosophila</i> gametes	University of North Carolina, Chapel Hill
Whiting, Anna R.	Contrasts in radiation-induced mutation rates at different meiotic stages	International Radiobiological Symposium, Radiation Effects and Milieu, sponsored by the National Swiss Fund for Research, Montreux, Switzerland
Wolff, Sheldon	Biophysical and biochemical aspects of radiation cytology (series of 5 lectures)	(1) University of Puerto Rico, Rio Piedras (2) University of Puerto Rico, Mayaguez
Wolff, Sheldon	The doubleness of the chromosome be- fore DNA synthesis as revealed by combined x-ray and tritiated thymidine treatments [<i>Radiation Research</i> 14, 517 (1961)]	Radiation Research Society, Washington, D.C.
Wolff, Sheldon	Some postirradiation phenomena that af- fect the induction of chromosome aberrations	Symposium on Recovery of Cells from In- jury, Gatlinburg, Tenn. (ORNL Biology Division Symposium)
Wust, C. J.	(1) Theories of antibody synthesis (2 lectures) (2) Theories of antibody synthesis (1 lecture)	Queens College, Charlotte, N.C. Pfeiffer College, Misenheimer, N.C.
Wust, C. J.	(1) Concepts of antibody synthesis (2) Dynamic aspects of biology (3) Divergence and convergence of biology disciplines in research (4) DNA synthesis and immunology in genetics (5) Immunological basis for cell aging (6) Microbial genetics (7) Protein synthesis	Winthrop College, Rock Hill, S.C.

Visiting Lecturers. – The following is a list of guest speakers from universities and scientific organizations at home and abroad who participated in the Visiting Lecturer Seminar Program during the six months preceding this report. Included were scientists from Italy, Sweden, Argentina, England, Czechoslovakia, and Brazil.

BIOLOGY PROGRESS REPORT

SPEAKER	AFFILIATION	SUBJECT
Herman M. Kalckar	Department of Biology, Johns Hopkins University, Baltimore, Md.	Galactose metabolism as a marker for studies in the regulation of gene action
R. E. Scossiroli	Istituto di Genetica, Universita de Pavia, Pavia, Italy	Selection after radiation
W. K. Baker	Department of Zoology, University of Chicago, Chicago, Ill.	The genetic control of pigment differentiation in somatic cells
Angelo Bianchi	Universita di Milano, Istituto di Genetica, Milano, Italy	The use of interchanges involving A and B chromosomes in studying artificial mutagenesis in maize
Nils R. Ringertz	Institute for Cell Research, Karolinska Institutet, Stockholm, Sweden	Acid polysaccharides of the tissue mast cell
Ruby M. Valencia	Departamento de Biologia Y Medicina, Comision Nacional de Energia Atomica, Laboratorio de Genetica, Buenos Aires, Argentina	Mutations induced in recently fertilized eggs of <i>Drosophila</i> , in X chromosomes of maternal vs paternal origin
Michael Fry	Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Ill.	The effects of aging on the duodenum of mice
Uzi Nur	Department of Biology, Vanderbilt University, Nashville, Tenn.	Cytogenetics of a supernumerary chromosome, with an accumulation mechanism, in the mealy bug <i>Pseudococcus maritimus</i>
Michael Feldman	Laboratories of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, Md.	Immunogenetics of tumors grown in radiation chimeras
Paul Plesner	Department of Bacteriology and Immunology, Harvard Medical School, Boston, Mass.	Nucleotide and protein metabolism during synchronized cell division in <i>Tetrahymena pyriformis</i>
Lucien G. Caro	The Rockefeller Institute, New York City, N.Y.	Electron microscopic radioautography of thin sections: the transport of newly synthesized proteins in pancreatic exocrine cells
Benjamin D. Hall	Department of Chemistry, University of Illinois, Urbana, Ill.	Specific RNA-DNA hybrid formation
Harry Rappaport	Department of Biophysics, Yale University, New Haven, Conn.	Bacterial transformation and the nucleic acid-protein problem
Robin H. Mole	Radiobiological Research Unit, Medical Research Council, Harwell, Didcot, Berkshire, England	Time as a factor in mammalian radiobiology
Ellis S. Kempner	National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.	Selection and utilization of metabolic analogs for nucleic acid synthesis
Samuel W. Luborsky	National Institute of Mental Health, National Institutes of Health, Bethesda, Md.	The physical properties of rabbit liver S-RNA

SPEAKER	AFFILIATION	SUBJECT
Karel Sebesta	Institute of Biochemistry, Czechoslovak Academy of Sciences, Prague, Czechoslovakia	The metabolism of pyrimidine analogs
O. B. Zaalberg	The Wistar Institute, Philadelphia, Pa.	Skin grafting and sex-linked tissue incompatibility
Frank Seto	Department of Biology, Berea College, Berea, Ky.	A phase in developmental genetics
Cornelius A. Tobias	Donner Laboratory, University of California, Berkeley, Calif.	Biological effects of heavy ions
Mortimer L. Mendelsohn	Department of Radiology, Hospital of the University of Pennsylvania, Philadelphia, Pa.	Autoradiographic studies of the growth of autochthonous C3H tumors
George Chapman	Department of Anatomy, Cornell University Medical College, New York, N.Y.	Comparative studies of cell fine structure
Oswaldo Frota-Pessoa	Department of General Biology, University of Sao Paulo, Sao Paulo, Brazil	New Approaches to the determination of mutation rates in man
Charley Steinberg	Biology Division, California Institute of Technology, Pasadena, Calif.	Gene recombination at the molecular level

Foreign Travel. – Alexander Hollaender gave 16 lectures in one month during his trip to Japan in May–June. Five were presented at Tokyo University, two at the National Institute of Radiological Sciences in Chiba, two at the University of Nagoya, two at the University of Kyoto, and others at Osaka University, the National Institute of Genetics in Misima, the Atomic Energy Station in Tokai, the University of Hiroshima, and the National Institute of Health in Tokyo. (Titles of his addresses are listed in this report under "Lectures.")

Other places visited were the Cancer Institute in Tokyo, the Institute for Protein Research at Osaka University, and the Atomic Bomb Casualty Commission in Hiroshima.

The purpose of Dr. Hollaender's trip was to visit laboratories and consult with Japanese scientists on the organization of Japanese radiation biology research, the use of radiation in genetic studies, radiation protection and recovery work, and cooperation between Japanese laboratories and the Oak Ridge National Laboratory on studies of the basic mechanism of radiation. Throughout the country interest ran high in the establishment of molecular biology, biophysical, and radiation biology institutes separate from the university centers although in some cases associated with them.

Five members of the Biology Division attended the Fifth International Congress of Biochemistry in Moscow, USSR, August 10–16. They were Lazarus Astrachan, Waldo E. Cohn, D. G. Doherty, K. B. Jacobson, and F. T. Kenney.

Cohn presented an invited paper at this congress. Earlier in the summer he presided at the sixth session of the Symposium on Ribonucleic Acids and Polyphosphates in Strasbourg, France. He also visited a number of laboratories in Moscow, in Dublin, Ireland, in Prague, Czechoslovakia, and in Warsaw, Poland.

Astrachan presented a paper entitled "Resemblance of bacterial RNA and DNA." He also consulted with investigators in other countries who are doing research on structure, analysis, and function of nucleic acids and the chemistry and genetics of bacteriophage.

Doherty attended the congress on a grant from the American Society of Biological Chemists. After the meeting he visited the Institute of Radiophysics and University of Uppsala in Stockholm,

Sweden, the Department of Clinical Biochemistry at the University of Oslo, and the University of Edinburgh, Scotland.

Kenney presented a paper called "Studies on the mechanism of adrenocortical control of hepatic transaminase activity" at the Moscow congress. He visited laboratories in France, Belgium, Italy, and Sweden to discuss his main field of interest – enzymatic mechanisms involved in biosynthesis of proteins, with particular emphasis on the synthesis of specific enzymes.

In addition to attending the congress in Moscow, Jacobson visited laboratories in seven countries where he consulted with other biochemists on the synthesis of RNA, how its amount in a cell can be regulated, and what relationship exists between RNA and protein synthesis.

R. F. Kimball, D. G. Doherty, and E. F. Oakberg presented invited papers last May at the International Radiobiological Symposium, "Radiation Effects and Milieu," sponsored by the National Swiss Fund for Research in Montreux, Switzerland. Anna R. Whiting presented a paper on the same occasion.

Kimball addressed the symposium on the subject "Chromosome duplication and mutation." He also spoke before the British Empire Cancer Research Unit of Mount Vernon Hospital, in Middlesex, England, before the Institute of Genetics, University of Pavia, Pavia, Italy, before the Institute of Genetics, University of Stockholm, Sweden, and before the Institute of Cell Research, Karolinska Institute, Stockholm, Sweden.

Doherty's paper at the Montreux symposium was entitled "Chemical protection to specific systems by AET and related compounds." After the meeting, he visited laboratories in Switzerland, Germany, France, Belgium, England, and the Netherlands to discuss chemical protection against radiation damage.

"Radiation response and the sensitivity of mammalian cells" was the subject of Oakberg's invited paper in Montreux. He also gave an invited paper at the Symposium on Dynamics of Irradiated Cell Populations during the meeting of the Association of Radiation Research in Oslo, Norway, last June. Oakberg later visited laboratories in England, Scotland, and the Scandinavian countries, where research is being done on the effects of radiation on germ cells with emphasis on cell killing and induction of mutations.

Sheldon Wolff gave a series of lectures last April on the subject "Biophysical and Biochemical Aspects of Radiation Cytology" at the Biology Department of the University of Puerto Rico.

W. L. Russell again served as an adviser to the United States delegation, this time at the Ninth Session of the United Nations Scientific Committee on the Effects of Atomic Radiation in Geneva, Switzerland, early in the year.

Last April, in London, Fred. J. de Serres gave a paper on "Some aspects of the influence of environment on the radiosensitivity of microorganisms" at the Eleventh Symposium on Microbial Reaction to Environment. He also held discussions with scientists in London, Edinburgh, Paris, Copenhagen, Rome, Milan, and Zurich.

John S. Kirby-Smith attended the International Biophysics Congress in Stockholm July 31–August 4, and presented an invited paper entitled "Synergistic action of x rays and ultraviolet radiation on chromosomal breakage in *Tradescantia* pollen." Before the congress, he gave a seminar on chromosome breakage at the University of Rome, Italy. At the International School of Physics, University of Naples, he presented lectures in their course on biophysics on "Electron spin resonance as applied to studies of free radicals in biological and biochemical systems" and on "Radiation-induced chromosomal aberrations." After meeting these responsibilities, Kirby-Smith visited a number of laboratories in England and Germany to discuss radiological physics and electron spin resonance.

Fifteenth Annual Biology Research Conference. – The 1962 Research Conference sponsored by the Biology Division of the Oak Ridge National Laboratory will be held at the Mountain View Hotel, Gatlinburg, Tenn., April 9th through 12th. The committee, comprised of C. C. Congdon (chairman), Takashi Makinodan, R. A. Popp, D. M. Prescott, and W. L. Russell has arranged the following tentative program:

SPECIFICITY OF CELL DIFFERENTIATION AND INTERACTION

Monday, April 9

Morning

Chairman – Ruggero Ceppellini, Istituto di Genetica Medica dell'Universita di Torino, Milan, Italy

Nucleic acids in development – J. Brachet, Faculté des Sciences, Université Libre de Bruxelles, Bruxelles, Belgium

Nuclear transplantation and problems of specificity in developing eggs – John A. Moore, Department of Zoology, Columbia University, New York, N.Y.

Afternoon

Chairman – J. P. Trinkaus, Department of Zoology, Yale University, New Haven, Conn.

Cell interactions in differentiation – Clifford Grobstein, Department of Biological Sciences, Stanford University, Stanford, Calif.

Specific demonstration of cell-to-cell interaction – T. Yamada, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.

Evening

Smoker – Host: Union Carbide Corporation

Tuesday, April 10

Morning

Chairman – P. A. Weiss, Rockefeller Institute for Medical Research, New York, N.Y.

Interactions of cells in culture – A. A. Moscona, Department of Zoology, University of Chicago, Chicago, Ill.

Specificity in the relation between nerve cells and their peripheral connections (tentative title) – Viktor Hamburger, Department of Zoology, Washington University, St. Louis, Mo.

Afternoon

Mountain climbing or tour through the Great Smoky Mountains. The group will leave from the Mountain View Hotel at 2:00 PM. Bus transportation will be provided for the tour.

Evening

Reception and dinner

Wednesday, April 11

Morning

Chairman – G. Hoecker, Instituto de Biologia "Juan Noe," Santiago, Chile

Specificity of ultrastructure in differentiating cells and its genetic control – C. H. Waddington, Institute of Animal Genetics, Edinburgh, Scotland

Phenotypic expression of mouse isoantigens – Göran Möller, Department of Tumor Biology, Karolinska Institutet, Stockholm, Sweden

Afternoon

Chairman – A. H. Coons, Department of Bacteriology and Immunology, Harvard Medical School, Boston, Mass.

The role of cellular division and maturation in antibody formation – T. Makinodan, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.

Phylogeny and ontogeny of the immune mechanism – M. Hašek, Biological Institute, Czechoslovak Academy of Sciences, Praha, Czechoslovakia

Evening

Round-table discussion: Subject to be announced

Chairman – D. M. Prescott, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.

Thursday, April 12

Morning

Chairman – R. W. Briggs, Department of Zoology, Indiana University, Bloomington, Ind.

The problem of single cell manipulations as applied to plant sciences – A. J. Riker and A. C. Hildebrandt, Department of Plant Pathology, University of Wisconsin, Madison, Wis.

Tolerance induction during development – R. E. Billingham, The Wistar Institute, Philadelphia, Pa.

Summary – Sir MacFarlane Burnet (tentative), The Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Melbourne, Victoria, Australia

1961 Biology Symposium. – The proceedings of the Fourteenth Annual Biology Symposium, *Recovery of Cells from Injury*, held in Gatlinburg, Tenn., in April, are being published as a supplement to the *Journal of Cellular and Comparative Physiology* and will be available in December 1961.

Chemical Protection and Bone Marrow Conferences. – Chemical protection and bone marrow transplantation conferences were first started in 1957 in Oak Ridge. Eleven meetings have been held in Oak Ridge and other cities up to the time of the present report period. The purpose of the conferences is to provide a means for prompt exchange of information about recent experimental results between clinical research workers and those doing animal research.

The plan of the conferences this year has been to hold highly specialized meetings about specific problems as well as occasional more general meetings of the original type. Many people and organizations besides the Biology Division staff participate in arranging these meetings.

Proceedings of the meetings are published by *Blood*, *The Journal of Hematology*, by the Biology Division, or by the sponsoring organization. A list of meetings held during and shortly after the present report period follows.

Chemical Protection Against Ionizing Radiation (screening for protective compounds, pharmacology of active compounds, protective effects on various systems, protective effects in mammals, and combined treatment). University of Rochester, March 16–17, 1961, Rochester, N.Y.

Conference on Bone Marrow Transplantation and Chemical Protection (general meeting). Atlantic City, N.J., April 10, 1961.

Bone Marrow Conference (cold injury to cells, toxicity of chemical additives, preservation of living tissues and organs, clinical uses of preserved living cells, instrumentation and equipment for preservation of living cells). University of Buffalo and Linde Company, August 18–19, 1961, Buffalo, N.Y.

International Symposium on Tissue Transplantation (general meeting). University of Chile, August 30–September 2, 1961, Santiago, Chile.

A specialized conference on *Blood-Bone Marrow Tissue Culture and Cell Separation* will be held at the National Institutes of Health in Bethesda, Md., October 20–21, 1961.

Education. – For the fourth consecutive summer the Biology Division, in cooperation with the Oak Ridge Institute of Nuclear Studies, provided training for a group of college students under the Student Trainee Program. Sixteen student trainees worked in the Division for approximately ten weeks and completed written reports on their research projects. The students, their affiliations, and the sections to which they were assigned, were:

STUDENT TRAINEE	INSTITUTION	ASSIGNED TO
Burrough, Martha K.	Longwood College, Farmville, Va.	Radiation Microbiology
Cannon, Patricia A.	North Georgia College, Dahlonega	Animal Facility, 9207
Conner, Mary A.	Winthrop College, Rock Hill, S.C.	Cell Physiology
Coughlin, Alice J.	Reed College, Portland, Ore.	Biophysics
Davis, Ruthanne	Tuskegee Institute, Tuskegee, Ala.	Mammalian Recovery
Hitchens, Lee M.	Wilson College, Chambersburg, Pa.	Pathology and Physiology
Howsden, F. Lester	Phillips University, Enid, Okla.	Pathology and Physiology
Medina Mendez, Jose J.	University of Puerto Rico, Rio Piedras, Puerto Rico	Pathology and Physiology
Newton, Isabella	Appalachian State Teachers College, Boone, N.C.	Pathology and Physiology
Oldham, J. H.	Middle Tennessee State College, Murfreesboro	Pathology and Physiology
Osterbind, Rosilie S.	Southwestern at Memphis, Memphis, Tenn.	Cytology and Genetics
Parks, Barbara L.	George Peabody College, Nashville, Tenn.	Plant Physiology and Photosynthesis
Price, Nancy J.	Winthrop College, Rock Hill, S.C.	Cell Growth and Reproduc- tion
Roberts, Gerald D.	Berea College, Berea, Ky.	Chemical Protection and Enzyme Catalysis
Sykes, Emily	Randolph-Macon Woman's College, Lynchburg, Va.	Mammalian Recovery
Simpson, Larry	Princeton University, Princeton, N.J.	Biophysics

In addition, several undergraduate groups visited the Division during the summer months, other visits are scheduled for this autumn (1961), and H. I. Adler, N. G. Anderson, Peter Mazur, and C. J. Wust have visited undergraduate colleges in the southeast to present lectures.

Contributing to the University of Tennessee seminar program, Department of Zoology and Entomology, C. C. Congdon, George Hodgson, and Paul Urso presented seminars entitled, respectively, "Histology of the immune mechanism," "Use of radioactive iron in the bioassay of the erythropoietic stimulating factor," and "Study of cell differentiation during the secondary precipitin response."

Seven lectures were given by members of the Biology Division as a part of the ten-week course given by the ORNL Health Physics Division. Participating in this program were G. E.

Cosgrove (2 lectures), M. S. Engel, E. F. Oakberg, Liane B. Russell, W. L. Russell, and R. C. von Borstel. At a Health Physics Research seminar in April, A. C. Upton spoke on "Effects of radiation on life span: relation to aging."

During his visit abroad (see "Foreign Travel" section), J. S. Kirby-Smith attended a course on Biophysics at the International School of Physics in Naples, Italy, and presented lectures entitled "Electron spin resonance as applied to studies of free radicals in biological and chemical systems," and "Radiation induced chromosomal aberrations."

D. G. Doherty gave a Medical Education for National Defense (MEND) lecture, "Chemical protection against ionizing radiation," at the University of Kentucky, Lexington, in May, and E. F. Oakberg participated in a two-week course on *Epidemiology of Radiation Injury* at the St. Louis School of Medicine, St. Louis, Mo. Dr. Oakberg's lecture was "Genetic effects of radiation in the mouse."

In August, T. T. Odell, Jr., lectured on "Mammalian radiobiology" at the ORINS-NSF summer course for high school teachers, and on "Acute and late somatic effects of radiation in mammals" to college professors of biology attending the ORINS Radiation Biology Institute.

A. C. Upton lectured on the general subject "Biological effects of radiation" to members of the Physics and Chemistry Divisions taking a course in Radiation Control. Another lecture of the same title was presented by Dr. Upton to the 79th session of the Radioisotope Techniques School, ORINS Radioisotope Course D.

Included as lecturers to students at the Oak Ridge School of Reactor Technology (ORSORT) this summer were H. I. Adler, Liane B. Russell, W. L. Russell, and A. C. Upton.

CYTOLOGY AND GENETICS

Paramecium

R. F. Kimball	Stella W. Perdue
O. Carolyn Wells ¹	H. Marine Scandlyn
D. B. Williams ²	Lindy Hatch ¹
	Wirtley Raine ¹

Tradescantia, Vicia, Hordeum

Sheldon Wolff	H. E. Luippold
	Sister Augustine Mattingly ³

Maize and Phage

Drew Schwartz ⁴	Rea M. Fulkerson
D. R. Krieg	C. B. Kincaid
Jane K. Setlow ³	Kathrine H. McGrath

Neurospora

F. J. de Serres	Ida R. Cox
H. E. Brockman ⁵	Arlee P. Teasley
B. B. Webber ²	Rosilie S. Osterbind ⁶

Cell Growth and Reproduction

D. M. Prescott	Romance F. Carrier
R. C. von Borstel	P. E. Eide
J. G. Carlson ⁷	Nancy J. Price ⁶
Douglas Davidson ⁸	
Mary E. Gauden ⁷	
B. C. Kluss ²	
E. A. Löbbecke ⁸	
Gladys S. Van Pelt ²	
Anna R. Whiting ⁷	
Tuneo Yamada ⁸	

Drosophila

D. L. Lindsley, Jr.	Guthrie T. Pratt
E. H. Grell	Ruby D. Wilkerson
Rhoda F. Grell ⁷	
Benjamin Hochman ²	
P. E. Thompson ⁵	
Mary Warters ¹	

¹Loanee.

²Research participant.

³USPHS Fellow.

⁴Leave of absence, NSF Senior Postdoctoral Fellowship, Jerusalem, Israel.

⁵Research associate.

⁶Student trainee.

⁷Consultant.

⁸Visiting investigator from abroad.

CYTOGENETIC EFFECTS OF RADIATION

Further Studies of the Effect of Chloramphenicol on the Induction of Mutations by X Rays in *Paramecium*

R. F. Kimball

Stella W. Perdue

H. Marine Scandlyn

Introduction. – Much of the work on modification of mutation in bacteria has been done with the protein-synthesis inhibitor, chloramphenicol. This agent has also been used to study the mutation process in *Paramecium* and has been shown to decrease mutation when used at high enough concentrations. Our information on its action on the mutation process in *Paramecium* has been rather limited, however, appreciably less than for several other agents. Consequently a major effort has been made to remedy this situation while at the same time making a start on certain types of experiments which previous work had suggested should give us a better understanding of the mutation process.

Methods. – The methods of obtaining paramecia at known times after division, x irradiating, and detecting recessive lethal mutations in the micronucleus were the same as those given in previous reports and publications. Micronuclear DNA was determined by microspectrophotometry of Feulgen-stained preparations, using the recently acquired Canalco microspectrophotometer.

Results and Discussion. – Three kinds of experiments, each with several replicates, have been carried out. In the first (Fig. 1a), log-phase paramecia were irradiated (4500 r) at 0.15 hr after division and transferred to 1.5 mg/ml of chloramphenicol at the times indicated in the graph. The exposure to the agent lasted 4 hr before transfer to fresh culture medium. At the time of transfer to chloramphenicol, specimens were also dried on slides for DNA determinations. The results show that the end point for effective modification of the mutation process coincides closely with DNA doubling, that is, chromosome duplication. This confirms our earlier conclusions⁹ (based on less direct evidence) and may represent a point of difference with the bacterial work, in which RNA rather than DNA synthesis has been taken to be the end point.¹⁰

In the second experiment (Fig. 1b), paramecia were transferred to 1.5 mg/ml of chloramphenicol at 0.1 hr after division, left in it for 4 hr, and irradiated (4500 r) at the times shown. The reverse of the slope of the line in the graph is a measure of the rate of loss of premutational damage.⁹ This slope is between one-third and one-half that found in normal log phase. Exploratory microspectrophotometer measurements have shown that the chromosomes do not duplicate during the 4 hr in chloramphenicol, but the time of duplication has not yet been determined. This experiment, plus data still to be obtained from irradiation at various times after the chloramphenicol treatment is ended, should give us a complete quantitative accounting of the change in premutational damage with the time under these conditions. We have previously concluded that chloramphenicol, as well

⁹R. F. Kimball, N. Gaither, and S. W. Perdue, *Internat. J. Radiation Biology* 3, 133–47 (1961).

¹⁰C. O. Doudney and F. Haas, *Genetics* 45, 1481–1502 (1960).

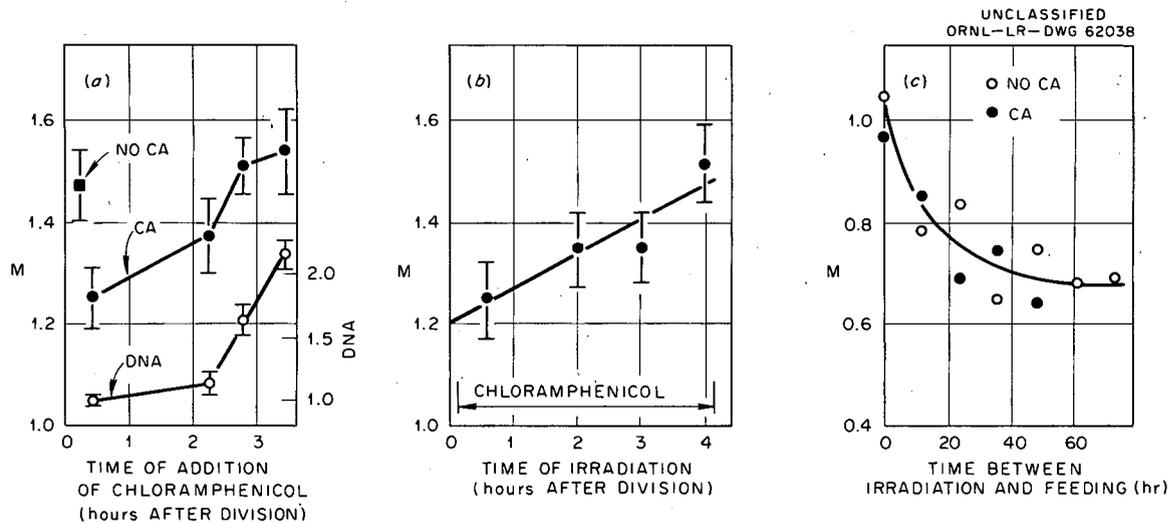


Fig. 1. Amount of Mutation (M) Plotted Against the Time of Various Treatments. Confidence limits (95%) given.

as other agents, decreases mutation by allowing more time for loss of premutational damage despite the fact that it also decreases the rate of loss. The data in Fig. 1b show that the decrease in rate of loss is not very great and make it plausible that a reasonable delay in DNA synthesis would account for decreased mutation.

In the third experiment (Fig. 1c), stationary-phase paramecia with or without 1.5 mg/ml of chloramphenicol were irradiated at various times before transfer to culture medium. The decrease in mutation with time measures the rate of loss of premutational damage under stationary-phase conditions.¹¹ The lower limit which is approached as time increases could represent mutation irrevocably produced at the time of irradiation but would also include any mutation that might be produced by conversion of premutational damage to fixed mutation during the stationary phase. The results, though somewhat variable, show no detectable effect of chloramphenicol on the rate of loss of premutational damage, in contrast to the log-phase findings. There is also no detectable effect on the lower limit, but the curve could not be carried out as far as desirable because stationary-phase paramecia begin to die in a little over two days in chloramphenicol, although they survive a week or more in its absence. Thus either the lower limit results entirely from mutation fixed at the time of irradiation, or chloramphenicol is not an appropriate agent for revealing mutation fixed during the intermediate period.

Long-Term X-Ray Injury in *Spathidium spathula*

D. B. Williams

Introduction. — *Spathidium spathula* is considerably more sensitive to x-ray-induced division delay than any ciliate reported.¹² When irradiated with 1 and 2 kr 1 hr after division, the genera-

¹¹R. F. Kimball, N. Gaither, and S. M. Wilson, *Proc. Natl. Acad. Sci. U.S.A.* 45, 833-39 (1959).

¹²D. B. Williams, submitted to the *Journal of Protozoology* (1961).

tion time is significantly lengthened. Besides initial-division delay, long-term injury characterized by gigantism, extra cytostomes at abnormal sites, and continued growth depression have been reported¹² for *Spathidium* after treatment with 4 to 25 kr. These injuries persist through several weeks of isolation culturing and continue through encystment. Long-term x-ray injury has been reported for other ciliates, particularly a high incidence of delayed death 4 to 14 fissions after exposure to 50 kr in *Tillina magna* and *Colpoda sp.*,¹³ and a permanently increased generation time in a strain of *Tetrahymena pyriformis*¹⁴ which became amiconucleate after receiving 285 kr. In these ciliates gigantism along with morphological abnormalities were not reported.

This report gives additional information on such persistent effects in irradiated spathidia and associates it with total micronuclear loss which occurs within four days after irradiation. Also, evidence is given for induction, by 15 kr, of a highly significant amount of deleterious and recessive lethal mutations.

Results and Discussion. – Daily observations were made on a large number of lines irradiated with 0, 6, 15, 25, and 55 kr. The division rate and presence or absence of gigantism and morphological abnormalities were recorded for each line. Table 1 gives the per cent of poor lines (0 to 2

Table 1. Per Cent Poor Lines on Days 1, 2, 3, 4, and 8 After Irradiation

	1	2	3	4	8	Number of Lines Observed
Controls	0	0	0	0	0	196
6 kr	13.3	5.0	5.0	5.0		60
15 kr	27.9	18.3	9.8	6.1	4.2	567
25 kr	74.1	60.9	41.4	13.3	8.5	85
55 kr	100	100		98.8	97.5	86

daily divisions associated with morphological abnormalities) for each dose on succeeding days of cultivation. At the three lowest doses most of the recovery occurred by the fourth day and then only a very small amount of recovery occurred up to 8 days. Continued cultivation of lines showing no recovery after 8 days demonstrated that the injury was of permanent nature. Representatives from permanently injured lines were stained with Azure-A and without exception proved amiconucleate, whereas the unirradiated controls averaged about 20 micronuclei per animal. The macronucleus was enlarged and in some cases fragmented in amiconucleate lines.

When does micronuclear loss occur? In most cases micronuclear loss occurred by the time of first division after treatment, but it also occurred up through the 15th division. An analysis of 15 lines which became amiconucleate after receiving 25 kr gave these results: amiconucleate by first division, 7; between 2nd and 5th, 3; between 6th and 10th, 3; between 11th and 15th, 2. In

¹³J. Bridgman and R. F. Kimball, *J. Cell. and Comp. Physiol.* 44, 437-45 (1954).

¹⁴C. Wells, submitted to the *Journal of Protozoology* (in press, 1961).

all cases micronuclear loss was completed within 96 hr of exposure. Analysis of the number of micronuclei in those lines showing only temporary injury gave an increase in number to about 30 on day 1, with a return to nearly the normal number of 20 on day 3. It is likely that micronuclear division can occur in the absence of cell division in these cases. It seems improbable that micronuclear loss is a result of direct radiation injury to the individual micronuclei because loss of all 20 nuclei would be an unlikely event. Rather some injury to the cell as a whole must lead to the loss of all nuclei.

Wells¹⁴ has presented evidence that shows that micronuclear loss in *Tetrahymena pyriformis* occurs in two steps after irradiation. In both species the amiconucleates have a longer generation time. *Spathidium* shows a marked increase in cell size and macronuclear volume which is apparently permanent, for amiconucleate lines have been subcultured 63 days with little if any recovery occurring. Selfing in these lines is followed by death of all exconjugants, but encystment has been observed as many as five times in some lines and excysted animals give no signs of recovery.

In an experiment to test for mutation induction by x rays, 24 lines which had recovered normal morphology and fission rate after 15 kr were allowed to self after they had passed through about 50 fissions. Ten selfing pairs were isolated from each original line and observed after 48 hr, with the result that 53.4% of the isolated pairs showed poor growth, gross abnormalities, or lethality. The 25 control lines yielded only 4.0% such exconjugants. The test was repeated after another 50 fissions and 52.9% of the irradiated lines gave recessive lethal or deleterious mutations as compared to 1.2% for the controls. If the effect were vegetative rather than genetic, the frequency of lethal and poor-growing exconjugants should decrease with the increase in number of fissions between tests. Selfing probably produces homozygosity of micronuclear mutations induced by radiation.

Fission Delay in *Tetrahymena pyriformis* After a Low Dose of X Rays¹⁵

Carolyn Wells Lindy Hatch

Introduction. — One manifestation of the general insensitivity of ciliate species to irradiation damage is the failure of low doses of ionizing radiation to produce a marked delay in vegetative fission. Although such division delay is obtained after the irradiation of most ciliates with high (50 to 200 kr) doses, only one ciliate¹⁶ has been shown to be sensitive to this form of damage after low (1 to 10 kr) doses. This ciliate, *Spathidium spathula*, undergoes prolonged fission delay after only 1 to 4 kr when irradiated 60 min after division.

Recent work in this laboratory¹⁷ has demonstrated the relative resistance of another ciliate, *Tetrahymena pyriformis*, to the killing action of x rays. The LD₅₀ of the most radiosensitive strain

¹⁵Work performed under Atomic Energy Commission Contract No. AT-(40-1)-2793.

¹⁶D. B. Williams, submitted to the *Journal of Protozoology* (in press).

¹⁷C. Wells, *J. Cellular Comp. Physiol.* 55, 207-19 (1960).

tested was around 100 kr. However, work described in this report indicates that, when division delay is used as a criterion for radiation damage, *T. pyriformis* seems to be one of the more radio-sensitive species.

Methods. – The cells used in this study, members of strain 1/II NT, *T. pyriformis*, were cultured in axenic proteose peptone (1%)–liver extract (0.1%) medium adjusted to pH 7.2. Groups of synchronized cells were obtained by removing cells in fission from a population of rapidly dividing log-phase organisms during a 10-min interval. Half of each group was irradiated; the other half served as controls. One set of organisms was irradiated at each of the following times: 30, 60, 90, 140, 150, 160, 165, and 170 min after the beginning of an isolation period. The 160-, 165-, and 170-min experiments were repeated several times. Each experimental group was given a total x-ray dose of 4 kr, delivered at a dose rate of 1.6 kr/min. The cells were irradiated in the presence of catalase to avoid possible peroxide effects. After treatment, the cells were placed in fresh medium and observed until the time of division could be recorded.

Results. – Most of the control cells divided 200 to 220 min after isolation. When cells were irradiated at 30, 60, 90, 140, and 150 min after isolation, their mean generation time was 60 to 100 min longer than that of the controls. A much greater fission delay was observed in cells irradiated 160 min after isolation – most of these organisms did not divide until 440 to 460 min after isolation, a fission delay of about 240 min. Cells irradiated 170 min into the cell cycle were delayed only about 100 min, similar to those cells irradiated earlier in the interdivision interval. Surprisingly, cells irradiated midway between 160 and 170 min (165 min) divided at about 190 to 220 min, *thus showing no fission delay whatsoever*. These remarkable results with 160-, 165-, and 170-min cells have been repeated several times.

Discussion. – A generalized fission delay of 60 to 100 min is induced in *T. pyriformis* cells when 4 kr of radiation is administered at most times during the cell cycle. Cells seem particularly sensitive to radiation-induced fission delay about 160 min after division. Approximately 5 min later, cells are remarkably insensitive to this form of radiation damage, but the period of insensitivity is short, since cells irradiated about 170 min after division show the generalized delay characteristic of about 100 min. The period of extreme sensitivity can be correlated cytologically with the time of micronuclear prophase, and the period of insensitivity occurs at about the same time as micronuclear metaphase. This correlation may be coincidental. The data neither support nor fail to support any supposition that micronuclear mitotic stages are responsible for the observed radiation responses. The results show clearly, however, that pronounced radiation effects, measured by fission delay, are produced in cells of this ciliate species by only 4 kr of x irradiation.

The Theoretical Kinetics for Two-Hit Chromosome Exchanges

Sheldon Wolff H. E. Luippold

Introduction. – The theoretical kinetics for the induction of two-break chromosome exchanges by sparsely ionizing radiation has been only imperfectly understood. The usual result, whether

viable translocations (in *Drosophila*) or inviable dicentrics (in *Tradescantia*) are scored, is to observe that exchanges increase as the $\frac{3}{2}$ power of the dose. Attempts to explain this phenomenon usually assume that the exchanges are two-hit phenomena that should increase as the square of the dose ($Y = kD^2$) and that the discrepancy is caused in *Drosophila* by the production of relatively larger numbers of inviable exchanges at high doses,¹⁸ and in *Tradescantia* by the production of one-hit exchanges in addition to the two-hit ones.¹⁹

Theoretically, however, exchanges are not expected to increase as the square of the dose. Consequently, we have derived expressions for the yield of two-hit exchanges. The theoretically expected values from these expressions give very good fits to existing data without necessitating the interposition of extraneous factors to account for the shapes of the curves.

Results. — In a cell there are only a limited small number of places where the chromosomes come close enough to rejoin and form an exchange if broken. Since breaks are distributed at random and are proportional to the dose of radiation, the chance of not breaking a chromosome in one of these sites is given by the first term of the Poisson expansion (e^{-kD}). The chance, therefore, of breaking a chromosome at a site at least once is $(1 - e^{-kD})$ and of breaking both chromosomes independently is $(1 - e^{-kD})^2$. This would be the yield of exchanges per site. If a cell has n effective sites, then the yield per cell is given by the formula $Y = n(1 - e^{-kD})^2$. A fit of *Tradescantia* data to this expression is given in Fig. 2. Table 2 gives the theoretically expected values for the yield per site at various values of kD .

Discussion. — The least-squares fit of the *Tradescantia* data indicates that in this organism there are 3.6 effective sites for exchange formation. It has been determined by other methods that the number was about 4.

The fit of these data to the simple equation $Y = kD^x$ indicates that exchanges increase as the $\frac{3}{2}$ power of the dose.

The values recorded in Table 2 show that the curve for exchanges has a constantly decreasing slope and will saturate when all sites have an aberration. At low doses, doubling the dose results in increasing the yield by a factor of 4. At higher doses this factor decreases. Dose curves for translocations induced in *Drosophila* are in the segment of the curve that shows an average of a threefold increase in translocation per doubling of the dose, hence the $\frac{3}{2}$ power.

At low doses, *Drosophila* translocations have been found to quadruple as the dose doubled. The change in slope of the curve and the apparent increase in exchanges as the $\frac{3}{2}$ power of the dose are not caused by extraneous factors such as an increase in the relative numbers of inviable aberrations but are expected on theoretical grounds.

¹⁸H. J. Muller, in *Radiation Biology*, ed. A. Hollaender, McGraw-Hill, 1954.

¹⁹D. G. Catcheside, D. E. Lea, and J. M. Thoday, *J. Genet.* 47 (1946).

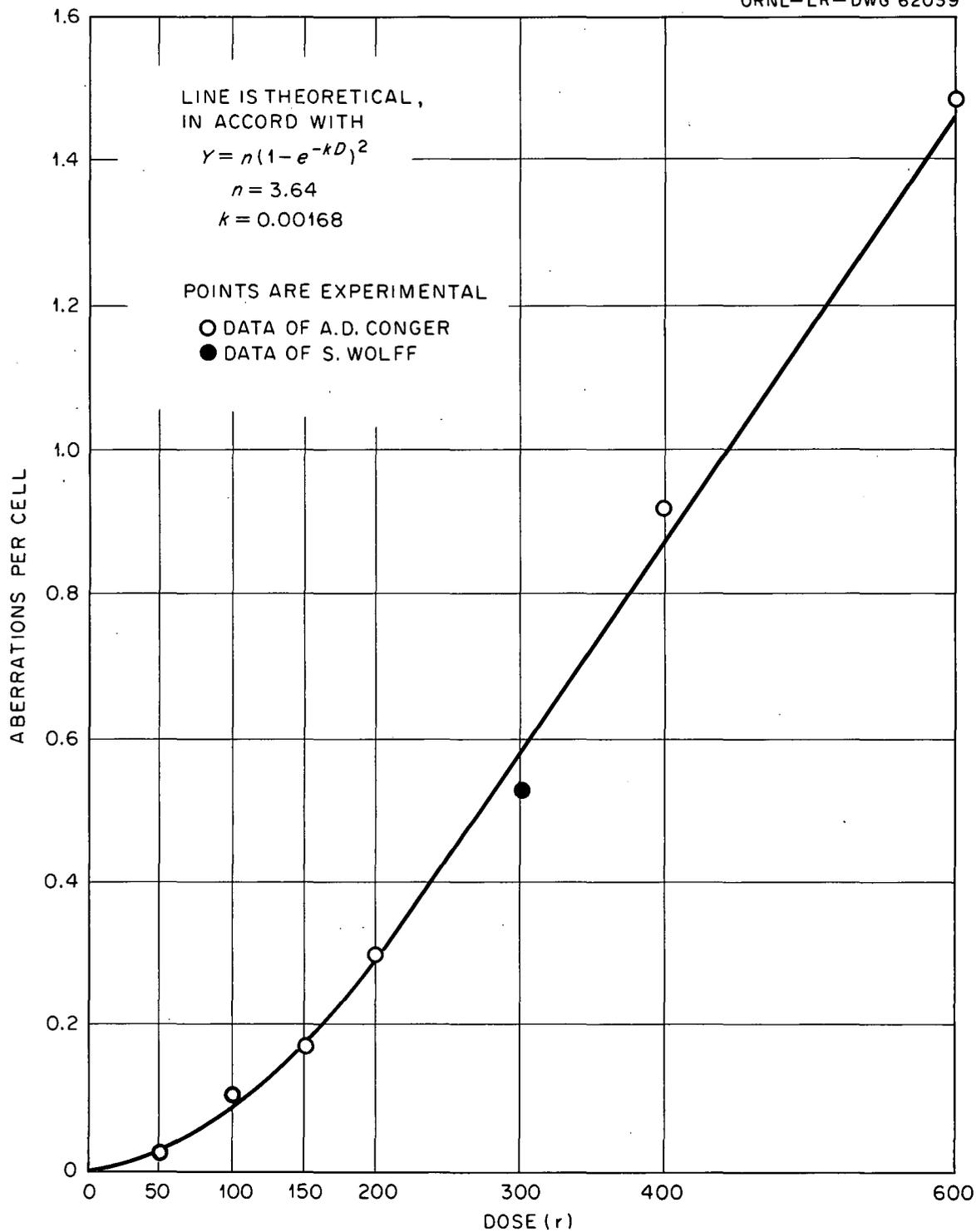


Fig. 2. Dose-Effect Curve for Chromosome Exchanges Induced in *Tradescantia* Microspores.

Table 2. Yields of Aberrations per Site at Various Doses

kD	Yield per Site $(1 - e^{-kD})^2$	Ratio of Yields When Dose Is Doubled $(1 - e^{-2kD})^2 / (1 - e^{-kD})^2$
0.001	0.000001	4
0.002	0.000004	
0.01	0.0061	4
0.02	0.0004	
0.1	0.009	3.6
0.2	0.0327	3.3
0.3	0.0671	3.0
0.4	0.109	2.8
0.5	0.154	2.6
0.6	0.203	
0.7	0.253	
0.8	0.304	
0.9	0.352	
1.0	0.399	1.9
2.0	0.748	
3.0	0.903	
4.0	0.964	
5.0	0.987	
6.0	0.995	
7.0	0.998	
8.0	0.999	

Basic Protein Synthesis in the Meristematic Nucleus

Sister Augustine Mattingly

Introduction. — The investigation of the relationship of nuclear basic protein to the process of differentiation has progressed chiefly through biochemical analyses of nuclei,²⁰ microspectro-photometric measurements of histone-specific alkaline fast green^{21,22} or the Sakaguchi reaction,²³

²⁰E. D. Hudack, A. P. Gibbons, and D. O. Brummond, *Exptl. Cell Research* **22**, 338–44 (1961).

²¹M. Alfert and I. I. Geschwind, *Proc. Natl. Acad. Sci. U.S.* **39**, 991–99 (1953).

²²D. P. Bloch and H. Y. C. Hew, *J. Biophysic. and Biochem. Cytol.* **8**, 69–81 (1960).

²³J. McLeish, *Chromosoma (Berl.)* **10**, 686–710 (1959).

and autoradiography.²⁴ A fundamental difficulty of the last technique is the masking of nuclear label by incorporation of label into cytoplasmic protein. This problem has been overcome by the development of a procedure for the rapid and efficient isolation of clean nuclei from neutral-formalin fixed roots of *Vicia faba*. This technique allows the investigator to utilize much more advantageously the excellent resolution afforded by tritium-labeled amino acids.

Autoradiographs of such nuclei have been prepared in order to determine the extent of nuclear incorporation of basic amino acids, their possible localization within the nucleolus, and the degree of correlation of nuclear label with the level of cellular differentiation as determined by nuclear morphology and position within the root.

Results. – Primary roots of *Vicia faba* were grown in tritiated lysine for 15 min; grain counts on isolated nuclei from the distal 0.5-cm section show that 65% of the nuclei are labeled. Furthermore, at this time 40% of the nucleoli have more than two grains associated with the organelle. Numerous isolated nucleoli with no visible Feulgen-positive material are heavily labeled.

Some roots were maintained in a cold lysine solution for intervals up to 24 hr. The mean nuclear label reaches a maximum approximately 30 min after removal from the radioactive solution, drops sharply in the following 30 min and then very gradually declines. At the end of 24 hr the nuclei remain labeled, and densely labeled nucleoli are found. The latter concurs with recently published observations of postmitotic nucleolar label in connective tissue cells grown in tissue culture.²⁵

Discussion. – In view of the apparent disparity of results in previous studies of nucleolar protein,^{26,27} the label produced in the nucleoli of meristematic cells after 15 min is significant. Furthermore, other observations indicate that in those nuclei whose morphology clearly indicates an origin from a differentiated cell, the incidence of nucleolar label is low. Consequently it appears that there is a real difference in the behavior of basic protein in the nucleus of an embryonic cell from that of a mature and differentiated cell.

The Mechanism of Ethyl Methane Sulfonate-Induced Gene Mutation

D. R. Krieg Rea M. Fulkerson

Introduction. – Any detailed explanation of mutagenesis involves gene structure and the mechanisms of gene action and duplication, and our continued study of the mutagenic action of ethyl methane sulfonate (EMS) has that objective. A previous report²⁸ outlined the impetus for,

²⁴J. Woodward, E. Rasch, and H. Swift, *J. Biophysic. and Biochem. Cytol.* **9**, 445–62 (1961).

²⁵H. Harris, *Nature* **190**, 1077–78 (1961).

²⁶J. Carneiro and C. P. Leblond, *Science* **124**, 391–92 (1959).

²⁷A. Ficq, *Experientia* **9**, 377 (1953).

²⁸D. R. Krieg *et al.*, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, pp 28–29.

methods of, and preliminary results in a comparative study of EMS-induced reversion at various *r* mutant sites of bacteriophage T4. Further measurements of the yields of induced revertants from a standard exposure of phage to the mutagen strengthen the generalizations advanced at that time, and it is now appropriate to offer further interpretation.

Gene mutations capable of spontaneous reversion may be assumed to involve any of three distinct hypothetical classes of changes at the level of a single nucleotide pair within DNA: (1) Transition – replacement of a guanine-cytosine pair (or in the case of phage T4, a guanine-hydroxymethylcytosine pair) by an adenine-thymine pair, or the reverse. The two transitions may be referred to as GC to AT and as AT to GC. (2) Transversion – replacement of a purine-pyrimidine pair by a pyrimidine-purine pair. There are four such changes in this class which may be summarized as GC or AT to CG or TA. (3) Insertion or deletion – addition or subtraction of a single pair from the sequence. It has been postulated that base analogs mutagenic to duplicating genomes produce only transitions. Chemicals which produce base analogs *in situ* by reaction with the normal bases within DNA may similarly be expected to produce transitions. The remaining classes of hypothetical changes might reasonably be produced by proflavine and agents which can destroy a base *in situ*.

Results and Discussion. – The 29 *r* mutants now tested for EMS-induced reversion had originally been induced from wild-type T4 by the action of the analogs 2-aminopurine (AP) or 5-bromuracil (BU), by EMS, or by proflavine. We found three AP-induced mutants which were strongly EMS-revertible; the estimated yields were respectively 64, 81, and 152 apparent wild-type phage per million progeny. The other AP-induced mutants and the BU-induced mutants checked (five each) showed little or no EMS-induced reversion. If the hypothesis is accepted that analog-induced mutants are based on transitions, we could hypothesize that one kind of transition is induced by EMS at fairly homogeneous frequencies far exceeding the other kind of transitions. That hypothesis, however, would not preclude the possibility that EMS may also induce one or more of the other classes of base-pair changes. That possibility was investigated in two ways. First, eight proflavine-induced mutants were tested and found to yield little or no EMS-induced wild-type revertants. We conclude that whatever the type of (nontransition) changes represented by these mutants, the reverse change is not strongly induced by EMS. Second, eight EMS-induced mutants were tested and also found to yield little or no EMS-induced revertants. The fact that these mutants had not been produced by any kind of base pair change which can be strongly reversed by EMS also accords with the hypothesis that EMS most frequently induces one kind of transition. In all the above observations, "little or no" reversion means that there was one or less induced wild-type phage per million progeny. The actual values for the low-level analog- and EMS-induced mutants was 0.4 ± 0.4 per million. For proflavine-induced mutants, the results were 0.2 ± 0.3 per million. In at least some of the cases, the low level observed appeared to be a real induced reversion.

While these results strengthen the internal consistency of the transition hypothesis of analog-induced mutation by demonstrating a sharp dichotomy between two groups of presumably transition-induced mutants (which could correspond to the GC to AT and the AT to GC transitions), other

lines of evidence must be provided to establish the interpretation and to determine which transition is the one strongly induced by EMS. Another argument from the above results can be mentioned, however, which would mitigate against any idea that the EMS-induced mutants we examined were produced by transversions. If transversions were the major class of EMS-induced gene mutations, some of them should have been strongly EMS-revertible since half the types of transversions (i.e., the GC to CG and the AT to TA changes) involve the same process for forward and reverse mutation.

Chemical results, presented and discussed elsewhere in this volume by B. C. Pal, show that several analogs may be produced by the ethylating action of EMS on normal purines and pyrimidines. Two of the analogs are especially attractive for speculation as to induced transitions. Formation of 3-ethyladenine in the DNA might produce AT to GC transitions since the analog in its most likely chemical state could pair by a single hydrogen bond either with T or with C. If the pairing with C is at least as likely as the pairing with T, the mutation would be expected to occur usually at a very early postexposure duplication. On the other hand, formation of 7-ethylguanine in the DNA might be expected to produce GC to AT transitions. The guanine analog can be expected to exist in either of two tautomeric states. The keto state should be able to pair with C by three hydrogen bonds, as does guanine. The enol state should be able to pair with T by two or three hydrogen bonds, and this would lead to the mutations. If the probability of a tautomeric shift to the enol form is small but constant at any of the occasions when the treated molecule serves as a template for DNA synthesis, then induced transitions would be equally likely to occur as delayed mutations at any postexposure duplication. This pattern of delayed mutation has been observed for one of the strongly revertible mutants we have studied and also for most "forward *r* mutations."²⁹

The hypothesis that the mutants we find most strongly EMS-revertible revert by a GC to AT transition is also supported by the correspondence between our two classes of mutants and the two distinct classes of mutants revertible by the action of hydroxylamine, a mutagenic chemical which reacts more rapidly with C than with T.³⁰ The idea that EMS can induce both kinds of transitions, with AT to GC producing earlier mutations but at a lower frequency, can be supported by comparison of the extent that induced revertants from different *r* mutants can be detected when the treated phage themselves infect a bacterial host requiring early action of the revertant gene. We find in such an experiment that only a few per cent of the potential revertants are detected from one of the highly revertible *r* mutants, while with one of the less revertible *r* mutants a large fraction is detected. As a result of this difference in efficiency of detection, the mutant which actually can produce about 200 times as many revertants as the other appears in such "direct plating" experiments to yield only about eight times as many reversions. This illustrates the hazards of quantitative interpretation of mutation rates estimated from experiments in which there is inadequate

²⁹D. M. Green and D. R. Krieg, *Proc. Natl. Acad. Sci. U.S.* **47**, 64 (1961).

³⁰E. Freese *et al.*, *Proc. Natl. Acad. Sci. U.S.* **47**, 845 (1961).

opportunity for mutation to occur before a selective assay, and helps to explain why in an earlier report³¹ the reversions studied were not so clearly identifiable as belonging to two distinct classes.

Our interpretation that transitions are produced by a tautomeric shift of 7-ethylguanine, rather than by the two-step process involving this analog which has been advanced elsewhere,³¹ is also supported by our preliminary data showing an approximately linear relationship between reversion of a strongly induced mutant and the duration of the EMS exposure.

In summary, various lines of evidence are available to support the hypothesis that the high frequencies of EMS-induced mutations we observe are due to GC to AT transitions, with the other types of base-pair changes occurring to a lesser extent if at all. This finding should be useful in the identification of the structural basis of a large class of gene mutants. Complementary purine-pyrimidine pairing, which is believed to underlie a semiconservative mechanism of DNA duplication, is intrinsic to this hypothesis. Thus, the verification of our hypothesis for EMS-induced mutagenesis could demonstrate the correspondence between a chemical mechanism of DNA synthesis and a genetic analysis of gene duplication.

Ultraviolet-Induced Mutation of Intracellular T4 Bacteriophage

Jane K. Setlow

Introduction. – It has been shown that the ultraviolet action spectrum for elimination of plaque-forming ability in T2-infected bacteria changes as a function of development of the virus.³² There is evidence that this shift in action spectrum reflects change in viral DNA from an organized to a disorganized state.³³ The action spectrum of disorganized DNA is similar in shape to the sum of the absorption spectra of the ultraviolet-sensitive components of DNA, the pyrimidines. This suggests that ultraviolet (UV) irradiation of disorganized DNA involves little energy transfer from purines to pyrimidines, and further that a biological function of DNA involving only one or two bases could have an action spectrum like the absorption of the particular base or bases involved. Thus it might be possible to assign a particular base to a particular function, since the absorption spectra of the individual bases are very different. Benzer³⁴ has genetically mapped thousands of point mutants in the *rII* region of the T4 bacteriophage genome, and has evidence that each of these mutants differs from the wild type by a small number of nucleotide pairs, possibly one. Action spectra for UV-induced reversion of these mutants, obtained during the disorganized stage, should show large differences, because of differences in the base pair altered from the wild type.

Results. – T4 complexes have been shown to have a disorganized-type action spectrum at 7 min after infection. A method has been developed for irradiating large numbers of T4-infected

³¹E. Bautz and E. Freese, *Proc. Natl. Acad. Sci. U.S.* **46**, 1585 (1960).

³²J. K. Setlow and R. B. Setlow, *Proc. Natl. Acad. Sci. U.S.* **46**, 791 (1960).

³³J. K. Setlow and R. B. Setlow, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, p 29.

³⁴S. Benzer, *Proc. Natl. Acad. Sci. U.S.* **47**, 403 (1961).

bacteria at this time of the latent period. After irradiation, these complexes are permitted to produce progeny phage, and the titers of revertant and total phage are measured. Preliminary experiments suggest (1) that it is possible to induce reversions under these conditions and (2) that two mutants tested differ markedly in their response to 2400-A radiation.

Modification of X-Ray Inactivation of Transforming DNA by Sonication

Jane K. Setlow

Introduction. – An attempt has been made to investigate the shape of the x-ray inactivation curve of transforming DNA in solution and the different sensitivities of different markers by examining the change in the curve resulting from previous sonication of the DNA. Experiments have been done with *Hemophilus influenzae* DNA containing a number of markers which confer drug resistance on recipient cells.

Results and Discussion. – The x-ray inactivation curve of untreated DNA on a semilogarithmic plot has an initial concave portion followed by a linear region. All markers showed a decrease in sensitivity after sonication (as judged by the slope of the linear part of the curve), and the concave portion of the curve was eliminated. DNA containing two different markers with the same initial sensitivity showed the same decrease in sensitivity as a result of sonication, but two markers which initially had different x-ray sensitivities had the same sensitivity after sonication. The possibility that sonication releases a protective substance into the medium was eliminated by finding that the presence of a sonicated DNA solution during irradiation did not alter the x-ray sensitivity of unsonicated DNA.

A possible explanation of these phenomena is that the typical x-ray inactivation curve represents two types of inactivation: (1) a decrease in probability of recombination of the markers, caused by a hit on the DNA molecule far from the genetic marker, and (2) elimination of successful incorporation of the marker by any hit within a region closer to the marker. The first mechanism could yield an inactivation curve with a steadily decreasing slope, accounting for the concave portion of the x-ray curve, and as observed for ultraviolet inactivation of *H. influenzae* DNA.³⁵ The second mechanism yields exponential survival, as observed in the higher-dose portion of the x-ray curve. Sonication is known to break DNA molecules.³⁶ If marker-bearing DNA molecules sonicated before irradiation retain little or no material outside the "direct-hit" region, then only the second (exponential) type of inactivation can take place. Furthermore, if the direct-hit region itself may be reduced by sonication, the sensitivity of the marker would be decreased. For the different markers the direct-hit regions may be postulated to be initially of different sizes, to account for the differences in the slopes observed for the exponential part of the typical inactivation

³⁵J. K. Setlow, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, p 30.

³⁶P. Doty, B. B. McGill, and S. A. Rice, *Proc. Natl. Acad. Sci. U.S.A.* 44, 432 (1958).

curves; however, these regions may tend to become more nearly the same size as a result of sonication.

Photoreactivation and the State of Organization of DNA

Jane K. Setlow R. B. Setlow

Introduction. – Evidence that only organized polynucleotides sustain photoreactivable ultraviolet (UV) damage comes from work on the photoreactivation of irradiated complexes of T2 phage and ϕ X174 with *E. coli*. Complexes of T2 irradiated early in the latent period (DNA organized³⁷) undergo more photoreactivation than those irradiated at intermediate times³⁸ (DNA disorganized³⁷). Our data on the irradiation of the virus ϕ X174 and its complexes indicate that irradiated free virus (DNA organized in the virus) and irradiated 3-min complexes (DNA organized in the bacterium³⁹) are photoreactivable but that irradiated 0.7-min complexes (DNA disorganized in the bacterium) are not. For a more critical test of the hypothesis that irradiation of organized DNA results in photoreactivable damage whereas irradiation of disorganized DNA does not, experiments have been performed on photoreactivation of ultraviolet irradiated *Hemophilus influenzae* transforming principle which was heated above the melting point either before, during, or after inactivation by 2537-A radiation. A photoreactivating extract was prepared from yeast.⁴⁰ The measure of biological activity of the DNA used was the ability to transform sensitive *H. influenzae* cells to streptomycin resistance.

Results and Discussion. – The experiments are summarized below. After the sequence of treatments indicated, each preparation was tested with the yeast extract for photoreactivability. The ultraviolet irradiation was at room temperature, except for the one case noted. The intervals of 98°C heat did not exceed 6 min, and were immediately followed by fast cooling (in some cases slow cooling was employed, with similar results).

Treatment	Photoreactivation
UV, no heat	+
UV at 98°C	-
UV, 98°C	-
98°C, UV	+
UV, 85°C	+
85°C, UV	+

³⁷J. K. Setlow and R. B. Setlow, *Proc. Natl. Acad. Sci. U.S.* **46**, 791 (1960).

³⁸N. Symonds and W. McCloy, *Virology* **6**, 649 (1958).

³⁹R. B. Setlow, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, p 138.

⁴⁰C. S. Rupert, *J. Gen. Physiol.* **43**, 573 (1960).

These data show that 98°C heat after as well as during ultraviolet exposure eliminates photoreactivability. Since heating below the melting point (85°C for 1 hr) did not prevent photoreactivation, it is apparently the melting of the DNA rather than the heat which eliminates the possibility of photoreactivation. The 98°C heat after irradiation does more than change the photoreactivability of the DNA. The survival is higher than for DNA which was heated to 98°C before irradiation.

It is concluded that melting of the DNA eliminates the fraction of ultraviolet damage that is photoreactivable, either by the removal of damages or by rendering them nonreactivable.

Experiments are planned in which DNA is melted at a much lower temperature in low salt concentration. If the same relations between irradiation, melting, and photoreactivation hold as at 98°C, then the hypothesis of elimination of photoreactivability by change to a disorganized state of the DNA will be greatly strengthened.

The Role of Extrinsic Heterokaryon-Incompatibility Factors in Heterokaryon Tests on *ad-3* Mutants of *Neurospora crassa* Derived from the Same Wild-Type Strain

F. J. de Serres

Previous data from crosses, and heterokaryon tests,⁴¹ and an analysis of an insertional translocation⁴² have shown that the *ad-3A* and *ad-3B* loci are separate, and functionally distinct. In more extensive tests on larger samples of *ad-3* mutants of different mutagenic origin,⁴³ no mutants were found that gave negative heterokaryon tests with all *ad-3A* and *ad-3B* testers. Certain mutants formed heterokaryons with some *ad-3A* and *ad-3B* tester strains so that they were represented initially on the complementation map⁴⁴ as partial overlaps into the *ad-3A* or *ad-3B* region. Since the existence of such a class of mutants is not expected in the absence of a class of true *ad-3A ad-3B* double mutants, the present experiments were planned to determine whether negative heterokaryon tests in such instances can be explained on any other basis. The experimental approach used was to cross such mutants to wild type and to compare the patterns of complementation of the original strains with those of the F₁ adenine-requiring progeny.

Results. — The interaction patterns of the original isolates and F₁ progeny in various pairwise combinations are given in Table 3. In all cases, F₁ strains were found that gave more vigorous heterokaryon tests with *ad-3A* or *ad-3B* tester strains than the original isolates. This indicates segregation of at least one (and in some crosses more than one) heterokaryon incompatibility factor. Positive heterokaryon tests were obtained with pairwise combination of heterokaryon-compatible F₁ progeny for every instance where a negative test was obtained with the original *ad-3A + ad-3B* strains.

⁴¹F. J. de Serres, *Genetics* 41, 668–76 (1956).

⁴²F. J. de Serres, *Genetics* 42, 366–67 (1957).

⁴³F. J. de Serres, this report.

⁴⁴F. J. de Serres, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, p 48.

Table 3. Number of Positive Heterokaryon Tests Obtained with Various Pairwise Combinations of *ad-3A* and *ad-3B* Mutants and Their F₁ Progeny (from a Cross to Wild Type) on Minimal Medium

<i>ad-3B</i> Mutant (Map Position)	<i>ad-3A</i> Mutant (Map Position)					
	1230-0200 (1,2)		1230-0049 (1 → 3)		2010-0194 (1 → 4)	
	Original	F ₁ Progeny	Original	F ₁ Progeny	Original	F ₁ Progeny
2017-0095 (2 → 10)						
Original	-	11/18	-	14/25	-	6/27
F ₁ progeny	4/25	+	4/25	+	25/25**	+
1152-0068 (3 → 28)						
Original	+		-	16/24	-	7/16
F ₁ progeny			7/16	+	0/17	+
1155-0055 (4 → 24)						
Original	+		+		-	14/16
F ₁ progeny					21/25	+
2017-0091 (4,5)						
Original	+		+		-	15/17
F ₁ progeny					17/17	+

*At least one pairwise combination was made of heterokaryon-compatible F₁ progeny from each cross.
 **9/25 combinations showed a markedly slower growth rate.

Conclusions. — The data from the present experiments show that the failure to obtain positive heterokaryon tests in pairwise combination of the original isolates of *ad-3A* and *ad-3B* mutants of common origin can be attributed to interaction of extrinsic heterokaryon-incompatibility factors. The presence of such factors in very large populations of mutants might be expected as a result of independent mutational events. However, the factors studied in the present experiments are unusual in that none prevented heterokaryon formation between these seven mutant strains and the majority of *ad-3A* or *ad-3B* testers. Negative tests were obtained only when such factors from different mutants were present in particular combinations. Since negative tests were obtained where at least weak positive tests might be expected, interaction of certain of these factors appears to be more than additive.

Correlation Between Mutagenic Origin of *ad-3* Mutants of *Neurospora crassa* and Allelic Complementation in Heterokaryons

F. J. de Serres

On the currently accepted theory of information transfer, some unique sequence of nucleotide pairs in a given region of the DNA molecule is believed to determine the sequence of amino acids in the polypeptide chain of a particular protein. Thus any mutagenic treatment that produces gross alterations (deletions, inversions, multiple site changes, etc.) in this region of the DNA molecule should produce gross alterations in the polypeptide chain, whereas treatment that alters only a single pair of nucleotides should produce a polypeptide chain in which there has been the erroneous substitution of only a single amino acid. Since all available evidence indicates that allelic complementation results from interaction of differentially altered polypeptide chains of the same enzyme, the proportion of complementing mutants in any given sample would be expected to be correlated with mutagenic origin. To test this hypothesis unselected samples of *ad-3* mutants from x-ray, ultraviolet, and HNO₂ treatment of wild-type strain 74A were used in heterokaryon tests to determine the fraction in each sample showing allelic complementation.

Results. – Each sample of mutants was tested with the 31 tester strains⁴⁵ as described previously.⁴⁶ The results of these experiments are presented in Table 4. Two main classes of mutants were found, as in previous analyses. No mutants were found that gave negative heterokaryon tests with all 31 tester strains, indicating that no true *ad-3A ad-3B* double mutants are produced with any of these mutagenic treatments. Some mutants were found that gave negative tests with certain *ad-3A* and *ad-3B* testers, and the significance of these results is described in another report.⁴⁷ No evidence was found for interaction of any of the *ad-3A* mutants with *ad-3A* tester strains in these or in separate tests where 80 were tested in all possible pairwise combinations. Among the *ad-3B* mutants, however, there is a marked correlation between mutagenic origin and the fraction showing allelic complementation. There is a direct correlation in each sample between the fraction showing allelic complementation and the fraction that might be expected to result from such a genetic change as alteration of a single nucleotide pair.

The difference in the relative frequencies of *ad-3A* and *ad-3B* mutants obtained with these three treatments may be associated with apparent differences in the locus sizes. Both crossing and heterokaryon data indicate that *ad-3A* locus is smaller in size than the *ad-3B* locus. One explanation of the data may be that after x-ray treatment a larger proportion of the *ad-3A* than the *ad-3B* mutations are lethal. This hypothesis is being subjected to a direct test with the use of a balanced heterokaryon between an *ad-3A ad-3B* double mutant and wild type in forward-mutation experiments in progress.

⁴⁵F. J. de Serres, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, p 48.

⁴⁶F. J. de Serres, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1960*, ORNL-2913, p 34.

⁴⁷F. J. de Serres, this report.

Table 4. Fraction of *ad-3A* and *ad-3B* Mutants Showing Allelic Complementation Among Samples of Different Mutagenic Origin

Mutagenic Treatment	Number of Mutants Tested	<i>ad-3A</i>		<i>ad-3B</i>		Number of Different Classes
		Per Cent of Total	Per Cent Complementing	Per Cent of Total	Per Cent Complementing	
X ray	146	8.2	0	91.8	6.0	7
Ultraviolet	125	15.2	0	84.8	16.0	16
HNO ₂	125	23.2	0	76.8	72.9	26

Conclusions. — These experiments show quite clearly that the loss of enzyme activity as a result of mutation in the *ad-3B* region is not always due to the production of grossly altered enzymes. That the loss of enzyme activity can result from many different types of alterations in the polypeptide chain seems likely from the wide variety of complementation patterns obtained. Both single and multiple complement mutants have been found in each of the three samples; no obvious difference in the complementation patterns of complementing mutants has been observed as a function of mutagenic origin. This may be due to present sample sizes, but the ability to show allelic complementation could be restricted to that class of mutations that produces single amino acid substitutions, so that no difference in the types of complementation patterns would be expected.

Frequency of *ad-3* Mutants of *Neurospora crassa* Producing Aborted Spores

H. E. Brockman F. J. de Serres

Introduction. — The fraction of *ad-3B* mutants which shows interallelic complementation varies as a function of mutagenic origin as follows: x-ray induced, 6.0%; ultraviolet induced, 16.0%; and nitrous acid induced, 72.9%.^{48,49} These results suggest that the frequency of interallelic complementation is a result of the major kind of genetic alteration induced by a particular mutagen. McClintock has demonstrated that *Neurospora* mutants which carry cytologically detectable reciprocal translocations produce aborted (white) ascospores when crossed to cytologically normal strains.⁵⁰ Furthermore, any strain carrying a chromosomal rearrangement that would produce chromosomal deficiencies following meiosis would be expected to produce some aborted ascospores. Thus, the production of aborted ascospores from crosses of *ad-3* mutants to a cytologically normal wild-type strain can be used as a screening method to estimate the frequency of this class of genetic alteration among *ad-3* mutants of different mutagenic origins.

⁴⁸F. J. de Serres, *Radiation Research* 12, 430 (1960).

⁴⁹F. J. de Serres, this report.

⁵⁰Barbara McClintock, *Am. J. Botany* 32, 671–78 (1945).

Results. – The frequency of aborted ascospores in two random samples from 158 *ad-3* × wild-type crosses was determined and then compared statistically with the control cross of wild-type × wild-type. The proportion of *ad-3* mutants which showed a greater than control frequency of aborted ascospores varied according to mutagenic origin, as shown in Table 5.

Discussion. – The results show that the frequency of the genetic event giving rise to aborted ascospores is inversely related to the frequency of interallelic complementation when x-ray, ultraviolet, and nitrous acid-induced *ad-3* mutants are compared. It would seem most likely that each *ad-3* mutant producing aborted ascospores has a chromosomal rearrangement associated with the *ad-3* locus, but the aborted spores could also possibly result from a chromosomal rearrangement in some other part of the genome or from a gene mutation effecting ascospore development.⁵¹ Therefore, these mutants are being further studied by genetic methods to determine if the production of aborted ascospores is always correlated with a chromosomal rearrangement including the *ad-3* locus. After this has been resolved, the complementation pattern of *ad-3* mutants associated with chromosomal rearrangements will be contrasted to those in which such a rearrangement is not demonstrable.

Table 5. Frequency of *ad-3* Mutants with Aborted Spores

Mutagen	Per Cent Survival	Frequency with Aborted Spores	
X-ray	21	13/23	56.5%
Ultraviolet	37	4/22	18.2%
Hydrogen peroxide	36	4/25	16.0%
Diepoxybutane	24	0/28	0.0%
Nitrous acid	25	0/50	0.0%
Spontaneous	100	2/10	20.0%

Induction of *Neurospora crassa ad-3* Mutants with Proflavine

H. E. Brockman B. B. Webber

Introduction. – Physical evidence⁵² has recently provided a basis for the hypothesis⁵³ that proflavine mutants in phage arise by a process involving intercalation of proflavine between adjacent nucleotide-pair layers in DNA molecules and a subsequent loss or insertion of base pairs during replication. On the basis of most acceptable theories of genetic coding, one would anticipate that a base-pair deletion or insertion would result in the production of an extensive section

⁵¹L. Garnjobst and E. L. Tatum, *Am. J. Botany* 43, 149–57 (1956).

⁵²L. S. Lerman, *J. Molecular Biol.* 3, 18–30 (1961).

⁵³S. Brenner *et al.*, *J. Molecular Biol.* 3, 121–24 (1961).

of "nonsense DNA." The hypothesis therefore predicts⁵³ that proflavine mutation in a genetic region determining amino acid sequence in a polypeptide would cause profound alterations in or complete absence of the determined polypeptide. Proflavine mutants in *Neurospora crassa* would, according to the above hypothesis, be expected to be extensive on a heterokaryon complementation map of the locus affected or completely noncomplementary with most or all of the mutants with which it is allelic.

Preliminary experiments have been undertaken to determine suitable concentrations and conditions for obtaining proflavine-induced mutants at the *ad-3* region in *Neurospora*.

Results. – Table 6 shows the unexpected effects of three different concentrations of proflavine in light upon macroconidial viability. A concentration of 0.0005% produces the fastest kill, while a tenfold increase or decrease in concentration causes a slower kill. An additional experiment indicated that concentrations of 0.05% and 0.000005% proflavine in the light also cause less killing than 0.0005%. An experiment comparing the killing effect of 0.0005% proflavine in light and in dark was also carried out. After 4 hr of treatment with proflavine in the dark the viability was twice that following the same exposure in the light, and the difference between treatments appeared to increase with time. This is in agreement with previous work with yeast.⁵⁴

Table 6. Macroconidial Survival (Percentage of Zero-Time Estimates)

	Zero Time	1 hr	2 hr	4 hr
0.005% Proflavine (light)	100	27.4	22.0	15.8
0.0005% Proflavine (light)	100	14.4	1.8	<0.08
0.00005% Proflavine (light)	100	83.0	44.9	19.9
No proflavine	100	66.5	51.4	38.2

It was assumed that proflavine killing was caused, at least in part, by nuclear inactivation and so it was anticipated that optimal conditions for obtaining *ad-3* mutations and for killing might be identical. Accordingly, an *ad-3* forward-mutation experiment with two concentrations (0.0005% and 0.00005%) of proflavine in the light was performed. The number of *ad-3* mutants recovered per 10⁵ survivors is as follows: 0.0005% proflavine, 1 hr (43.5% survival), 2.5 mutants; 0.0005% proflavine, 2 hr (4.7% survival), 7.5 mutants; 0.00005% proflavine, 4 hr (37.7% survival), 3.4 mutants; 0.00005% proflavine, 8 hr (43.6% survival), 1.3 mutants. Heterokaryon complementation analyses permitting a test of the hypothesized mechanism of proflavine-induced mutation in *Neurospora* are planned.

⁵⁴D. Freifelder and R. B. Uretz, *Nature* 186, 731–32 (1960).

CELL GROWTH AND REPRODUCTION

Protein Synthesis and the Loss of RNA from Anucleated Cells

D. M. Prescott

Romance F. Carrier

Previously reported experiments demonstrated that anucleated *Tetrahymena* were incapable of RNA synthesis but were able to incorporate amino acids into proteins. An extension of this work has revealed that this capacity for protein synthesis declines rapidly after anucleation and can be correlated with a loss of RNA.

Tetrahymena were sectioned into nucleated and anucleated fragments and incubated for 30 min in H³-histidine medium at various times after anucleation. During the first 30 min after sectioning, anucleates incorporate approximately half as much H³-histidine as nucleated fragments. The capacity for H³-histidine incorporation in anucleates declines rapidly during the first 1 to 2 hr and then falls more slowly. By 8 hr after anucleation, virtually all protein synthesis has stopped.

In order to follow the loss of RNA from anucleated fragments, *Tetrahymena* were incubated in H³-cytidine for 1 hr, transferred to nonradioactive medium, sectioned into fragments, fixed at various times over the next 10 hr, and autoradiographed. Loss of RNA was measured as the decrease in the amount of H³-cytidine in RNA. The decline in radioactive RNA was rapid during the first 1 to 2 hr and slow thereafter. At the time when H³-histidine incorporation into protein has ceased, approximately 50% of the labeled RNA has been lost. The experiments suggest that a fraction of RNA is "used up" during protein synthesis and that protein synthesis ceases when this fraction is exhausted.

Synthesis of RNA and Protein During Mitosis in Mammalian Tissue-Culture Cells

D. M. Prescott

M. A. Bender

If the interphase chromosome serves as a template to designate base sequences in RNA, RNA production might be depressed during two stages of the cell life cycle: (1) when the nucleus is engaged in syntheses concerned with nuclear replication (DNA-histone synthesis) and (2) during stages of mitosis, when the chromosomes are condensed by tight coiling. Recently completed work demonstrated that RNA synthesis is absent in nuclear regions of DNA-histone synthesis.⁵⁵ To test whether RNA (and protein) synthesis is depressed during mitosis, we measured the incorporation of tritiated precursors into RNA and protein during interphase and various stages of mitosis in mammalian cells in *in vitro* culture.

Chinese hamster tissue-culture cells were exposed to H³-uridine for 5 min, fixed, acid extracted, and autoradiographed. Labeled RNA was found in the nuclei of all interphase cells, but the cytoplasm was unlabeled. RNA labeling was less in early prophase cells, and no radioactive

⁵⁵D. M. Prescott and R. F. Kimball, *Proc. Natl. Acad. Sci. U.S.*, 47, 686 (1961).

RNA was detectable in any cells in stages between midprophase and middle telophase. This cessation of RNA synthesis occurred before the prophase disappearance of the nucleoli and before detectable disintegration of the nuclear membrane. Synthesis resumed in middle-to-late telophase but before nucleoli were visible. Hamster cells arrested in metaphase with colchicine synthesized no RNA, although colchicine had no detectable effect on the rate of H³-uridine incorporation by interphase cells.

In HeLa cells exposed to H³-uridine for 15 min, three classes of cells were clearly defined by autoradiography: (1) cells having a heavy incorporation of radioactivity in nuclear RNA but no trace of incorporation in the cytoplasm; (2) cells in mitotic stages between late prophase and late telophase, with no trace of incorporation of H³-uridine in either chromosomes or cytoplasm; and (3) a very few cells in prophase with radioactive RNA in both the nucleus and cytoplasm. The latter class of cells was at a sufficiently early stage of prophase, at the beginning of the 15-min incubation in H³-uridine, to incorporate radioactivity into nuclear RNA. During the 15-min period, such cells apparently progressed to the point of nuclear membrane breakdown – pouring out nuclear RNA into the cytoplasm.

H³-histidine incorporation in hamster cells declined by 75% between early prophase and middle telophase.

The experiments constitute evidence that (1) the dispersed chromosome of interphase is an essential participant in all RNA synthesis; (2) a mass release of RNA to the cytoplasm occurs in prophase at the time of nuclear membrane breakdown; and (3) as a consequence of the complete lack of RNA synthesis during mitosis, protein synthesis declines rapidly.

Incorporation of Nucleotides into Isolated *Euplotes* Nuclei by DNA Polymerase

R. C. von Borstel

D. M. Prescott

F. J. Bollum

Introduction. – The macronucleus of *Euplotes* has morphologically distinct band regions that make it possible to define microscopically the exact area of DNA synthesis.⁵⁶ The enzyme, calf thymus polymerase, requires denatured or single-stranded DNA as a primer for DNA synthesis and is inactive on native DNA preparations.⁵⁷ It therefore seemed possible that the presence of primer DNA could be detected in such cytological preparations by allowing the enzyme and tritiated substrates to react with fixed preparations prior to autoradiography. The formation of silver grains would then indicate the presence of DNA primer in the preparation.

Results. – When nuclei are isolated from *Euplotes*, part of the procedure must be performed in the presence of high concentrations of acetic acid. It is known that low pH will denature DNA. Therefore, squash preparations of *Euplotes* were also done in alcohol, without complete isolation of nuclei. In all cases, low-pH control squashes and isolations were prepared for comparison.

⁵⁶D. M. Prescott, R. F. Kimball, and C. H. Shappert, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, pp 32–33.

⁵⁷F. J. Bollum, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1959*, ORNL-2813, pp 154–55.

When the nuclei were acid-isolated and treated with the enzyme reaction mixture, tritium-labeled nucleotides were incorporated all over the nuclei, in both low-pH controls and the experimental preparations. In the alcohol squashes, nucleotides were incorporated in the low-pH control nuclei only. Interestingly enough, it was not possible in any case to observe incorporation of nucleotides into the band region where DNA synthesis was in progress at the time of fixation.

Discussion. — This method appears to be the most sensitive yet devised for detection of single-stranded or denatured DNA in fixed cells and tissues. The extreme sensitivity of the test requires that much care must be taken with tissue preparation to avoid denaturation of native DNA prior to assay.

Although these preliminary experiments have failed to demonstrate primer DNA in the region of DNA synthesis of *Euplotes* nuclei, they demonstrate the applicability of the method. Further experiments are expected to show whether the band regions where DNA synthesis occurs in *Euplotes* have priming action for distances only a few nucleotides long, or whether the priming DNA is somehow protected from the reaction mixture used in these experiments.

Root Growth Following Treatment with Colchicine and Indoleacetic Acid

Douglas Davidson

Introduction. — The growth of primary roots of *Vicia* is inhibited by colchicine. The loss of growth capacity is accompanied by changes in the chromosome constitution of the meristem cells (many of them become polyploid) and probably by a temporary cessation of the synthesis of the natural auxins necessary for growth.⁵⁸ The study to be described here was carried out to determine whether roots whose growth had been inhibited by colchicine could be stimulated to grow by an exogenous supply of auxin.

Results. — (1) The growth of primary roots was followed, over 7 days, in 3 groups, each of 40 roots. Treatment with 0.025% or 0.05% colchicine for 1 hr reduced the total growth in 7 days to 33.8% and 23.7% of that of the untreated roots. (2) Colchicine is effective in inhibiting root growth whether the whole root or only 3 mm at the root apex is immersed. This result agrees with previous reports that agents which inhibit growth do so by affecting the apical meristem. (3) Roots were treated with 0.05% colchicine for 3 hr. One day later, half the roots (of 32 beans) were treated with a 10^{-5} M solution of indoleacetic acid (IAA) for 24 hr. The growth of both groups of beans was followed over the succeeding 8 days. The total growth of roots treated only with the colchicine was 54% of that of roots also treated with IAA. The difference in the total growth in the 8-day period reflects the earlier onset of growth and the faster increase in the growth rate of the IAA treated roots. (4) Roots were treated with 10^{-5} M IAA for 24 hr. They showed a 56% inhibition of growth when compared with untreated roots.

⁵⁸D. Davidson, *Chromosoma* (in press).

Discussion. — The stimulation of slow-growing roots by IAA in concentrations that are inhibitory to normal roots leads to the following conclusions: (1) The sensitivity of roots to IAA is lost when their growth has been inhibited by colchicine; IAA becomes a growth stimulator, not an inhibitor. This may be related to changes in the amounts of natural auxins secreted after a colchicine treatment. (2) The stimulating effect of IAA does not result in an immediate effect. The inhibited roots do not resume growth at once, but they do so faster than colchicine-treated roots that lack IAA. The delay in the expression of a response to exogenous IAA reveals (1) that original inhibition of growth was due to more than the failure of synthesis of one growth factor and (2) also that the IAA is facilitating the recovery of the meristem of the root to a condition in which it will maintain growth in all its phases, not only in cell expansion.

Differential Sensitivity of *Habrobracon* Oocytes in the First Meiotic Prophase and Metaphase to Chemical Mutagens

E. A. Löbbecke R. C. von Borstel

Introduction. — From experiments on bacteriophage it is thought that ethyl methane sulfonate (EMS) acts principally at the molecular level of the genome. From this, the concept of mutagenesis at the nucleotide level is being re-examined. It seemed to be worth while to use the system of dominant and recessive lethals in the wasp *Habrobracon* to investigate the specific mode of action of EMS in higher organisms. The special feature of the wasp is that the effects on the haploid and diploid chromosome set can be compared directly and that it is possible to compare the induced mutations of clearly distinguished stages of oogenesis (first prophase and first metaphase of meiosis). Another reason to use *Habrobracon* is that x-ray and ultraviolet radiation data are available for comparison. Nitrogen mustard (HN₂) was used to provide a standard of comparison for EMS with a known potent chemical mutagen.

Results. — The difference in stage sensitivity to the treatment by aerosols of solutions of EMS and HN₂ can easily be demonstrated if the total hatchability of the unmated groups is compared. The dominant embryo lethal frequency is 100% hatchability. Plotted on semilogarithmic paper these data show approximately the same slopes of curves as have been observed in x-ray experiments.⁵⁹ Data on hatchability and the frequency of occurrence of recessive lethals are given in Tables 7 and 8.

The difference in sensitivity is also obvious, when recessive-lethal frequency is measured. This frequency is tested by setting surviving female offspring and counting as induced-recessive lethals those females that produce 50% or fewer inviable offspring.

Discussion. — From these data it can be concluded that after treatment with these chemical mutagens the first meiotic metaphase is more sensitive than the first meiotic prophase when either

⁵⁹R. C. von Borstel and M. L. Rekemeyer, *Genetics* 44, 1053-74 (1959).

Table 7. Hatchability

After EMS Treatment			After HN ₂ Treatment		
Dose (% Concentration)	Metaphase (%)	Prophase (%)	Dose (% Concentration)	Metaphase (%)	Prophase (%)
0.5	66	79	1	46	76
				62	96
1	2.5	49	2		42
	9.6	89	10	0.9	96

Table 8. Recessive-Lethal Frequency

After EMS Treatment				After HN ₂ Treatment					
Dose (% Concentration)	Metaphase		Prophase		Dose (% Concentration)	Metaphase		Prophase	
0.5	7/114	6.1%	5/229	2.1%	1	7/46	5.2%	4/170	2.3%
1			1/107	0.9%		2/49	4.1%	2/195	1.9%

dominant or recessive lethality is the criterion. In this respect both chemical mutagens are similar to x radiation. However, a 20-fold difference in recessive-lethal frequency between metaphase I and prophase I is observed with x radiation. With the chemical mutagens used, only a two- to threefold difference obtains.

The Cantaloup Locus of the Wasp *Habrobracon*

Gladys S. Van Pelt

Introduction. – The eye-color pigments of the wasp *Habrobracon* afford the opportunity to study genetically controlled biosynthetic pathways in an insect unrelated to *Drosophila*. There are eleven mutant types existing, six having been described and mapped, five being untested. The work to be reported here was undertaken to localize and characterize the untested mutants, to study the chemistry of the eye-color pigments of *Habrobracon*, and to compare the pigments with those found in *Drosophila* and other insects.

Results and Discussion. – All the five untested mutants, *garnet* of Whiting (c^g^W), *garnet* of Clark (c^g^C), *garnet* of von Borstel (c^g^B), *rose* of Clark (c^r^C), and *rose* of Whiting (c^r^W), were found to be allelic to *plum* (c^p) and *cantaloup* (c) at the *cantaloup* locus. At standard rearing

conditions (30°C) the order of dominance within this allelic series parallels the order of eye pigmentation: $c^+ > c^b > c^g > c^r = c$. Tests are being performed to localize the c alleles with respect to other genes on chromosome 1.

The garnet members of the series present additional problems. Although they have arisen in different laboratories, there is no certain way of distinguishing them at present. Homozygous females have an eye color generally lighter than hemizygous males. It is not known whether this difference is solely a matter of gene dosage or is dependent upon sex. The phenotypic expression of the garnet mutants is variable, with temperature a major contributing factor to this variation.

Wild-type and the c -locus mutants were raised at 37, 32, 30, 25, and 17°C to elucidate the temperature effect. Wild-type remains unchanged throughout the temperature range except at 37°C where the ocelli are lighter than normal. *Plum* is indistinguishable from wild-type at 37°C except for ocelli differences; at 30°C its color is dark red-brown; at 25 and 17°C it is dark garnet. The garnets retain the dosage effect at all temperatures. At 37°C females are a very pale pink; males are pink. The darkest garnet color (overlapping the expression of the *plum* allele) appears at 25°C. Eye color of the garnets at 17°C is paler than at 25°C but darker than that seen at 37°C. The roses and *cantaloup* are the same color at all temperatures except 17°C. At this low temperature, c^r is characterized by a dosage effect similar to that described above for the garnets; that is, females are lighter than males.

The complexity of the locus can be seen by the existence of three temperature effects: (1) The normal eye color of wild-type, the roses, and *cantaloup* remains relatively unchanged over the temperature range. (2) The *plum* mutant produces continuously darker eyes as the temperature is raised. (3) The garnets have the darkest color at an intermediate temperature, with a lighter expression at the temperature extremes.

Pteridine and ommochrome pigments are responsible for eye colors in insects. Chromatographic analyses of the pteridines present in heads of 25 individuals indicate a basic pattern in wild-type consisting of five major fluorescing spots. Alleles at the c locus and o^i (ivory) have additional fluorescing spots. One of these fluorescing spots may be comparable to *Drosophila* pteridines. Spectrophotometric examination of *Habrobracon* pteridine extracts yields absorption curves in the visible and ultraviolet range similar to that of the *brown (bw)* mutant of *D. melanogaster*. Since this eye-color gene blocks formation of the red pigments (pteridines), the resulting eye color is due to the brown pigments (ommochromes). Despite the extraction of the pteridines from the wild-type and mutants of *Habrobracon*, no color change was observed. On the other hand, extraction of the ommochromes removed all color from the eyes of the c -locus mutants as well as wild-type. Extracts of ommochromes have not yet been analyzed spectrophotometrically.

Although the biochemical analyses are incomplete, the evidence indicates that ommochromes are the principal eye-color pigments in *Habrobracon*, with pteridines having only a minor role. In *Drosophila*, the pteridines and ommochromes are independent and complementary pigments.

DROSOPHILA

Sex-Linked Male-Sterilizing Mutations

D. L. Lindsley Cloe Camba⁶⁰

Berg (1937)⁶¹ compared the incidence of induced recessive mutations to male sterility and lethality in the first and the second chromosomes of *Drosophila*. Assuming that genes capable of undergoing such mutations occur randomly within the genome, one might expect that their distribution among chromosomes would be related to chromosome length, that is, in the ratio of approximately 1:2 for the X chromosome vs chromosome II. The results for recessive lethals do not depart greatly from this expectation as she recovered 0.182 in the X and 0.441 in the second. Sex-linked recessive steriles on the other hand, rather than being recovered half as frequently as those on chromosome II, were almost three times as frequent, that is, 0.123 on the X vs 0.045 on the second. She interpreted this excess of sterility changes associated with the X chromosome as evidence for an excess of genes associated with male fertility and inferentially with the determination of maleness. We believe that, whereas a mutation of a sex-determining gene might lead to decreased fertility, it does not follow that a mutation to sterility necessarily involves a sex-determining locus. Thus an excess of sex-linked genes for sterility does not necessarily imply an excess of sex-linked sex-determining genes.

Berg was able to recognize translocations between the X and second chromosomes and noticed a strong correlation between T(X;2) and sex-linked male sterility. She attributed this correlation to mutation associated with the X-chromosome break. Lindsley, Edington, and Von Halle⁶² have shown, however, that the male sterility associated with reciprocal X-autosome translocations cannot be attributed to position effect or break associated mutation. Thus the incidence of induced change to sex-linked male sterility overestimates the incidence of genes concerned with male fertility to the extent that X-autosome translocations are involved. To estimate this involvement we have undertaken the recovery and cytogenetic analysis of a group-induced, sex-linked, male-sterilizing alteration.

From a group of 2109 X chromosomes irradiated with 4000 r, 132 cases of sex-linked male sterility were encountered. By two different methods we are determining which of these are translocations. The first method is the measurement of primary nondisjunction in FM6/ms(1) females [ms(1) = male sterile on first chromosome], and the second is by salivary gland chromosome analysis. The results of these studies to date are presented in Table 9. It can be seen that of the 79 cases so far determined 47 have cytologically detectable translocations, and of the remaining 32

⁶⁰Departamento de Biologia Geral, Universidade de São Paulo, São Paulo, Brasil.

⁶¹R. L. Berg, *Genetics* 22, 223-39 (1937); and *Genetics* 22, 240-48 (1937).

⁶²D. L. Lindsley, C. W. Edington, and E. S. Von Halle, *Genetics* 45, 1649-70 (1960).

Table 9. Cytogenetic Analysis of 79 Male-Sterilizing X Chromosomes

Cytological analysis	Primary Nondisjunction in FM6/ms(1) Females		
	≤ 0.05	> 0.05	Σ
Translocation observed	14	33	47
Translocation not observed	15	17	32
Σ	29	50	79

cases 17 show high primary nondisjunction characteristic of X chromosome translocation heterozygotes. This leaves 15/79 cases which do not appear to be translocations by either criterion employed.

It appears that less than 20% of sex-linked male sterilizing alterations are caused by gene mutations; thus of the 12.3% sex-linked changes observed by Berg, perhaps 2.5% may be attributed to gene mutation and almost 10% to reciprocal translocation. Now the mutation rate in the X (0.025) versus that of chromosome II (0.045) is more in agreement with expectations based on chromosome length. We conclude, therefore, that the genes that control male fertility are probably randomly distributed within the genome.

A Study of Gene Dosage and Enzyme Concentration in *Drosophila melanogaster*

E. H. Grell

Introduction. – In addition to carrying the information for the structure of an enzyme, genetic material must also carry information for the regulation of the amount of the enzyme. Among the factors that influence the concentration of enzymes in higher organisms is the dosage of genes. Several cases are known in humans where a homozygous mutation causes the absence of an enzyme activity and where persons heterozygous for the mutation have less enzyme activity than individuals homozygous for the normal allele.

In *Drosophila*, the mutation *ma-l* (maroon-like) causes the absence of xanthine dehydrogenase activity. Since *ma-l*⁺ is a sex-linked locus, normal females have two doses of the locus, and males have one. In order to test the effect of other doses of *ma-l*⁺ on xanthine dehydrogenase concentration, a centric duplication of *ma-l*⁺ and an X-chromosome deficient for *ma-l*⁺ were produced with x rays. These two altered chromosomes also allowed the production of females with one and three doses and males with two doses of *ma-l*⁺.

Results. – Xanthine dehydrogenase activity was measured in extracts of the various genotypes. The enzyme activity was the same for all genotypes irrespective of sex or the number of doses of *ma-l*⁺.

Discussion. – The expression of many characters in *Drosophila* is influenced by gene dosage. The *ma-l*⁺ locus represents a type that is uncommon among the mutants that have been studied. One possibility is that the *ma-l*⁺ locus contains the information for enzyme structure, but another locus controls the concentration of xanthine dehydrogenase.

**The Frequency of Lethals on Chromosomes II and IV in a Tennessee Population
of *Drosophila melanogaster***

Benjamin Hochman T. Lopashinsky⁶³

Introduction. — It has long been known that recessive lethal genes situated in the large autosomes contribute significantly to the total concealed genetic variability present in natural populations of *Drosophila*.^{64,65} Work with *D. melanogaster* has heretofore centered on the second chromosome, and findings of evolutionary and ecological importance have emerged.⁶⁶⁻⁶⁸

The fourth chromosome (microchromosome) of this species is unlike the other two autosomes (II and III) in several respects. Most obvious among these disparities is the size difference. For example, the IV:II ratio of larval salivary chromosome bands is approximately 1:38 (50 bands in chromosome IV and nearly 2000 in II). Other properties unique to chromosome IV are (1) an absence of crossing over (under cytologically normal conditions) and (2) the survival of haplo-IV and triplo-IV individuals. The net effect of these fourth-chromosome peculiarities on the occurrence and maintenance of lethal genes in nature is as yet unknown. The present investigation represents an initial attempt to solve this problem.

Results and Discussion. — Second and fourth chromosomes were extracted from a wild population inhabiting an orchard near Powell, Tennessee. The frequency of recessive lethal genes found on these autosomes is presented in Table 10.

Table 10. Lethals Derived from a Natural Population

Chromosome	Number Tested	Number of Lethals	Per Cent Lethals	Allelism Frequency
II	132	36	27.27	1.37
IV	104	3	2.88	0.00

The chromosome II results demonstrate that the population sampled is a "typical" one; that is to say, the measured lethal frequency is consistent with the north-south gradient ascertained by Ives^{66,67} for the second chromosome in American populations of *melanogaster*. That the breeding size of the population is fairly large is indicated by the low frequency of allelism obtained in cross tests of the lethals.

Chromosome IV lethals appear much more frequently than expected, considering the presumed paucity of loci within this microchromosome. The observed ratio of lethals on IV and II is 1:10,

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⁶⁴N. P. Dubinin, *J. Exp. Biol. (Moscow)* 7, 463-79 (1931).

⁶⁵A. H. Sturtevant, *Biol. Bull.* 73, 542-51 (1937).

⁶⁶P. T. Ives, *Genetics* 30, 167-96 (1945).

⁶⁷P. T. Ives, *Proc. Natl. Acad. Sci. U.S.* 40, 87-91 (1954).

⁶⁸H. T. Band and P. T. Ives, *Proc. Natl. Acad. Sci. U.S.* 47, 180-85 (1961).

a value more in accordance with the relative sizes of the two autosomes at somatic metaphase (0.3 to 3 μ) than with the estimated ratio of loci (1:38), derived from salivary chromosome band counts.

Although the data are not extensive, they suggest the testable hypothesis that, within a natural population, a chromosome-IV locus is more likely to contain a lethal than one in the larger second chromosome. Various explanations for this presumed difference include higher lethal mutation rates and/or lowered selection against these extremely detrimental genes in the smaller autosome. There is also a possibility that an inaccurate evaluation of the relative number of loci present in the two chromosomes is obtained when their salivary band totals are compared. Future investigations, enlarged in scope and improved methodologically, will be undertaken to determine whether or not the size and recombinational differences between chromosome IV and the other autosomes are accompanied by distinctions of evolutionary significance and basic chromosomal organization.

A Protective Role of Chromosome Synapsis During Spermatogenesis

P. E. Thompson

Introduction. — In males of *Drosophila*, the association of normal chromosomes with inverted homologs results in appreciable increases in the frequency of both spontaneous and induced lethal mutations, compared with controls lacking inversions.⁶⁹ This increase in mutability is most striking in a spermatogenic stage interpreted as meiosis, and evidence has been found that the incidence of mutations is related to the pairing of homologs. Two components of the aberrant pairing in inversion heterozygotes have seemed worthy of evaluation: the stress or torsion associated with inversion-loop configurations and the partial asynapsis that must accompany structural heterozygosity. A test of the effect of asynapsis without torsion was made possible by the use of SM5, a complex intrachromosomal rearrangement having 13 inversion or transposition breaks, which does not pair appreciably with its homolog.

Results. — SM5/+ males and isogenic control males were given 1000 r of x rays and mated to fresh females at 2-day intervals. This method permits the successive recovery of offspring from sperm which were progressively earlier in spermatogenesis at the time of irradiation. Recessive lethals were scored in the normal chromosome II from both types of male. The fractions of lethals among tested chromosomes, by mating period, are shown in Table 11. The difference in frequency during the period interpreted as meiosis (6 to 8 days) was highly significant ($P < 0.001$), indicating that asynapsis itself is an important factor in mutability. The interpretation that this mutability is limited to meiosis was supported by the pattern of induced crossovers and sterility in these experimental lines.

⁶⁹P. E. Thompson, *Genetics* 45, 1567-80 (1960).

Discussion. – The magnitude of the meiotic effect of asynapsis after irradiation suggests that the usual intimate synapsis of homologs contributes to a high level of repair which has broken down in these inversion systems.

Table 11. Frequency of Lethals from Asynaptic and Control Lines, by Mating Period

	Days After Irradiation							
	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16
SM5/+	5/88	5/101	44/367	67/341	8/332	10/480	0/67	0/51
+/+	4/101	5/89	35/388	34/396	11/241	7/364	0/69	0/55

MAMMALIAN GENETICS AND DEVELOPMENT

Section Chief – W. L. Russell

Genetic Effects of Radiation in Mice

Elizabeth M. Kelly	Jean W. Bangham
Louis Wickham	Patricia A. Burd
J. R. Inman	Mary B. Cupp
Josephine S. Gower ¹	Georgia M. Guinn
	Patricia R. Hunsicker
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	D. Patricia Wright

Effects of Radiation on Mammalian Gametogenesis

E. F. Oakberg	Evelyn Clark
V. C. Monesi ²	

Developmental Effects of Radiation in Mice

Liane B. Russell	Clyde L. Saylor
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Mammalian Cytogenetics

W. J. Welshons	Virginia H. Carlson
E. H. Y. Chu	Bobbie J. Scandlyn
Barbara S. Blander ³	Elena R. Monesi ²
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Effect of Radiation Dose and Dose Rate on Mutation Frequency

W. L. Russell	Elizabeth M. Kelly
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Further experiments have been undertaken to try to analyze the peculiar lack of linearity between mutation rate and dose in mice exposed to large doses of x rays. The effect first found⁵ was that a

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⁵W. L. Russell, *Genetics* 41, 658–59 (1956).

single acute dose of 1000 r gave a lower mutation frequency than that obtained with 600 r. The most likely explanation for this odd result seemed to be that a dose of 1000 r was killing more of the sensitive cells than 600 r and that the mutation frequency observed was characteristic of the more resistant cells, which were also assumed to have a lower sensitivity to mutation induction.

This hypothesis was tested in an experiment already reported⁶ in which the total dose of 1000 r was given in two steps: first 600 r, and then, more than 15 weeks later, a second dose of 400 r. The time interval was long enough to allow cell replenishment and the restoration of a normal pattern in the spermatogonial population. The mutation frequency observed was significantly higher than that obtained when the total dose was given at one time. As far as it went, this result supported the view that cell selection was the explanation for the lower mutation frequency obtained with a single acute 1000-r dose. However, further analysis by additional experiments was obviously needed. The preliminary results of one such experiment are described here.

Mutation rate was determined by our standard specific-locus method. The irradiated males received, on the posterior third of the body, a total dose of 1000 r of acute (90 r/min) x rays delivered in five fractions of 200 r each, with intervals of 24 hr between fractions. The design of this experiment was based on the rationale that the intervals of 24 hr between fractions were too short to allow repopulation of spermatogonia.

The first important result noted was that the length of the radiation-induced temporary sterile period in this experiment exceeded by several weeks that observed in the earlier work with a single dose of 1000 r. It was concluded that the fractionated irradiation had caused a more extreme depletion of the spermatogonia than had the single exposure and that cell selection had presumably been at least as severe. Therefore, if cell selection really accounted for the low mutation frequency with a single dose of 1000 r, the fractionated irradiation would, on the simplest hypothesis, be expected to give as low a mutation frequency or even lower.

The results obtained to date do not fit this expectation. The present mutation frequency in the new fractionated experiment is 25.0×10^{-5} per locus per 1000 r. This is significantly ($P = 0.015$) higher than the frequency of 10.3×10^{-5} observed in the original single exposure experiment and not significantly below the frequency of 29.1×10^{-5} found in the experiment in which the dose was given in two fractions separated by more than 15 weeks.

It is clear that the differences in mutational responses in these experiments cannot be explained simply on the degree of cell killing in the spermatogonia. On the other hand, cell selection is not entirely ruled out as the cause for the low mutation frequency in the single-exposure experiment. The failure to get a low mutation frequency in the current fractionation experiment could, theoretically, be attributed to a disruption, by the first fraction or two of the dose, of the normal pattern of the spermatogonial population. Such disruption could lead to a situation in which the average sensitivity of the cell population was high for the remainder of the dose. The total cell survival would then be low, but the mutation rate in the surviving cells could remain high.

⁶W. L. Russell, L. B. Russell, and E. M. Kelly (symposium on "Immediate and Low Level Effects of Ionizing Radiation," Venice, 1959), *Intern. J. Radiation Biol.*, suppl, 311-20 (1960).

Although cell selection cannot yet be ruled out as the cause for the low mutation frequency in the single-exposure experiment, the present results do suggest that one should entertain other hypotheses. Perhaps the possibility should be considered that, at high single doses, the irradiation is, in some way, actually interfering with the completion of the mutational process. Thus, on this view, a dose that exceeded 600 r would, in these mouse experiments, start to interfere with some of the mutational processes that would have been completed if the irradiation had stopped at 600 r.

Differences Between Mouse and Guinea Pig in Radiation Response of the Ovary

E. F. Oakberg Evelyn Clark

The mouse ovary is extremely sensitive to x radiation, and in the 101 × C3H F₁ hybrid female only 50 r is required to induce permanent sterility after an initial fertile period during which an average of 4 litters is produced.⁷ This response has been shown to result from killing of early oocyte stages by 50 r.⁸ Genther, however, reported no loss of early oocytes in the guinea pig after 360 r, and estimated the minimum sterilizing dose at 2160 r.⁹ Recent work has shown no effect of 300 r on the fertility of female beagles.¹⁰ In the human female, doses of 500 to 624 r are required to sterilize 90% of exposed individuals.¹¹ The above results have been interpreted as indicating that the radiation response of the mouse ovary is atypical of mammals in general. We have initiated experiments to compare the mouse and guinea pig, in the hope that the basis for some of the above species differences may be elucidated.

Young adult female guinea pigs were given 200 r of x rays at 78 r/min and then were killed at intervals ranging from 12 hr to 14 days after irradiation. Serial sections were prepared for each irradiated and control ovary, stained with iron hematoxylin, and examined for histological and cytological changes. Our preliminary observations confirm the finding of Genter⁹ that many of the oocytes in early stages of follicular development survive doses of several hundred roentgens. This is, indeed, in marked contrast to the mouse, where only 50 r is required to kill all the earliest oocytes in the adult ovary.

The more interesting observation, however, was the obvious difference in cytological appearance of the young oocyte in controls of the two species. In the mouse, the nuclear structure of this stage is characterized by two nucleoli and an open, reticulated chromatin network of the dictyate nucleus. In the guinea pig, oocytes in the earliest stages of follicle development have small nuclei which stain heavily, nucleoli are present, and the chromosomes are partially condensed. Detailed cytology

⁷L. B. Russell, K. F. Stelzner, and W. L. Russell, *Proc. Soc. Exptl. Biol. Med.* 102, 471-79 (1959).

⁸E. F. Oakberg, *Proc. 10th Internatl. Congr. Genet.* 2, 207 (1958).

⁹I. T. Genter, *Am. J. Anat.* 55, 1-45 (1934).

¹⁰A. C. Andersen and F. T. Shultz, *Radiation Research* 12, 417-18 (1960).

¹¹W. S. Peck, J. T. McGreer, N. R. Kretzschmar, and W. E. Brown, *Radiology* 34, 176-86 (1940).

of these cells is difficult, and final determination of the exact meiotic prophase stage represented will require sequential study of the ovary in earlier stages of development. It is clear, however, that marked differences in structure of the oocyte nucleus occur in the mouse and guinea pig.

The above observations on nuclear stage may bring the explanation of species differences into agreement with the known differences in sensitivity of different meiotic stages within a species. Thus the dictyate nucleus in early follicle stages may be particularly sensitive to radiation, and the high sensitivity of the mouse may result from most oocytes being arrested in this stage of meiosis. When possible differences in the stage at which oocyte development is arrested are added to the variation in rate of oocyte development and utilization in different species, ample basis for wide species differences in fertility of irradiated females is provided. Yet, identical cell stages, in comparable stages of follicular development, could have closely similar radiation sensitivity in different species.

Induction of Paternal Sex-Chromosome Loss by Irradiation of Spermatozoa

Liane B. Russell

Clyde L. Saylor

In experiments already reported^{12,13} we were able to induce loss of a sex chromosome by irradiating during the interval between fertilization and the first cleavage. This interval is relatively long (almost a full day), and it was possible to show that, during a 4½-hr span within it, there is a change in sensitivity to sex-chromosome loss and to lethal effects. It, therefore, appears that there is a change with time in the chromosomal state of the two haploid pronuclei within the zygote. For this reason it seemed of interest to determine the frequency of sex-chromosome loss from irradiation of the haploid sperm nucleus prior to its entry into the oocyte. Sex-chromosome loss induced in spermatozoa may, furthermore, be useful in the future as a measure of chromosome damage in comparative experiments (e.g., dose rate). Finally, the results obtained in the present experiment will be of interest in comparison with data now being obtained on nondisjunction frequencies from the irradiation of meiotic stages in both males and females.

Method. — Wild-type (+/Y) males were irradiated with 600 r of x rays and mated for two weeks immediately following irradiation to females bearing dominant sex-linked markers. As shown by Oakberg and DiMinno,¹⁴ spermatozoa ejaculated during this interval were irradiated as spermatozoa or spermatids. Since, however, contribution from spermatids would come only from matings made in the latter portion of the interval, and since almost all matings in the present experiment occurred in the first week following irradiation, we feel that the results obtained concern mostly irradiated spermatozoa. To keep the number of progeny approximately equal in irradiated and control groups, about twice as many matings were set up in the former as in the latter to allow for loss due to dominant lethals.

¹²L. B. Russell and C. L. Saylor, *Science* 131, 1321-22 (1960); *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, pp 48-49.

¹³L. B. Russell, *Science* 133, 1795-1803 (1961).

¹⁴E. F. Oakberg and R. L. DiMinno, *Intern. J. Radiation Biol.* 2, 196-209 (1960).

Females used were $Ta/+$, $Blo/+$, and $Ta+ / + Blo$ (cross-over frequency $Ta-Blo$ is about 4%). Loss of the paternal sex chromosome is thus indicated by the occurrence of Ta/O , Blo/O , or $TaBlo/O$ daughters. Since $+ / O$ is not detectable by phenotype ($+/+$ being a normal segregant), the detectability of loss of X^P or Y is, therefore, 50% when the mates used are $Ta/+$ or $Blo/+$. It is 100% when the mates used are $Ta+ / + Blo$, provided that the rare wild-type cross-over females are further tested to determine whether they are $+ / + +$ or $+ / + O$. All offspring were examined on the day of birth and at least once a week thereafter until the time of weaning.

Results and Conclusions. – Table 12 lists results to date. On combining the data from all types of matings used, a statistically significant difference ($P = 0.01$) in estimated frequency of paternal sex-chromosome loss is obtained. The values were 0.14% in controls and 1.32% in the group that received 600 r to sperm. However, it should be noted that these are probably minima, since they are based on frequency of $X^M O$ progeny. It is known that XO is somewhat less viable than XX and XY

Table 12. Frequency of Loss of the Paternal Sex Chromosome Following Irradiation of Sperm

Treatment	Mate	Progeny									Total Classified	% Loss of X^P or Y^a
		♂♂				♀♀						
		Ta Y	Blo Y	$++$ Y	$TaBlo$ Y	Ta +	Blo +	$++$ ++	$TaBlo$ ++	X^M O		
Control	Ta +	175		160		163		155		1 ^b	654	0.31
	Blo +		136	127			108	141		0	512	
	$Ta+$ + Blo	42	26	3	2	29	17	0	0	0	119	
										Total 1	1285	0.14
600 r to sperm	Ta +	152		149		121		138		5 ^b	565	1.77
	Blo +		107	119			121	95		1 ^c	443	0.45
	$Ta+$ + Blo	21	22	1	1	33	23	0	1	2 ^b	103	1.94
										Total 8	1111	1.32

^aEstimated frequency of occurrence calculated by taking account of detectability which, in the groups where $Ta/+$ or $Blo/+$ mates are used, is only $1/2$.

^b Ta/O .

^c Blo/O .

even when the X is wild type.^{15,16} Viability may be even further reduced in the case of Blo/O, as also indicated by the lower frequency of Blo/O than Ta/O exceptionals in the present experiment (Table 12).

It is not unreasonable to assume that the mechanisms that lead to the loss of a paternal sex chromosome following irradiation of spermatozoa are the same as those which, when affecting autosomes, cause dominant lethality. If this is the case, then the ratio of frequencies of sex-chromosome loss to dominant lethals should be approximately the same as the ratio of average length of X and Y to total length of autosomes. The former ratio is about 1:40 in the present experiment. The latter ratio – if relative chromosome lengths in sperm are proportional to metaphase lengths (which may, of course, not be the case) – is of approximately the same order of magnitude, since Y is the smallest chromosome,^{17,18} X of only medium length, and there are 19 autosomes.

A comparison may be made in the ease with which paternal sex-chromosome loss is induced in spermatozoa, on the one hand, and in the male pronucleus shortly postfertilization, on the other. With 600 r to spermatozoa, the induced rate (experimental minus control) is 1.2% (Table 12). With only 100 r delivered at 11:00 AM postfertilization, the induced rate (for paternal losses only) is 2.3%.¹³ It therefore appears that postfertilization irradiation may be more effective by an order of magnitude than irradiation of sperm in causing loss of a paternal sex chromosome.

Skeletal Abnormalities in Offspring of Irradiated Male Mice

U. H. Ehling M. L. Randolph

Over-all genetic damage from radiation, which cannot be estimated accurately from mutational information on a limited number of genes, must be measured empirically. In a series of experiments, skeletal damage was measured in the offspring after irradiation of either spermatogonial or post-spermatogonial stages with 600 rads of x rays, 200 rads of monoenergetic 14-Mev neutrons, or 80 rads of monoenergetic 3-Mev neutrons. Irradiated 101-strain males were mated to C3H-strain females. Litters were sacrificed at the age of 26 to 28 days. Whole skeletons were examined in detail in alizarin-red preparations.

Abnormalities were tabulated as class 1, abnormalities that were observed only once in the whole experiment; or as class 2, abnormalities that were found in two or more animals. Pooling the present results from all experiments showed no difference in the frequencies of class-2 abnormalities derived from irradiated and nonirradiated males. The frequency of class-1 abnormalities in the two groups differs significantly (22 out of 443 offspring from irradiated males and 7 out of 525 offspring from control males, $P = 0.0018$). The distinct increase of abnormalities in offspring from irradiated males

¹⁵W. L. Russell, L. B. Russell, and J. S. Gower, *Proc. Natl. Acad. Sci. U.S.* **45**, 554–60 (1959).

¹⁶W. J. Welshons and B. J. Scandlyn, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, p 57.

¹⁷E. H. Y. Chu and V. C. Monesi, *Genetics* **45**, 981 (1960).

¹⁸L. B. Russell and E. H. Y. Chu, *Proc. Natl. Acad. Sci. U.S.* **47**, 571–75 (1961).

demonstrates that skeletal effects are suitable for measuring part of the over-all damage from presumed dominant mutations. More data are being collected to evaluate the response of different gametogenic stages.

Induction by Ultraviolet Radiation of Chromosome Aberrations in Mammalian Cells

E. H. Y. Chu Elena R. Monesi

Introduction. — The geometry of mammalian cells grown in monolayer *in vitro* permits a study of the direct effects of ultraviolet (UV) radiation on cells and chromosomes because the single cells are thin and flattened, allowing penetration and absorption of the UV energy. By employing monochromatic wavelengths and by manipulating the physicochemical environment of the cells in culture, it may be possible to obtain information on the structure and behavior of chromosomes as well as on other cell properties.

Material and Methods. — An actively proliferating, fibroblast-like, clonal cell line (CHEF-125), which was derived from a female Chinese hamster embryonic cell, was used. It had an average generation time of less than 20 hr under the experimental conditions. Cells were grown in single layers in quartz dishes and, without removing the nutrient medium, irradiated from below with monochromatic UV light at wavelengths of 2652 and 2804 Å. Doses ranging from 50 to 400 ergs/mm² were administered at a uniform intensity by varying the time of exposure. Medium was changed immediately following irradiation. Photoreactivating wavelengths were absent during the course of experiment. In order to test the relationship between protein synthesis and aberration production, chloramphenicol was used in some experiments. Varying concentrations (10^{-4} to 10^{-2} M) of chloramphenicol were included in the medium at $\frac{1}{2}$ hr before, during, and for 2 hr after exposure to UV of 2652 Å. Cells were treated with colchicine and hypotonic solutions, fixed, air-dried, and then stained with aceto-orcein.

Results and Discussion. — Over 95% of the cells had an identical quasidiploid karyotype, each consisting of 22 chromosomes but having one normal medium-sized acrocentric chromosome (number IX) replaced by a new, very long (only slightly shorter than chromosome II) acrocentric chromosome. The remaining cells consisted of aneuploids having either 21, 23, or, rarely, 24 chromosomes, and of a very few polyploid cells. The population structure had been relatively stable in over 15 weekly transfer passages. Most remarkable was the exceedingly low spontaneous chromosome-aberration frequency (about 0.01 aberration per cell in 200 control cells examined), which was distinctly different from most normal mammalian cell lines *in vitro*.

All metaphases accumulated up to 24 hr after UV treatment exhibited chromatid type of aberrations. In two experiments in which cells were fixed at 48 hr (following 16 final hours with colchicine), only chromosomal types were found. In both categories, the aberration types induced by UV were qualitatively indistinguishable from those induced by x rays in both plant and mammalian materials.

In the limited quantitative data obtained to date, aberration frequencies increased with dose and seemed to follow the kinetics expected from the number of breaks involved. It also appeared that an unusual preponderance of chromatid exchanges were present in all dose series.

At 2652 A, most metaphase chromosomes were smooth in outline and aberrations scorable. At 2804 A, on the other hand, in addition to the scorable metaphases showing no or a few of the usual types of aberrations, there was a small proportion of metaphases in which the chromosomes were faintly stained and fuzzy or sticky in appearance. In fact, most irradiated interphase nuclei also appeared more faintly stained than the control, and the staining intensity seemed to be dose dependent. Furthermore, there were increasing percentages (proportional to dose) of metaphases in which multiple exchanges and extreme chromosome shattering occurred, making enumeration of breaks almost impossible. The same phenomena were observed in cells irradiated with UV of 2652 A and treated with chloramphenicol, but not in cells having received UV of 2652 A alone.

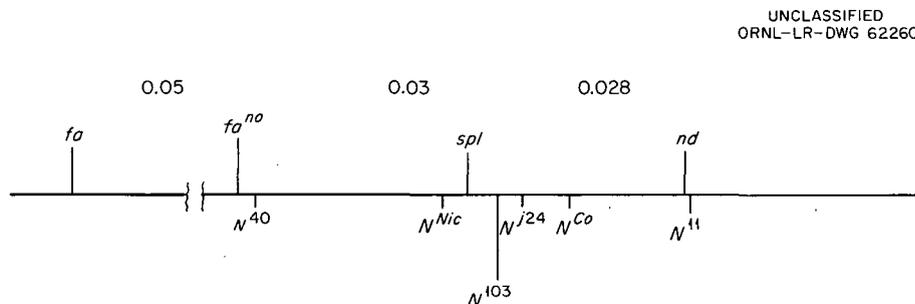
The scorable aberration frequencies were roughly comparable in magnitude when induced by the same doses of both wavelengths. The much higher frequencies of chromosome breakages, expressed as fragmentations and multiple exchanges, that were induced at 2804 A may indicate a more efficient absorption of energy by the protein components of mammalian chromosomes. Results from the chloramphenicol experiments suggest that protein synthesis is involved in the final production of aberrations. It seems reasonable to assume, therefore, that proteins play a more important role in preserving the structural integrity of chromosomes than has been attributed to them.

The Comparison of Recessive Lethal Pseudoalleles in *Drosophila* with Pseudoallelic Loci of Microorganisms

W. J. Welshons Elizabeth S. Von Halle

Introduction. – In previous publications pertaining to the pseudoallelic nature of the *Notch* locus in *Drosophila*, a tentative map of the locus was presented.¹⁹ Recently, a series of experiments have critically demonstrated the separability of loci that were given a tentative position, and four new mutant sites have been added to the series. Since the *Notch* locus is composed of both recessive visible and recessive lethal mutants, it is instructive to look upon the locus as one composed of a pseudoallelic recessive lethal series superimposed upon a series of recessive visible pseudoalleles. In this way one can compare and contrast the pseudoallelic systems at the *Notch* locus with other pseudoallelic loci in *Drosophila* and microorganisms.

Results. – For convenience, a map of the *Notch* locus is presented below (numerals above the line indicate map distances between recessive visibles):



¹⁹W. J. Welshons, *Cold Spring Harbor Symposia Quant. Biol.* 23, 171-76 (1958).

The linear order of recessive visible pseudoalleles is represented by the loci placed above the base line. The linear arrangement of recessive lethals relative to each other and relative to the recessive visible loci is indicated by the *Notch* (*N*) symbols below the base. Every locus shown on the map has been separated by crossing over from the next adjacent locus, with one exception: the separability of *notchoid* (*nd*) and *Notch*⁻⁸¹¹ (*N*⁻⁸¹¹) has not been demonstrated as yet.

All heterozygous combinations of recessive lethals are noncomplementary, as are combinations of recessive lethals with recessive visibles. Transheterozygotes of recessive visibles are variable in this respect. The *fa*^{no}/*nd* combination is clearly noncomplementary, while *fa/fa*^{no} and *fa/nd* show only a very slight degree of noncomplementarity; all combinations of *spl* with *fa*, *fa*^{no}, and *nd* are complementary.

Conclusions. – Since the recessive lethals promise to map continuously, as do the mutant sites of pseudoallelic loci in microorganisms, it is suggested that recessive lethals in *Drosophila* are analogous to the mutants normally used for genetic investigations of phage and *Neurospora*. On the other hand, the recessive visible loci of *Drosophila*, many of which are hypomorphic mutations, are probably analogous to the leaky mutants of microorganisms. Thus, it would appear that the continuous mapping of mutant sites observed in microorganisms, as opposed to the discontinuous mapping of mutant sites in *Drosophila*, is a consequence of the type of mutant (hypomorphic or amorphic) selected for use by geneticists. In *Neurospora*, for example, the amorphic mutants (recessive lethals) are easily utilized for genetic investigation; the hypomorphs (leaky mutants) are less desirable. In *Drosophila* the situation is exactly reversed because the amorphic recessive lethals are difficult to manipulate in the course of a pseudoallelic investigation since the lethality of transheterozygotes often precludes the genetic study.

The discontinuous mapping of recessive visibles in *Drosophila* probably results from the inadvertent selection of phenotypically similar mutants at a locus. Consequently, one can postulate that the gaps between recessive visible pseudoalleles will eventually be found to contain the mutant sites of recessive visibles displaying atypical phenotypes, wild-type isoalleles, and recessive lethals as well.

RADIATION MICROBIOLOGY

Mutagen Mechanisms – Bacterial Metabolism

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Bacterial Genetics

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Effect of AET on the X-Ray Sensitivity of Microorganisms

Alexander Hollaender A. Marie McCarthy

Earlier work with *Aspergillus terreus* showed differences in x-ray sensitivity, depending upon the nature of the inorganic ion present in the AET used for treatment. AET·Br·HBr (rearranged at pH 7 to MEG) caused an increase in sensitivity to x ray at concentrations above 0.01 M. However, no such increase was observed when AET·Cl·HCl was used.⁵

In the present investigation two strains of *Escherichia coli*, B/r ORNL and B ORNL, were treated with AET·Br·HBr and AET·Cl·HCl both prior to and during x irradiation. The experiments were repeated several times, and, in every case, maximum protection was obtained at concentrations of between 0.04 and 0.06 M. No sensitization to x ray similar to that found with *A. terreus* was observed with either strain of *E. coli* when treated with AET·Br·HBr or with AET·Cl·HCl, even when concentrations as high as 0.36 M were employed.

Radiation Microbiology – Bacterial Genetics

H. I. Adler J. C. Copeland

As a result of work by Taylor and Adelberg⁶ there are now available mating strains of *E. coli* that transfer the entire bacterial genome at high frequency. These strains, designated very high

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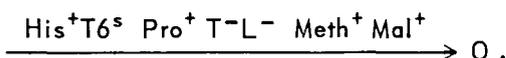
⁵A. Hollaender and A. M. McCarthy, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1960*, ORNL-2913, p 64.

⁶A. L. Taylor and E. Adelberg, *Genetics* 45, 1233-43 (1960).

frequency donors (VHF), are extremely useful for mapping new genetic markers and detailed analyses of linkage. We are using one of these, AB312, in an attempt to study the genetic control of radiation behavior in *E. coli*.

X irradiation of AB312 yields a sigmoid survival curve that cannot be attributed to cell clumping or multicellular forms. Strain AB531, an F⁻ (recipient) strain suitable for matings with AB312S, yields a curve which begins as a steeply sloped exponential but contains a somewhat more resistant component that becomes evident at higher doses. The AB312 strain is referred to as "resistant" and the AB531 strain as "sensitive."

The experiments are designed to answer the questions: (1) Is the difference in radiation behavior of these strains due to genes located on the known bacterial chromosome? (2) If so, how many genes are involved and where are they located? Matings are made and recombinants selected for well-mapped genetic properties. These recombinants are then exposed to x irradiation, and their survival is assayed to determine if they have inherited the "resistant" property of the VHF donor or retained the sensitivity of the recipient. Strain AB312 possesses genetic material in the following order:



Selections for methionine-positive recombinants and histidine-positive recombinants have been made. Among 60 methionine recombinants assayed, none have inherited the resistance characteristic of the donor parent. We have demonstrated that markers between methionine and the origin (O) were incorporated at high frequency (approx 60%) in this group of recombinants and have concluded that the resistant behavior is therefore not associated with the region of the chromosome between methionine and the origin. Selection for histidine-positive recombinants has also been made. Among 60 histidine recombinants assayed, five have inherited the resistant behavior characteristic of the donor parent. Analysis of unselected markers in these five indicates that the resistant property may be located close to the histidine markers. The analysis of histidine-positive recombinants has also suggested the existence of recombinants having a sensitivity intermediate between that of the donor and recipient parents. This may mean that we are studying a polygenic phenomenon. Larger samples are required before a definite statement can be made on this point.

The experiments to date give an affirmative answer to the first question posed: Radiation behavior can be associated with genes in the bacterial chromosome. The second, and more complex, question is now under study.

Mechanisms of X-Ray Protection with β -Mercaptoethylamine

H. L. Cromroy H. I. Adler

Four strains of *Escherichia coli*, B/r ORNL, B ORNL, B Hill, and Bs Hill, were used in a series of experiments measuring the protective capacity of β -mercaptoethylamine (MEA) compared with that due to oxygen removal by nitrogen bubbling. MEA afforded greater protection in all four

strains. The increment of MEA protection above that achieved by nitrogen bubbling was approximately identical for all strains studied. This led to the hypothesis that protection by MEA involves a mechanism other than oxygen removal. Experiments with the strain Bs Hill, in which MEA concentration is varied at a dose of 20 kr, support this hypothesis.

Further studies were done by varying the incubation period of MEA with the organisms vs survival to a constant x-ray dose. The initial studies indicate that most of the protection by MEA occurs immediately after contact with the bacteria. Increase in protection above the initial large increment does not occur until after a 10-min incubation period.

At present, studies are being done by using polarographic techniques to determine quantitatively the amount of oxygen removed from solution by MEA and the time required for oxygen removal to determine if the rate of oxygen removal is concurrent with the rate at which protection develops. Preliminary results indicate that oxygen removal by MEA oxidation is a relatively slow process and cannot account for the immediate protective effect of MEA observed.

MAMMALIAN RECOVERY

Hematopoietic Recovery

C. C. Congdon
R. R. Bigelow¹
Gino Doria²
W. H. McArthur³
Stanfield Rogers¹
S. G. Wilson¹
Patricia G. Delker

Bone Marrow Physiology

L. H. Smith⁴
Joan W. Goodman
G. S. Hodgson²
T. W. McKinley, Jr.
Ruthanne Davis⁵
Emily Sykes⁵

Mammalian Cytology and Genetics in Vitro

M. A. Bender
P. Carolyn Gooch

Immune Tissue Reaction in Irradiated Mice⁶

C. C. Congdon

A cellular reaction lasting three days in spleen and lymph nodes of lethally irradiated mice given rat bone marrow was attributed to antigenic stimulation of residual antibody-forming cells in the irradiated recipient.⁷ Attempts are being made to use the spleen reaction as an assay for the general class of transplantation antigens, since many antigenic materials that cause lymphatic tissue changes and serum antibody formation in normal animals did not cause the three-day spleen reaction in irradiated mice. Foreign tissues, however, such as homologous mouse, hamster, and rabbit marrow did cause the cellular reaction in the irradiated spleen. One exceptional lot of horse serum was active. This result led to an investigation of the soluble material from boiled human saliva which was active in the system. The active material was attributed to the salivary mucin. Strong activity was present in the mucoprotein fraction from bovine submaxillary gland. Mucoprotein from rat urine was also active. Water-soluble extracts prepared from rat parotid, brain,

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⁶In collaboration with R. D. Owen, G. D. Novelli, and C. J. Wust.

⁷C. C. Congdon, *Radiation Research* 9, 102 (1958).

spleen, lung, and kidney, and suspensions of erythrocyte stroma were active. Rat liver microsomes were active in one preparation, but water-soluble extracts of whole homogenized liver showed variable activity.

Immune Reaction by Chimera Spleen Cells

Gino Doria

Introduction. – A study is being undertaken on the immune status of bone marrow chimeras. The chimeras are lethally irradiated C57BL/6 or (C57BL/6 × DBA/2)F₁ mice given isologous or semi-isologous bone marrow, and the immune reaction studied is one of parent against F₁ cells.

Method. – Spleen cells from F₁ hybrid mice sensitized against rat RBC are transplanted into lethally irradiated parental strain (P) mice, which also receive the test antigen. Antirat RBC agglutinins produced in the serum of the irradiated recipients are due to the secondary response of the transferred spleen cells. If the recipients are also injected with isologous (P) spleen cells, the agglutinin production by the F₁ cells is impaired; the reduction in titer is much greater if the P cell donor has been preimmunized against F₁ cells. Hence, it is possible to distinguish between primary and secondary response by P cells. Therefore, when chimera spleen cells are transferred into the lethally irradiated recipients, it should be possible to detect a secondary response type of reaction by P against F₁ cells if any primary immune reaction had been going on in the chimera. If so, the reduction in agglutinin titer produced by F₁ cells should be much greater when the reacting cells are from semi-isologous, as compared with isologous, chimeras.

Results and Discussion. – Preliminary results show that spleen cells from semi-isologous chimeras, in which (C57BL/6 × DBA/2)F₁ is the host and C57BL/6 the bone marrow donor, are capable of giving a secondary response when challenged by F₁ cells. This reaction has not been found when spleen cells from chimeras of reversed constitution were tested. Furthermore, this donor-against-host immune reaction was detectable only at 30 days after the irradiation and bone marrow injection. The fact that this feature is concomitant with a mortality peak of the semi-isologous chimeras indicates that this immune reaction plays a role in the pathogenesis of the secondary disease.

Study of Oxygen Consumption in Irradiated Mice Given Foreign Bone Marrow⁸

W. H. McArthur

Oxygen consumption of the mouse was measured by means of a spirometer, a sensitive instrument capable of measuring extremely small volume changes in a closed system. The inlet fitting was connected to an animal chamber containing the animal, a CO₂ absorbing material (sodium calcium hydrate), and a water vapor absorbent (anhydrous copper sulfate). As the animal used O₂ it produced

⁸In collaboration with A. Kretchmar, Oak Ridge Institute of Nuclear Studies.

CO₂, which was absorbed; this resulted in a reduction of volume which was compensated by the piston of the instrument moving to the right, into the cylinder. Thus, the pen motion records the O₂ uptake on a strip chart. The calibration figure was $\frac{2}{3}$ cc of O₂ uptake per mm of pen travel.

The irradiated recipients (900 r) were LAF₁/Jax mice. They received 40×10^6 C₃BF₁/Cum bone marrow cells. Donor bone marrow from the isologous strain of LAF₁ female mouse was also used. Measurements were made over a period of 38 days.

The average number of cubic centimeters of O₂ consumed per minute by two normal mice was 1.40 and 1.57. Oxygen consumption by two isologous animals for 36 days averaged 1.23 and 1.37 cc/min. Average O₂ consumption of three homologous animals was 1.29, 0.99, and 1.15.

In these preliminary experiments the homologous animals used slightly less than the isologous mice. The two homologous bone-marrow-treated mice had very low O₂ consumption and died at 13 and 26 days after irradiation.

The weight of the normal mice remained constant. The isologous mice lost weight the first two weeks after irradiation, and within a month regained their original weight. The weight of the homologous mice fluctuated; they lost weight, then gained, only to be followed by a loss of weight. Two animals continued to lose weight until they died.

During O₂ uptake, it was noted that at times a great deal of moisture accumulated on the walls of the chamber in which the mouse was placed. Moisture was noted only when isologous and homologous marrow treated animals were in the chamber. Accumulation of moisture was noted on the twelfth day after irradiation with the homologous animals, whereas with the isologous animals moisture accumulation was noted on the twenty-third day after irradiation.

Oxygen-uptake experiments on a second group of animals are now in progress to check the results of the first experiment. In addition, CO₂, nitrogen balance, and water balance studies are being undertaken on irradiated mice treated with foreign bone marrow.

Competence of Donor Bone Marrow Cells After Sublethal X Irradiation in Vitro and Frozen Glycerol Preservation for Promoting Recovery of Supralethally Irradiated Mice⁹

Patricia G. Delker

Preliminary experiments by G. Cudkowicz indicated that sublethal x irradiation exposures (400 to 500 r) to mice just before their bone marrow was collected for injection into lethally irradiated homologous recipients greatly reduced the incidence of secondary disease. In the present work the homologous donor bone marrow was irradiated in vitro instead of in vivo; then, the marrow was frozen and thawed in a glycerol medium before injection into lethally irradiated recipients. It turned out that the homologous combination chosen did not show a significant amount of secondary disease, and, hence, a comparison with the original experiments of Cudkowicz was not obtained. However,

⁹Work done in collaboration with G. Cudkowicz, Pathology and Physiology Section.

the results (Table 13) did show that sublethally irradiated bone marrow could be frozen and thawed and still retain its ability to keep lethally irradiated mice alive.

The bone marrow cells were suspended in Tyrode's solution and given 400 r of x radiation. The portion of the sample to be frozen was diluted 1:2 with 30% glycerol and slowly frozen to -25°C . It was then thawed in a 37°C water bath and diluted to a concentration of 40×10^6 cells/ml for intravenous injection. The recipient mice ($\text{C}_3\text{BF}_1/\text{Cum}$) were given 950 r and 40×10^6 LAF₁ marrow cells.

Irradiation (400 r) of the bone marrow did have a substantial detrimental effect on the ability of the marrow to promote survival of lethally irradiated mice, as would be expected from earlier studies. Freezing and thawing in glycerol did not prove to be damaging for this large donor cell dose, as would also be expected from previously reported work on marrow preservation.

It is interesting that the irradiated and frozen-thawed cells did better than those given 400 r only. Whether the reduction in mortality in the irradiated recipient is significant or not would require further experimentation.

Table 13. Mortality in Mice Treated with Irradiated and Frozen-Thawed Bone Marrow Cells

Treatment of Homologous Donor Bone Marrow Cells	Number of Mice	Mortality (%)	
		Day 30	Day 60
400 r and freezing-thawing in glycerol	106	10	14
Freezing-thawing in glycerol	58	2	12
400 r	61	16	34
Untreated	52	2	7

Guinea Pig RBC Life Span

L. H. Smith T. W. McKinley, Jr.

Introduction. – Life span studies of Cr^{51} -labeled RBC in strain 2 and 13 guinea pigs have continued. In this report, more definitive data for autologous RBC are presented together with survival values for isologous and homologous RBC. Methods used are the same as previously reported.¹⁰

Results and Conclusions. – Table 14 shows the times when 50 and 95% of the original radioactivity remained in the circulation. These times were estimated from freehand curves derived from plots of the means of radioactivity remaining against time after injecting the Cr^{51} -labeled RBC. The $T_{1/2}$ (50% depletion) for autologous RBC was the same as previously reported on a different group of animals.¹⁰ Survival of isologous RBC was essentially the same as for autologous cells,

¹⁰L. H. Smith and T. W. McKinley, Jr., *Biol. Div. Semiann. Progr. Rept.* Aug. 15, 1960, ORNL-2997, pp 106-7.

Table 14. Survival Data for Autologous, Isologous, and Homologous Guinea Pig RBC

Recipient	Donor	Days ^a		Number of Animals
		50% Depletion	95% Depletion	
Strain 2	Self, autologous	14-18	59-70	10
Strain 2	Strain 2, isologous	15-19	57-68	9
Strain 2	Strain 13, homologous	9-13	41-50	10

^aEstimates based on the root mean square deviation from the mean.

but the survival time of homologous RBC was reduced. Despite this reduction there was no indication of an immune clearance of homologous cells, and the shapes of autologous, isologous, and homologous RBC curves were similar, that is, curvilinear. That all three types of RBC disappeared exponentially is suggested by the observation that for each type a reasonably straight line was obtained when the radioactivities were plotted on a log scale.

Proliferation of Peripheral Leucocytes in Radiation Chimeras

Joan W. Goodman

Introduction and Methods. – A renewed interest in the physiologic activities and ontogenic potentials of nucleated cells in peripheral blood has developed recently. Results of several experiments suggest that peripheral blood contains immunologically competent cells.¹¹ The present experiments demonstrate that peripheral leucocytes can transplant and proliferate in lymph nodes of heavily irradiated mice.

When heavily irradiated mice are treated with homologous bone marrow, lymph nodes become progressively smaller within the first week. Suspensions prepared from such nodes contain very few cells, and these are almost all nonspecifically killed in normal serum and complement. It has not been possible to identify the strain of origin of these cells soon after irradiation. However, in the present experiments, when blood was also injected at the time of marrow treatment, lymph nodes were normal in size or somewhat enlarged within a few days time. Cell yield in suspensions was improved by comparison with marrow-only treated mice, and nonspecific death, although still much higher than in control suspensions from normal mice, was reduced to a level making cytotoxic identification possible. In order to distinguish lymph node cells derived from the injected blood from those having their origin in injected marrow cells, blood from a third mouse strain was used. To reduce background cell death to a minimum, one of the parent strains of the F₁ hybrid marrow-donor was chosen as blood donor.

¹¹J. W. Goodman and C. C. Congdon, *A.M.A. Arch. Pathol.* 72, 18 (1961).

F₁ hybrid mice were given a lethal dose of x rays and then, within 24 hr, an injection of homologous F₁ hybrid bone marrow (10⁷ nucleated cells) and 0.5 ml blood (around 2.5 × 10⁶ nucleated cells) from an inbred parent strain. At intervals thereafter, mice were killed and lymph node and bone marrow cell suspensions were typed by the cytotoxic method.¹²

Results and Discussion. – Table 15 shows qualitatively the results from a single experiment. As soon as six days after treatment, the lymph node contained donor-type cells that could be identified by their reactivity with cytotoxic sera specific for the blood-donor strain. Bone marrow cells showed the specificities of the hybrid marrow-donor type. The presence of a small number of parent strain (blood-donor derived) cells in the marrow suspension would not have been detected in these tests.

Although lymph node cell suspensions from these chimeras contained few cells compared with those from unirradiated control mice, by the second week after irradiation as many as eight million could be obtained from the easily resected nodes of a single mouse. This is approximately three times the total number of leucocytes of all types present in the inoculum of 0.5 ml of blood. Because the cells counted in such a suspension actually constitute a small percentage of all lymphoid tissue present in the intact mouse, considerable proliferation of blood-donor type leucocytes must have occurred.

In five additional experiments involving three different donor-recipient combinations, 21 chimeras were individually tested in a similar way, and the proliferation of lymphoid elements derived from donor peripheral blood was confirmed.

¹²P. A. Gorer and P. O’Gorman, *Transplantation Bulletin* 3, 142 (1956).

Table 15. Reactivity of Chimeric Bone Marrow and Lymph Node Cells

Mouse ^b	Tissue	Serum ^a				
		C3H Anti B6D2F ₁	B6D2F ₁ Anti C3H	D2 Anti B6	B6 Anti D2	C3F ₁ Normal (Nonimmune)
B6	Bone marrow or lymph node	+	0	+	0	0
D2	Bone marrow or lymph node	+	0	0	+	0
B6D2F ₁	Bone marrow or lymph node	+	0	+	+	0
1C3F ₁	Bone marrow or lymph node	0	+	0	+	0
Chimera ^c	Bone marrow	+	0	+	+	0
	Lymph node	+	0	+	0	0

^a+: Percentage dead (antiserum – nonimmune) > 15.

0: Percentage dead (antiserum – nonimmune) < 10.

^bAt least seven animals tested for each mouse designation.

^cChimera: 950-r 1C3F₁ ← B6D2F₁ bone marrow (10⁷ nucleated cells) + B6 blood (2.5 × 10⁶ leucocytes). Bone marrow and lymph node represent one chimera tested on each of days 6, 13, 15, 18, 20, 22, and 26 after x irradiation and treatment.

Studies on Erythropoietic Tissue Repopulation and on Tissue Transplantation Antigens¹³

G. S. Hodgson

Erythrocyte Fe⁵⁹ Uptake as a Function of Bone Marrow Dose in Lethally Irradiated Mice. –

Lethally irradiated mice have been used as an in vivo medium for culturing stem cells responsible for repopulation of erythropoietic tissue. The relationship between bone marrow dose and 24-hr uptake of Fe⁵⁹ in erythrocytes has been established. The magnitude of Fe⁵⁹ uptake in red cells depends on the number of bone marrow cells injected and on the time of Fe⁵⁹ injection. When Fe⁵⁹ is injected five days after irradiation, erythrocyte Fe⁵⁹ uptake is a linear function of bone marrow dose in the range of 1×10^6 to 10×10^6 bone marrow cells. When Fe⁵⁹ is injected on day 7, Fe⁵⁹ uptake is a linear function of marrow dose in the range 6×10^4 to 1×10^6 cells. When Fe⁵⁹ is injected on day 9, Fe⁵⁹ is a linear function of marrow dose in the range of 5×10^4 to 5×10^5 cells. This technique has been applied to a comparison of efficiency in repopulating erythropoietic tissue in irradiated mice of isologous, homologous, and heterologous (rat) bone marrow. Homologous (LAF₁ → BC₃F₁) marrow is only slightly less efficient (~1.4 times) than isologous marrow; heterologous marrow is ~80 times less efficient. A comparison has also been made of the effectiveness of peripheral leukocytes and bone marrow in repopulating erythropoietic tissue of lethally irradiated mice. About 100 to 200 times more leucocytes than marrow cells are required to obtain a given Fe⁵⁹ uptake.

Radiosensitivity of Marrow Cells Responsible in Re-establishing Erythropoiesis in Lethally Irradiated Mice. – Use has been made of the previously described relationship between marrow dose and Fe⁵⁹ uptake to study the radiosensitivity of bone marrow cells responsible for repopulating erythroid tissue in lethally irradiated mice. In these studies, marrow was irradiated in vitro (250-kv x rays), and the iron uptake of lethally irradiated mice injected with irradiated marrow compared with that of animals injected with aliquots of nonirradiated marrow. Analysis of the data indicates a survival curve with an extrapolation number of ~3 and a D₃₇ of ~65 r. These values are similar to those obtained by Makinodan *et al.*¹⁴ for immunologically competent cells: extrapolation number of 27 and a D₃₇ of 70 r. However, they differ from those obtained by Till and McCulloch for radiosensitivity of cells responsible for survival¹⁵ of lethally irradiated mice and for spleen colonization.¹⁶ In the latter case, the extrapolation number was ~2, and the D₃₇ was 115 rads (Co⁶⁰ gamma). A similar value for D₃₇ was obtained from the mouse survival data.

These findings suggest that immunologically and erythropoietically competent cells may differ in radiosensitivity from the cell or cells competent in inducing survival and spleen colonization.

¹³Some of these studies were carried out in collaboration with J. W. Goodman and C. C. Congdon.

¹⁴T. Makinodan, M. A. Kastenbaum, and W. J. Peterson, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, p 73.

¹⁵E. A. McCulloch and J. E. Till, *Radiation Research* 13, 115 (1960).

¹⁶J. E. Till and E. A. McCulloch, *Radiation Research* 14, 213 (1961).

Studies of the radiosensitivity of stem cells of platelets and granulocytes, using specific methods, would help to contribute to the explanation of this difference.

Effect of Pretreatment with Rat Urine Extracts on the Transplantation of Rat Bone Marrow in Lethally Irradiated Mice. – Previous observations^{17,18} have indicated that urine from rabbits and rats contained potent antigenic materials. It was thus thought possible that transplantation antigens were present in urine. Experiments were designed to test this hypothesis. It is known that if mice are pretreated with rat marrow before irradiation, they will reject rat marrow injected after a lethal dose of x rays. This observation was the basis for the assay. Mice were injected with rat urine extracts one week prior to irradiation, and within 3 hr after irradiation they received 50×10^6 rat bone marrow cells. Controls were run, using (1) mice “preimmunized” with rat bone marrow, (2) non-preimmunized mice treated with rat bone marrow, (3) preimmunized mice treated with isologous bone marrow, (4) isologous marrow-treated mice, and (5) irradiated controls. Nine days after irradiation, mice were injected with Fe^{59} , and Fe^{59} uptake in erythrocytes was measured one day later.

The results of experiments to date show that rats injected with dialyzed rat urine (3 mg of protein), frozen rat dialyzed urine, ethanol extracts of rat urine, and fractions of rat urine not soluble in 90% phenol reject rat bone marrow. Studies are now under way to further purify and characterize the materials responsible. Previous studies of similar fractions of urine indicate that these contain large quantities of acid glycoprotein.

Types and Rates of X-Ray-Induced Chromosome Aberrations in Human Blood Irradiated in Vitro

M. A Bender P. Carolyn Gooch

Introduction. – Previous investigations of radiation-induced chromosome aberrations in human cells have been made either on tissue-cultured cells^{19,20} or on cells from persons who had received radiation doses some time before examination.²¹ To provide more accurate information for the estimation of human radiation hazards and to facilitate the use of somatic chromosome aberrations as a biological dosimeter, we have studied the chromosomes of leucocytes from freshly drawn human blood.

Method. – Blood from four individuals was used for these experiments. The blood samples were irradiated with hard (HVL = 2 mm Cu) x rays immediately after they were drawn, in order to approximate the in vivo situation as closely as possible. Short-term cultures were then made of the leucocytes, by a modification of the method of Moorhead *et al.*²² Three days later, the cultures were

¹⁷G. Hodgson *et al.*, *Proc. Soc. Exptl. Biol. Med.* 103, 745 (1960).

¹⁸C. C. Congdon *et al.*, *Radiation Research* 14, 458 (1961).

¹⁹M. A. Bender, *Science* 126, 974 (1957).

²⁰M. A. Bender, pp 103–18 in *Immediate and Low Level Effects of Ionizing Radiations* (ed. by A. A. Buzzati-Traverso; proceedings of symposium held in Venice, June 22–26, 1959, published as a supplement to the *International Journal of Radiation Biology*) Taylor and Francis, London, 1960.

²¹M. A. Bender and P. C. Gooch, *Radiation Research* (in press, 1961).

²²P. S. Moorhead *et al.*, *Exptl. Cell Research* 20, 613 (1960).

colchicine-treated and then fixed, stained, and mounted as previously described.²¹ The cells were then scored for chromosome aberrations.

Results. – The results are presented in Table 16. From the lack of induced chromatid-type aberrations, it is obvious that virtually all the cells examined were in their pre-DNA-synthesis (G_1) phase at the time the blood was drawn. This has been independently confirmed by experiments using tritium-labeled thymidine and autoradiography.²³ A single sample was analyzed for the period of the first appearance of mitoses in cultures from 200-r irradiated blood. As is expected, the aberration frequency was higher than in the samples fixed at 72 hr. Total breaks and rings and dicentrics increased as a function of dose; the rings and dicentrics increased roughly as the square of the dose.

Discussion. – Assuming for simplicity that the frequency of breaks increases linearly, the data yield a breakage rate of 0.0029 break per cell per roentgen. This value is in excellent agreement with the value calculated previously for human epithelioid cells in vitro.²⁰ Assuming that rings and dicentrics increase as the square of the dose, the data yield a coefficient of production for these aberrations of 7.2×10^{-4} ring and dicentric per cell per roentgen.²⁰ Since little if any difference in aberration rate is expected between blood irradiated before or after it is drawn, these rates are probably valid for the blood of irradiated humans.

²³M. A Bender and D. M. Prescott, *Exptl. Cell Research* (in press, 1961).

Table 16. Aberrations in Human Blood Irradiated in Vitro

Dose (r)	Cells Scored	Cells with $2n = 46$	Chromatid Aberrations	Chromosome-Type Aberrations					
				Deletions	Rings	Dicentrics	Exchanges	Breaks (%)	Rings and Dicentrics (%)
Analyzed as 72-hr Cultures									
Control	430	15	6	1(?)	0	0	0	1.6	0.0
50	300	11	1	17	0	8	0	11.3	2.7
100	402	17	13	44	2	26	8	32.9	7.0
200	400	18	9	79	12	66	14	68.0	19.5
Analyzed as 54-hr Culture									
200	100	2	3	18	6	24	2	85.0	30.0

RADIATION IMMUNOLOGY

Takashi Makinodan	Rachel R. Carter
J. F. Albright	W. J. Peterson
E. H. Perkins	Martha A. Robinson
E. E. Capalbo ¹	Patricia F. Lincoln ²
Franco Celada ¹	Paul Urso ^{2,3}
R. D. Owen ²	

Relative Antibody-Forming Capacity of Spleen Cells as a Function of Age

Takashi Makinodan W. J. Peterson

Introduction. — Our past studies have shown that the *in vivo* culture model is most suitable to assess quantitatively the antibody-forming capacity of lymphoidal cells.⁴ That is, a linear \log_2 relation with a slope of 1.0 can be demonstrated between activity, as expressed in terms of 6-day antibody titer, and cell number. Using this model we found that there exists in the intact animal an autoregulatory mechanism which permits only a fraction of the total population of competent cells to participate in a given immune response.⁵ Thus, for example, one-tenth of a spleen from a pre-immunized mouse can be shown to produce as much antibody as an intact mouse in response to an optimum secondary dose of antigen. In view of this, it would seem that data obtained from studies on the antibody-forming capacity of individuals throughout their life span, although informative, could not be readily interpreted. With this in mind, preliminary studies were carried out utilizing the *in vivo* culture model to determine the primary antibody-forming capacity of 76×10^6 spleen cells (anti-sheep RBC response) from (C3H/Anf Cum ♀ × 101/Cum ♂) donors varying in age from 0.25 to 29 months.

Results. — As shown in Fig. 3, the maximum activity was obtained with spleen cells from 8-months-old mice. Relative to this $2^{-(\text{titer}_{\text{max}} - \text{titer}_x)}$, the activity of cells from 1-week-old mice was about 1%, from 1-month-old mice about 10%, from 3-months-old mice about 40%, and from 29-months-old mice about 15%.

Discussion. — As we would expect, these results show that during the first month of life there is an accelerated increase in the primary antibody-forming capacity of spleen cells. Surprising, however, is our finding that spleen cells from the so-called “immunologically mature” 3-months-old

¹Visiting investigator from abroad.

²Consultant.

³ORINS Fellow.

⁴T. Makinodan, *Fed. Proc.* 19, 586–89 (1960).

⁵T. Makinodan, M. A. Kastenbaum, and W. J. Peterson, *Journal of Immunology* (in press).

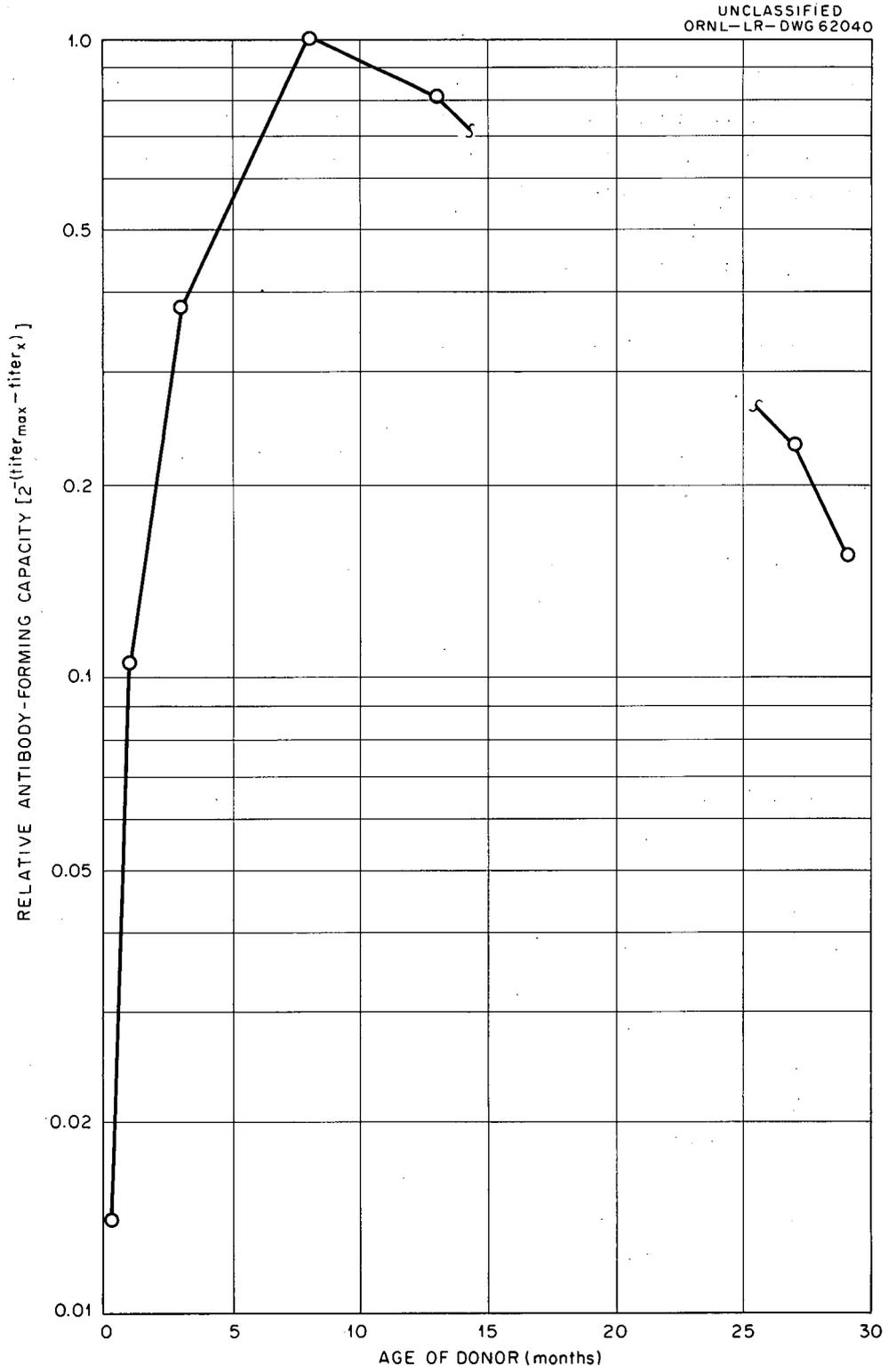


Fig. 3. Primary Sheep RBC Agglutinin Response of 76×10^6 Spleen Cells as a Function of Age.

mice possessed only about 40% activity relative to those of the 8-months-old mice. Another interesting observation is that those from the terminal stage of life were only about 15% as active. Although it is very tempting to speculate as to the cause of the rise in the relative antibody-forming capacity of spleen cells during the early stage of life and the decline during the terminal stage of life, these preliminary data do not justify our doing so. More elaborate studies are now being carried out to determine whether the change in the relative primary antibody-forming capacity of spleen cells throughout the life span of mice can be associated with a shift in the population of cell type or with a quantitative change in the protein-synthesizing capacity of the competent cells.

Phagocytosis as an Initial Cellular Event Leading to Antibody Formation

E. H. Perkins

Martha A. Robinson

Introduction. – It is possible that phagocytosis and intracellular antigen degradation are prerequisite to antibody production. If this is so, then the phagocytic process should reveal elements of the same selectivity and recognition of "foreignness" as is true of antibody production when judged by other criteria. The present report presents some results of experiments which show that phagocytosis is indeed highly selective with regard to erythrocyte antigens and that this selectivity can be modified by the presence of specific erythrocyte antibodies.

Results. – The per cent phagocytosis (per cent of cells engulfing one or more erythrocytes) by mouse peritoneal exudate mononuclear cells, when incubated in the presence of mouse normal sera or type-specific antisera, is shown in Table 17. Attention is directed to the following: (1) The more distant the genetic relationship between erythrocyte and phagocyte the greater is the per cent phagocytosis. (2) Unabsorbed mouse serum contains phagocytosis-enhancing factors (opsonins, natural antibodies) the action of which is particularly apparent on more closely related erythrocytes. (3) Erythrocyte-specific antiserum increases the degree of phagocytosis, although the influence of genetic relationship between erythrocyte and phagocyte is still maintained. Not included in the table are data which show that a higher per cent of phagocytosis is accompanied by an increase in number of erythrocytes incorporated per phagocyte.

When phagocytes are exposed to a mixture of erythrocytes, that is, to chicken and rabbit erythrocytes, either in the presence or absence of specific mouse antisera to both types of erythrocytes, the majority of cells incorporate the chicken red cells. Some phagocytes contain only rabbit red cells and a few contain both types. When, however, the mixture of erythrocytes is added to phagocytes in the presence of mouse anti-rabbit erythrocyte serum only, the engulfment of rabbit erythrocytes is highly favored. Some phagocytes contain chicken erythrocytes and again only a few contain both types.

Discussion. – It is clear that the selectivity of phagocytosis of erythrocytes is in agreement with general cellular antigenicity, the intensity of which depends upon the genetic relationship between donor and recipient (degree of foreignness). The expression of intrinsic genetic differences may be modified by the presence of preformed antibody; this effect, however, may be related to the

Table 17. Phagocytosis^a of Erythrocytes from Various Species as Influenced by Normal and Type-Specific Antisera

Erythrocyte Species	Per Cent Phagocytosis in:		
	Normal Sera, Specifically Absorbed	Normal Sera, Unabsorbed	Specific Antisera
Chicken	18.33	19.20	23.40
Sheep	5.9	13.7	26.1
Rabbit	1.9	17.7	20.4
Guinea Pig	1.4	11.7	12.1
Hamster		0.65	7.9
Rat	1.1	1.2	2.6
Mouse (homologous)		0.6	1.2

^aErythrocyte-phagocyte ratio 10:1; 30-min incubation at 37°C.

enhanced antigenicity of substances when in the form of antigen-antibody complexes. Further experiments in progress are designed to test the possibility that phagocytic cells may be involved in: (1) a morphological transformation to a cell in which antibody can be readily demonstrated, and (2) the transfer of "information" to a different type of cell capable of antibody synthesis.

Maximum Generation Time of Spleen Cells During Primary Antibody Formation

E. E. Capalbo

Introduction. – A twofold reduction in the generation time of lymphoid cells, labeled in vivo with H³-thymidine (H³T), occurs when these cells are transferred into in vivo cultures (heavily irradiated isologous recipients) during the inductive phase of secondary agglutinin response.⁶ In order to study the activity of these cells in an in vivo culture, but kept isolated from the recipient, a modified diffusion chamber technique was used. This method was shown to be suitable for the study of agglutinin formation by spleen cells.⁷ The present report concerns the rate of division of cells, cultured in vivo in diffusion chambers, as a function of time after primary antigen stimulation.

Results. – Twelve million spleen cells from nonimmunized C31F₁/Cum (C3H/Anf Cum × 101/Cum) 12-weeks-old mice were cultured with six million sheep red blood cells (SRBC), using the diffusion-chamber technique previously described⁷ and labeled with H³T by injecting the recipients intraperitoneally at 0, 4, and 6 days after antigen stimulation. By determining the percentage of labeled cells among reticular, lymphoid, and plasmacytic cells the H³T index curves were established in experimental (cells plus antigen) and in control (cells minus antigen) groups. Figure 4 shows the following:

⁶E. E. Capalbo and W. D. Gude, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, p 115.

⁷E. E. Capalbo, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, p 74.

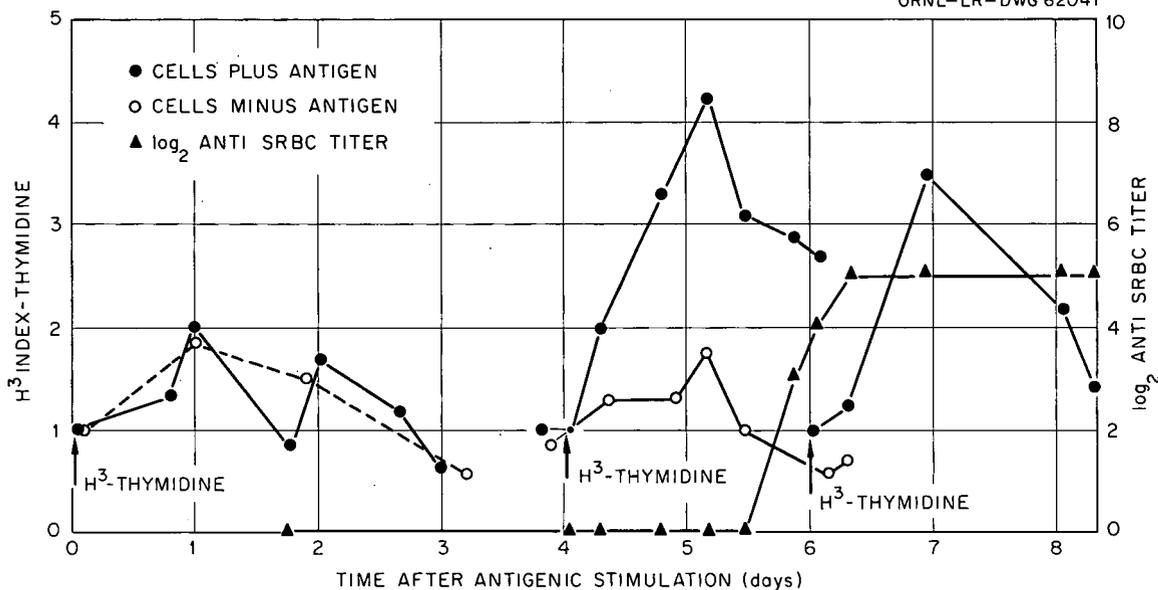


Fig. 4. Spleen Cell Generation as a Function of Time After Primary Antigen Stimulation.

1. During the early part of the inductive phase (0 to 3 days) there is no change in the H³T index of the experimental group from that of the control group.
2. During the latter part of the inductive phase (4 to 6 days) a twofold decrease in the generation time of the stimulated cells occurs.
3. During the productive phase (6 days and more) a twofold decrease of the generation time of the stimulated cells also occurs.

During secondary agglutinin response we also found a twofold decrease in the generation time of the stimulated cells in both the inductive and productive phases, using the same culture technique as described above.

Discussion. – These results show that during the inductive and the productive phases of antibody formation, both in primary and secondary agglutinin responses, there is a higher frequency of proliferation of the antigen-stimulated cells. Furthermore, in the inductive phase of the primary agglutinin response two different parts can be recognized: the first, from 0 to 3 days in which no modification of the generation time of the stimulated cells is observed; the second, from 4 to 6 days in which a higher frequency of proliferation is observed in the stimulated cells.

Radiosensitivity of Antihomograft Reactivity Studied on Isolated Spleen Cells

Franco Celada Rachel R. Carter

Introduction. – Previous experiments⁸ have shown that in the intact animal the homograft reaction mechanism is more radioresistant than the agglutinin synthesizing capacity. In order to study sys-

⁸F. Celada and T. Makinodan, *J. Immunol.* 86, 638 (1961).

tematically and in a quantitative manner this phenomenon, it is necessary to determine the radiation inactivation rate of a controlled number of cells. This has been possible by the use of a recently described model⁹ which enabled us to study the homograft-rejecting capacity of isolated cells. Essentially, known numbers of spleen cells from homologous true-breeding mouse strains (P_1 and P_2) are transferred simultaneously into irradiated ($P_1 \times P_2$) F_1 hybrid recipients, which are considered as in vivo test tubes, being for all practical purposes immunologically inert. The "killing" effect of the "active" P_1 on the "target" P_2 cells is measured by determining the decrease of anti-rat RBC agglutinin titer for which the latter cells (that had been preimmunized with this antigen) are responsible. Since the decrease in titer is linear with the increase in number of the active P_1 cells introduced into the system, it is possible to determine radiation inactivation of homograft-rejecting capacity at a cellular level.

Results. - Two sets of experiments were performed: (1) A fixed equal number of 24×10^6 active (C3H) and target (C57BL) spleen cells were transferred into (BL \times C3H) F_1 mice. The BL cells were preimmunized against rat RBC. The C3H cells were preimmunized against BL transplantation antigens and exposed in vitro to 0, 50, 100, 150, 200, 250 r. The inactivation rate with respect to the O-r control was determined. (2) A fixed number of 24×10^6 spleen cells from C57BL and C3H mice immunized against RBC were transferred separately into culture mice after exposure in vitro to the same radiation doses. The inactivation of their agglutinin-forming capacity was determined. The results, shown in Fig. 5, indicate that there is a significant difference in radiosensitivity between homograft reactivity

⁹F. Celada and Rachel R. Carter, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, pp 77-78.

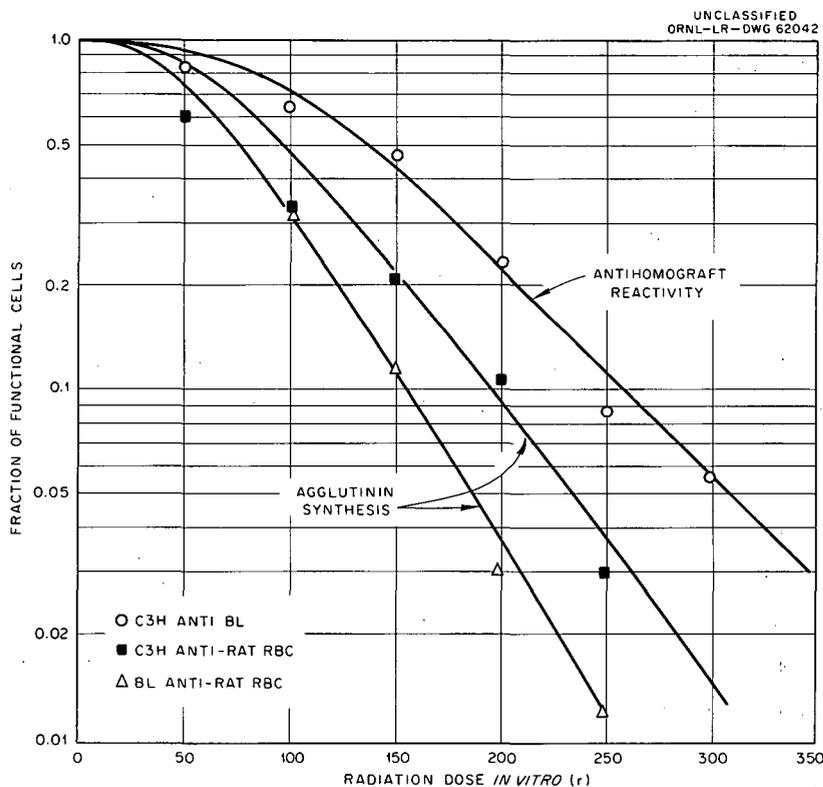


Fig. 5. Radiosensitivity of Homograft-Rejecting and Agglutinin-Forming Capacity of Isolated Spleen Cells.

and agglutinin synthesis of cells from the same strain (C3H). There is also a strain difference in the sensitivity of agglutinin formation (C3H vs C57BL).

Conclusion. – It can be concluded that the mechanisms of homograft rejection (and possibly of hypersensitivity) differ from circulating antibody production at a cellular level. The object of present investigations is to determine whether two cell lines are involved in the two functions or a different role is played by maturation and cell division with respect to the two immunological expressions. It should be emphasized that in light of these data it is probably incorrect to extrapolate results from one immune reaction to another and even from one mouse strain to another.

Intracellular Localization of Antibody

Paul Urso

Introduction. – In a previous report¹⁰ it was shown (1) that cells from an immunologically active population divide preferentially over nonactive cells during the inductive and productive phases of antibody formation and (2) that antibody-synthesizing cells of the proplasma cell type divide preferentially over nonsynthesizing cells during the logarithmic rise (3 to 6 days) in extracellular antibody. This report describes the intracellular distribution of the sites of antibody formation as a function of time after antigen stimulation.

Results. – Cells from the spleen and popliteal lymph nodes of preimmunized rabbits were cultured in 0.1- μ diffusion chambers with BSA as antigen and at intervals after culturing determinations on intracellular antibody activity were made as described previously.¹⁰ Antibody was found localized in the following areas of the cell: (1) as rods or dots in the nucleus, (2) as an island in the nucleus (nucleolus), (3) as an island located partly in the nucleus and the cytoplasm, (4) as an island in the cytoplasm in juxtaposition with the nucleus (Golgi region), and (5) as lobed areas in the cytoplasm and then diffusely throughout the cytoplasm. Table 18 represents the relative frequency of these intracellular sites. The greatest frequency of nuclear and cytoplasmic islands containing antibody occurred on the third and fifth days after antigen stimulation and during the logarithmic rise in antibody production, peak in mitotic activity, and peak in plasmablasts, proplasma, and plasma cell production. Nucleoli containing antibody could not be found after the third day. The nuclear rods or dots, the Golgi region, and lobed areas of the cell containing antibody were observed in relatively low frequency beginning on the fifth day and throughout the duration of the experiment (10 to 15 days).

Discussion. – The results show that the most likely sequence of intracellular antibody synthesis is as follows: (1) in the nucleolus, (2) in the Golgi region, and (3) in lobed areas of the cytoplasm and then diffusely throughout the cytoplasm. The close temporal relation of these cytological events to cellular maturation, mitosis, and antibody synthesis suggests that they are all interrelated and essential for antibody formation.

¹⁰P. Urso, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, pp 75-76.

Table 18. Relative Frequency of Location of Intracellular Antibody

Time (days)	Number of Cells Showing Localized Areas of Antibody			
	Nuclear Rods or Dots	Nuclear Island	Cytoplasmic Island	Antibody Diffused in Cytoplasm
3	2 (3.2) ^a	10 (16.1)	18 (29.0)	32 (51.6)
5	3 (2.9)	1 ^b (1.0)	7 (6.7)	94 (89.5)
6	0 (0)	0 (0)	0 (0)	24 (100)
7	2 (2.8)	0 (0)	1 (1.4)	68 (95.8)
8	0 (0)	0 (0)	1 (3.1)	31 (96.9)
9	0 (0)	0 (0)	1 (3.3)	30 (96.8)
10-15	2 (3.7)	0 (0)	3 (5.6)	49 (90.7)

^aFigures in parentheses represent per cent of antibody-containing cells examined.

^bIsland located partially in the nucleus and cytoplasm.

PATHOLOGY AND PHYSIOLOGY

Section Chief – A. C. Upton

Pathological Effects of Neutrons and High-Energy Radiation in Mammals

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Gustavo Cudkowicz	M. L. Davis
H. E. Walburg, Jr.	Audrey Eisenstadt
W. H. Benedict ¹	V. K. Jenkins
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Physiological and Chemical Effects of Radiation

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⁶Temporary summer employee.

Inhibition of the Foreign Bone Marrow Reaction by Irradiation of the Marrow Donor

Gustavo Cudkowicz

Introduction. — Bone marrow donor mice were exposed *in vivo* to an x-ray dose lethal to all but 2 to 6% of their total marrow cell population⁷ in an attempt to deprive their marrow of immunologically active cells but not of all blood-forming elements capable of continuous proliferation. It was expected that after transplantation of such irradiated marrow into lethally irradiated homologous recipients, the surviving donor elements would repopulate the recipients' hemopoietic sites but show reduced graft-vs-host immunological reactivity responsible for secondary disease.

Methods and Results. — After total-body exposure to 950 r of x rays, adult (101 × C3H/Anf)F₁ mice were injected intravenously with 40 to 50 × 10⁶ nucleated (C57L × A/He)F₁ or (C57BL/6 × DBA/2)F₁ marrow cells. Donor mice were exposed to 400 to 500 r of x rays 30 min before sacrifice. In some instances, the donor mice were given 9 mg of the radioprotective compound AET intraperitoneally 20 to 30 min before irradiation. Mortality among recipients was recorded for 90 days after treatment, the incidence of secondary disease being estimated only among mice surviving more than 21 days after irradiation. To ascertain whether the irradiated donor cells had transplanted, peripheral blood hemoglobin in the recipients was characterized,⁸ since the hemoglobins of the host and donor strains differed in solubility. The experimental results (Table 19) can be summarized as follows: (1) The preirradiation of donor mice with 400 to 500 r reduced the ability of their marrow to

⁷J. E. Till and E. A. McCulloch, *Radiation Research* 14, 213 (1961).

⁸R. A. Popp and G. E. Cosgrove, *Proc. Soc. Exptl. Biol. Med.* 101, 754 (1959).

Table 19. Survival of Lethally Irradiated Mice Following Implantation of Homologous Marrow (30 Mice per Group)

Recipient Strain	Donor		Number of Surviving Mice			Incidence of Secondary Morbidity and Mortality (%)
	Strain	Treatment	21 days	90 days Secondary Disease Present	90 days Secondary Disease Absent	
(101 × C3H/Anf)F ₁	(C57BL/6 × DBA/2)F ₁	None	30	2	0	100
(101 × C3H/Anf)F ₁	(C57L × A/He)F ₁	None ^a	27	0	0	100
		None	30	6	0	100
		AET	30	3	0	100
		400 r	24	0	15	37
		AET + 400 r	30	6	18	40
		500 r	20	2	18	10
		AET + 500 r	28	0	28	0

^a1.5 × 10⁶ nucleated cells injected instead of 30 × 10⁶.

protect irradiated recipients against early radiation lethality; however, recipients of such marrow showed a reduced incidence of secondary disease. (2) The administration of AET to donor mice before irradiation protected hemopoietic cells, as judged by effectiveness of the marrow in preventing early death among lethally irradiated recipients; however, the incidence and severity of secondary disease in such recipients was as low as when donor mice were irradiated in the absence of AET, suggesting that the compound did not protect antibody-forming cells in the marrow. (3) The treatment of unirradiated donor mice with AET had no detectable effect on the occurrence of secondary disease among lethally irradiated recipients. (4) Variations in the severity and incidence of secondary disease were not detectably influenced by the number of marrow cells transplanted. (5) All chimeras surviving 90 days after irradiated marrow transplantation had donor type hemoglobin in their peripheral blood.

Discussion. – The reduction of secondary disease in homologous recipients of irradiated bone marrow cells is tentatively attributed to the elimination of immunologically competent cells from donor marrow to an extent adequate to inhibit the development of clinically significant graft-vs-host immune reactions.

Relative Inability of AET⁹ and APMT¹⁰ to Protect Immunologically Competent Cells

Gustavo Cudkowicz

Audrey Eisenstadt

Introduction. – Published data suggest that AET is not as effective a radioprotective agent for antibody-forming cells¹¹ as for other cell types.¹²⁻¹⁴ In support of this idea, the following experiments show that AET and APMT do not modify the radiosensitivity of immunologically competent cells in adult parental strain mice, as judged by the ability of such cells to induce wasting disease on transplantation into sublethally irradiated F₁ hybrid recipients.

Methods and Results. – Young adult (C57BL/Cum × 101/Cum)F₁ and (101/Cum × C3H Anf/Cum)F₁ hybrid mice were exposed to 600-r total-body x irradiation (an LD_{0/30} for these strains) and subsequently injected intraperitoneally with parental strain liver cells, as previously.¹⁵ The C3H donor mice were preimmunized against 101 spleen cells before the liver cell transfer. Donors of both strains were exposed to 50 to 400 r of whole-body x irradiation immediately before killing, and AET or APMT was administered to some of them 30 min before irradiation in doses and routes proved to be effective for protection of the whole animal.¹³ Recipient mice were kept under observation for 90 days to record the occurrence of wasting disease (ruffling of hair, weight loss, and diarrhea) and related mortality. It was assumed that any impairment by radiation of the viability of the donor antibody-forming

⁹AET: *S*, β -aminoethylisothiuronium·Br·HBr.

¹⁰APMT: *S*, γ -aminopropyl-*N*'-methylisothiuronium·Br·HBr.

¹¹T. Makinodan, I. C. Shekarchi, and C. C. Congdon, *J. Immunol.* 79, 281 (1957).

¹²L. H. Smith, *Exptl. Cell Research* 13, 627 (1957).

¹³D. G. Doherty, p 45 in *Radiation Protection and Recovery*, ed. by A. Hollaender, Pergamon Press, 1960.

¹⁴G. Cudkowicz, *Proceedings of the Society for Experimental Biology and Medicine* (in press, 1961).

¹⁵G. Cudkowicz and G. E. Cosgrove, *Proc. Soc. Exptl. Biol. Med.* 105, 366 (1960).

cells would be detectable by a reduction in the incidence of wasting disease and mortality among recipients. The x-ray dose to the donor required to reduce mortality among F_1 hybrid recipients to 50% was 206 r (95% confidence limits, 183 to 229 r) for C57BL donors and 363 r (95% confidence limits, 330 to 402 r) for C3H donors (Fig. 6). The strain difference in dose possibly reflects differences in the absolute numbers of cells involved in primary and secondary immunological responses, since the C3H donors were preimmunized against the recipient. Under the conditions of these experiments, administration of AET or APMT to donor mice did not modify the incidence of wasting disease among recipients. Hence, the survival of immunologically competent cells in the irradiated liver was not altered by chemical radioprotective treatment.

Discussion. – The survival of several irradiated cell types,¹³ including that of marrow hemopoietic cells^{12,14} is increased by chemical radioprotective treatment. It is noteworthy, therefore,

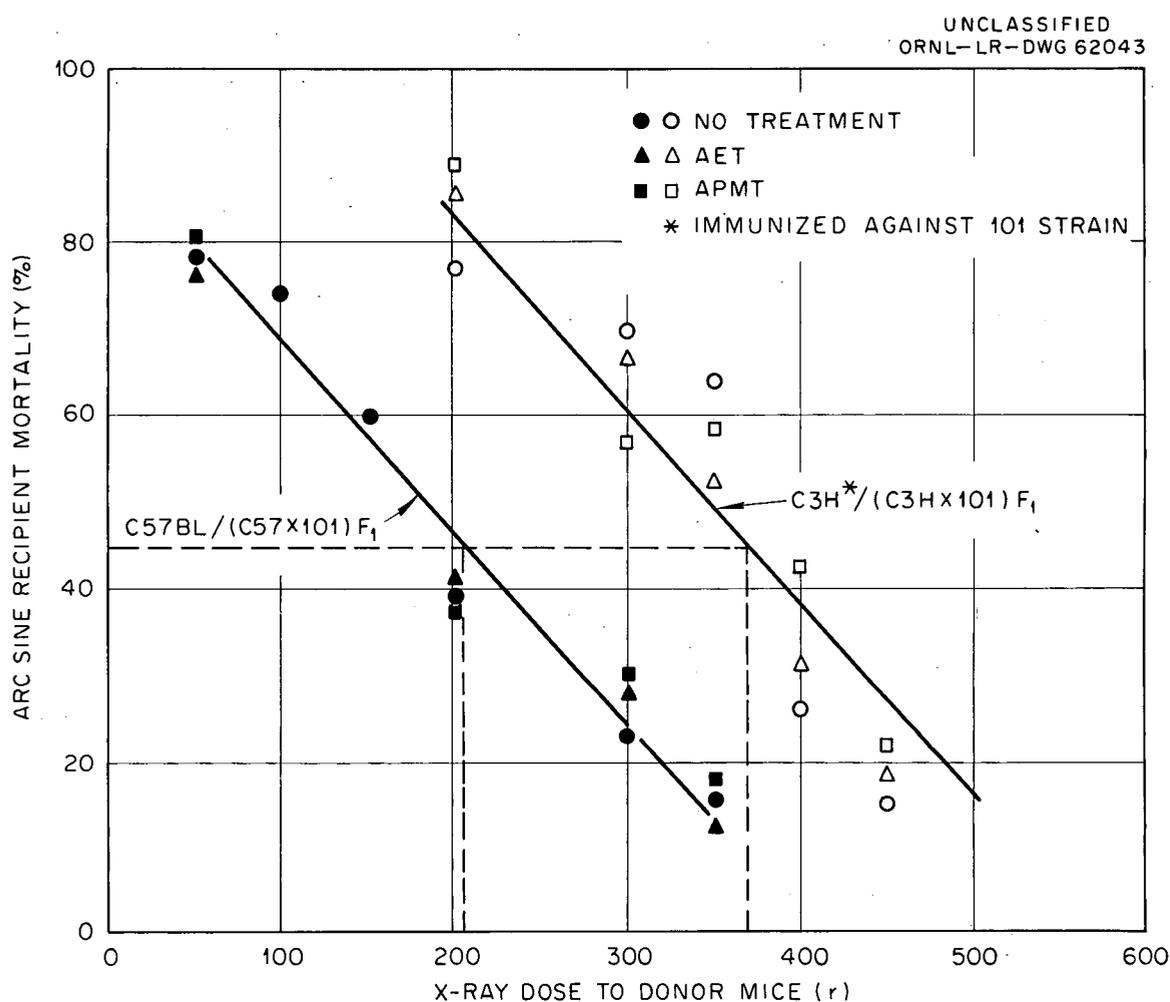


Fig. 6. Sixty-Day Mortality of Sublethally Irradiated F_1 Hybrid Mice Given Parental Liver Cells (Number of Cells Equivalent to 0.25 Whole Liver) Exposed *In Vivo* to X Radiation, With and Without Chemical Protection. (C57BL \times 101) F_1 recipients, C57BL liver donors: see "solid" data points. (101 \times C3H Anf) recipients, C3H Anf liver donors: see "open" data points.

that the viability and function of immunologically competent cells in the liver was not detectably protected against radiation injury by AET and APMT. This finding indicates a possible method by which radiation may be used selectively to destroy immune cells in tissues for transplantation.

Liver Changes in Sublethally Irradiated F_1 Hybrid Mice Receiving Parental Strain Spleen Cells

M. L. Davis

W. D. Gude

G. E. Cosgrove

Introduction. – Histopathologic changes in the liver have been noted in mice undergoing the foreign-spleen reaction, consisting of liver-cell vacuolation, focal necrosis, and the periportal infiltration of leucocytes. Since these changes may be of considerable severity, they prompted us to investigate liver function in the affected mice.

Methods and Results. – Young adult male (101/Cum \times C3H/Anf Cum) F_1 mice were given 300 to 500 r of 250-kvp whole-body x rays in three separate experiments (Table 20). Half the mice in each group were used as radiation controls, while the other half received intraperitoneally a suspension of

Table 20. Liver Changes in Mice with the Foreign-Spleen Reaction

Experiment Number	X-Ray Dose (r)	Spleen-Donor Strain	Day Tested	BSP Retention (%)	Results of Histologic Stains of Liver ^a	
					Fat	Cell Vacuolation
1	500	101/Cum	7	4.1	1	1
			8	6.7	2	1
			9	25.0	3	4
			10	74.0	3	4
	500	None	7	1.7	2	2
			8	2.8	1	0
			9	1.7	1	1
			10	1.4	0	0
2	500	101/Cum	6	1.4	1	1
			8	1.4	3	4
			9	8.3	2	3
			10	75.5	3	4
	500	None	6	1.8	0	2
			8	1.9	1	0
			9	1.8	1	1
			10	1.5	0	0
3	300	C3H/Anf Cum	8	2.2	2	3
			11	1.9	1	1
			12	49.0	4	4
	300	None	8	1.9	0	0
			12	2.8	0	0
4	0	None		3.1	0	0

^aSeverity graded on basis of 0 to 4, 0 being normal and 4 representing the greatest severity of abnormality.

15×10^6 parental-strain spleen cells (donor previously sensitized to reciprocal parent). At intervals thereafter, animals were subjected to the bromsulphalein (BSP) liver-function test (receiving a dose of 100 mg of BSP per kg of body weight), then killed and sectioned for histologic examination. No animal receiving foreign spleen survived beyond 12 days, and from 6 to 12 days, as the reaction increased, such individuals became progressively sicker. The degree of liver cell vacuolation, increase in stainable fat, and increase in retention of BSP were roughly parallel (Table 20).

Discussion. — The fatty degeneration of the liver in animals with the foreign-spleen reaction is not considered to be a specific lesion but merely a manifestation of general illness, although other possibilities cannot be excluded. The systemic illness is presumed to be related to an immunological reaction between grafted antibody-forming cells and the tissues of the recipient. Details of the pathogenesis of the various lesions remain, however, to be elucidated. Hence, the extent to which the observed liver changes may have contributed to morbidity and mortality are not yet known.

Susceptibility of Parental and F_1 Hybrid Mice to Leukemia Induction by X Irradiation

V. K. Jenkins W. D. Gude

Introduction. — Preliminary evidence suggests that spontaneous and radiation-induced lymphoid and myeloid leukemias in the AKR and RF strains are associated with filterable leukemogenic agents. In the search for evidence of common etiologic factors, the present study was undertaken to compare the incidence of such diseases in these strains and their F_1 hybrids.

Results. — Exposure of young adult mice to 300-r whole-body x irradiation decreased the incidence of leukemia in AKR mice but increased it in the other strains (Table 21). Lymphoma induction was

Table 21. Spontaneous and Radiation-Induced Leukemia in Parental and F_1 Hybrid Mice of the RF and AKR Strains

Strain	Sex	X-Ray Dose (r)	Number of Mice	Median Survival Time (months)	Leukemia Incidence (%)	
					Thymic Lymphoma	Myeloid
AKR	F	0	92	10	96	2
	F	300	84	10	79	0
	M	0	79	10	70	0
	M	300	93	12	52	2
$(AKR \times RF)F_1$	F	0	86	21	24	3
	F	300	97	12	43	3
	M	0	78	20	17	1
	M	300	88	14	24	16
$(RF \times AKR)F_1$	F	0	94	22	27	0
	F	300	85	15	40	12
	M	0	83	24	7	0
	M	300	95	18	22	9
RF ^a	F	0	97	20	5	3
	F	300	101	12	43	12
	M	0	314	19	5	3
	M	300	104	12	16	38

^aRF data previously published [A. C. Upton *et al.*, *Cancer Res.* 19, 842-48 (1958)].

greater in females and myeloid leukemia induction greater in males, as noted earlier in RF mice. The incidence of both types of leukemias in F_1 hybrids was intermediate between that of the parental strains, lymphomas being higher in $(AK \times RF)F_1$ than in $(RF \times AK)F_1$ mice.

Discussion. – The pattern of leukemia incidence in F_1 hybrids suggests that susceptibility factors are inherited from each parental strain, as might be anticipated in a simple Mendelian system. It would appear, however, that susceptibility to lymphomas is transmitted to a greater degree via AKR females than via AKR males, in accordance with earlier observations. The virological implications of these results are being explored further.

Increase in the Platelet Count by Injection of Activated Serum and Foreign Agents

T. T. Odell, Jr.

T. P. McDonald

T. C. Detwiler

Introduction. – We previously reported that the serum of rats rendered platelet-deficient by bleeding caused an increase in the number of circulating platelets on injection into homologous-recipient assay rats, the peak occurring five days after the start of a two-day course of injections.¹⁶ Serum of untreated control rats did not cause any increase. We have since found that serum of rats depleted of platelets by injection of antiserum produces a similar platelet response. In addition, we have observed that various foreign agents also cause a similar pattern of response.

Methods. – Platelet levels of serum donors were reduced to less than 10% of normal by intraperitoneal injection of 0.25 to 0.50 ml of antiserum specific to rat platelets. Blood was collected about 24 hr after injection of the antiserum, and the serum was separated and frozen for storage. Assay rats were injected subcutaneously twice a day for two days with 6 ml of serum per injection. Other agents injected on a similar schedule included normal human plasma, soluble egg albumin, and ground glass. Peripheral platelet counts and total white blood cell counts were taken before the initial injection and at intervals thereafter.

Results and Discussion. – The number of platelets and white blood cells in the peripheral circulation five days after initiation of the injections are shown in Table 22 as a percentage of the initial counts that were taken before the injections were begun. The increase in platelet counts in response to serum of platelet-deficient donors and the absence of this increase in response to serum of normal rats suggests the presence of a specific platelet-stimulating agent in the serum of treated donors. Injection of this serum did not alter the total white cell counts either at five days or earlier. It is apparent, however, that foreign substances are capable of initiating a similar platelet response, although egg albumin, and probably the ground glass too, also caused an increase in the total white cell counts.

These results raise the question whether or not the final pathway in platelet stimulation is the same with foreign material as with the indigenous material. They demonstrate that nonhumoral sub-

¹⁶T. T. Odell, Jr., T. P. McDonald, and T. C. Detwiler, *Biol. Div. Semiann. Progr. Rept.* Feb. 15, 1961, ORNL-3095, pp 88-90.

Table 22. Platelet and White Blood Cell Response to Various Agents 5 Days After Initial Injection

Treatment ^a	Number of Rats	Platelets (Per Cent of Initial Count)	White Blood Cells (Per Cent of Initial Count)
Normal rat serum (~1.68 g ^b)	6	110	101
Active rat serum (~1.68 g)	14	141	103
Normal human plasma (~1.68 g)	4	138	112
Ground glass	9	144	131 ^c
Soluble egg albumin			
2% solution, 3 ml × 4 (0.24 g)	4	139	119
10% solution, 3 ml × 4 (1.2 g)	4	151	169
7% solution, 6 ml × 4 (1.68 g)	3	140	220

^aInjection volume was 6 ml injected each of four times except where noted otherwise (see dose schedule for egg albumin).

^bApproximate dry weight of substance injected.

^cWhite cell counts on only 3 of the 9 rats.

stances may induce, perhaps indirectly, responses like those we believe to be humoral in nature and thus emphasize the difficulties in identifying and characterizing the control factors involved in normal homeostasis.

Age Changes in Mouse Collagen Fibers

E. B. Darden, Jr. M. B. Bradley J. W. Conklin

Introduction. – In a study of the effects of radiation and senescence on mouse collagen fibers, preliminary results¹⁷ showed that the temperature for maximum thermoelastic contraction (TMC) of tail-tendon fibers increased smoothly with age in virgin RF mice and that fibers from animals which had received whole-body radiation several months previously showed similar behavior. By contrast, there was no pronounced correlation between TMC and age in animals from the breeding colony. The results, now essentially complete, confirm these findings, although the final interpretation awaits statistical analysis.

Results. – Of approximately 300 virgin female and male RF/Up mice housed separately by sex in groups of 8 to 10 per cage, about half received 450-r whole-body x irradiation at 2 to 3 months of age. At intervals thereafter, animals were killed, and fibers were taken for examination. The points from animal to animal are scattered, but a trend of increasing TMC with age is present both in controls (Fig. 7a) and in irradiated mice (Fig. 7b). On the other hand, results from breeding animals show little if any correlation between TMC and age (Fig. 7c), even though the breeders generally

¹⁷E. B. Darden, Jr., and M. B. Bradley, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1959*, ORNL-2813, pp 137-39.

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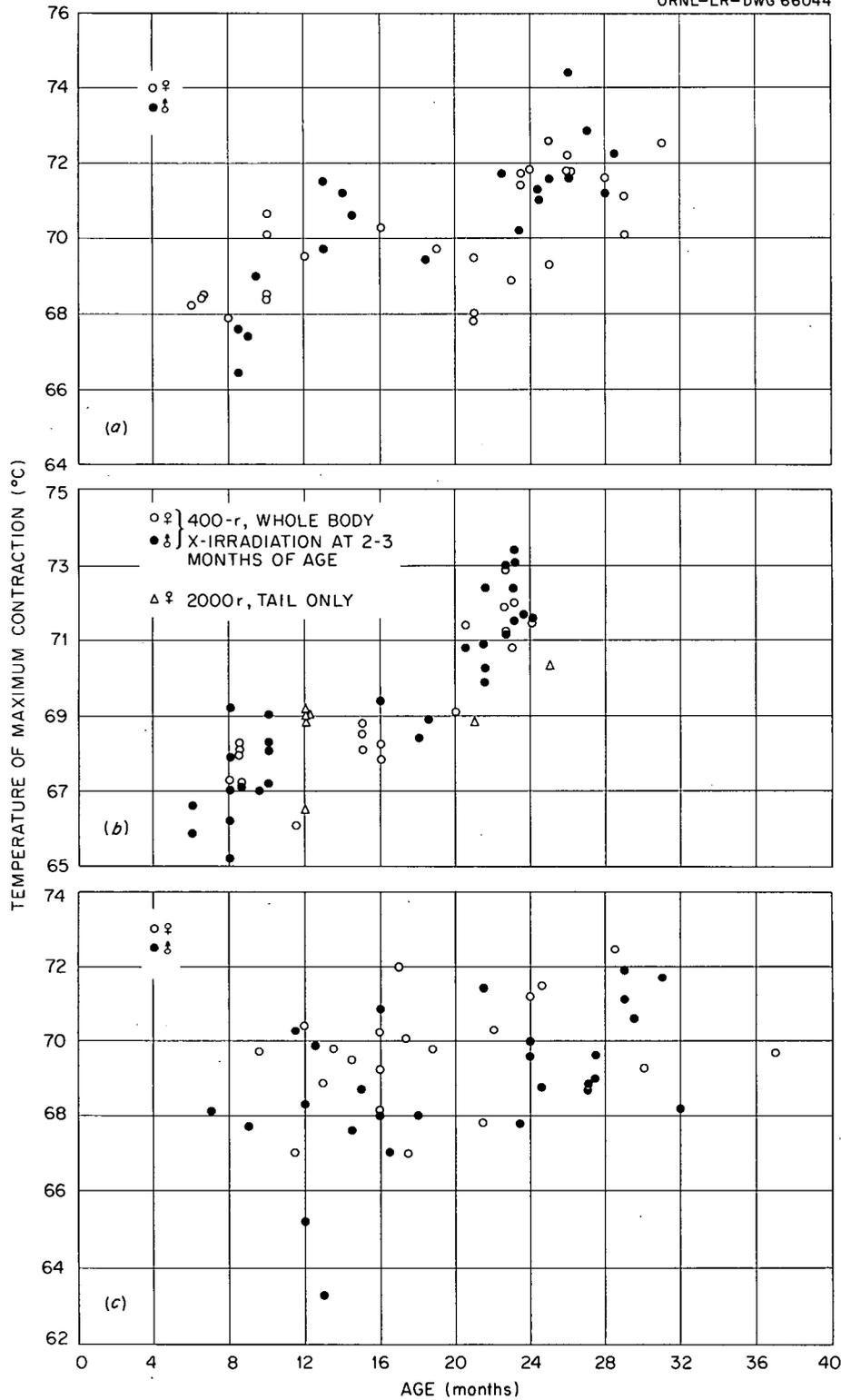


Fig. 7. Thermal Contraction as a Function of Age in Tail-Tendon Fibers of RF Mice. Each point is the mean of 2 to 4 fibers from one animal. (a) Unirradiated nonbreeders; (b) irradiated nonbreeders; (c) mice from breeding colony, fresh fibers.

live longer than the nonbreeders. Data obtained with fibers from mouse tails stored weeks to months at 0°C show a larger scatter of points but are in basic agreement with the results obtained with fresh fibers.

Discussion. – Thermal contraction of collagen fibers from nonbreeding mice, as measured by this method, show an age-dependent relation to temperature. The results provide strong evidence that the underlying aging process is not appreciably affected by life-shortening doses of ionizing radiation. Fibers from animals maintained under breeding conditions, on the other hand, apparently undergo little or no age-dependent change as measured by this criterion. Whether this difference is attributable simply to external factors, such as less crowded conditions in the breeding cages, or is related to endocrine, nutritional, or other factors is a subject for future study.

Studies of Aging in Blood Platelets

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Introduction. – Isotopic studies have indicated that circulating blood platelets have a predetermined life span and are not (under normal circumstances) lost by random removal. The mechanisms of aging of platelets and their removal from the circulation are not known. Studies of red blood cells have shown that their glycolytic capability declines with age, until the production of ATP is impaired. (The concentration of ATP then reaches a level too low to supply the energy necessary for the maintenance of cellular integrity.) A similar condition might exist in platelets, although platelets and red blood cells may differ in this respect, since platelets have a high concentration of ATP. We have therefore measured the concentration of ATP and the platelet volume of young and old platelets and of populations of platelets having a normal age distribution in an attempt to establish an age-related difference which could serve as a point of departure for further studies of the aging of platelets.

Methods. – Young platelets were obtained from rats that had been injected with anti-rat-platelet rabbit serum in amounts sufficient to destroy all platelets. Two days after injection, the newly formed platelets, all less than two days old, were collected (rat platelets have a life span of approximately 4.5 days). To obtain old platelets, rats x-irradiated with 900 r to inhibit platelet production were used as in vivo "culture tubes" for aging normal platelets injected into them. On the sixth day after irradiation, the irradiated rats were injected with freshly collected platelets in sufficient numbers to raise their platelet counts to normal levels. On the eighth day after irradiation, the remaining platelets were collected; they were thus at least two days old. Control rats that received irradiation but no platelets had platelet counts that averaged 3% of the normal counts on the eighth day. Washed platelets were suspended in saline, and counts were made of platelets, white blood cells, and red blood cells. The average contamination of white blood cells was 0.11% (calculated as volume/volume); the maximum was 0.55%. In the majority of the samples no red blood cells were observed, although there was a small red-blood-cell contamination in one sample of young platelets. The ATP concentration of this sample was not unusual. The packed volume of platelets was measured in microhematocrit tubes with a cathetometer. ATP was extracted from platelets by heating aliquots of the suspension in 0.01 M glycine, pH 7.4, at 100°C for 20 min. It was measured by the firefly-luminescence method.

Results and Discussion. — The results are presented in Table 23. They suggest a decrease in the size of platelets (an increase in numbers per milliliter) with increasing age. This change is small, the old platelets being about 75% as large as the young platelets, so that additional experiments will be necessary to verify this observation. Although the content of ATP (amount of ATP per platelet) in the old platelets is less than in the young platelets, the decrease in size of the platelets results in a constant concentration of ATP (amount of ATP per volume of platelets). It thus appears that platelets, unlike red blood cells, maintain a constant concentration of ATP throughout their life.

Table 23. The ATP Content of and Size of Platelets in Relation to Their Age

Age of Platelets	Number of Samples	ATP Content (μ moles per 10^{11} Platelets) ^a	Number of Platelets per Milliliter of Packed Platelets ^a
			$\times 10^{10}$
Young (<2 days)	4	4.2 (± 0.3)	8.8 (± 0.3)
Normal distribution	12	4.2 (± 0.6)	10.6 (± 1.4)
Old (>2 days)	4	3.6 (± 0.4)	11.8 (± 0.8)

^aOne standard deviation.

Studies on Plasma Lactic Dehydrogenase Activity in Mice with Radiation-Induced Myeloid Leukemia

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Introduction. — Elevation of the plasma lactic dehydrogenase (LDH) activity has been noted in mice with a variety of neoplasms, including leukemia,^{19,20} and preliminary data suggest that the elevation may be caused by a filterable agent or agents. The present study was undertaken to determine whether the LDH was increased in RF mice with radiation-induced myeloid leukemia and to explore the pathogenesis of any such increase encountered.

Results and Discussion. — Plasma LDH levels were generally increased in mice with transplanted radiation-induced myeloid leukemia. The degree of elevation varied irregularly with the transplant generation (Fig. 8), the mean of all values being about 13 times normal (Table 24). On comparison of the plasma LDH with that of various tissues, through the use of diphosphopyridine (DPN) analogs,²¹ the LDH of leukemic plasma was found to differ from that of normal plasma and to resemble more closely that of the liver (Table 25). From preliminary observations, the extent of LDH increase seems to be correlated with the severity of leukemic infiltration of the liver.

¹⁸Enzymology Group.

¹⁹V. Riley, *Science* 132, 545-47 (1960).

²⁰F. Wroblewski, *Ann. N.Y. Acad. Sci.* 75(1), 322-37 (1958).

²¹N. O. Kaplan, *Science* 131, 392-97 (1960).

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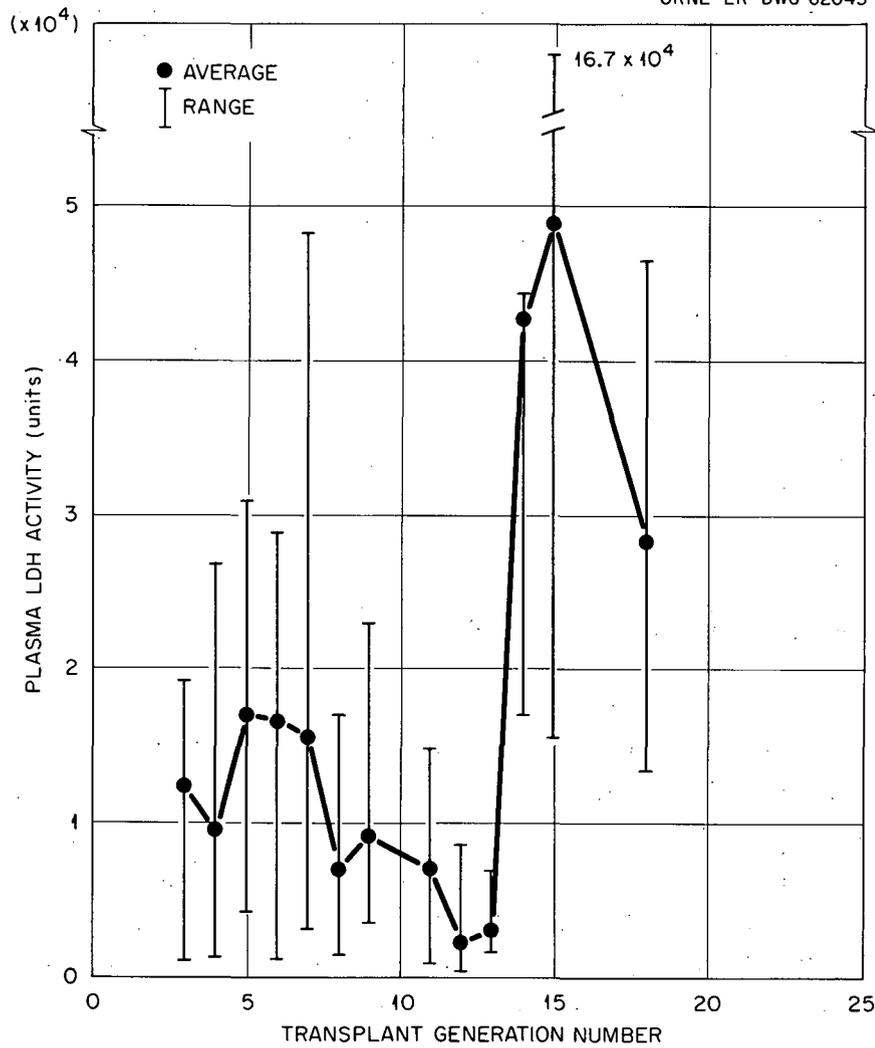


Fig. 8. Plasma LDH in Mice with Transplanted Myeloid Leukemia in Relation to the Transplant Generation.

Table 24. Plasma LDH Activities in Mice with Transplanted Myeloid Leukemia

Group	Number of Mice	Activity ^a (units)
Normal mice	12	1,260 (445-1,870)
Leukemic mice	117	16,200 (375-167,000)

^aAverage of all values; range shown in parentheses.

Table 25. Reactivity of Leukemic Plasma LDH with DPN Analogs

DPN Analogs	Leukemic Plasma	Normal Plasma	Normal Organ LDH					
			Heart	Liver	Kidney	Bone Marrow	Thymus	Spleen
			Ratio ^a					
Diphosphopyridine nucleotide	2.5	1.8	2.1	2.5	1.8	2.6	2.2	2.3
Deamino-DPN	3.1	2.5	2.3	2.9	2.5	3.0	2.7	2.7
3-Acetylpyridine-DPN	1.1	1.1	3.3	1.4	1.5	1.1	1.9	1.3
3-Pyridine aldehyde-DPN	2.8	2.6	1.8	3.1	2.5	1.7(?)	1.7	2.6
Thionicotinamide-DPN	3.8	3.0	3.4	3.6	1.8	5.9	3.5	4.4

^aValues are expressed as the ratio of activity in high substrate concentration (0.10 M) to that in low substrate concentration (0.0125 M).

Repopulation of Thymus by Immunologically Competent Cells Derived from Donor Marrow

R. A. Popp

Introduction. — Recovery of the thymus in irradiated mice may be promoted by injection of isologous^{22,23} or parental²² bone marrow cells. It is not known, however, whether this results from repopulation of the thymus by the implanted cells²⁴ or from facilitated growth of surviving autologous cells.²⁵ The following experiment was designed to determine the preponderant type of cell in regenerated thymuses of irradiated F₁ hybrid mice given parental marrow.

Methods and Results. — Although a single dose of 650 r of x rays was sublethal for B1F₁ mice and killed only 13% of B6D2F₁ mice, 50 to 80% of such recipients died within 90 days after receiving an intraperitoneal injection of the thymus cells from a parental strain donor (Table 26). Thymus cells from (C57BL × 101)F₁ mice which had received C57BL marrow cells also killed all sublethally irradiated B6D2F₁ recipients (Table 26). Histological examination of such recipients showed granulomatous reticular cell hyperplasia in lymph nodes, splenic white pulp, and Peyer's patches.

Discussion. — The mortality in 650-r-irradiated F₁ mice injected with parental lymphoid cells is attributed to immunogenetic differences between the recipient and donor.²⁶ Since the data suggest that our experimental mice died from a similar immunological reaction, it is inferred that the regenerated thymus is repopulated by donor cells in lethally irradiated F₁ hybrids given parental marrow.

²²B. B. Hirsch *et al.*, *Radiation Research* 5, 52 (1956).

²³P. Urso and C. C. Congdon, *Blood* 12, 251 (1957).

²⁴N. Gengozian *et al.*, *Proc. Soc. Exptl. Biol. Med.* 96, 714 (1957).

²⁵H. S. Kaplan *et al.*, *Proc. Soc. Exptl. Biol. Med.* 98, 384 (1958).

²⁶D. E. Uphoff, *J. Natl. Cancer Inst.* 19, 123 (1957).

Table 26. Survival of Sublethally Irradiated F₁ Hybrid Mice Injected with Thymus Cells

Donor Strain	Recipient		Survival (%)			
	Strain	Number	Days After Treatment			
			10	30	60	90
	B1F ₁	48	100	100	100	100
B1F ₁	B1F ₁	48	98	98	98	98
C57BL	B1F ₁	48	85	48	25	17
101	B1F ₁	48	100	69	58	50
C57BL/C57BL ^a	B1F ₁	30	93	37	23	17
101/101 ^a	B1F ₁	64	94	36	23	9
	B6D2F ₁	52	100	87	85	83
B1F ₁	B6D2F ₁	24	96	92	92	92
C57BL	B6D2F ₁	24	96	0	0	0
B1F ₁ /C57BL ^b	B6D2F ₁	56	100	5 ^c	0	0

^aIsologous bone marrow chimeras.

^bParent-F₁ bone marrow chimeras.

^cAll 30-day survivors were dead 40 days after treatment.

Inheritance of Differences in Serum Esterase Among Inbred Strains of Mice

R. A. Popp Diana M. Popp

Introduction. – The pattern of serum esterases separated by starch gel electrophoresis is different in C57BL and C57L mice from that in AKR, RF, 101, C3H, BALB/c, and SEC mice. The sera of C57BL and C57L mice reveal one band of esterase near albumin, whereas two esterases appear in serum preparations of the others. Observations on the inheritance of the various esterases are reported herein.

Methods and Results. – C57BL mice possessing the single band variant were mated with 101, BALB/c, and SEC mice possessing the double esterase. Sera of the F₁ hybrids revealed two bands on starch gel preparations; however, the heterozygote could be distinguished by a photometric assay, since the level of esterase activity in F₁ serum was intermediate between that in either parental strain. The classification of F₂ and BC₁ progeny of the three F₁ hybrids for serum esterase traits is shown in Table 27.

Discussion. – The electrophoretic and photometric assays reveal that the factors governing the esterase differences are codominantly expressed. Results from the genetic studies (Table 27) suggest, moreover, that the various esterase types are controlled by allelomorphous differences at a single locus.

Table 27. F_2 and BC_1 Progeny Classified for Serum Esterase Traits

Matings	Genotype of Progeny					
	Es^1/Es^1^a		Es^1/Es^2		Es^2/Es^2	
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
$(C57BL \times 101)F_1 \times (C57BL \times 101)F_1$	12	10.75	22	21.5	9	10.75
$(C57BL \times 101)F_1 \times C57BL$	27	20.5	14	20.5		
$(C57BL \times 101)F_1 \times 101$			31	33.5	36	33.5
$(C57BL \times BALB/c)F_1 \times (C57BL \times BALB/c)F_1$	24	21.75	42	43.5	21	21.75
$(C57BL \times BALB/c)F_1 \times C57BL$	20	20	20	20		
$(C57BL \times BALB/c)F_1 \times BALB/c$			12	13	14	13
$(C57BL \times SEC)F_1 \times (C57BL \times SEC)F_1$	8	11	24	22	12	11
$(C57BL \times SEC)F_1 \times C57BL$	25	27	29	27		
$(C57BL \times SEC)F_1 \times SEC$			37	36	35	36

^a Es^1/Es^1 , Es^1/Es^2 , and Es^2/Es^2 indicate homozygous-single, heterozygous, and homozygous-double esterase types respectively.

Electron Microscope Studies of Radiation Damage in the Young Oocyte of the Mouse

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Introduction. – The young oocyte of the mouse shows two features of special interest. First, it goes through a stage in which it is extremely sensitive to x rays (7 r kills approximately half of the cells in 18 hr). Secondly, meiosis is arrested in the middle of prophase, and the chromosomes disperse. Meiosis is only resumed just before ovulation. In the present investigation, the morphology of the young oocyte was investigated, with particular attention to structures affected by radiation.

Results and Discussion. – Young oocytes irradiated with doses of 7 to 200 r were examined at intervals shortly after irradiation. With doses of 30 to 200 r there was a drop in the numbers of mitochondria per oocyte within 2 min from the time of irradiation. The numbers were restored during the next few minutes. The normal process of multiplication of mitochondria by fission appeared to be momentarily arrested. Five to six hours following a dose of 7 r, all the irradiated oocytes contracted in size. In the next 2 hr some cells shrunk further and became pycnotic. Other cells expanded and either recovered or were killed by karyolysis, the necrosis being complete by 8 to 12 hr. By 24 hr, half of the oocytes appear to have effected complete recovery.

No other cell type in the ovary was detectably affected by x rays in the dose range used. In both karyolytic and pycnotic degenerating cells, the nucleolus showed severe shrinkage. Similar appearances were seen in some of the nucleoli of nearly mature oocytes of unirradiated ovary, in which there is a high natural death rate. After a phase of radiosensitivity, oocytes rapidly passed into a

phase of radioresistance. Morphological comparisons between the two stages showed, apart from the increase in number of follicle cells associated with the resistant oocyte, differences in the structure of the nucleolus and of the Golgi apparatus. It is suggested that the nucleolus is the principal target in irradiated oocytes, although at doses somewhat higher than 7 r the changes in mitochondria may be important.

In the resting (dictyate) phase of meiosis, the chromosomes were dispersed into 0.1- μ -wide bundles containing 100-A microfibrils. In a few cases, two 40-A units were seen inside the microfibrils. The microfibrils appeared to run into the nucleolus from all directions, and it is speculated that they were continuous with the fibrils of the nucleolus.

CELL PHYSIOLOGY

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The Density-Gradient Ultracentrifuge

N. G. Anderson

Introduction. – It is our purpose to develop a general method for the separation of particles, the method being based on differences in rates of sedimentation through liquid density gradients. In principle such a method is applicable to the separation of cells, subcellular particles, protein or nucleic acid molecules, and the isolation of viruses. In practice, however, the separations which may be achieved are severely limited by the restrictions imposed by the use of swinging-bucket centrifuges. These of necessity have very small capacities and are subjected to sedimentation artifacts where sector-shaped tubes cannot be employed.

Experimental. – Four low- and medium-speed centrifuge systems have been built in the development of the density-gradient ultracentrifuge. The first system⁷ demonstrated that excellent separations of nuclei, mitochondria, and microsomes could be achieved with sucrose gradients in sector-shaped tubes. Eighteen hours, however, were required to fractionate 2 to 3 ml of material. The second system⁸ demonstrated that gradients could be run into large centrifuge tubes very rapidly if the stabilizing effect of centrifugal force were employed, that is to say, if the gradient is run into the centrifuge while it is running. The third system^{9,10} employed a tubeless hollow rotor to demonstrate that density gradients could be introduced into such rotors and recovered while spinning at speeds up to 3000 rpm. Unfortunately, considerable spraying occurred in the system used to recover

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⁷N. G. Anderson, *Exptl. Cell Research* 9, 446–59 (1955).

⁸J. F. Albright and N. G. Anderson, *Exptl. Cell Research* 15, 271–81 (1958).

⁹N. G. Anderson, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1955*, ORNL-1953, p 117.

¹⁰J. R. C. Brown, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1955*, ORNL-1953, p 119.

the gradient at the end of the run, and the centrifuge head was almost impossible to keep in balance. A fourth system, designed around the Spinco model K centrifuge, allowed gradients of 621 ml to be introduced into a tubeless rotor at speeds up to 18,000 rpm. Very little difference was seen between the shape of the density gradient introduced into the rotor and that recovered after centrifugation.

On the basis of this work, a density-gradient ultracentrifuge was constructed on subcontract by the Spinco Division of Beckman Instruments, Inc. The centrifuge was planned to spin in a refrigerated vacuum chamber at speeds up to 30,000 rpm. Fluid can be pumped into and out of the rotor at all speeds. The first rotor (rotor I) has a flexible upper shaft. While it is very stable when empty or when filled with solid material, it is extremely unstable when filled with water or sucrose solution. The cause of the instability has not yet been determined. It is thought that the fluidity and compressibility of water allow the center of rotation of the fluid and metal masses to differ, giving rise to instability. Experimentally, increasing the number of vertical, radially arranged septa increases stability. Rotor II was made longer and narrower, with 36 septa and a capacity of 1625 ml. The partially disassembled rotor is shown in Fig. 9. The designed speed is 40,000 rpm with



Fig. 9. Rotor II, with Top Removed To Show Internal Septation.

$90,952 \times g$ at the outer edge of the gradient. This rotor has a stiff upper-bearing shaft and cannot be perfectly balanced. Upper-bearing problems have limited this rotor to 22,500 rpm, at which speed excellent performance has been obtained. Rotor temperature is held to $5 \pm 0.5^\circ\text{C}$. The sample volume is variable up to 400 ml, depending on the resolution required.

Discussion. – While many problems concerned with rotor stability, better seals for the liquid-containing lines running into the rotor, and higher-speed drive motors remain to be solved, the feasibility of building density-gradient ultracentrifuges has now been demonstrated. While this work has been done independent of AEC-sponsored work on the gas ultracentrifuge, it now appears that many of the principles and problems involved in the DGU-1 and the gas ultracentrifuge are identical. Future design, development, and testing will therefore be carried out in collaboration with the gas ultracentrifuge group.

Separation of Liver-Cell Particulates in the Density-Gradient Ultracentrifuge

N. G. Anderson C. L. Burger

Introduction. – The key procedure in the proposed Cell Fractionation Project is the quantitative separation of subcellular particulates from living cells. None of the previous methods allows the quantitative separation of gram quantities of each component. The resolution obtainable with the density-gradient ultracentrifuge has been examined in a series of twenty runs using rat liver.

Experimental. – The rotor was filled with a 1200-ml gradient extending linearly with radius from 17 to 55% w/w sucrose solution, followed by a 66% sucrose "cushion" at the rotor edge. To avoid overloading the continuous recording systems used to analyze the gradient at the end of the run, a relatively small sample was used. It consisted of a 50-ml linear gradient of a 20% liver homogenate prepared in 8.5% sucrose, and 17% sucrose. Distilled water was used to push the sample into the rotor at about 2000 rpm. In the separation shown in Fig. 10 the rotor was then accelerated to 19,539 rpm and run for 3 hr. After decelerating to about 2000 rpm, the gradient was displaced out of the rotor with 66% sucrose through a 0.2-cm quartz cell for continuous recording of optical density at $295 \mu\mu$. Part of the effluent was diluted with an alkaline buffer and run through a system recording optical density at 260 and $410 \mu\mu$. The results show that an excellent separation of the usual components was obtained, and, in addition, a new peak composed of what appear to be cell-wall fragments was obtained.

Discussion. – It should be emphasized that rotor II used in these experiments was designed to study (1) rotor and gradient stability, (2) performance of the fluid-line seals which allow liquids to be pumped into and out of the centrifuge during operation, (3) the fractionation of cell populations into different cell types, (4) the separation of subcellular organelles and viruses, and (5) the fractionation of protein and nucleic acid mixtures on the basis of sedimentation rate. The rotor is therefore a compromise design which is not ideally suited to doing any single one of the above. The separations observed with liver, while superior to any large-scale separations which we have

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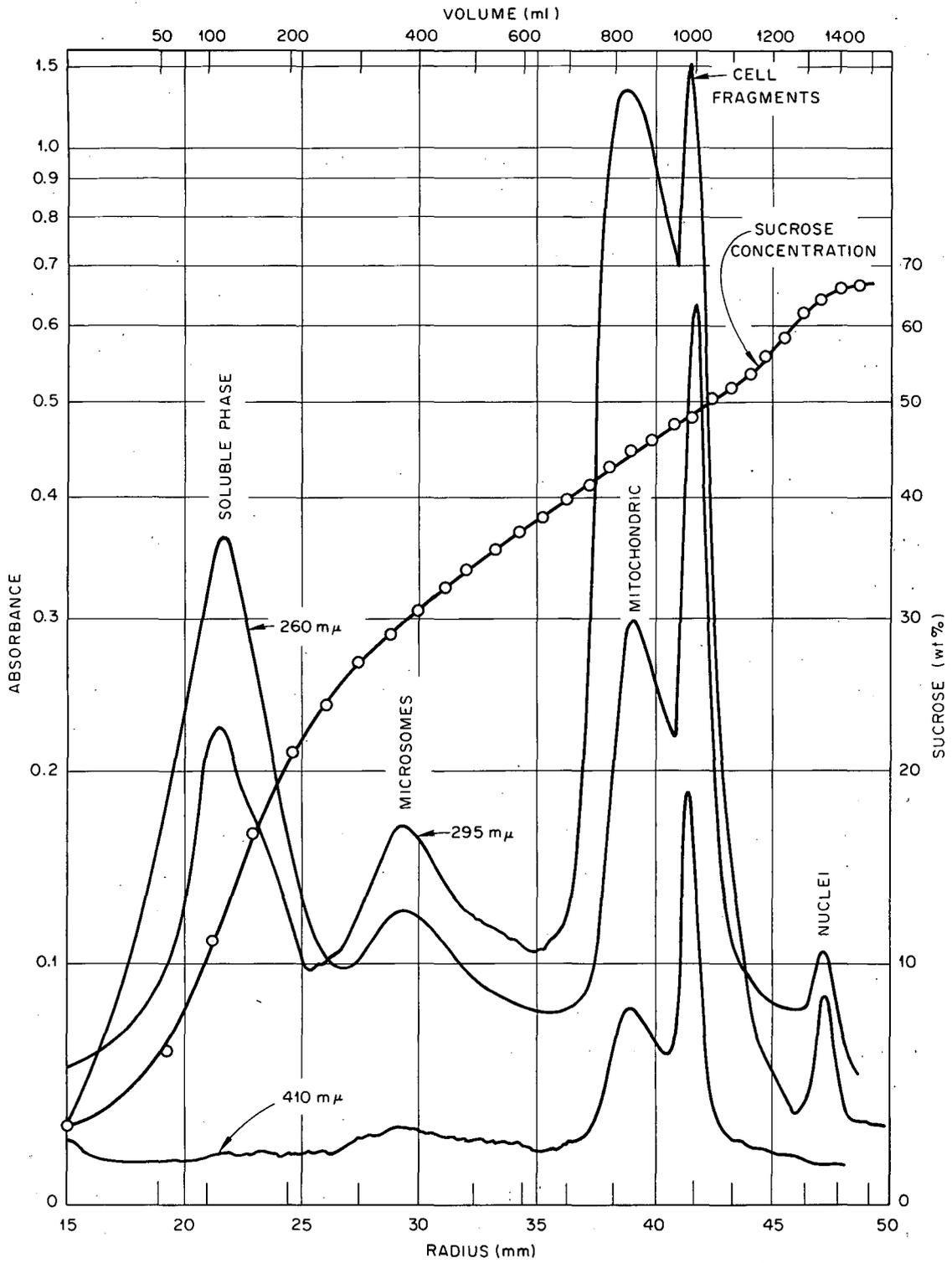


Fig. 10. Scan of Density Gradient from DGU-I Rotor.

obtained previously, do not represent the resolution ultimately obtainable with rotors designed for a single purpose. The direct isolation of viruses from suspensions of broken cells is now being studied.

The Isolation and Characterization of Tissue Proteins

H. E. Bond N. G. Anderson

Introduction. – A protein component, designated as a sex-associated protein, normally present in the male rat liver and absent from that of the female has been described in previous reports. Preliminary investigation involving the relationships of the male and female hormones with the production of the sex-associated protein was discussed in the last report. Further investigations conducted in a systematic manner have substantiated the previous findings. Presently the data are being prepared for publication.

Results. – Ten groups of experimental rats were prepared. One of four types of animals, intact males and females and castrated males and females, was used in each group. Experiments were conducted in a manner to investigate either the induction or disappearance of the sex-associated protein in response to the presence or absence of male and female hormones. Commercial preparations of testosterone propionate, testosterone phenylacetate, and estradiol dipropionate were administered in experiments requiring treatment with exogenous hormones. No significant increase in sex-associated protein is induced in the male treated with testosterone. The protein normally absent from the female is induced following treatment with testosterone. The protein component does not develop in the untreated female castrate, but it is induced in this animal with testosterone treatment in much the same manner as it is in the intact female treated likewise. The protein disappears slowly from the male castrate, but comparatively rapidly from both the intact and castrated males treated with estradiol. In the untreated male castrate which has depleted most of its sex-associated component, the protein is restored to nearly normal amounts following treatment with testosterone. All experiments were related to time. Two to three weeks were required for significant changes to manifest themselves following hormone treatment.

An additional study relating the amount of sex-associated protein to the age of the male rat is presently being completed. Preliminary results show the amount of the protein to be very much less in the young animal than in the sexually mature adult.

Discussion. – The data suggest that the sex-associated protein is both induced by the action of testosterone and repressed by the action of estradiol. Other androgens and estrogens have not been investigated. The biological significance of the sex-associated protein has not been assessed, and the function of the protein in the physiology of the animal is presently unknown. Elucidating this function will form the basis for much of the future research on the sex-associated protein. Additional avenues of research open to investigation are the opportunities to study specific hormonal effects and differentiation at the macromolecular level.

Effect of Subzero Temperatures on Yeast Cells

Peter Mazur

Introduction. – Two hypotheses are currently in vogue as to the cause of death in cells exposed to subzero temperatures. One, the so-called “salt-death” theory, states that death is due to the high concentration of solutes that are formed during the progressive solidification of a solution. The other theory is that death is related to the direct effects of ice crystal formation, particularly intracellular ice. In the case of yeast¹¹ and other microorganisms,¹² the evidence strongly favors the second alternative and seems incompatible with the first. The hypothesis based on the effects of intracellular ice rests, however, on indirect evidence, and direct confirmation or refutation is desirable.

One direct approach seemed to be the following: A suspension of yeast cells in deionized water can, as a first approximation, be considered as small packets of aqueous solution suspended in water and isolated from the water by the cell membrane (Fig. 11, schematic drawing I). The external water freezes when this system is cooled below 0°C; the internal water either freezes inside the cell (II) or it does not (III). If it does not, it must either remain supercooled (III a) or must leave the cell in

¹¹P. Mazur, *Biophys. J.* 1, 247–64 (1961).

¹²P. Mazur, *Ann. N.Y. Acad. Sci.* 85, 610–29 (1960).

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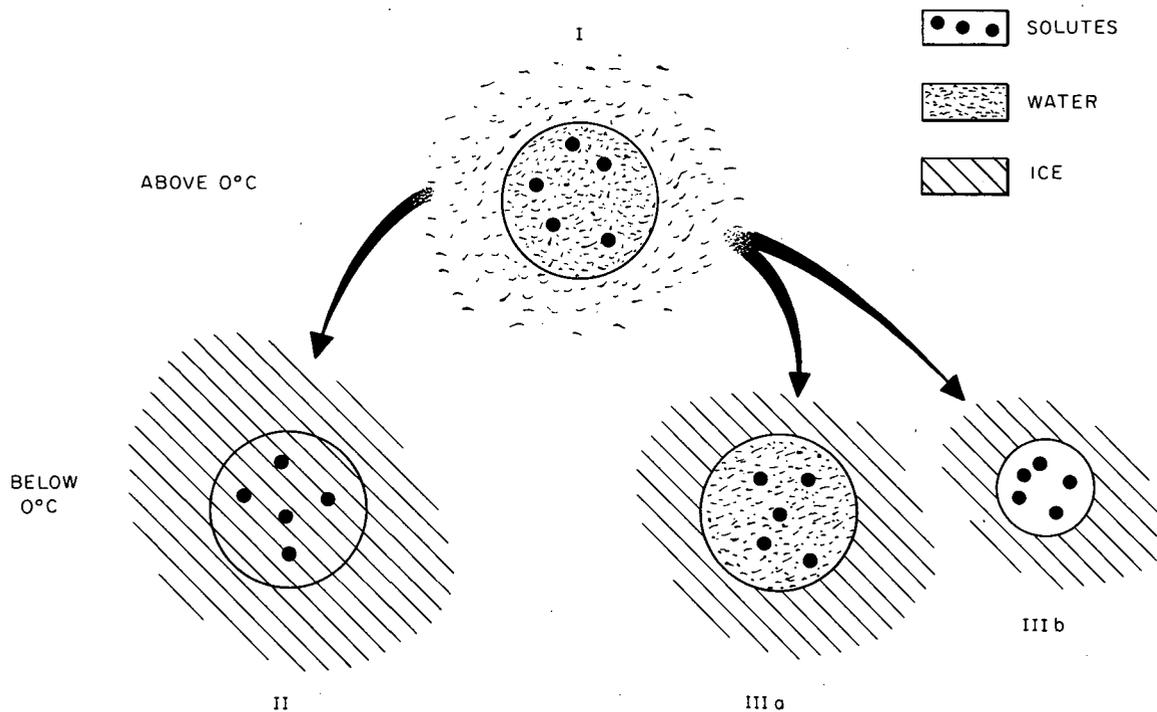


Fig. 11. Alternative Possibilities for the State of Water in Yeast Cells at Subzero Temperatures.

response to the higher vapor pressure of supercooled water and freeze externally (III*b*). In all three cases, the solutes remain in the cell, for the cell membrane is impermeable to them; and even if it were not, the solutes could not diffuse at significant rates into a surrounding solid matrix. Consequently, in both III*a* and III*b* the solutes will be separated from pure ice, and when this system is warmed, the only melting observed will be that of pure ice beginning at 0°C.

On the other hand, if the cells freeze internally (Fig. 11, schematic drawing II), the internal solutes *will* be in contact with ice, and the cell interior will be a frozen solution. When a frozen solution is warmed, the melting of ice begins considerably below 0°C. Since, in melting, every gram of ice absorbs 80 cal, this absorbed heat will retard the rate of temperature rise of the suspension relative to a system containing only pure ice.

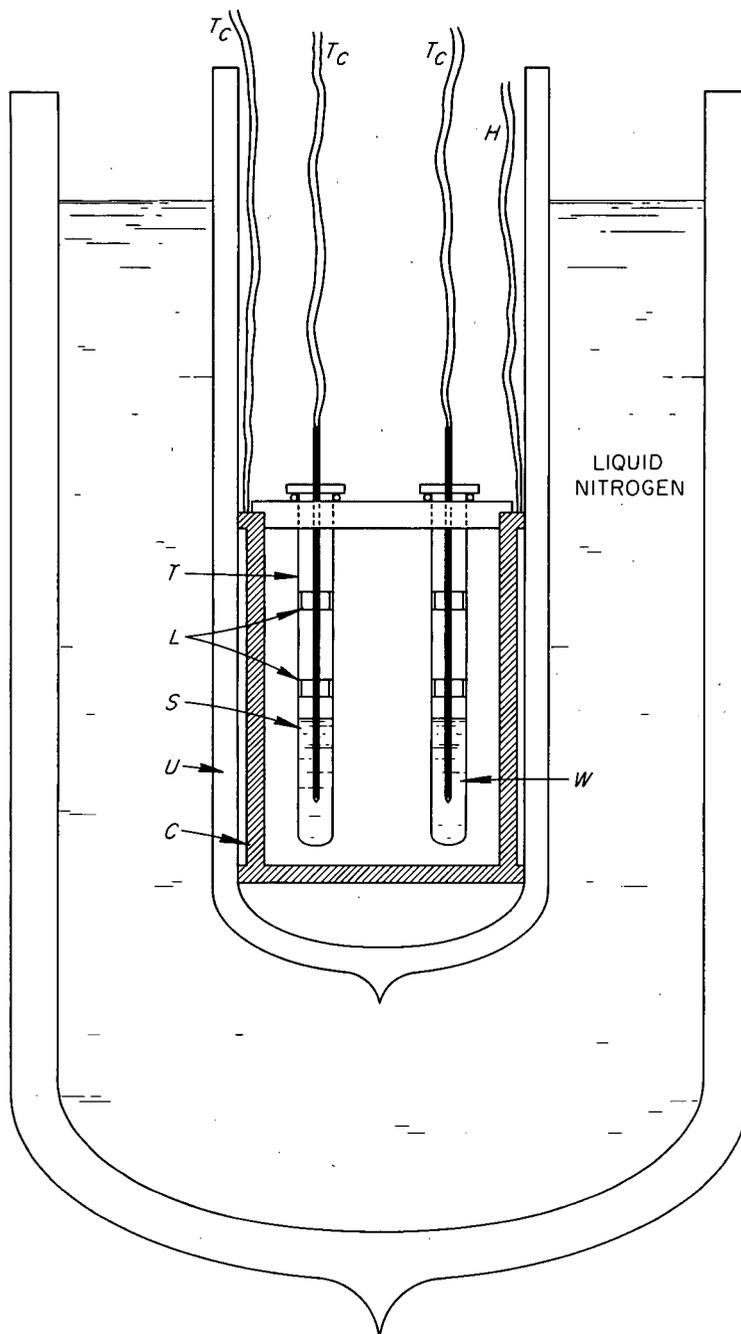
Procedure. – The procedure being used to see if such heat absorption occurs is the following: Three Lusteroid tubes are symmetrically placed in a holder. To one is added 1.50 ml of cell suspension (~40% cells by hematocrit $\approx 4 \times 10^9$ cells per milliliter); to the other two are added 1.50 ml of pure water. Fine 40-gage thermocouples are positioned coaxially in the center of the tubes and to a fixed depth of immersion in the liquid samples by use of Lucite spacing washers (Fig. 12). One thermocouple measures the actual temperature of one pure water sample (T_i) to within $\pm 0.2^\circ\text{C}$. The other two form a single thermocouple circuit and measure the difference in temperature (ΔT) between the cell suspension and the other water sample to within $\pm 0.02^\circ\text{C}$. The liquid samples are frozen under controlled conditions, equilibrated at a given temperature, and then warmed at 1.20 to 1.30°C/min. Equilibration and warming take place in a brass cylinder arranged as shown in Fig. 12. The temperature difference ΔT and T_i are followed as a function of time until all melting is completed. One can then plot ΔT as a function of T_i to yield a so-called “differential thermal analysis” plot.

Results. – Solutions of KCl were used to calibrate the apparatus. Figure 13 gives differential thermal plots (ΔT vs T_i) for 0.001, 0.01, and 0.1 *m* KCl. Negative deflections mean that the solution is absorbing heat relative to the sample of pure ice. Heat absorption is first detectable at about -11°C , a temperature agreeing with the published value of -10.7°C for the eutectic point of KCl solutions, and continues with increasing warming as the ice progressively melts, until finally ΔT reaches a maximum, ΔT_{max} . The maximum occurs when the pure ice sample reaches 0°C. Its temperature remains at 0°C during melting, whereas the KCl solution continues to warm slowly to produce a gradual decrease in ΔT with time.

The greater the concentration of the solution, the greater the maximum difference in temperature, ΔT_{max} . In fact, $\log (\Delta T_{\text{max}})^2 \approx \log C + \log K$, where C is the molal KCl concentration. This observed relation agrees with that derivable from published freezing point–composition phase diagrams for KCl solutions.

A rapidly frozen cell suspension also absorbs excess heat during warming (Fig. 13). The amount absorbed is roughly equivalent to that absorbed by 0.05 *m* KCl. In other words, a rapidly cooled suspension of yeast cells in pure water behaves thermally during warming like a frozen aqueous solution. This behavior is that predicted by model II of Fig. 11, which assumes intracellular freezing. It is not compatible with either model III*a* or III*b*, which assume no intracellular freezing.

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C = BRASS CYLINDER WRAPPED WITH NICHROME WIRE
H = LEAD FROM NICHROME WIRES TO VARIAC
L = LUCITE SPACERS
S = CELL SUSPENSION OR TEST SOLUTION

T = LUSTEROID TUBE
T_C = THERMOCOUPLES
U = UNEVACUATED, UNSILVERED DEWAR
W = WATER SAMPLE

Fig. 12. Apparatus Used in Differential Thermal Analysis.

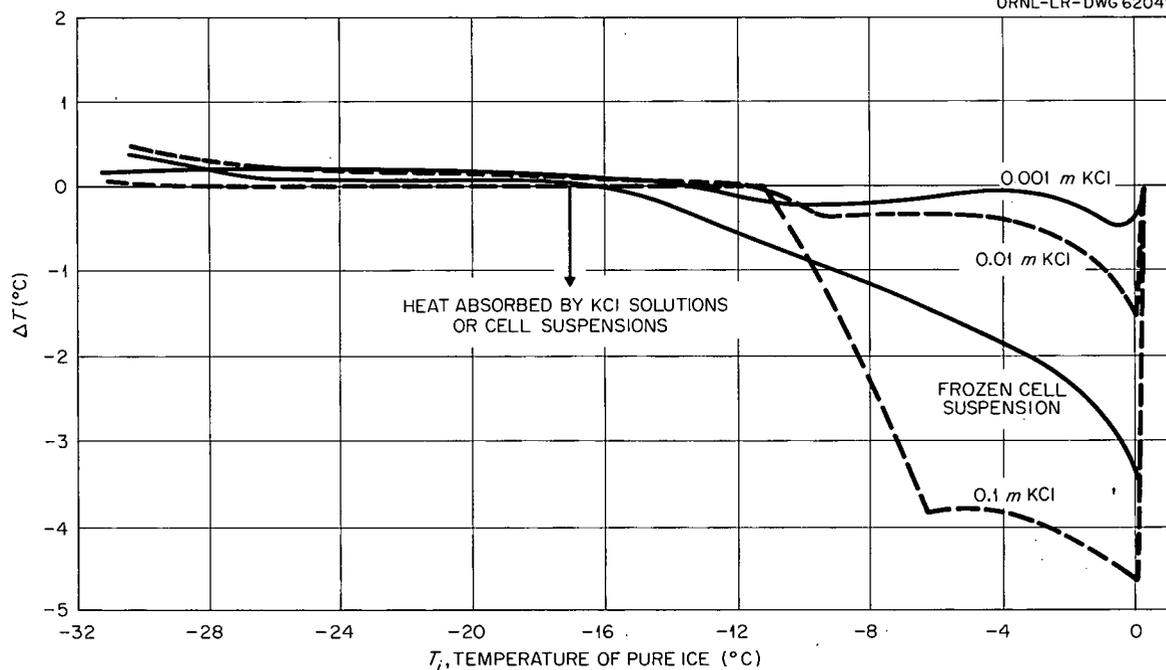


Fig. 13. Differential Thermal Analysis During the Warming of Frozen KCl Solutions and a Frozen Yeast Suspension. Samples were frozen rapidly in liquid nitrogen.

On the other hand, this thermal behavior cannot be said to prove intracellular freezing until two complications not dealt with in Fig. 11 have been investigated. The nature of these complications and the experiments being done to evaluate their thermal contributions to the system will be reported on in the next semiannual report.

A Study of Enzymatic Constituents in the Frog Embryo

R. A. Wallace

Previous work¹³ has indicated the quantitative parameters of a number of enzymes during a developmental sequence. Further effort has now been devoted toward the development of methods for elucidating the relationships of individual members of an enzyme complex during this sequence. Similar studies have frequently employed starch gels as media for the zonal separation of enzymes, but the opacity of the starch and the slowness of separation have frequently hindered sensitive quantitative analysis of small amounts of material.

Based upon preliminary reports,^{14,15} an investigation of acrylamide gels was undertaken, and a number of their properties were found superior to starch gels – completely transparent, accurately

¹³R. Wallace, *Developmental Biology* 3 (in press, 1961).

¹⁴S. Raymond and Y. Wang, *Anal. Biochem.* 1, 391 (1960).

¹⁵M. White, *J. Phys. Chem.* 64, 1563 (1960).

reproducible, less brittle, and more rapidly separable into finely resolved zones under high voltage. Furthermore, many gels could be prepared in distilled water and stored indefinitely until just before use, at which time they were preincubated overnight in the desired buffer. By using frog hemoglobin and various enzymes, conditions for the optimal resolution of proteins have been clarified. Conditions for the aerobic determination of several dehydrogenases, using phenazine methosulfate and a sensitive tetrazolium salt,¹⁶ have also been defined. Photometric scanning of the incubated gels (after alcohol treatment to increase absorption at 570 m μ) revealed an excellent linear response proportional to enzyme concentration over a tenfold range (lactic dehydrogenase in 10 μ l of 1 to 10% frog-liver homogenates). At least two components displaying malic dehydrogenase activity and four with lactic dehydrogenase activity have been electrophoretically resolved and evaluated in various tissues of the adult frog and embryo. The developmental relationships of these components in the frog embryo will now come under study.

Rat Serum Proteins

R. E. Canning

Introduction. – Work has continued on the isolation of rat serum proteins, determination of physical characteristics of the isolated proteins, and the production of antibodies against the proteins.

Results. – By use of a thin layer of 1.5% agar (pH 8.6, on a glass slide) the previously¹⁷ isolated rat macroglobulin was immuno-electrophoresed for 4 hr. It was observed that the isolated macroglobulin was slightly contaminated by two other proteins which migrated slower than the macroglobulin. The macroglobulin preparation was then electrophoresed at pH 8.6, $\mu = 0.1$, in a starch block. At the end of 20 hr, the starch was cut into $\frac{1}{2}$ -in. strips and these strips were eluted with cold 0.15 M NaCl. The eluates were then tested by immuno-electrophoresis for purity. One of the eluates contained pure rat macroglobulin, as determined by the above procedure.

Rat serum albumin and β globulin were prepared by a combination of Charlwood's¹⁸ method and continuous-curtain paper electrophoresis. The purity was checked by the immuno-electrophoresis method.

The three isolated proteins were injected into rabbits, and antisera was obtained. The antigens and antibodies were tested for purity by the immuno-electrophoresis method, and it appears that all antigens and antibodies are pure.

Discussion. – The determination of sedimentation coefficient, partial specific volume, and amino acid analysis of rat albumin and globulin are being done. Gamma globulin is also being isolated and

¹⁶M. M. Dewey and J. L. Conklin, *Proc. Soc. Exptl. Biol. Med.* 105, 492 (1960).

¹⁷R. E. Canning, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, p 101.

¹⁸P. A. Charlwood, *Biochem. J.* 78, 163 (1961).

we expect to have a sufficient quantity for injection into rabbits soon. Once the antibodies are obtained against the gamma globulin, we will have four pure antigens and antibodies. Future work involves determining the amounts of these antigens in whole rat serum and in the soluble protein preparations of liver, kidney, testis, and brain.

Polypeptidyl Proteins

L. M. Krausz

Florence C. Scaduto

R. R. Becker

Introduction. – The investigation of polypeptidyl proteins as useful models for the study of the biological and physical properties of proteins has been continued with special emphasis on the problem of hydrophobic interactions. Amino acid and end-group analyses of a series of chymotrypsin derivatives have been completed. Continuing experiments on the physical properties of poly-L-valyl chymotrypsin and poly-L-valyl ribonuclease by several techniques will also be reported. Ultracentrifuge experiments on poly-L-valyl chymotrypsin and poly-L-valyl ribonuclease have been carried out, and spectrophotometric titrations of this ribonuclease derivative have been obtained. Results of NMR studies of ribonuclease and poly-L-valyl ribonuclease appear in the Biophysics section of this report.

Results and Discussion. – The series of chymotrypsin derivatives were analyzed for total amino acid composition (Table 28) after hydrolysis periods of 24, 48, 72, and 120 hr in constant-boiling HCl, since the valyl, isoleucyl, and *t*-leucyl derivatives are very resistant to acid hydrolysis. Even 120 hr appears to be an insufficient hydrolysis period for the *t*-leucyl derivative in that only 10 of the 13 lysines present were recovered. The value given for the number of moles of *t*-leucine added is therefore somewhat low. Since the polyglycyl derivative prepared in this series was unaccountably low in extent of modification, an earlier preparation which also showed complete activity but which is heavily modified is included. The sites acylated were calculated according to the analysis of the DNP-protein derivatives after complete hydrolysis for both lysine and ϵ -DNP-lysine and according to the assumption that two α -amino groups have been acylated. The data recorded under DNP-ileu, DNP-ala, and DNP-peptides were obtained from 16-hr hydrolyses. It is apparent that the *N*-terminal isoleucine was not acylated in the modification reaction. The data show that the *N*-terminal alanine has been acylated (except in cases of the very poorly modified polyglycyl derivative). The average length of the attached peptides is relatively low for most of the derivatives. It is of interest that no significant differences were found among the L-, D-, and DL-valyl derivatives in terms of extent of acylation, average chain length, or in peptide isolation. A discussion of the enzymatic properties of these derivatives is included in a paper in press.¹⁹

¹⁹R. R. Becker, *Symposium on Poly- α -Amino Acids*, University of Wisconsin Press (in press).

Table 28. Polypeptidyl Chymotrypsins

	Moles Added per Mole ^a	Sites Acylated per Mole ^b	Average Chain Length	DNP-ileu per Mole ^c	DNP-ala per Mole ^c	DNP- Peptides per Mole ^c
α -Chymotrypsin	0	0	0	0.5	0.7	0
Polyglycyl chymotrypsin ^d	55	10	5			
Polyglycyl chymotrypsin	5.6	3	1.7	0.7	0.4	0
Poly-DL-ala chymotrypsin	22.8	6	3.6	0.5	6.0	0
Poly-D-val chymotrypsin	34.1	10	3.4	0.5	0	0.6
Poly-DL-val chymotrypsin	31.7	9	3.5	0.5	0	0.7
Poly-L-val chymotrypsin	33.0	9	3.7	0.5	0	0.5
Poly-DL-ileu chymotrypsin	28.0	8.6	3.3	4.3	0	0.5
Poly-DL- <i>t</i> -leu chymotrypsin	17.3	9.8	1.8	1.5 ^e	0	2.1

^aDetermined by method of D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.* 30, 1190 (1958).

^bDetermined by method of Sanger.

^c16-hr hydrolysis.

^dW. H. Konigsberg and R. R. Becker, *J. Am. Chem. Soc.* 81, 1428 (1959).

^eDNP-*t*-leu and DNP-ileu combined.

Native ribonuclease contains three normal and three abnormal tyrosine residues; the latter cannot be titrated below pH 12.²⁰⁻²² The high value for the pK of the three abnormal tyrosines has been attributed to tyrosyl-carboxylate hydrogen bonding and to hydrophobic bonding. Poly-L-valyl ribonuclease was titrated to see whether the introduction of apolar peptides might increase the number of abnormal tyrosine residues. As in the case of the native enzyme, two groups of three residues each were found, although the pK observed for those titrating at the lower pH was slightly higher than that observed for the native enzyme (10.7 compared with 10.2).

We have examined a preparation of poly-L-valyl chymotrypsin, using the Archibald method for determining molecular weights under the same conditions used by Rao and Kegeles²³ in studying the polymerization of the native enzyme. That the poly-L-valyl chymotrypsin is a highly polymerizing system is shown by the following data: In approximately 1% solution, α -chymotrypsin gave a molecular weight of 28,000; the value for the poly-L-valyl chymotrypsin was approximately 120,000. Preliminary experiments with ribonuclease and poly-L-valyl ribonuclease gave values for S_{20} of 1.6 and 2.0 respectively. These data support the suggestion that those polypeptidyl proteins modified with nonpolar amino acids aggregate, probably through hydrophobic interactions. Hydrophobic bonding is also suggested by the observations that several poly-*t*-leucyl and polyvalyl chymotrypsin derivatives form turbid solutions at room temperature which clear upon cooling to 0°.

²⁰D. Shugar, *Biochem. J.* 52, 142 (1952).

²¹C. Tanford, J. D. Hauenstein, and D. G. Rands, *J. Am. Chem. Soc.* 77, 6409 (1955).

²²C. C. Bigelow, *J. Biol. Chem.* 236, 1706 (1961).

²³N. Rao and G. Kegeles, *J. Am. Chem. Soc.* 80, 5724 (1958).

NUCLEIC ACID CHEMISTRY

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A. Jeannine Bandy
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Pilot-Scale Preparation of Calf Thymus Polymerase

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Patricia A. Williams

Gradient elution chromatography of a partially purified DNA-polymerase from calf thymus gland has indicated that nuclease activity (measured as destruction of *Hemophilus influenzae* transforming DNA by Jane Setlow) can be completely separated from synthetic activity. The chromatographic process, while providing a very desirable purification step, resulted in total activity losses of about 80%. Since analytical-scale chromatography consumes partially purified enzyme from about 1 kg of thymus gland, it is apparent that an adequate supply of partially purified material must be readily available for further purification studies. The published procedure⁴ cannot be scaled up directly because the ultra- and high-speed centrifugation steps used are limited to rather small fluid volumes (2 to 3 liters), and it is desirable to be able to carry out several steps on 30 to 100 liters in a reasonable period of time.

A process was therefore designed using only filtration and decantation as the means of separating precipitates from solutions. Six such preparations on a 10- to 14-kg scale (30 to 45 liters of extract) have been performed, and it is now possible to process 10 kg in about two weeks. The enzyme product obtained is comparable in activity to that obtained from the laboratory-scale process, and the percentage yield is somewhat less, probably due to the rather prolonged processing period. It would be desirable to increase the batch throughput to about 100 liters (30 kg of thymus gland) and reduce handling time to four days. The means whereby this chemical operating capacity may be obtained are currently under consideration.

¹USPHS Fellow.

²Visiting investigator from abroad.

³Research participant.

⁴F. J. Bollum, *J. Biol. Chem.* 235, 2399 (1960).

Reaction of Methylamine with Periodate-Oxidized Adenosine 5'-Phosphate To Form a Cyclic Tertiary Amine

J. X. Khym A. Jeannine Bandy

Introduction. — It has been demonstrated that a wide variety of amino compounds react with periodate-oxidized 5'-ribonucleotides to form addition complexes whose stability is dependent upon pH.⁵ The complex formed by methylamine and oxidized adenosine 5'-phosphate can be stabilized by reduction with sodium borohydride. This compound (I) was isolated in crystalline form, and its structure was determined by acid hydrolysis, which eliminates adenine, leaving a compound (II) which was characterized by further periodate oxidation studies.

Results and Discussion. — Elementary analysis showed that in the formation of compound I only one molecule of methylamine was combined. Compound I reacted negatively to tests for primary, secondary, and acyclic amine residues. Hydrolysis in 6 N HCl at 100°C removes adenine, leaving a structure (II), containing the added methylamine and the original phosphate group, that reacted positively to an aldehyde-group test. When II is reacted with periodate, two moles of this reagent are consumed, releasing one mole of formaldehyde and one mole of formic acid, and there is produced one mole of *N*-methylformamide and one mole of glycolaldehyde phosphate. If dephosphorylated first, II consumes three moles of periodate and produces two moles of formaldehyde, two moles of formic acid, and one mole of *N*-methylformamide. Hence, I is a substituted morpholine derivative, 2-adenine-3-hydroxy-4-methyl-6-phosphorylmethylene-morpholine.

An Approach to Determining the Size Range of the Smallest Self-Duplicating DNA Units

N. B. Furlong, Jr.

Extracts of calf thymus enriched in DNA polymerase catalyze the formation of diester linkages in two modes.^{6,7} One mode of reaction results in the *addition* of monomer units on the ends of DNA or oligodeoxynucleotide chains used as primers in the reaction. The second mode results in the *replication* of entire DNA chains which are probably identical with the DNA of the primer. Tests using homogeneous thymidylate polymer primers up to nine units in length have shown that addition is the only reaction detectable with this type of primer. With primers consisting of high-polymer DNA or DNA breakdown products hundreds of units in length, however, replication occurs many times faster than addition. With heterogeneous polymers derived from DNA, replication requires that all four-component deoxynucleoside triphosphates be present as substrates; thus, replication and addition can be differentiated by measuring the alteration in reaction rate caused by the omission of one or more component substrates.

⁵J. X. Khym, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, pp 102-3.

⁶F. J. Bollum, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, p 103.

⁷F. J. Bollum, p 60 in *The Cell Nucleus*, ed. by J. S. Mitchell, Butterworths, London, 1960.

Measurements comparing the priming ability of enzymatic digests of DNA in the two reaction modes suggested that replication was still the dominant reaction even with extensively degraded DNA primers. To achieve a more quantitative idea of the chain length of primers capable of replication, the following technique was developed: P^{32} -DNA from *E. coli* was hydrolyzed by pancreatic DNase for varying lengths of time. The products of these degradations were then used as primers in polymerase reactions under two conditions: in the first, only H^3 -dCTP was present; in the second, H^3 -dCTP along with the other three triphosphate substrates were present. These reaction mixtures, after incubation, were chromatographed on DEAE paper. We have shown that homogeneous thymidylate polymers up to 12 units in length have R_f values on DEAE paper which diminish with increasing length. It was therefore predicted that in the pH range of 5 to 8 heterogeneous polydeoxyribonucleotides of any length would have mobilities directly proportional to chain length. The average length of the DNA pieces at a given point on the dried chromatogram was determined by cutting out slips along a radioactive trace, measuring the total P^{32} and the phosphatase-labile P^{32} . This latter determination was carried out directly on the slip by the action of *E. coli* phosphatase followed by extraction of the inorganic phosphate into *N*-butanol as phosphomolybdate. Parallel slips along the trace were analyzed by scintillation counting conditions set to discriminate between H^3 and P^{32} . The molar ratios of H^3/P^{32} were determined from the data and correlated with the average length measurement. If the H^3/P^{32} ratio for products of the same length was higher under replication conditions than for addition, this was taken as evidence that replication was occurring in that size range.

Results of these measurements to date indicate that replication is the dominant reaction in the range of oligodeoxynucleotides as short as 20 units, but gives way to addition as the average length of primer decreases to 10 to 12 units. This preliminary interpretation does not take into account more subtle effects such as the possibility of polycytidylic polymerizations or preferential addition onto the ends of chains. Further experiments are planned to calibrate the DEAE paper under a set of standard eluting conditions which will allow the use of H^3 -DNA with each of the P^{32} -labeled deoxynucleoside triphosphates.

The Utilization of Pseudouridine by *Tetrahymena*

Keiichi Kusama

Introduction. — Investigations of the biosynthesis of pseudouridylic acid in rat liver RNA⁸ and *Tetrahymena* RNA⁹ have been initiated in this laboratory. In the work cited components of RNA were examined after the RNA itself was isolated from various fractions and then was subsequently degraded to yield the compounds of interest. In this work cell fractions, from which the RNA had

⁸L. O. Frøholm, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, pp 105-6.

⁹L. O. Frøholm, unpublished data.

been removed, of *Tetrabymena* were examined for relative specific activities to determine the distribution of activity among various purine and pyrimidine derivatives.

The cells of *Tetrabymena* were incubated in a synthetic medium containing H^3 -pseudouridine (ψ -Urd) for 20 hr. Following the incubation period both the supernatant solution of the cell-culture medium and the "acid-soluble fraction" from the cells were used to study the metabolism of ψ -Urd.

Results and Discussion. – Of the many nucleic acid components present in both fractions, the following compounds have been identified: high-activity compounds ψ -Urd (122) (ref 10), uracil (33), uridine (13); low-activity compounds (2–4) inosine, xanthosine, guanosine, AMP, ADP, ATP; no-activity compounds hypoxanthine, xanthine, guanine, and adenine. Since the specific activity of uridine is one-tenth that of ψ -Urd, it is not likely that an intramolecular rearrangement of ribose from the 5 to the 1 position of uracil had taken place.

The observation that the purine bases contained no activity whereas the purine nucleosides and nucleotides did contain some activity seems to suggest that the ribose moiety of ψ -Urd is utilized in the synthesis of purine nucleosides.

The Reactions of Ethyl Methanesulfonate with the Base Constituents of Bacteriophage T4 Nucleic Acid

Bimal C. Pal

The reaction products of ethyl methanesulfonate (EMS) with the purine and pyrimidine bases found in phage T4 have been investigated as part of a study of the mechanism of action of this mutagen.¹¹

Adenine on treatment with EMS forms three products which have been identified as 1-ethyladenine, 3-ethyladenine, and 9-ethyladenine, the yields being 8.4, 25.4, and 8.8% respectively. Guanine forms two major derivatives, one of which has been identified as 7-ethylguanine, and traces of two other products. One product is formed by 5-hydroxymethylcytosine. Thymine does not react with EMS.

Methylation of the 7-N of adenine had previously been reported,¹² but our results, both with EMS and the alkylating agents previously employed, indicate that the presumed 7-methyladenine actually corresponds spectroscopically with 3-alkyladenines.

The relative extent of ethylation we find for 1- and 3-N atoms of adenine is in the reverse order of their basicity as calculated by Nakajima and Pullman¹³ and also contrasts with the findings of Brooks and Lawley¹⁴ that 1-methyladenosine was formed with three times the yield of 3-methyladenosine.

¹⁰Counts per minute per millimicromole.

¹¹D. R. Krieg *et al.*, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, pp 45–46.

¹²B. Reiner and S. Zamenhoff, *J. Biol. Chem.* **228**, 475 (1957).

¹³T. Nakajima and B. Pullman, *Bull. soc. chim. France* **1958**, 1502–6; B. Pullman, *J. Chem. Soc.* **111**, 1621–23 (1959).

¹⁴P. Brooks and P. D. Lawley, *J. Chem. Soc.* **112**, 539–45 (1960).

Interactions within purines between the imidazole and the pyrimidine ring components are shown by these investigations in two ways: (1) the influence of the 2- and 6-carbon substituents on the relative reactivities of the 7- and 9-nitrogens of guanine and adenine, and (2) the apparent effect of the 9-glycoside bond on the relative reactivities of the 1- and 3-nitrogens of adenine.

NUCLEIC ACID ENZYMOLOGY

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T2 Specific RNA Associated with the RNA-DNA Hybrid

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Hall and Spiegelman, at the University of Illinois, demonstrated that a metabolically active species of RNA from T2-infected bacteria formed a hybrid with T2 DNA. The technique employed was the method of slow cooling (annealing) a mixture of denatured T2 DNA with RNA partially purified by the sucrose-gradient centrifugation method. In order to demonstrate whether the RNA associated with DNA was compositionally similar to the T2 DNA (see "Nucleic Acid Enzymology" section in previous semiannual reports) and therefore probably complementary in structure to the DNA, the following experiment was carried out.

Experimental. — *E. coli* B was grown from low inoculum to about 4×10^8 cells per milliliter in synthetic medium containing a high level of P^{32} orthophosphate. The bacteria were infected with T2 bacteriophage in high multiplicity, given a short pulse of tritiated uridine, then chilled with medium containing sodium azide to stop phosphorylative reactions. Total RNA of the culture was prepared by the phenol method. Enrichment of labeled RNA was accomplished by centrifugation through a sucrose gradient. This partially purified RNA was subjected to slow cooling with denatured T2 DNA and the resultant RNA-DNA peak collected. The latter product and a sample of the original RNA from the phenol preparation were each mixed with carrier RNA, then hydrolyzed with alkali, and the resultant mononucleotides analyzed by ion exchange chromatography.

This procedure permits a mononucleotide analysis of the metabolically active RNA in the T2-infected cell even though the amounts of products are too small to be measured with classical analytical approaches. Thus, since P^{32} orthophosphate was present in constant concentration throughout the experiment, the relative amounts of P^{32} in the individual RNA mononucleotides will be a measure of their relative content in the RNA. The tritium marker merely serves as a convenient guide for estimating the presence of the newly synthesized RNA formed after infection.

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Results and Discussion. – The results shown in Table 29 illustrate that, although RNA partially purified by sucrose-gradient centrifugation does not have a composition similar to T2 DNA, the material from the hybrid is remarkably similar in composition to T2 DNA. These data add strong support to our previous conclusions that an RNA similar in structure to T2 DNA is formed immediately after infection. This RNA we believe to be the intermediate in carrying out genetic information from DNA for the synthesis of specific proteins.

Attempts at purification of the T2-specific RNA in larger quantities are being undertaken. While the results are encouraging, they remain too preliminary to describe in detail. Eventual success in purification would allow a description of the size and general properties of the RNA and permit *in vitro* experiments for assaying the mechanism of its biologic action.

Table 29. Distribution of P³² Activity in Nucleotides

Nucleotide	Distribution of P ³² Activity (Ratios)	
	RNA from Phenol Preparation	RNA from Hybrid
Cytidylic	25.1	17.2
Adenylic	26.2	33.6
Uridylic	22.5	31.1
Guanylic	26.2	18.1

Nucleic Acids of Amphibian Eggs

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M. Helen Jones

Elliot Volkin

The discovery that ovarian egg RNA has the unique property of consisting in large measure (about 50%) of acid-soluble RNA has prompted us to investigate methods for the separation of various RNA species.

Results and Discussion. – Separations using ECTEOLA-SF columns have been partially successful in fractionating the RNA. Three distinct fractions of RNA can be obtained reproducibly, albeit no claim can be made for molecular homogeneity within each fraction. Typical results are shown in Fig. 14. The first peak (unlabeled) is a contaminant free of RNA. Peak RNA₁ is largely acid-soluble RNA, while RNA₂ is mostly acid-insoluble RNA. The peak labeled DNA-RNA has attracted our attention because of the constancy of its composition with a number of different frog-egg nucleic acid preparations. This fraction invariably contains two moles of DNA for each mole of RNA. With preparations from mature eggs, the RNA in this fraction is totally acid soluble, while the RNA in this fraction from preparations from young eggs is totally acid insoluble. The nucleotide composition of the RNA in this fraction is characterized by an extremely low uridylic acid content (around 10%) and a high guanylic acid content (over 50%). Experiments are under way to determine whether this fraction represents a bona fide RNA-DNA complex. Although some DNA from frog-liver nucleic acid preparations can be eluted at the region corresponding to this peak, no RNA is found associated with the liver DNA.

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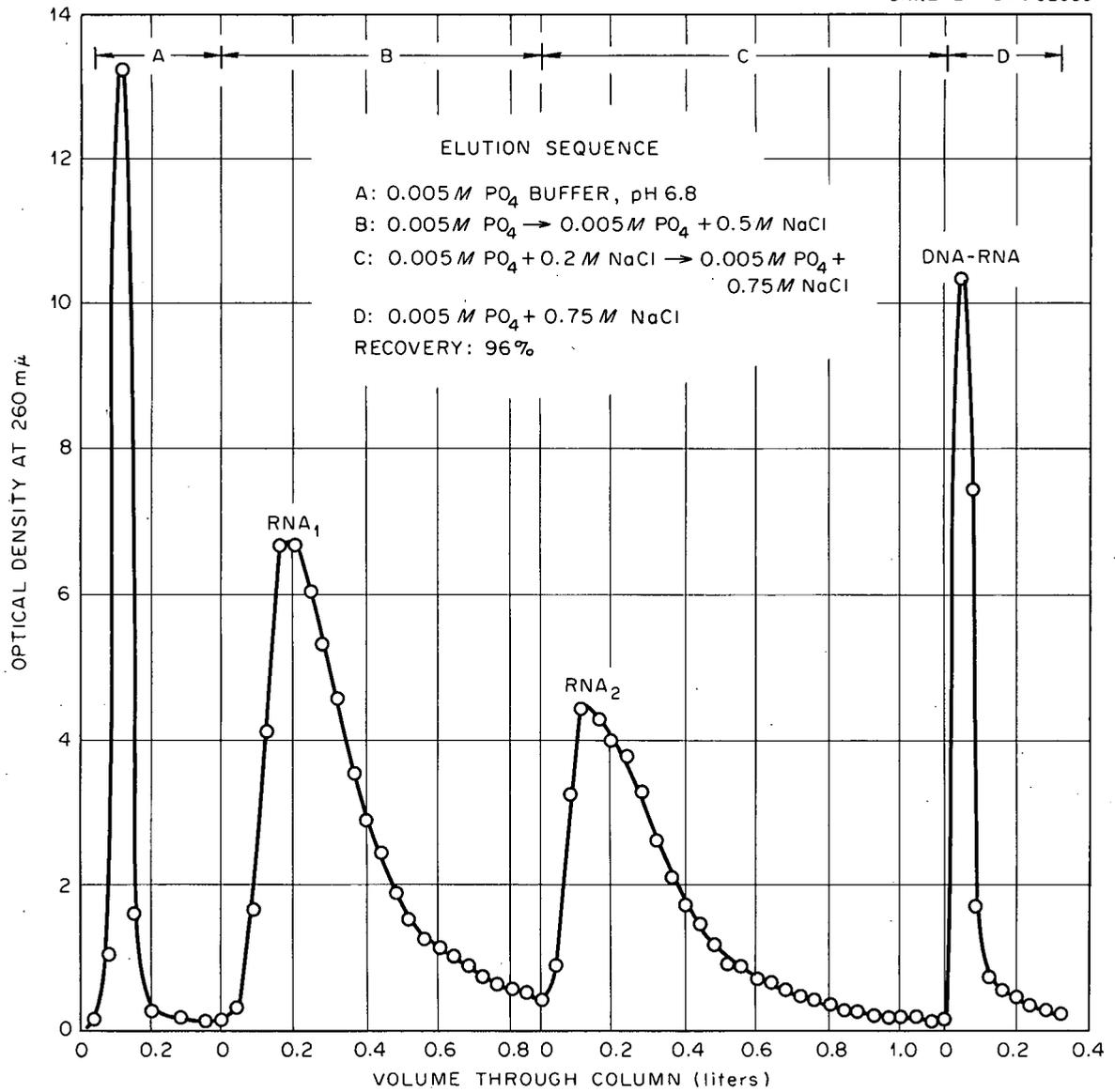


Fig. 14. Separation of Amphibian-Egg Nucleic Acids.

CHEMICAL PROTECTION AND ENZYME CATALYSIS

Chemical Protection

D. G. Doherty

Cynthia M. Park

Structure of Mucoproteins

Anatoly Bezkorovainy¹

G. D. Roberts²

Enzyme Catalysis

F. C. Grimm¹

J. D. Bales, Jr.

Father J. A. Duke³

Chemical Protection

D. G. Doherty

J. D. Bales, Jr.

Cynthia M. Park

Introduction. – The investigation of the pharmacological properties in mice of a series of protective compounds related to and including 2-mercaptoethylamine and *S*,2-aminoethylisothiourea dihydrobromide (AET) by DiStefano, Leary, and Hodge⁴ revealed considerable similarity of action. One effect was a marked reduction of oxygen tension in the spleen and presumably in other tissue that could be reversed by subsequent treatment with dibenamine. This observation offered a way to determine the role of low oxygen tension in the protection afforded by *S*,2-aminoethylisothiourea and to test the hypothesis that these sulfhydryl compounds act via the production of tissue hypoxia.

Results and Discussion. – The combined toxicity of AET and dibenamine precluded testing at the highest intraperitoneal dose of AET alone (360 mg/kg). It was found that (C₅₇BL × C₃H)F₁ mice would tolerate a maximum of 200 mg of AET per kilogram intraperitoneally [either as AET in water or as mercaptoethylguanidine (MEG)] and 20 mg of dibenamine per kilogram intravenously. Dibenamine alone did not alter the radiation LD₅₀ of the mice (700 r). The radiation LD₅₀ for the combined treatment was determined at three dose levels of AET and MEG (100, 150, and 200 mg/kg) and 20 mg of dibenamine per kilogram. Corresponding controls were done with AET and MEG alone, using mice of the same age group. The approximate LD₅₀'s for the various treatments are shown in Table 30. It can be seen that spleen anoxia had very little effect upon the short-term mortality of the animals, the 25-r difference being regarded as barely significant. Thus hypoxia, at best, contributes a very minor amount to the protection afforded by AET at supralethal radiation dose levels.

¹Research associate.

²Student trainee.

³Research participant.

⁴V. DiStefano, D. Leary, and H. Hodge, personal communication.

Table 30. Approximate LD₅₀'s for the Various Treatments

Dose (mg/kg)	LD ₅₀ 30 Days			
	AET		MEG	
	Combined	Control	Combined	Control
100	900	925	925	950
150	1100	1125	1050	1075
200	1250	1250	1225	1225

Structure of Glycoproteins

Anatoly Bezkorovainy

D. G. Doherty

Introduction. – The majority of the structural studies of plasma glycoproteins reported so far have been confined to those of human plasma. It seemed to us worth while from a comparative biochemical viewpoint and because of a more accessible source of supply to investigate the isolation of these glycoproteins from bovine plasma.

Results and Discussion. – Bovine plasma was found to contain a protein negatively charged at pH 4.5, but not as acidic as the well-known orosomucoid. It was also found that this plasma component bound radioactive thyroxine when labeled plasma was subjected to paper electrophoresis and autoradiography. This protein was purified to an apparently homogenous state from bovine plasma by the ammonium sulfate precipitation of a fraction containing some 10% of this acidic protein (M-2 glycoprotein) followed by chromatography of this fraction on carboxymethyl cellulose. The M-2 glycoprotein was eluted from the column in a homogeneous state (as shown by electrophoretic and ultracentrifugal analyses) with acetate buffer at pH 5. It had a molecular weight of 59,000 (determined by sedimentation-viscosity measurements) and contained some 20% carbohydrate in a 1:1:1 ratio of hexose:hexosamine:sialic acid. The N-terminal end group was leucine. An apparent absence of tryptophan was noted, which explains the low extinction coefficient at 280 m μ of 5.3.

In electrophoretic studies using M-2 glycoprotein and bovine serum albumin labeled with ¹³¹I thyroxine it was found that the M-2 glycoprotein removed thyroxine from labeled albumin but that unlabeled albumin could not remove thyroxine from labeled M-2 glycoprotein to any great extent. Preliminary experiments utilizing the equilibrium dialysis method of Doherty and Vaslow⁵ have indicated that at 27°C both the M-2 glycoprotein and albumin have free energies of binding of about -6 kcal/mole. Although the ΔF of binding between thyroxine and the M-2 glycoprotein was expected to be lower than that of thyroxine-albumin, it is, nevertheless, possible that the difference is too small to be accurately determined by the method used. Further studies on this problem are in progress.

⁵D. G. Doherty and F. Vaslow, *J. Am. Chem. Soc.* **74**, 931 (1952).

Enzyme Substrate Complex Formation

F. C. Grimm D. G. Doherty

Introduction. – We have continued our investigation of the nature of the catalytic site of bovine heart mitochondrial malic dehydrogenase. The present report concerns an attempt to elucidate the nature of the coenzyme binding site, using a reagent, iodoacetamide, that reacts with the sulfhydryl groups of the enzyme.

Results. – Malic dehydrogenase purified from bovine heart⁶ was completely inactivated by the reaction of the enzyme with 4 to 5 moles of iodoacetamide at pH 8.5. Presence of the coenzyme (0.001 M DPNH) did not prevent the reaction and inhibition of the enzyme by iodoacetamide. The iodoacetamide-treated malic dehydrogenase (acid hydrolysate) revealed that 1.3 residues of cysteine were carboxymethylated per mole of enzyme. These findings and those obtained with *p*-chloromercuribenzoate (refs 7 and 8) are consistent with the view that a "free" sulfhydryl group on the enzyme reacts directly with the coenzyme during catalysis. Although 4 to 5 moles of iodoacetamide reacted with the enzyme, no other amino acid derivatives were detected. The present work should now permit the determination of the amino acid sequence in the vicinity of the *S*-carboxymethylcysteine.

⁶F. C. Grimm and D. G. Doherty, *J. Biol. Chem.* **236**, 1980 (1961).

⁷E. S. G. Barron and T. P. Singer, *J. Biol. Chem.* **157**, 221 (1945).

⁸R. G. Wolfe and J. B. Nielsands, *J. Biol. Chem.* **221**, 61 (1956).

ENZYMOLOGY

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Biosynthesis of RNA

K. B. Jacobson G. D. Novelli

Introduction. – The interrelationship between protein synthesis and the function of RNA has been indicated for a number of years, and more and more evidence is accumulating to support the hypothesis that RNA has an essential role in protein synthesis and, indeed, may be the template on which the amino acids are arranged. Recently a system was established in this laboratory⁶ in which a protein is synthesized by subfractions of ruptured cells of *E. coli*. This system has a virtually absolute requirement for the presence of RNA and DNA during its operation. It therefore offers the opportunity to examine the interrelationship between RNA and protein synthesis.

Results. – The incorporation of nucleoside triphosphates into RNA was obtained by using the fraction of *E. coli* that does not sediment at $105,000 \times g$ in 1 hr. This system is like that of Hurwitz⁷ and will be useful when the cell-free system is investigated for the synthesis of RNA associated with protein synthesis.

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⁶T. Kameyama, W. H. Taylor, and G. D. Novelli, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, p 167.

⁷J. Hurwitz, A. Bresler, and R. Diring, *Biochem. Biophys. Research Commun.* 3, 15 (1960).

To know better what species of RNA is actually associated with the synthesis of a specific protein, some studies with living cells of *E. coli* were undertaken. The induced production of β -galactosidase results in the formation of the enzyme predominantly in soluble form but also of a small fraction of the total enzyme that is bound to the ribosomes. This latter circumstance allows for the selection of those ribosomes associated with β -galactosidase synthesis and thus allows the search for the RNA associated with enzyme production to be restricted. Operationally this group of ribosomes, bound to β -galactosidase, are precipitated from suspension by the antiserum to the enzyme. Presumably the specific radioactivity of this ribosome fraction should exceed that of the general population if the labeled precursor to RNA is presented to the cell for a short period of the initial stage of induction of the enzyme. This expectation has been upheld in several experiments and, in addition, evidence was obtained indicating a difference in base composition of the RNA. Some doubt was cast on the earlier results through a finding which indicates a radioactive contaminant was included in some, but probably not all, of the RNA preparations. A means of avoiding such contamination has been attained and the previously observed information will be re-examined.

Conclusion. – It appears that a means is available to examine selectively a specific fraction of the general population of ribosomes. By characterizing the turnover rate and chemical composition of the RNA of this fraction the way will be opened for investigating the behavior of a similar RNA associated with the cell-free synthesis of a specific protein.

Immunochemical Analysis of the Induction of Tyrosine- α -Ketoglutarate Transaminase of Rat Liver

F. T. Kenney

Introduction. – Initial studies of this induction, using antibody prepared against enzyme purified from noninduced livers, had indicated that enzyme antigen was independent of induction and that it also failed to incorporate radioactive amino acids during induction. These findings were interpreted as indicating that induction involves activation of an immunologically similar but enzymically inactive precursor rather than *de novo* synthesis.⁸ However, when a more specific antiserum, prepared against a more highly purified enzyme preparation, was employed in specific competition assays, it became apparent that no cross-reactive material was present in noninduced liver extracts. Thus the concept of induction involving activation of a precursor became suspect insofar as the precursor could not be detected. The present report describes a complete reappraisal of carrier experiments which lead to the definitive conclusion that induction does, indeed, reflect *de novo* enzyme synthesis.

Results and Discussion. – Specific assays were developed in which the content of enzyme antigen of liver extracts was determined in terms of immunological competition. Particulate liver fractions were also assayed, using variations in technique which should have permitted detection of even weakly cross-reacting antigens. The results of these experiments can be summarized as follows: (1) There

⁸F. T. Kenney, *Biochem. Biophys. Research Commun.* 2, 333 (1960).

is an increase in soluble antigen which is equivalent to the increase in enzyme activity. (2) Particulate fractions contain only negligible amounts of either enzyme or antigen. (3) Pyrophosphate extracts of ribosomes contain higher antigen activity than can be accounted for as enzyme activity. Thus there is no apparent source of preformed antigen to serve as precursor, and the only fraction containing what may be inactive enzyme is the ribosome fraction. The latter could be interpreted as indicating a small, steady-state level of what may be partially completed enzyme and strongly suggests that the protein synthetic mechanism of the liver is operative in this induction.

Experiments in which the hepatic amino acid pool was labeled with C^{14} amino acids during induction were repeated. The enzyme was precipitated by the addition of an equivalent amount of antibody rather than by a large excess, as used previously.⁸ Using these highly specific conditions, radioactivity measurements indicated that amino acids were incorporated into the enzyme at a rate about 5 times greater than that of the average soluble proteins. Excess antibody precipitates unrelated proteins which are apparently not radioactive, because their inclusion drastically lowers the specific radioactivity of the antigen-antibody precipitate.

It is thus apparent that transaminase induction involves the *de novo* formation of enzyme protein. We have recently established⁹ that adrenocortical hormone is the actual inducer of this enzyme. Hydrocortisone (and other glucocorticoids) thus stimulate the synthesis of a particular enzyme in a fashion quite analogous to that of substrate in microbial enzyme induction. Experiments planned and in progress are aimed at further delineation of the role of adrenal hormone in the control of specific enzyme synthesis.

Studies on Protein Synthesis by Cell-Free Preparations from Maize

R. J. Mans G. D. Novelli

Introduction. — A moderately stable, cell-free system capable of incorporating added radioactive L-leucine into deoxycholate-treated particle protein has been isolated from maize seedlings.¹⁰ The system demonstrates an absolute requirement for dialyzed (2 hr) supernatant, ATP, and magnesium. Although the amount of isotope incorporated into particle protein is significant, the system is unable to show a net increase in protein. This inability can be ascribed to several factors, two of which seem experimentally most opportune to pursue at present.

First, a lack of component(s) in the maize preparations seems probable, especially in the light of the recent findings of Novelli.¹¹ Whereas the bacterial supernatant fraction which stimulates *E. coli* particles to synthesize β -galactosidase contains a significant amount of DNA, the maize supernatant is devoid of detectable levels of DNA. It is conceivable that the maize-incorporating system lacks a

⁹F. T. Kenney and R. W. Flora, *Journal of Biological Chemistry* (in press).

¹⁰R. J. Mans and G. D. Novelli, *Biochim. et Biophys. Acta* 50, 287–300 (1961).

¹¹G. D. Novelli, *Fifteenth Annual Symposium on Fundamental Cancer Research*, 1961 Symposium Monograph, Houston, Texas, Feb. 23–25, 1961.

nucleic acid component derived from the nucleus or a specific product of the nucleus and is thus unable to complete the biosynthesis of protein.

Second, from a quantitative aspect, the failure to detect net protein synthesis may be due to the lack of a sensitive assay of the newly formed protein. Incubation of the maize system with L-leucine results in the incorporation of approximately 0.8 μmole of radioactive leucine per milligram of particle protein. Assuming that the protein is about 10% leucine, the net increase in protein is about 1 $\mu\text{g}/\text{mg}$ of added particle protein, much below the level of sensitivity of the assay now used.

Results and Discussion. – In accord with the first postulation, attempts have been made to isolate the DNA and also the intact nuclei from various maize sources for addition to the incorporating system. Native DNA is notoriously difficult to obtain from higher plant tissues, primarily because of the small amount of DNA relative to other components of the tissue. DNA's prepared from other sources were found inactive in the system. Efforts to extract DNA from homogenates of seedlings and fresh or frozen kernels by salt or phenol treatment have been unsuccessful to date. The failure seems to be due to the large amount of starch and other polysaccharides present in the homogenates. Efforts to predigest the starch with amylases as well as remove it by differential centrifugation have not been effective.

Attempts to isolate intact nuclei from either seedlings or kernels have been only moderately successful. "Gentle" homogenization of seedling shoots in 0.25 M sucrose and subsequent centrifugation across a sucrose gradient has yielded fractions enriched in nuclei; however, these fractions are by no means clean enough for use in the incorporating system.

In an attempt to overcome the lack of sensitivity of the assay for newly formed protein, studies are being undertaken to identify and isolate a protein which increases appreciably during the germination process. Preferably, the protein should be a specific enzyme whose synthesis can be controlled by the investigator much the same as the induced enzymes of bacteria. With the isolation of such an enzyme it should then be possible to ascertain if the incorporation observed at present represents net protein synthesis and, furthermore, what additional factors, if any, are required for net synthesis.

The Mechanism of Thiosulfate Oxidation by *Thiobacilli*

H. D. Peck, Jr. M. P. Stulberg

Introduction. – Cell-free extracts of *Thiobacillus thioparus* have been prepared that oxidize thiosulfate to sulfate and concomitantly produce ADP from AMP and P_i .¹² Santer¹³ has shown that intact cells of this organism transfer O^{18} from P_i to the sulfate produced during the oxidation of thiosulfate, and the mechanism of this transfer of O^{18} has been studied in cell-free extracts.¹⁴ It was shown that

¹²H. D. Peck, Jr., and E. Fisher, Jr., *Fed. Proc.* 20, 219 (1961).

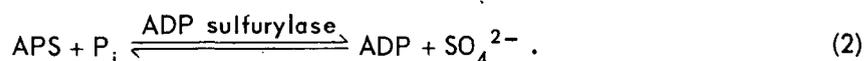
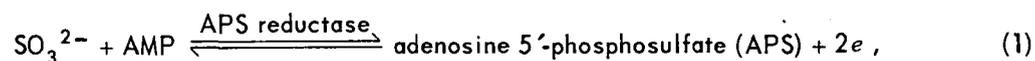
¹³M. Santer, *Biochem. Biophys. Research Commun.* 1, 9 (1959).

¹⁴M. P. Stulberg and H. D. Peck, Jr., *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, p 123.

O^{18} was transferred from P_i to sulfate during the oxidation of thiosulfate by extracts; however, the transfer was small due to a dilution of the O^{18} in the P_i during the course of the incubation. Since it was postulated that this transfer of O^{18} proceeded via AMP, it was proposed to examine the transfer of O^{18} from P_i to AMP and from AMP to sulfate in these extracts. Previously O^{18} was demonstrated to be transferred from AMP to sulfate during the oxidation of thiosulfate.¹⁴ In order to complete these studies, it was also necessary to demonstrate that O^{18} could be transferred from P_i to AMP.

Results. – Thiosulfate was oxidized to sulfate by a cell-free extract of *T. thioparus* in the presence of O^{18} labeled P_i . The ADP formed by this reaction was isolated by paper electrophoresis and degraded to AMP by adenylic kinase in the presence of glucose and hexokinase. The AMP was isolated and the O^{18} content of the terminal phosphate determined. The observed content of O^{18} in the phosphate agreed reasonably well with that calculated from the postulated reaction sequence.¹⁴

Discussion. – During the oxidation of thiosulfate to sulfate in extracts of *T. thioparus*, O^{18} is transferred from P_i to sulfate; from P_i to adenosine monophosphate, and from AMP to sulfate. These results are consistent with the following reaction sequence for the oxidation of sulfite to sulfate:

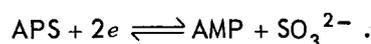


In addition the data demonstrate that in the reaction catalyzed by ADP sulfurylase (Eq. 2) there is a cleavage of the P-O bond, and the S-O bond of the sulfate produced remains intact.

The Purification and Properties of Adenosine 5'-Phosphosulfate Reductase

H. D. Peck, Jr. T. E. Deacon

Introduction. – Adenosine 5'-phosphosulfate (APS) reductase is involved both in the reduction of sulfate by the "sulfate-reducing bacteria"¹⁵ and in the oxidation of reduced sulfur compounds by the *Thiobacilli*.¹⁶ The reaction catalyzed by this enzyme is reversible¹⁷ and proceeds as follows:



The reduced form of the dye, methyl viologen, can be utilized as electron donor for the reduction of APS, and $Fe(CN)_6^{3-}$ can be employed as electron acceptor in the oxidation of the sulfite. Since this reaction represents a new mechanism for the conversion of chemical energy into biologically utilizable energy, the mechanism of action of APS reductase is being studied in detail.

¹⁵H. D. Peck, Jr., *Proc. Natl. Acad. Sci. U.S.* **45**, 701 (1959).

¹⁶H. D. Peck, Jr., *Proc. Natl. Acad. Sci. U.S.* **46**, 1053 (1960).

¹⁷H. D. Peck, Jr., *Biochim. et Biophys. Acta* (in press).

Results. – APS reductase has been reproducibly purified in good yield some fiftyfold from extracts of *Desulfovibrio desulfuricans*. The extent of purification was followed by employing both APS reduction with reduced methyl viologen and APS formation with $\text{Fe}(\text{CN})_6^{3-}$ as measures of activity. Since the ratio of these two activities remained constant, it seems that both activities are catalyzed by the same enzyme. This enzyme preparation is unstable at 0°C but can be stored for several months at –20°C. No evidence has been obtained for an easily dissociable cofactor; however, the preparation at this level of purification is yellow and has an absorption spectrum typical of flavin-containing enzymes. The fact that the yellow color is partially bleached by the addition of sulfite (in the absence of AMP) suggests that APS reductase may contain a flavin as its prosthetic group. Since the reduction of flavin by sulfite occurs only in protein fractions containing APS reductase, it seems that this phenomenon is related to the enzyme under consideration. Attempts to resolve this enzyme for flavin by classical procedures have been unsuccessful.

Discussion. – Further purification of APS reductase will be necessary before definitive experiments on the mechanism of action of this enzyme. However, the reduction of flavin in the absence of AMP can be interpreted to indicate that sulfite is oxidized to sulfate to yield a stable enzyme-sulfate intermediate. This reaction, if it actually occurs, might be employed to label and to determine the amino acid sequence at the “active site” of APS reductase.

Studies on Antibody Synthesis

C. J. Wüst G. D. Novelli

Introduction. – Experiments designed to develop a cell-free system for antibody synthesis have been continued. This report will be concerned with three aspects of this study: (1) incorporation of isotopically labeled amino acid into protein by a spleen particle-liver supernatant system; (2) the antigen, glyceraldehyde-3-phosphate dehydrogenase, or triose phosphate dehydrogenase and its assay and (3) particle-bound antibody isolated from the immunized animal.

Results and Discussion. – In the previous report¹⁸ some properties of a cell-free incorporating system were presented. This system consists of particles obtained from spleen homogenates after differential centrifugation and isolation at $105,000 \times g$ and soluble components derived from liver. Both the rat and mouse have been used. The rat system was examined in an effort to improve the efficiency of incorporation. The particles were washed and treated with sodium desoxycholate in order to eliminate the incorporating activity of particles alone. The liver supernatants were dialyzed against several solutions, the best to date being 0.25 M sucrose in 7×10^{-3} M 2-mercaptoethanol. Table 31 is presented to show the relative efficiencies which were obtained in several experiments and to compare this system to an all-liver system. Isolation of desoxyribonucleic acid (DNA) was made from spleens by Duponol and salt extraction followed by deproteination by phenol or Sevag

¹⁸C. J. Wüst, *Biol. Div. Semiann. Progr. Rept.*, Feb. 15, 1961, ORNL-3095, pp 119–20.

Table 31. Efficiency of Incorporation of C¹⁴ Leucine onto Rat Liver and Spleen Particles in Cell-Free Systems

Particles	Supernatant	Specific Activity (counts min ⁻¹ mg ⁻¹ of particle protein)
Spleen, washed	None	20-30
Spleen, DOC ^a	None	0
Spleen, washed	Spleen	20-40
Spleen, washed	Liver	260
Spleen, DOC ^a	Liver	220-270
Spleen, DOC ^a	Liver, dialyzed ^b	400
Liver, washed	Liver	780
Liver, washed	Spleen	170
Liver, washed	Liver, dialyzed ^b	1530

^aTreatment with 0.8% sodium desoxycholate at pH 7.7.

^bDialysis against 0.25 M sucrose and 7×10^{-3} M 2-mercaptoethanol.

treatment. The addition of this material to the incorporating system increased the efficiency about 15% in some instances, especially when the DNA was isolated from immunized animals. This finding was not consistently reproducible and is being investigated further. Similar results have been found with the system derived from (C3H/Anf Cum ♀ × C57BL/Cum ♂) hybrid mice.

The enzyme triose phosphate dehydrogenase was isolated from rabbit muscle and used as antigen. Assay of this enzyme was made by using the methods described by Velick.¹⁹ The enzyme-stabilizing component of this assay system is cysteine. The pH used for cysteine-containing solutions is 8.5. At this pH, cysteine is unstable and is oxidized to cystine. Another source of the necessary enzyme-stabilizing sulfhydryl group is 2-mercaptoethanol. The use of this compound in solutions containing the enzyme and in the assay mixture yielded a threefold or better increase in titratable enzyme activity over assays employing cysteine. In addition, solutions containing less than 80 μg of enzyme protein per milliliter retained 70 to 80% of their activity after 24 hr in an ice bath. The optimal concentration of 2-mercaptoethanol was found to be 2×10^{-2} M.

Mice and rats immunized to the enzyme triose phosphate dehydrogenase were sacrificed at several time intervals after injection of the antigen in order to determine the presence of enzyme-inhibiting activity on the spleen and liver particles. Previous experience indicated that the enzyme elicited a precipitating and neutralizing antibody in rats, mice, and chickens. Using cysteine in the assay mixture, a large amount of inhibiting activity was 5 times the relative amount found in serum. Unimmunized animals were sacrificed and particles obtained from the livers and spleens.

¹⁹S. F. Velick, pp 401-6 in *Methods in Enzymology*, vol 1, ed. by S. P. Colowick and N. O. Kaplan, Academic Press, New York, 1955.

These particles showed about 5% of the inhibiting activity of particles from immunized animals. Although some of the activity demonstrated on particles from immunized animals was removed by treatment of the particles with sodium desoxycholate, the residual inhibiting capacity had the same relative efficiency to neutralize enzymic activity in terms of units per milligram of protein.

The material solubilized by desoxycholate was dialyzed to remove the bile salt and then concentrated by flash evaporation. Using the Ouchterlory agar-diffusion technique, this material reacted specifically with anti-mouse or anti-rat gamma globulin, depending upon the animal used. Three lines of antigen-antibody reactivity were normally observed. The reactivity of soluble protein from particles with anti-gamma globulin could also be demonstrated by the precipitin test. No reactivity was demonstrated with the antigen. The substitution of mercaptoethanol for cysteine was made in the assay solutions, as indicated above. No neutralizing activity could be demonstrated with particles obtained from immunized animals or in immune serum when this change was made. Whether the inhibition observed when cysteine was used to stabilize the enzyme was the result of antibody neutralization is not certain at this time. It should be emphasized that only the sera and particles obtained from immunized animals showed marked inhibition of enzymic activity. The negative results obtained with mercaptoethanol may indicate a protection phenomenon by sulfhydryl groups of the enzymic site from antibody neutralization. This problem is being pursued.

**Studies on the Role of DNA in the Synthesis of β -Galactosidase by
Cell-Free Extracts of *Escherichia coli***

J. M. Eisenstadt G. D. Novelli

Introduction. – Studies have been continued in order to elucidate the role of DNA in protein synthesis.²⁰ Preliminary experiments had determined that cell-free preparations from x-ray-treated cells of *Escherichia coli* were unable to synthesize β -galactosidase in vitro when incubated under conditions where extracts from unirradiated cells were active. Mixing the 105,000 \times g supernatants and particles from x-irradiated cells and those from active extracts established that the site of x-ray inactivation was contained in the supernatant fraction. A nucleic acid extract prepared from the active supernatant by precipitation with streptomycin sulfate was able to restore the ability of x-irradiated supernatants to catalyze enzyme synthesis in the cell-free system. Treatment of the nucleic acid extracts with ribonuclease (RNase) and desoxyribonuclease (DNase) demonstrated that DNase inactivates this action while RNase has no effect.

Therefore, purified native DNA was prepared from various sources and tested for its ability to restore x-ray-inactivated materials in the synthesis of β -galactosidase.

²⁰J. M. Eisenstadt and G. D. Novelli, *Biol. Div. Semiann. Progr. Rept.* Feb. 15, 1961, ORNL-3095, p 111.

Results and Discussion. – Native DNA was prepared from bacterial sources by a combination of detergent²¹ and phenol²² treatments. The resulting preparations were highly purified, as determined by chemical and physical measurements.

It remained, then, to determine if the effect of DNA in restoring enzyme synthesis to the inactivated system was specific for the gene responsible for the synthesis of β -galactosidase (z^+). The observation that DNA from another bacterial source was ineffective suggested that the DNA effect was not due to its polyanionic nature. Therefore, DNA was prepared from the inducible strain, the constitutive strain, and the lactose-negative strain (not containing the β -galactosidase gene).

The test system consists of the particles and supernatant derived from x-ray-treated cells. The results show that DNA from the inducible strain which had not been induced has little effect in restoration of activity, while DNA from similar cells that were preinduced is effective in restoring enzyme synthesis. DNA's prepared from strains lacking the β -galactosidase gene are completely ineffective. Constitutive DNA (from the strain which makes the enzyme at all times), by contrast, permits some enzyme synthesis to occur in the absence of inducer but is more effective when inducer is present. These studies suggest that the synthesis of this enzyme is controlled at the level of the gene.

Other experiments indicate a requirement for the nucleoside di- and triphosphates in the DNA-restored, x-ray-inactivated system. These studies suggest that the synthesis of RNA may be necessary for enzyme formation and are consistent with the notion that DNA may be responsible for the directed synthesis²³ of a "messenger" RNA which contains the information for the amino acid sequence of enzyme protein.

These observations suggest that it may be possible to use this cell-free enzyme-forming system as a bio-assay for a specific gene as well as an assay for a specific repressor. Studies are being continued in an effort to determine the optimum conditions required for the expression of gene action in order to make the system a specific and reproducible bio-assay.

Studies Concerning the Control of β -Galactosidase Synthesis in Cell-Free Extracts of *Escherichia coli*

W. R. Finnerty G. D. Novelli

Introduction. – Mechanisms involved in the control of cellular function fall generally into two categories, repression and induction. These mechanisms represent the effects of specific molecules on the synthesis of enzymes and most likely other structural proteins. Repression and induction were more closely allied when Vogel²⁴ suggested that a definite relationship exists not only in terms

²¹S. Zamenhof *et al.*, *J. Biol. Chem.* **219**, 165 (1956).

²²K. S. Kirby, *Biochem. J.* **66**, 495 (1957).

²³J. J. Furth, J. Hurwitz, and M. Goldmann, *Biochem. Biophys. Research Commun.* **4**, 431 (1961).

²⁴H. J. Vogel, *Proc. Natl. Acad. Sci. U.S.* **43**, 491 (1957).

of cellular function but also in the underlying molecular events associated with the interference or promotion of the release of products from templates. Pardee²⁵ presented evidence that induction of β -galactosidase represented an antagonism toward the action of an endogenous repressor.

Repression has been observed in growing organisms by the addition of a wide variety of substances, including amino acids, purines and pyrimidines, carbohydrates, and other organic and inorganic types of compounds. Accordingly a wide range of enzymes are subject to repression, the activity appearing to occur regardless of the type of catalytic activity possessed by the enzyme. This repressor phenomenon seems to be fundamental with biological systems, and examples of specific repression have been studied in a variety of bacterial species as well as mammalian cells. Therefore, repression, specifically the mechanism(s) involved and the nature of the biological repressor compounds formed by the living organism, appears to be of particular relevance in the control of cellular function.

Results and Discussion. – Previous investigations in this laboratory have been concerned with studying the cell-free synthesis of β -galactosidase in *Escherichia coli* (inducible strain, $i^+ z^+ y^+$).²⁶ Initial experiments were undertaken to evaluate cell-free synthesis of β -galactosidase in the constitutive mutant *E. coli* ML 308 ($i^- z^+ y^+$). Results indicated cell-free synthesis of β -galactosidase with enzyme units increasing four- to sixfold.

Constitutive and induced supernatants were fractionated by streptomycin precipitation, and both precipitates and supernatants were dialyzed. Streptomycin-treated supernatants were ineffective in supporting enzyme synthesis, but their initial activity was restored by addition of the material precipitated by streptomycin.

Results indicate that cell-free synthesis of β -galactosidase can be increased by adding native DNA extracted from constitutive *E. coli* cells. This increase can also be shown with streptomycin-treated supernatants (streptomycin removes the DNA from the supernatant). These observations indicate that the system synthesizing β -galactosidase is DNA sensitive and that the levels of enzyme produced can be controlled by the addition of DNA. DNA extracted from an induced culture of *E. coli* ($i^- z^+ y^+$) was observed to exert a stimulatory increase of β -galactosidase as long as methyl-thio- β -D-galactoside was present in the reaction. This suggested that the "i" gene is important to the mechanism(s) involved in the synthesis of β -galactosidase.²⁷

DNA extracted from an inducible strain of *E. coli* ($i^+ z^+ y^+$) but noninduced was tested to determine its biological activity. Results showed that β -galactosidase synthesis was inhibited or repressed after the addition of noninduced DNA to a reaction which had been actively carrying out synthesis. A control reaction was observed to continue enzyme synthesis during the period of enzyme repression by

²⁵A. B. Pardee and J. L. Prestidge, *Biochim. et Biophys. Acta* **36**, 545 (1959).

²⁶T. Kameyama and G. D. Novelli, *Biochem. Biophys. Research Commun.* **2**, 393 (1960).

²⁷J. M. Eisenstadt and G. D. Novelli, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, p 112.

noninduced DNA. This experiment suggested that the "i" gene was exerting its repressor action on the synthesis of β -galactosidase since the cells had not been previously induced prior to DNA extraction to relieve the repressor activity in the cell.

An experiment designed to balance the effects of constitutive DNA and noninduced DNA was done with the following results. A reaction mixture synthesizing β -galactosidase for 30 min was repressed by adding noninduced DNA. After 30 min, constitutive DNA was added to the reaction; and after a slow lag period, β -galactosidase synthesis began again. Another reaction which was repressed at zero time by the presence of noninduced DNA was allowed to remain repressed for 30 min. At this time, constitutive DNA was added to the reaction. The repression was relieved by the addition of constitutive DNA, and a net increase in β -galactosidase was followed for 30 min. At this time, non-induced DNA was added to the reaction, and β -galactosidase synthesis was repressed. A control reaction run simultaneously with these two reactions continued to carry out enzyme synthesis for the entire period. These results indicate that a specific factor present in the DNA preparation, whether it be a small molecule attached to a portion of the DNA or whether it is the DNA itself, is exerting a positive effect on the cell-free synthesis of β -galactosidase.

Studies on the Transfer of Amino Acids from SRNA to Ribosomes by *Escherichia coli*

Maria D. Garcia-Pineda G. D. Novelli

Introduction. – This is a continuation of the previously reported investigation²⁸ that is designed to learn something about the nature of the transfer of amino acids from SRNA to ribosomes.

Results and Discussion. – Transfer RNA was charged with C¹⁴ amino acids as previously described.²⁸ Washed and unwashed ribosomes were used in the presence and absence of GTP and the supernatant soluble factor. The ability of this factor to transfer amino acids from SRNA to ribosomes was determined. In no case was a transfer greater than 2.5% obtained. The specificity of the transfer reaction was tested by the substitution of maize ribosomes for the *Escherichia coli* ribosomes. Some specificity is evident since the maize ribosomes were less effective as acceptor of the amino acid.

The efficiency of incorporation from free amino acids was compared with the efficiency of transfer from amino acid charged SRNA. The incorporation rate was about the same in both cases. Incorporation of amino acids was tested with ribosomes and supernatant from the constitutive and the inducible strain. The inducible system was about 4 times more efficient than the constitutive system. Crossing experiments between the two systems revealed that the deficiency of the constitutive system was in the ribosomes. The nature of the deficiency is being investigated.

²⁸M. D. Garcia-Pineda and G. D. Novelli, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, p 117.

Effect of X Irradiation and Homologous Bone Marrow on a Cell-Free Amino Acid Incorporating System from Mouse Liver

Charlotte R. Lea G. D. Novelli

Introduction. – As previously reported,²⁹ studies of a mouse liver amino acid incorporating system were initiated with the hope of obtaining information on the rate-limiting step(s) in protein synthesis. Kretchmar and Congdon³⁰ had observed an increase in liver weight in mouse bone marrow chimeras. It was hoped that this increase in liver weight was a reflection of increased protein synthesis in this organ. Experiments were designed to determine whether this was the case and, if so, which component of the cell-free system showed the earliest increase.

Results and Discussion. – Ribonucleoprotein (RNP) particles and whole supernatant were prepared, as described previously,²⁹ from the livers of mice sacrificed 4, 8, 12, 16, and 24 days after x irradiation. Two groups of mice were used, one irradiated only, and the other treated with homologous bone marrow after irradiation.

Comparison of the recombined systems with supernatants and particles tested separately with standard particles and supernatants indicated a rise above normal in the ability to incorporate amino acids in both systems at 4 to 8 days, followed by a drop below normal in both groups at 12 days. After 12 days all the untreated animals had been sacrificed or died. The cell-free systems from the treated animals remained somewhat below normal at 16 days and then returned to nearly normal levels by day 24. The changes were never much greater than 20% and were approximately equivalent for the particles and supernatants. The results of these preliminary experiments suggest that the increase in liver weight observed by Kretchmar and Congdon was not reflected in a faster rate of protein synthesis but must be attributed to other causes.

The Effect of DNA on a Mouse Liver Amino Acid Incorporating System

Charlotte R. Lea G. D. Novelli

Introduction. – A recently developed addition to the theory of protein synthesis suggests that a specific RNA, called "messenger RNA" (mRNA)³¹ is formed under the direction of DNA. This mRNA may then participate in the formation of a specific RNP particle or template for protein synthesis or may aid in removing the protein which has been formed so that a new molecule can be made on the same particle. A cell-free system from *Escherichia coli* for the synthesis of β -galactosidase, developed in this laboratory³² and dependent on the joint presence of specific DNA as well as RNA precursors, appears to support this theory. Extending this theory to mammalian systems might allow

²⁹C. R. Lea and G. D. Novelli, *Biol. Div. Semiann. Progr. Rept. Aug. 15, 1960*, ORNL-2997, p 171.

³⁰A. L. Kretchmar and C. C. Congdon, *Am. J. Physiol.* **200**, 102 (1961).

³¹F. Jacob and J. Monod, *J. Mol. Biol.* **3**, 318 (1961).

³²J. M. Eisenstadt and G. D. Novelli, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, p 111.

explanation of a puzzling aspect of these systems. With two reported exceptions,^{33,34} the incorporation reaction appears to be essentially complete in 15 to 20 min, with no indication of release of newly formed protein into the soluble phase. In the preparation of cell-free mammalian systems, the nuclei, containing all of the DNA, are discarded; thus any possible communication between DNA and particles via mRNA is disrupted.

Results and Discussion. — Several samples of active *Escherichia coli* DNA prepared by J. M. Eisenstadt as well as a sample of mouse liver DNA, prepared by a Kirby procedure,³⁵ failed to stimulate incorporation.

The recent reports of DNA-dependent RNA synthesizing systems raised new hope for this theory. Accordingly, this enzyme was prepared from mouse liver nuclei by a variation of the procedure of Weiss.³⁶ This also failed to stimulate incorporation.

It is interesting that only the microbial incorporating system has a specific requirement for DNA. It is possible that the mammalian and plant incorporating systems being investigated represent simply the fraction of the protein synthesizing machinery that has stable mRNA. However, if the dogma that DNA contains the information determining the amino acid sequence in a protein is to be universally applied, some form of messenger must be operative in the mammalian system. Experiments to explore this possibility are being continued.

Cell-Free Synthesis of Tryptophan Synthetase

O. H. Smith G. D. Novelli

Introduction. — The demonstration by Ingram³⁷ that a single genetic difference is expressed through the substitution of one amino acid for another in genetically distinct forms of human hemoglobin offers important evidence for the previously postulated correspondence of nucleotide sequences in DNA with amino acid sequences in proteins. A number of enzymatic reactions with amino acids believed to be involved in protein synthesis have been described, but little information is yet available as to the biochemical mechanism by which genetic information is ultimately expressed in amino acid sequences. A logical approach to examining these intermediary reactions in protein synthesis appeared to be through the study of an "in vitro" protein synthesizing system. The demonstration of cell-free synthesis of β -galactosidase with *Escherichia coli* fractions³⁸ further amplified the feasibility of this experimental approach. The tryptophan synthetase complex of *E. coli*, consisting of two distinct proteins, was chosen for study. These proteins have been

³³A. Korner, *Biochim. et Biophys. Acta* **35**, 554 (1959).

³⁴J. B. Lingrel and G. Webster, *Biochem. Biophys. Research Commun.* **5**, 57 (1961).

³⁵K. S. Kirby, *Biochem. J.* **66**, 495 (1957).

³⁶S. B. Weiss, *Proc. Natl. Acad. Sci. U.S.A.* **46**, 1020 (1960).

³⁷V. M. Ingram, *Nature* **180**, 326 (1957).

³⁸T. Kameyama and G. D. Novelli, *Biochem. Biophys. Research Commun.* **2**, 393 (1960).

characterized genetically, biochemically, and immunologically, and it is felt that this backlog of information will contribute substantially to studies on the mechanism of specific protein synthesis.

Results and Discussion. — A number of preliminary experiments, employing conditions similar to those used for β -galactosidase synthesis, have demonstrated what appears to be cell-free synthesis of the tryptophan synthetase proteins. These promising results will be subjected to further examination with the intention first of demonstrating that net protein synthesis is taking place and second to study the intermediary reactions of protein synthesis as a biochemical mechanism for the transfer of genetic information.

The Role of Ribosomes in Protein Synthesis

W. H. Spell, Jr. G. D. Novelli

Evidence indicates that ribosomes are the templates upon which protein synthesis takes place. It would be of considerable value to study the formation of protein on the ribosomes and the subsequent release.

Kameyama has presented evidence for the presence of a ribosomal-bound β -galactosidase fraction in crude extracts of induced *Escherichia coli*.³⁹ This fraction showed little mobility when subjected to zone electrophoresis on Geon resin. Attempts to develop this observation for preparative purposes were unsuccessful. Several lots of Geon resin and starch were used in addition to varying the potential and method of extract preparation.

Separation by centrifuging a ribosomal suspension through a sucrose density gradient in the Spinco ultracentrifuge⁴⁰ would serve not only to isolate the ribosomal-bound enzyme but to study the possible roles of various ribosomal fractions in the synthesis of β -galactosidase in *E. coli*. Accordingly, this method has been adopted for further work. Preliminary studies indicate that density-gradient centrifugation will prove fruitful. We have obtained evidence for the existence of a β -galactosidase fraction which has the same sedimentation velocity as 70 S ribosomes. In addition there are apparently two soluble fractions, one of which sediments at roughly the same velocity as 30 S ribosomes. This fraction is most likely the dimer reported by Wallenfels.⁴¹ Addition of 2-mercaptoethanol to the buffer in which the cells were ruptured and then washed eliminated this peak. The amount of enzyme sedimented with 70 S ribosomes is very small ($\ll 1\%$). Further studies involving the effect of Mg^{2+} concentration and spermine in the growth media on the incorporation of leucine- C^{14} into ribosomal fractions are in preparation.

³⁹T. Kameyama, *Biol. Div. Semiann. Progr. Rept.* Aug. 15, 1959, ORNL-2813, p 184.

⁴⁰R. J. Britten and R. B. Roberts, *Science* 131, 32 (1960).

⁴¹K. Wallenfels *et al.*, *Biochem. Z.* 331, 459 (1959).

**The Stimulation of the Incorporation of Amino Acids into Proteins of
Escherichia coli by an Unknown Factor**

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J. M. Eisenstadt

Audrey N. Best

G. D. Novelli

Introduction. – During studies on the incorporation of amino acids into protein by a cell-free system from *Escherichia coli*, we observed a marked stimulation of incorporation by the addition of DNA. Further investigation of the stimulation by DNA gave results suggesting that a new and novel type of incorporation was taking place.

Results and Discussion. – The cell-free system used for these studies is the same as the one used for the synthesis of β -galactosidase. The addition of 50 to 100 μ g of DNA brought about a 3- to 6-fold increase in the incorporation of C^{14} leucine. The stimulation, however, was found to be insensitive to DNase, suggesting that the stimulatory factor was probably a contaminant of the DNA. This suggestion was further strengthened by the finding that only certain preparations of DNA were active. The stimulatory factor is heat stable and slowly dialyzable. The incorporation requires the presence of an energy generator, ribosomes, and supernatant, and is inactivated by ribonuclease. Unlike other incorporating systems from *E. coli*, this system is not inhibited by chloramphenicol or by treatment with DNase. One of the most interesting features of this system is that more than 75% of the radioactive protein is soluble. In all other incorporating systems from bacteria, plants, and animals, 90% of the radioactive protein remains associated with the particles.

We are now attempting to determine the nature of the stimulatory factor.

Analysis of a Sulfated Bacterial Heteropolysaccharide-Protein Complex

Mary-Lowell B. Taylor

G. D. Novelli

Introduction. – As we reported,⁴² no sulfur-containing amino acids were found in a bacterial, extracellular, sulfated heteropolysaccharide-protein complex. Thus, we have concentrated on the separation of the heteropolysaccharide from protein in order to isolate the sulfated components.

Results and Discussion. – Previous attempts to dissociate the heteropolysaccharide and protein had suggested that these components were linked by covalent bonds. We have further attempted to separate the heteropolysaccharide and protein portions with sodium hydroxide and hydrochloric acid.

Treatment of the $S^{35}O_4$ -polysaccharide-protein complex with 1 N NaOH at room temperature or at 100°C, with subsequent passage of the hydrolysis mixture through a charcoal column, showed that 65% of the original polysaccharide was recovered, containing from 30 to 60% of the original protein. The amount of radioactivity remaining with the recovered material was also extremely variable. The protein and polysaccharide adsorbed by the charcoal could not be recovered.

Treatment of $S^{35}O_4$ -polysaccharide-protein complex with 0.1 N HCl at 100°C for 10 min resulted in denaturation of the protein portion such that 70% of the original protein could be recovered, free of

⁴²M.-L. B. Taylor and G. D. Novelli, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, p 125.

polysaccharide, by centrifugation. The radioactivity recovered in the precipitated protein represented 70% of the initial radioactivity.

Since as previously reported there are no sulfur-containing amino acids in the protein portion, and since free $S^{35}O_4$ can be demonstrated after incubation of $S^{35}O_4$ -polysaccharide-protein complex with *Limax* digestive gland or treatment at 100°C in 1 N HCl for 30 min, we are now attempting to isolate the sulfated components from the acid denatured protein by enzymatic methods.

Assay, Purification, and Properties of Dihydroorotic Dehydrogenase in *Escherichia coli*

W. H. Taylor, Jr. G. D. Novelli

Introduction. – We have continued a study of the repressible dihydroorotic dehydrogenase (DHO dh) of *Escherichia coli*. Previous studies^{43,44} have shown that this enzyme is associated with the cell membrane. This report describes a new assay for DHO dh, solubilization of the enzyme, and a twentyfold purification procedure.

Results and Discussion. – Our procedure for the assay of DHO dh is a measure of the rate of ferricyanide reduction in the presence of dihydroorotic acid (DHO) and potassium cyanide at pH 8. The reaction measured is as follows:



We have confirmed this stoichiometry and shown that the assay is valid and linear with enzyme dilution. Experiments with potassium cyanide have indicated that cytochromes are involved in DHO oxidation by the particulate enzyme. Potassium cyanide stimulates ferricyanide reduction in the presence of particulate enzyme by allowing all the electrons from DHO oxidation to flow to ferricyanide. Thus, the advantage of this assay procedure over an aerobic assay previously employed is that it is more direct and does not require an intact electron-transport system.

The ferricyanide reduction assay has been used to measure the solubilization of the enzyme from membrane fractions. DHO dh has been solubilized from membrane fractions by treatment with 3% sodium desoxycholate and centrifugation at $100,000 \times g$ for 2 hr. The soluble enzyme did not sediment at this force. The dialyzed supernatant was fractionated with ammonium sulfate at pH 8.6. The fraction precipitated between 40 and 50% saturation by ammonium sulfate was twentyfold higher in specific activity than the crude extract. After desoxycholate treatment the enzyme activity was no longer stimulated by cyanide, indicating that oxygen does not compete as an electron acceptor for the soluble enzyme. Further purification of the enzyme should reveal what natural electron acceptors are involved in the oxidation of DHO.

⁴³W. H. Taylor and G. D. Novelli, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, p 121.

⁴⁴W. H. Taylor and G. D. Novelli, *Bacteriol. Proc.*, p 84 (1961).

MICROBIOLOGY

Enzyme and Tracer Studies on Bacterial Metabolism

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Lethal Action of H₂O₂, X Rays, and Radon on *Rhodopseudomonas spheroides*

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Wild-type and high-catalase mutant³ *Rhodopseudomonas spheroides* were studied with regard to the lethal action of H₂O₂, of x rays, and of alpha particles from radon. The radon was dissolved in cell suspensions to a concentration of 0.2 mc/ml, giving a dose rate of about 10 kr/hr of 6-Mev alpha particles.

From information on the permeation of H₂O₂ into *R. spheroides* cells,⁴ and the intracellular destruction of H₂O₂ by catalase,⁵ one can compute the intracellular concentration of H₂O₂ in a given experimental arrangement. Whether peroxide is generated within the cells or diffuses in from the outside, the intracellular level in the high-catalase mutant is predicted to be 100-fold lower than that in the wild type. This prediction was confirmed through direct spectrophotometric observation of catalase-H₂O₂ compounds in living cells of *R. spheroides* (experiments made in collaboration with B. Chance).

The survival of *R. spheroides* exposed to H₂O₂ is in accord with the foregoing considerations: the wild type is 100-fold more sensitive than the mutant to killing by H₂O₂.

The high-catalase mutant of *R. spheroides* was found to be fully as sensitive as the wild type toward killing by x rays and alpha particles. Furthermore, oxygen had a protective action on cells exposed to H₂O₂, whereas it had no effect in the case of alpha-particle irradiation and aggravated the killing of cells exposed to x rays. Finally, the steady-state level of intracellular H₂O₂ that could be maintained by these radiations is far too small to account for the observed rate of killing.

Two reservations must be entertained in the interpretation of these experiments. One is that the killing by alpha particles might be mediated by a transitory concentration of H₂O₂ (about 1 M) along the ionizing track. This transient peroxide would be dissipated in less than 10⁻⁷ sec as a result

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³R. K. Clayton and C. Smith, *Biochem. Biophys. Research Commun.* **3**, 143 (1960).

⁴R. K. Clayton, *Biochim. et Biophys. Acta* **36**, 35 (1959).

⁵R. K. Clayton, *Biochim. et Biophys. Acta* **36**, 40 (1959).

of diffusion; its lifetime would not be affected by the presence of catalase. Since *R. spheroides* can withstand intracellular peroxide concentrations up to 3 M for as long as 3 sec without loss of viability, this explanation for the lethal action of alpha particles appears to be ruled out.

Another reservation is that the radiation-sensitive sites in the cells might be enclosed in sub-cellular compartments that contain no catalase and are extremely impermeable to H₂O₂. There is no morphological evidence for such compartments in bacteria.

Aside from these reservations our experiments show that H₂O₂ is unimportant in mediating the lethal action of x rays and alpha particles on *R. spheroides*.

Catalase in Iron-Deficient *Rhodopseudomonas spheroides*

R. K. Clayton Carolmarie Smith

In high-catalase mutant *Rhodopseudomonas spheroides*⁶ the catalase content ranges from 3% (anaerobic cultures) to 15% (aerobic cultures) of the total dry cell mass. About 10% of this catalase is bound to subcellular particles, but only about 1% remains bound after repeated washing.

In cells grown under iron deficiency the catalase content is depressed about fourfold. In these the presence of apocatalase was sought by two methods: (1) precipitation with antibody specific for the catalase of *R. spheroides*, and (2) addition of hemin in an attempt to convert the apoenzyme to its active form. Neither method revealed the presence of apocatalase; it is concluded tentatively that the continuing synthesis of apocatalase in *R. spheroides* requires its conversion to catalase.

Polynucleotide Phosphorylase of *Clostridium perfringens*

M. I. Dolin

The polynucleotide phosphorylase of *Clostridium perfringens*^{7,8} has now been brought to a state of purity in which it catalyzes a unidirectional substrate-specific synthesis of polyadenylate from adenosine diphosphate (ADP) in a reaction which is completely dependent upon the presence of a high-molecular-weight polybase. The enzyme does not catalyze the phosphorolysis of polyadenylate nor does it use cytidine diphosphate (CDP) or uridine diphosphate (UDP) as substrates. Basic poly- α -amino acids are the most effective activators, although polyvinylamine shows significant activity.

With cruder enzyme preparations, which do not show complete dependence on polybasic activators, the effect of basic polypeptides is to decrease the K_s for ADP about thirtyfold. These partially purified enzyme preparations are able to catalyze the synthesis of polycytidylate and polyuridylate, as well as the phosphorolysis of polyadenylate. All these reactions, however, are almost completely inhibited by basic polypeptides. Polyadenylate does not reactivate the enzyme preparations which show either partial or complete dependence on the polybasic activators.

⁶R. K. Clayton and C. Smith, *Biochem. Biophys. Research Commun.* 3, 143 (1960).

⁷M. I. Dolin, *Biol. Div. Semiann. Progr. Rept. Feb. 15; 1961*, ORNL-3095, p 128.

⁸M. I. Dolin, E. Godiniaux, and M. Grunberg-Manago, *Bacteriol. Proc.*, p 180 (1961).

An interesting feature of the clostridial system is the change in specificity for activators as a result of enzyme purification. The most highly purified preparation, unlike the cruder enzyme, cannot be activated by protamine, histone, or high concentrations of inorganic salts. This change in response indicates that nonspecific charge effects do not explain the activation phenomenon. In particular, the inactivity of protamine suggests that polyadenylate binding is not the mechanism of the activation.

The behavior of the enzyme on purification suggests that the native polynucleotide phosphorylase is held in an active configuration by a polybase. Purification of the enzyme would remove the polybase and thereby cause structural changes in the enzyme, which would vary in extent with the degree of purification. These configuration changes would then be reversed, or partially reversed, on addition of an appropriate polybase.

These findings raise once again the question of the physiological significance of polynucleotide phosphorylase, especially with regard to those enzymes which apparently catalyze only homopolymer formation.⁹ It is possible that in certain bacteria the enzymes may be involved not in RNA synthesis but rather in the formation of storage material from the riboside diphosphates.

Methylmalonyl Isomerase

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

Introduction. – Methylmalonyl isomerase converts methylmalonyl-CoA to succinyl-CoA by causing a migration of the thioester group of the CoA ester to its β -methyl carbon. Eggerer *et al.*¹⁰ proposed that this is a free-radical reaction involving the cobamide coenzyme as a reduced intermediate. We tested this theory by use of a variety of compounds which could react with the free radicals.¹¹ A group of related compounds were effective inhibitors of the system; these were methylene blue and Nile blue (excellent electron acceptors) and chlorpromazine (an excellent electron donor). These inhibitors apparently complexed the apoenzyme rather than competitively reacting with a free-radical intermediate.

Chemical means of implicating free radicals have had to be employed, as our existing electron-spin-resonance equipment is too insensitive to measure "spin" in a "live" enzyme preparation (due to the presence of water).

Hence, continuing chemical studies have employed Fenton's reagent (ferrous iron and hydrogen peroxide) to generate free radicals which could complex the suspected radical intermediates in the isomerase reaction.

⁹P. S. Olmstead and G. L. Lowe, *J. Biol. Chem.* **234**, 2965 (1959).

¹⁰H. Eggerer *et al.*, *J. Am. Chem. Soc.* **82**, 2643 (1960).

¹¹E. F. Phares, M. V. Long, and S. F. Carson, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, p 127.

Experimental and Discussion. – Radical production by Fenton's reagent is initiated with hydrogen peroxide. Two procedures for addition of the peroxide were used: a rapid (single) addition, or an equal amount over a period of 20 sec. With the complete system (enzyme plus substrate) a 50% inhibition resulted from either rapid or slow peroxide addition. When either the enzyme or the substrate was initially exposed to a *slow* peroxide addition and then mixed with the missing component, a 50% inhibition also resulted. However, if either the substrate or enzyme was subjected to *rapid* addition of peroxide and then mixed with the missing component, there was little or no inhibition observed.

The results of the experiments with rapid addition of peroxide suggest that the complete system is more sensitive than are the separate components. If this sensitivity were the result only of a reaction with an "intermediate," recovery of the enzyme activity should be observable. The slow-addition experiments, which were designed to detect this recovery, produced permanent damage to the components. Thus the concentration of Fenton's reagent and the duration of its contact with the enzyme system appear to control the mechanism of damage to the system.

PLANT PHYSIOLOGY AND PHOTOSYNTHESIS

Plant Physiology

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Photosynthesis

W. A. Arnold

Pigment Formation and Photosynthesis in Dormant Lettuce Seeds⁴

A. H. Haber W. L. Carrier

Introduction. – Dormant seeds can perform many complex metabolic processes^{5,6} and, in certain instances, mitosis and cytokinesis.⁷ Very little attention has been given, however, to the possibility that tissue differentiation may occur within dormant seeds.

In the course of other studies we noticed that seeds of lettuce (*Lactuca sativa* L., var. New York) became green even though germination was prevented either by preirradiation of the dry seeds with 300 to 1000 kr Co⁶⁰ gamma rays or by imbibition of otherwise untreated seeds in solutions of maleic hydrazide (MH). When the seeds are sown on filter paper in petri dishes containing 0.03 *m* MH at pH 5.7 under 30 to 100 ft-c white light at 23°C, about 60 to 70% remain dormant. After 7 days, however, about 25 to 30% of the dormant seeds are visibly green. Corresponding dark-imbibed controls in MH do not become green. The color is localized in an internal sector near the outer epidermis of the cotyledon which faces the light. Within this sector are chloroplasts of 4 to 5 μ diameter, indistinguishable under the microscope from the chloroplasts in the expanded cotyledons of growing seedlings.

Eighty per cent acetone extracts were prepared from green dormant seeds that had been in 0.03 *m* MH and exposed to 100 ft-c continuous white light for 7 days. These extracts showed the chlorophyll a peak at 663 $m\mu$. This peak is absent in the extracts of dark-imbibed controls in MH.

¹Research participant.

²Consultant.

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⁴Digest of article in *Plant Physiol.* 36(3), 351–53 (1961).

⁵A. H. Haber and N. E. Tolbert, *Plant Physiol.* 34, 376 (1959).

⁶E. H. Toole *et al.*, *Ann. Rev. Plant Physiol.* 7, 299 (1956).

⁷A. H. Haber and H. J. Luippold, *Plant Physiol.* 35, 486 (1960).

The optical density of the nonchlorophyll pigments in the extract of light-exposed seeds at 440 to 450 μ is several times greater than that in the extract of the dark-imbibed control seeds at these wavelengths. Consequently the dormant seeds exposed to light also synthesized pigments other than chlorophyll. Presumably these nonchlorophyll pigments are carotenoids, since they have maximal absorption in the blue region of the visible spectrum and since carotene synthesis accompanies chlorophyll synthesis in developing chloroplasts.⁸

The green dormant seeds also developed the capacity to perform photosynthesis, as shown by a light- and chlorophyll-dependent fixation of C^{14} -bicarbonate with label appearing in sugar phosphates and sucrose.

Discussion. – Lettuce-seed dormancy resulting from either gamma irradiation or MH treatment seems similar to dormancy resulting from other experimental treatments and from naturally occurring environmental conditions. In all these cases the dormancy can be broken by similar chemical agents and light.^{9,10} This is not intended to suggest, however, that dormancy resulting from gamma irradiation or MH treatment necessarily must be identical in all respects with other possible types of dormancy.

The formation of apparently normal, functional chloroplasts probably involves the synthesis of specific types of protein.¹¹ The purpose of this communication is to point out that a failure to germinate in general cannot be caused by a complete incapacity to perform developmental processes and differentiation. This conclusion is consistent with recent studies on cell division and cell expansion in seed germination, showing that the initiation of germination can be correlated only with expansion.⁷ Seed dormancy must therefore be attributed to a block that specifically prevents elongation of the embryonic axis, and not to a general prevention of developmental processes and differentiation.

Anatomic Studies of Gamma-Irradiated Wheat Growing Without Cell Division¹²

D. E. Foard¹³ A. H. Haber

Introduction. – Wheat grown from gamma-irradiated (800 kr) grain can germinate and produce small seedlings ("gamma-plantlets") without undergoing any desoxyribonucleic acid (DNA) synthesis, mitosis, and cell division. Such growth is sustained by metabolism¹⁴ and gives typical growth responses to gibberellic acid.¹⁵ The studies described in this paper were undertaken to

⁸T. W. Goodwin, *Biochem. J.* **70**, 612 (1958).

⁹A. H. Haber and H. J. Luippold, *Int. J. Radiation Biol.* **1**, 317 (1959).

¹⁰A. H. Haber and J. D. White, *Plant Physiol.* **35**, 495 (1960).

¹¹M. De Deken-Grenson, *Biochim. et Biophys. Acta.* **13**, 203 (1954).

¹²Digest of article in *Am. J. Botany* **48**(6) Pt 1, 438–46 (1961).

¹³Research participant.

¹⁴A. H. Haber, W. L. Carrier, and D. E. Foard, *Am. J. Botany* **48**, 431 (1961).

¹⁵A. H. Haber and H. J. Luippold, *Am. J. Botany* **47**, 140 (1960).

see to what extent gamma-plantlets undergo differentiation. In addition to furnishing information concerning the sensitivity of differentiation to ionizing radiation, such studies indicate capacities of the cells in the unsown embryo for differentiation without replication of DNA and cell division.

Summary. – A morphological and histological study has been made of wheat seedlings growing without DNA synthesis, mitosis, and cell division after gamma irradiation of the grain. The development of these seedlings parallels the normal development of unirradiated wheat in correlative growth of primordia and organs and in the production of highly differentiated cell and tissue types (e.g., trichoblasts, vascular elements, chlorenchymatous mesophyll, and cortex). The absence of cell division makes these seedlings differ from unirradiated plants in several respects: no initiation of new organs, abnormal maturation of regions corresponding to the meristems, and greater cell lengths. Guard cells, subsidiaries, and hair-bearing cells are lacking except in the apical millimeter of the first leaf. Anatomic similarities of these plants to those treated with maleic hydrazide are discussed, including maturation to the tip of the roots. The central cylinder of the upper pair of lateral seminal root primordia becomes highly differentiated with negligible elongation of the primordia. The occurrence of a high degree of differentiation after doses of radiation that produce extensive chromosome breakage indicates that in the absence of mitosis the chromosomes need not remain intact for the cells to continue differentiation. These studies show that such seedlings can be used to indicate the capacities of the cells in the embryo for differentiation without DNA synthesis, mitosis, and cell division.

Photosynthesis

W. A. Arnold E. B. Gassner

Introduction. – The problem is to understand the initial steps in photosynthesis. Experiments in the last few years have shown that the chlorophyll, in chloroplasts or chromatophores, acts as a semiconductor in several different ways. One of these is a sudden change in the dielectric constant of dried chromatophores upon illumination. The time course of this change in dielectric constant has been under study since last September.

Results. – The relaxation time for the change in dielectric constant seems to be 0.2 sec.

Discussion. – It was expected that the relaxation time would be approximately 0.001 sec, as is found for the delayed light production. What this new result means is not yet understood.

BIOPHYSICS

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Compared Effects of Oxygen, β -Mercaptoethylamine, and Photoreactivation on Killing of *Escherichia coli* by Ionizing and Nonionizing Photons

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Introduction. — Although it is well known that x rays lose the majority of their energy in tissue through ionization and events subsequent to ionization, whereas ultraviolet energy loss involves only electronic excitation, yet it is curious that practically every biological effect that can be produced by one is also produced by the other radiation. Apparently, at some unknown point in the chain of developing radiation damage in the cell, their actions become similar. The present study was an effort to obtain some information, in one biological system, on the extent to which the early effects of these two agents may overlap.

It is generally thought that oxygen and β -mercaptoethylamine (MEA) affect only x-ray damage and that photoreactivation (PR) affects only ultraviolet (UV) damage. The literature on these matters, however, is either nonexistent (MEA), sparse (oxygen, PR), or contradictory (chemical protection from UV).

Our experiments were done with *Escherichia coli* strains B and B/r, which show quite different sensitivity, both to x rays and UV. In addition, B/r shows a very high MEA protection,³ a large oxygen effect,⁴ and both B and B/r show high UV photoreactivation.⁵ In our oxygen studies, we avoided bubbling, which has been shown⁶ to be a hazardous technique with UV. In the protection experiments, we used MEA, one of the best protective agents known, and avoided the problem of physical shielding of UV by using very thin (0.26 mm) samples. Whenever possible, parallel experiments with x rays and UV were done on the same cultures and at the same time. Thus the experiments were deliberately set up to reveal the smallest possible effects.

We used 250-kvp x rays, with 3-mm aluminum filtration, and 2537-A UV. Both the bacteriology and the irradiations were conducted in a manner to provide the highest possible reproducibility.

¹Student trainee.

²Loanee.

³C. Elias, *Radiation Research* (in press, 1961).

⁴H. L. Cromroy and H. I. Adler (in preparation, 1962).

⁵J. Jagger, *Bacteriol. Revs.* **22**, 99–142 (1958).

⁶D. M. Ginsberg and J. Jagger (in preparation, 1962).

Results. – No *oxygen effect* at all was found with either B or B/r for killing with UV. The variation in these experiments was very low, perhaps a few per cent.

No *MEA protection* from UV killing was found with B or B/r at concentrations of 0.006, 0.012, and 0.02 M, all of which gave protection from x-ray killing. A small protection, with dose-reduction factor (DRF, as defined by Jagger;⁵ the inverse of DRF as defined by Alexander⁷) of 0.92, was found with 0.04 M MEA, which provides almost maximum protection from x-ray killing.³ Experiments at higher concentrations were not attempted because even our very thin samples would absorb an appreciable fraction of the radiation, thus complicating interpretation. Much higher concentrations (above 0.1 M) are toxic to the cells,⁴ and it appeared unlikely that one would get higher UV protection with them.

No *photoreactivation* from x-ray killing of B was found at any survival level, but a very slight effect (DRF of 0.99) was found for B/r at 10^{-3} survival.

Discussion. – We found that the PR of UV damage to either B or B/r gives a DRF of about 0.30. Our experiments show a maximum of 1% PR with x rays. PR has been shown to occur with equal efficiency after wavelengths in the range 2400–2900 Å,⁵ and hence 70% of any damage should be photoreactivable. A 2% PR in our system would be easily detected. Therefore, although about 37% of x-ray *primary energy loss* goes into excitation,⁸ *not more than 4% of the x-ray lethal damage is caused by excitations in the 2400–2900-Å energy range.*

X irradiation of B/r in nitrogen gives a DRF of about 0.30, and in MEA a DRF of 0.12.³ Our experiments show a lack of oxygen effect with UV and only a slight MEA protection with UV (DRF of 0.92). Thus, *not more than 20% of the x-ray lethal damage is similar to 2537-Å lethal damage.* The reason why this overlap can be higher than the 4% mentioned above lies in the possibility that x rays may produce some ionizations or vacuum-UV excitations that are capable of producing a lethal damage similar to the nonphotoreactivable portion of 2400–2900-Å damage.

Finally, one may conclude that, in this system: (1) lethal damage caused by ionization cannot be photoreactivated; (2) oxygen does not affect lethal damage caused by excitation at 2537 Å; and (3) MEA can protect only slightly from lethal damage caused by such excitation.

Free-Radical Kinetics

M. L. Randolph

Introduction. – Electron-spin-resonance (ESR) studies on crystalline amino acids have been directed toward putting the observed phenomena into a logical pattern describable in physical and mathematical terms.

⁷P. Alexander, pp 3–44 in *Radiation Protection and Recovery*, ed. by A. Hollaender, Pergamon Press, 1960.

⁸E. Pollard, *Adv. Biol. Med. Physics* 3, 153–89 (1953).

Results and Discussion. – Previously reported⁹ saturation of electron spin resonances and their more rapid decay after heavy x irradiation of glycine and glutamic acid were extended to 22-Mev protons for glycine. As expected, decay in air was found to be markedly increased at temperatures up to 100°C, but the current lack of a proper mathematical model to fit decay data has precluded determining activation energy by an Arrhenius plot. Elementary considerations indicate that radiation-produced free radicals must occur in pairs, $R_i\cdot$ and $H_i\cdot$. ESR techniques often measure only one resonance, usually presumed to be the heavier ($R\cdot$) radical, while the lighter ($H\cdot$) radical, thought to be hydrogen in some cases, is undetected. Preliminary attempts to correlate the gas pressure generated and number of electron spins produced by heavy irradiation of glycine *in vacuo* have yielded a very rough estimate of $\frac{1}{10}$ as many diatomic molecules as spins. Failure of the gas to escape the crystalline lattice or combination with contaminants on the wall of the static vacuum irradiation tube could explain this disparity. Assuming that only two radicals $R\cdot$ and $H\cdot$ are produced at a rate K_0I by irradiation at dose rate I of a material, and that the only possible recombination mechanisms are $R_i\cdot + H_i\cdot$, $R_i\cdot + H_j\cdot$, $H_i\cdot + H_j\cdot$, and $R_i\cdot + R_j\cdot$ (where subscripts i and j designate different parent molecules), one can set up the differential equations to describe the time variations of concentrations $R\cdot$ and $H\cdot$. Unfortunately the solutions become so complex that only certain cases have been solved. If the recombination $R_i\cdot + H_i\cdot$ is energetically probable, very rapid decay of resonances produced by ultraviolet radiation, which can give a radical pair little kinetic energy for separation, would be expected. In preliminary experiments with a dose of about 150 megarads from a 1000-w mercury arc lamp, filtered by NiSO_4 and CuSO_4 , no long-lived resonances were observed in glycine. This could indicate failure to form the radicals or their rapid decay by $R_i\cdot + H_i\cdot$ recombination. Rapid decay with lifetimes of the order of a few seconds has been found characteristic of photo-induced ESR signals in chloroplasts.¹⁰

Electron-Spin-Resonance Studies on Succinic Acid and Succinic Acid Peroxide

J. H. Fränz M. L. Randolph

Introduction. – Latarjet^{11,12} has discussed the possibility that disuccinoylperoxide might play an important role in irradiation. Heller and McConnell¹³ have recently given electron-spin-resonance (ESR) data on the formation of free radicals in succinic acid. Our work on production efficiency with radiation, resonance curve shape, and decay complement and extend Heller and McConnell's studies.

⁹M. L. Randolph, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, pp 132–33.

¹⁰P. B. Sogo, L. A. Carter, and M. Calvin, in *Free Radicals in Biological Systems*, ed. by M. S. Blois, Jr., *et al.*, Academic Press, New York, 1961.

¹¹R. Latarjet, in *Ciba Foundation Symposium on Ionizing Radicals and Cell Metabolism*, 1956, pp 275–97.

¹²R. Latarjet, *Strahlentherapie* **110**, 443–50 (1959).

¹³C. Heller and H. M. McConnell, *J. Chem. Phys.* **32**, 1535 (1960).

Methods and Materials. — Our previously described ESR spectrometer and x-ray irradiation facilities have been used to study free-radical production.¹⁴ Materials examined have included: (1) succinic acid of analytic grade, as supplied by Mallinckrodt Chemical Works; and (2) disuccinoylperoxide prepared in the laboratory of Latarjet and from the Lucidol Division of Wallace & Tiernan, Incorporated. All materials were polycrystalline, irradiated, stored, and measured in air at room temperature.

Results and Conclusions. — Measurements of the radiochemical G values (resonances per 100 ev absorbed) gave a gradual decrease of G from 0.64 to 0.44 in the dose range from 300 kilorads to 5.4 megarads for disuccinoylperoxide, and from 1.62 to 0.43 in the dose range from 40 kilorads to 5.4 megarads for succinic acid. The differential peak heights for the peroxide decreased by almost a factor of 2 in the period from 15 to 120 min after 1-hr irradiations. The decay rate of the succinic acid was of the same order. Decay was nonexponential. For the peroxide, the roughly quintuplet absorption curve lost its multiplicity within a week after irradiation. No differences were detected between the two peroxide samples. Shortly after irradiation of the succinic acid we see some fine structure, but much less than Heller and McConnell report, which becomes indistinguishable about two days after irradiation. This disparity is thought to be real, probably a result of different preparation techniques for the crystalline samples, a possibility we are now investigating. Although by no means conclusive, the radiochemical G values found tend to support Latarjet's hypothesis.

The Effects of Ultraviolet Light on Nucleic Acids. I. Uridine and Deoxycytidine

R. B. Setlow

Introduction. — The shapes of the action spectra for inactivation of viruses and transforming principle DNA may be used to determine the states of organization of the nucleic acids of these entities.^{15,16} It would be useful if the analysis of action spectra could be further refined so as to yield the relative contribution to inactivation of quanta absorbed in the individual bases. It is first necessary to know if the action spectra for the destruction of the bases are similar to their absorption spectra.

Results. — The two nucleotides most readily destroyed by ultraviolet light, uridine and deoxycytidine, have been irradiated in 0.01 M PO_4^{3-} , pH 7, by monochromatic light of high intensity so as to minimize reverse reactions. As a result of irradiation the pyrimidine rings take up water and lose their characteristic absorption at 2650 Å (ref 17). The values obtained for the

¹⁴M. L. Randolph and A. H. Haber, in *Proceedings of the Symposium on The Effects of Ionizing Radiation on Seeds and Their Significance for Crop Improvement*, Karlsruhe, Aug. 8-12, 1960.

¹⁵R. B. Setlow, *Biochim. et Biophys. Acta* **39**, 180 (1960).

¹⁶J. K. Setlow and R. B. Setlow, *Proceedings of the National Academy of Sciences of the United States* (in press).

¹⁷D. Shugar, in *The Nucleic Acids*, vol III, ed. by E. Chargaff and J. N. Davidson, Academic Press, New York, 1960.

quantum yield for this reaction are shown in Table 32. The quantum yield is constant, indicating that action and absorption spectra are similar, except at the lowest wavelengths.

Table 32. The Quantum Yields for the Alteration of Uridine and Deoxycytidine

Wavelength (A)	Uridine	Deoxycytidine
2800	0.016	0.0061
2650	0.017	0.0059
2480	0.019	0.0059
2380	0.017	0.0062
2300	0.022	0.0062
2260	0.038	0.0071 ^a

^aNot completely reversible.

The Effects of Ultraviolet Light on Nucleic Acids. II. The Reversible Production of Thymine Dimers in DNA

R. B. Setlow Alice J. Coughlin

Introduction. — Thymine and thymidine in solution are very resistant to ultraviolet (UV) irradiation but if they are irradiated dry or in frozen solution dimers are formed (see ref 17 for a review). The dimers are split by UV of shorter wavelength.¹⁸ Such dimers have been isolated from the desoxyribonucleic acid (DNA) of irradiated bacteria.¹⁹ The formation of dimers in irradiated DNA may be detected by the absorption decrease at 2700 A. Ultraviolet inactivation of DNA may be associated with the formation of such dimers.

Results and Discussion. — Irradiation of native or denatured thymus DNA in solution by 2800-A radiation results in the formation of thymine dimers. If one assumes that dimers can be formed only between adjacent thymines in a polynucleotide chain, then in denatured DNA one observes an absorption decrease corresponding to the formation of approximately all possible dimers; but, in native DNA only one-third of the possible dimers are formed. The dimers made by 2800-A light are split by radiation of shorter wavelength, and subsequent 2800-A radiation forms them again. The decrease in the melting temperature of DNA after irradiation is not reversible. Preliminary experiments on the UV-inactivation of the virus $\phi \times 174$ indicate that the light-reversible reaction of thymine dimers participates in the inactivation of the virus.

¹⁸R. Setlow, *Biochim. et Biophys. Acta* **49**, 237 (1961).

¹⁹A. Wacker, H. Dellweg, and D. Weinblum, *Naturwissenschaften* **20**, 477 (1960).

The Effects of Ultraviolet Light on Nucleic Acids. III. T4 Phage Containing 5-Bromouracil

R. B. Setlow R. Boyce²⁰

Introduction. — Phage in which about 80% of thymine is substituted by 5-bromouracil (BU) show a fourfold increase in sensitivity to 2537-A radiation and a 75-fold increase toward "visible" light.²¹ We have compared the action spectra for inactivation of normal and BU-substituted T4 phage from 2300 to 3130 Å.

Results and Discussion. — The action spectrum for normal phage is similar to that of T2.²¹ The sensitivity of the BU phage is 4 times greater than the normal at wavelengths below 2900 Å. At longer wavelengths the sensitivity of the BU phage increases to 100 times that of the normal at 3130 Å. A detailed comparison of the two spectra, using the fact that the BU absorption spectrum is shifted to longer wavelengths compared to thymine, leads up to the conclusion that not only is the BU-DNA more susceptible to UV damage but that above 2900 Å quanta absorbed in BU residues have a much greater chance of inactivating the virus than quanta absorbed in other bases.

Further Studies on the Modification of Ultraviolet-Induced Aberration Frequencies in *Tradescantia* Pollen

H. L. Cromroy²²

Introduction. — Previous research²³ had indicated that exposure of *Tradescantia* pollen to light in the region 4000 to 6000 Å prior to ultraviolet (UV)²⁴ irradiation (1×10^6 ergs/cm², 2537 Å) reduced the aberration frequency to one-third that of pollen which had received UV irradiation alone. At that time, speculation was made that the mechanism involved appeared to be a photoprotective one similar to that found in some bacteria. To study this effect more fully, a 1000-w high-pressure xenon-arc lamp (OSRAM XBO-1001) which provided a continuous and relatively constant spectrum throughout the visible wavelengths in conjunction with appropriate Corning cut-off filters and Bausch & Lomb interference filters was used as a light source. The wavelengths used for pretreatment effects were 3650, 4360, 4860, 5170, 5780, 6440, and 7680 Å. Initial studies were made with a standard 15-min pretreatment exposure dose at every wavelength. The exposure dose varied from 1.2 to 1.9×10^6 ergs/cm². In order to test the feasibility of obtaining an action spectrum for the "photoprotective" effect, dose-response curves were run for each pretreatment at 5-min intervals up to 25 min.

Results. — Pretreatment with the visible and near-visible wavelengths produced several effects. A wavelength of 3650 Å followed by UV produced an increased aberration frequency over

²⁰Associated with Yale University.

²¹F. W. Stahl *et al.*, *Virology* 13, 98 (1961).

²²Consultant, Radiation Microbiology Group.

²³H. L. Cromroy, *Biol. Div. Semiann. Progr. Rept. Feb. 15, 1961*, ORNL-3095, p 135.

²⁴UV refers to the 2537-Å waveband.

that produced by UV alone. A wavelength of 5170 Å appeared to be the most protective wavelength, reducing the frequency of aberrations by 30%. A wavelength of 7680 Å, although it produced no aberrations by itself, when combined with UV, more than doubled the frequency obtained with UV alone.

Discussion. – Maximum protection occurs with pretreatment in the 5000 to 6000 Å band, peaking at 5170 Å. Maximum sensitization occurs at 7680 Å. This would lead to an interpretation of a dual effect. This is further substantiated by the shape of dose-response curves. These curves all peak at a definite dose and then decline instead of plateauing. This would appear to indicate that more than one biologically active compound is involved in the pretreatment effects with visible and near-visible light.

NMR Studies on AET and Its Derivatives

H. G. Jones J. S. Kirby-Smith

Introduction. – The successes of *S*,2-aminoethylisothiourea (AET) and its derivatives as chemical protective compounds against biological damage from ionizing radiation justify thorough fundamental studies of these compounds and their interrelations. Doherty, Shapira, and Burnett²⁵ have suggested that the reaction products of AET are formed through a cyclic intermediate which depends on both pH and time. To check such possibilities, nuclear magnetic resonance (NMR) studies of aqueous AET and its derivatives were performed.

Methods and Materials. – The NMR spectra were obtained using the Varian nuclear magnetic resonance spectrometer described elsewhere.²⁶ Chemical shifts with respect to the water peak were measured using an audio oscillator and the modulation-frequency technique.²⁷ The pure compounds, AET, 2-aminothioazoline (2-AT), 2-mercaptoethylamine (MEA), and guanidoethyldisulfide (GED) were prepared by D. G. Doherty²⁸ of the Chemical Protection Group. All samples were prepared from these compounds using D₂O, with pH varied from 2.5 to 12 by NaOH and phosphate buffer.

Results and Discussion. – Figure 15 illustrates the time sequence of spectra of a 0.71 M sample of AET in D₂O at its normal pH. The NMR spectra for the reaction products of AET are given in Fig. 16. The 2-AT, MEA, and GED solutions were prepared by dissolving the crystalline compounds in D₂O. The MEG solution was prepared by dissolving 1 g of AET in 20 ml of 0.18 N NaOH and, after a 5-min equilibration, stopping the reaction by acidifying with 0.2 N HCl. Comparison of Figs. 15 and 16 show that by 30 days AET at normal pH completely reacts to form 2-AT. Similar studies in the pH range 2.5 to 4 indicated the favored reaction product to be 2-AT.

²⁵D. G. Doherty, R. Shapira, and W. T. Burnett, Jr., *J. Am. Chem. Soc.* **79**, 5667 (1957).

²⁶See Jones, Becker, and Scaduto, this report.

²⁷J. T. Arnold and M. E. Packard, *J. Chem. Phys.* **19**, 1608 (1951).

²⁸R. Shapira, D. G. Doherty, and W. T. Burnett, Jr., *Radiation Research* **7**, 22–34 (1957).

At pH 7 the predominant product is MEG, and the solution is reasonably stable; MEG seems to be the only product at higher pH values up to about 12, where MEA appears as a product.

Conclusions. – The NMR results seem to confirm those obtained previously by classical chemical techniques.²⁹ These nondestructive NMR techniques enable one to follow such reactions easily as they progress, with some description of their changing molecular structure also being obtainable.

²⁹J. X. Khym, D. G. Doherty, and R. Shapira, *J. Am. Chem. Soc.* **80**, 3342 (1958).

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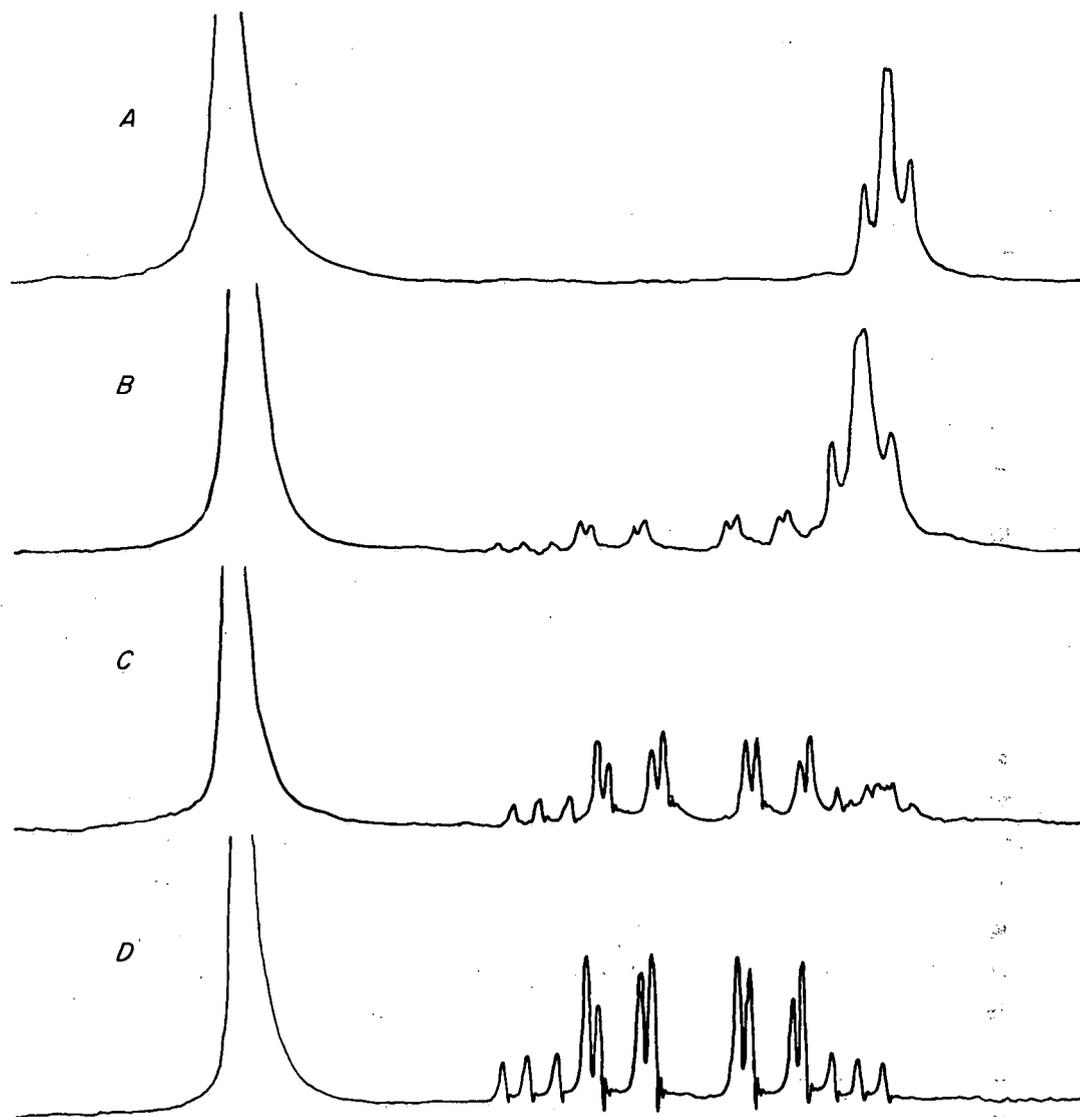


Fig. 15. Time Sequence of NMR Absorption Spectrum of 0.71 M AET in D_2O at pH 4.5: Curve A, immediately after preparation; curve B, after 3 days equilibration; curve C, 18 days; curve D, 30 days.

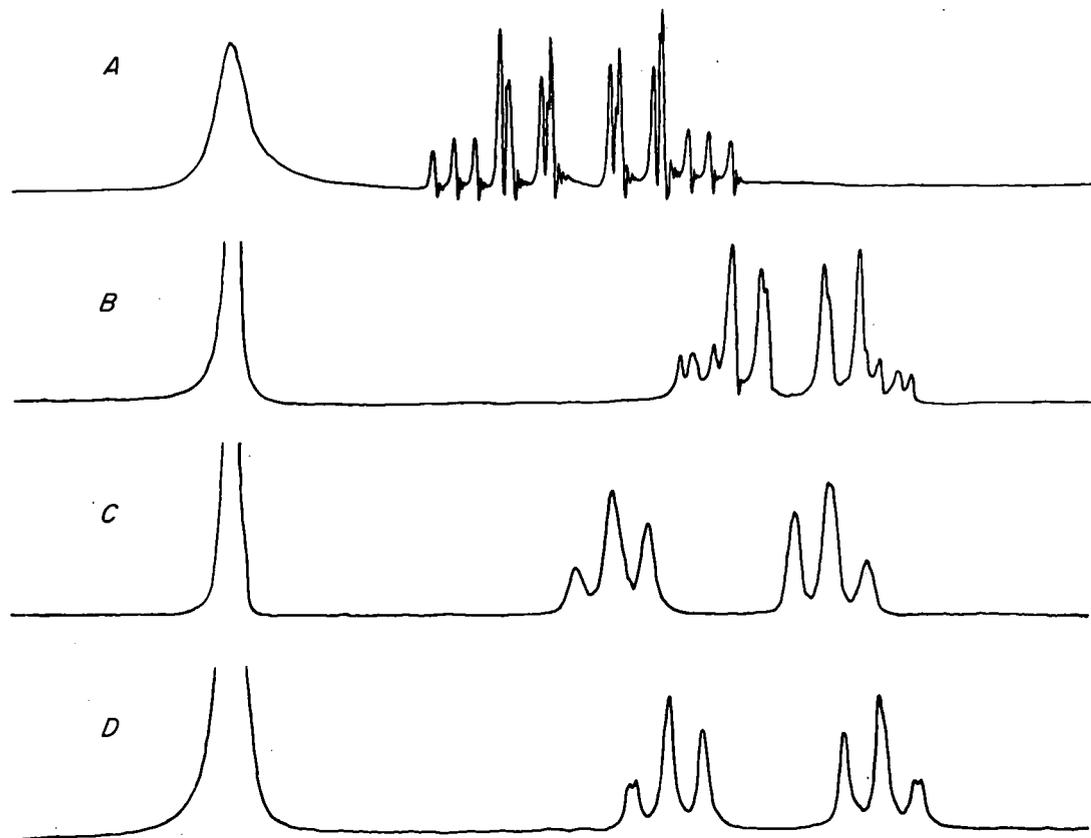
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Fig. 16. NMR Absorption Spectra of AET Derivatives. Curve A, 2-AT; B, MEA; C, GED; D, MEG.

NMR Spectra of Polypeptidyl Proteins

H. G. Jones R. R. Becker³⁰ F. C. Scaduto³⁰

Introduction. — Nuclear magnetic resonance (NMR) spectra of proteins in general provide limited information, owing to the broadness of the lines obtained. However, Kowalsky and Boyer^{31,32} have demonstrated that a marked sharpening of the spectra of several proteins could be brought about by the oxidation of disulfide bridges, or by using 8 M urea as a denaturing agent. The sharpening effect was explained largely as an increase in configurational freedom of the various residues brought about by the unfolding of the molecule. Therefore we examined several polypeptidyl proteins by this technique to obtain information on the extent to which the added residues

³⁰Cell Physiology Group.

³¹A. Kowalsky and P. D. Boyer, *Federation Proc.* 19, 337 (1960).

³²P. D. Boyer, p 1 in *Brookhaven Symposia in Biology*, No. 13, (1960).

are restricted in their motion. Ribonuclease and poly-L-valyl ribonuclease were studied first since spectra of the native enzyme have been reported and the polypeptidyl derivative was available from other studies.³³

Materials and Methods. – The spectra presented were obtained using a Varian Associates' model V4302 NMR spectrometer with 60-Mc frequency oscillation and 14.1-kilogauss field. The proton resonance absorption spectra are printed by a model G-10 graphic recorder. Observations were made with that radio-frequency level yielding maximum amplitude of the water signal with minimum saturation. The samples were examined in spinning cylindrical Pyrex glass tubes of 5-mm outside diameter. Solutions were prepared from the various compounds, using D_2O as solvent.

Poly-L-valyl ribonuclease was prepared by reaction of the enzyme with the *N*-carboxyamine acid anhydride of L-valine. Amino acid analysis indicated the attachment of 22 valine residues per mole.

Results and Discussion. – The expected spectrum of poly-L-valyl ribonuclease would be rather broad if strong interactions of the added valyl side chains occur. Figure 17 demonstrates this as well as a marked sharpening of the lines in the presence of 7 M urea. Solutions of poly-L-valyl ribonuclease in D_2O at the concentrations used (10 to 20%) show a turbidity that can be

³³R. R. Becker, in *Symposium on Poly- α -Amino Acids*, University of Wisconsin Press (in press).

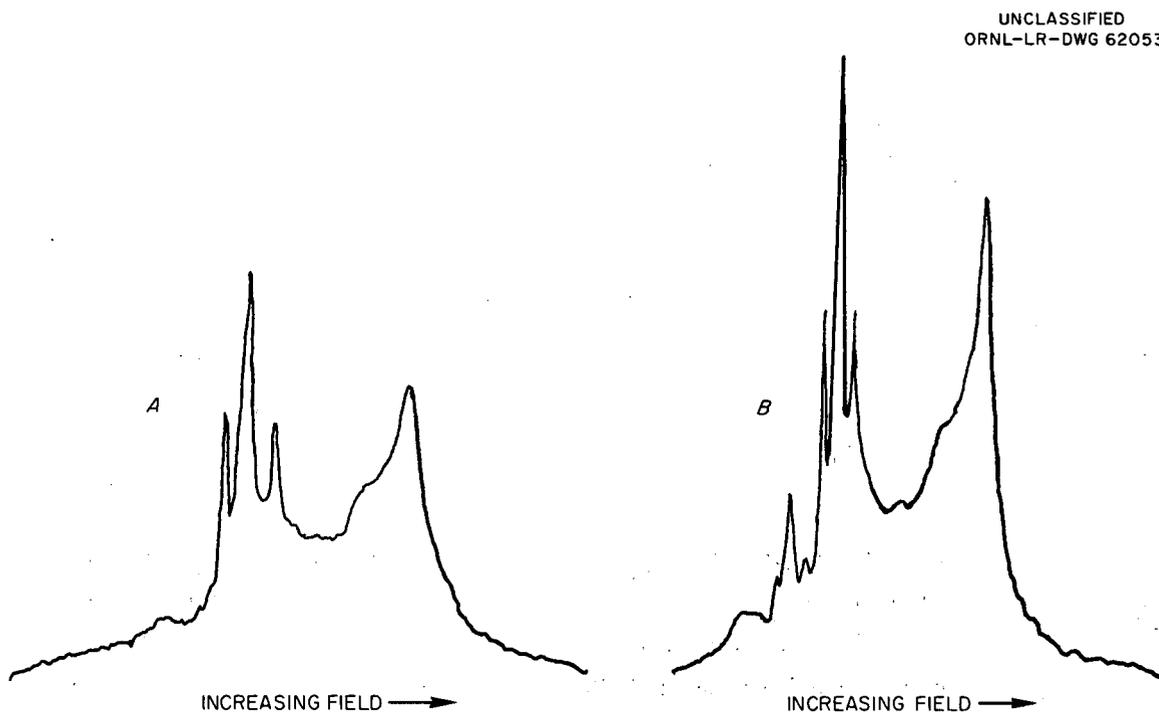


Fig. 17. NMR Absorption Spectra of Poly-L-valyl Ribonuclease in D_2O . Curve A, 10% protein in D_2O ; curve B, 12% protein in D_2O in presence of 7 M urea.

removed by cooling or by the addition of urea. In addition, the NMR spectrum of this derivative is sharpened on cooling and broadens as the sample is permitted to warm to room temperature. This behavior is consistent with other properties of the modified protein in regard to hydrophobic interactions. Figure 18 shows the spectra of physical mixtures of ribonuclease and L-valine and L-valyl-L-valine. In these cases no broadening of the usual ribonuclease spectrum occurs, but there is a marked increase in those peaks associated with the added protons. These studies are being continued on a series of polypeptidyl derivatives of ribonuclease, with special emphasis on the effects of temperature.

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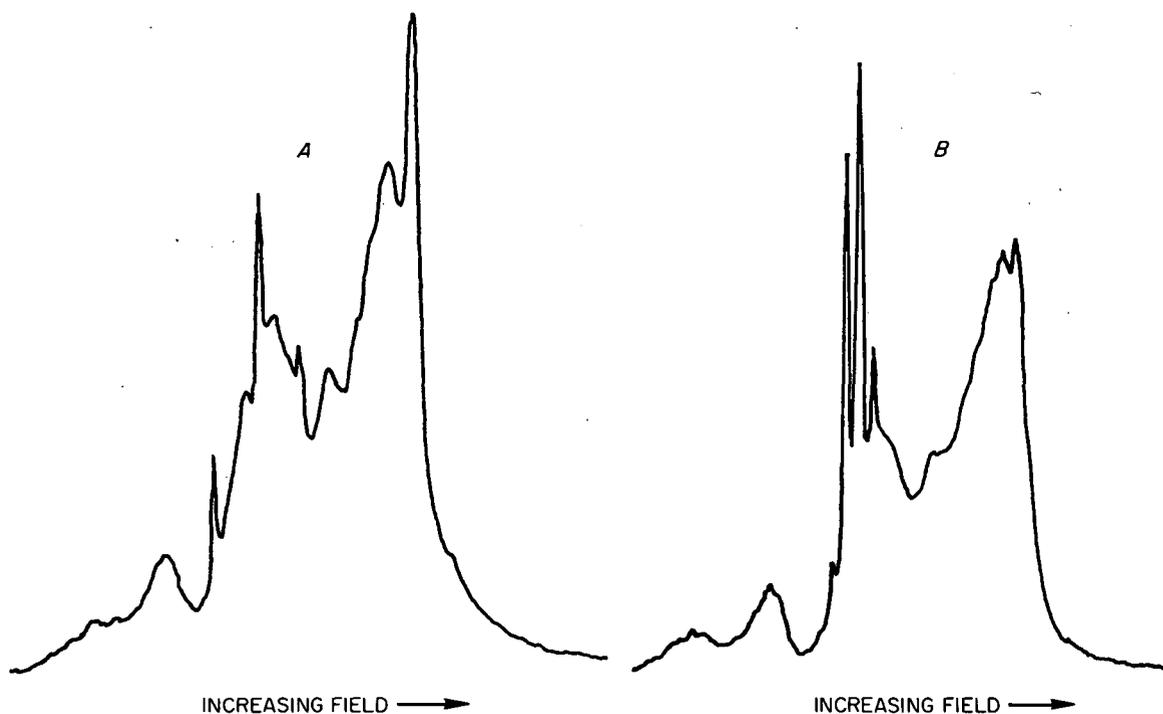


Fig. 18. NMR Absorption Spectra of Ribonuclease in D_2O . Curve A, 20% protein in D_2O plus 22 moles of L-valine per mole of RNase; curve B, 10% protein in D_2O plus 5 moles L-valyl-L-valine per mole of RNase.

Electron-Spin-Resonance Studies on Fluorescent Dyes

L. P. Simpson

J. S. Kirby-Smith

M. L. Randolph

Introduction. — A body of electron-spin-resonance (ESR) data on fluorescent dyes has been collected for evaluation in terms of the intimate mechanisms of energy absorption and transfer in the photo-oxidation process. Fluorescent dyes alone, organic substrates, and mixtures, both mechanical and lyophilized, were studied before and after visible irradiation. Effects of water, oxygen, and heat were examined.

Results and Discussion. — Several normal dyes, which have an unknown past history of exposure to light, heat, and moisture, were found to contain stable free radicals in concentrations

from 9.5×10^6 (neutral red) to 6.6×10^{18} (methylene blue) spins per gram, with half-widths of 6 to 10 gauss. In fluorescein and phenosafranin very broad signals of width over 500 gauss were observed. These are not free radicals but may be other paramagnetic species. Of all resonances observed only that in normal fluorescein had a spectroscopic g value markedly different from diphenylpicrylhydrazyl.

The ESR spectra were modified by exposure to visible light, heat, and evacuation, that is to say, removal of oxygen and water vapor. At about 30°C , irradiation for an hour from tungsten lamps through NiSO_4 apparently increased the free-radical concentrations seen in normal dyes. Irradiated fluorescein isothiocyanate exhibited the only fine structure seen in our studies. Heating, which may have involved concurrent evaporation of moisture, greatly increased radical concentrations. For example, in neutral red, heated for an hour to 68, to 95, and to 150°C , the number of spins per gram increased from 1.6 at room temperature to 2.4, 7.7, and 13.4×10^{17} respectively. Evacuation of any of the dyes tested into the micron range of pressure increased the ESR signals; this may be the result of removal of water or oxygen. Addition of water decreased the signals, whereas readmission of air produced no change. Figure 19 presents a typical decay curve for free radicals produced by a combination of light, heat, and possible loss of water.

In the substrates, egg albumin, tryptophan, potassium iodide, resorcinol, and 1-naphthol, no free radicals were seen following visible irradiation. In the substrate-dye complex, DNA-acridine orange, only the very broad signal of DNA was seen both before and after irradiation.

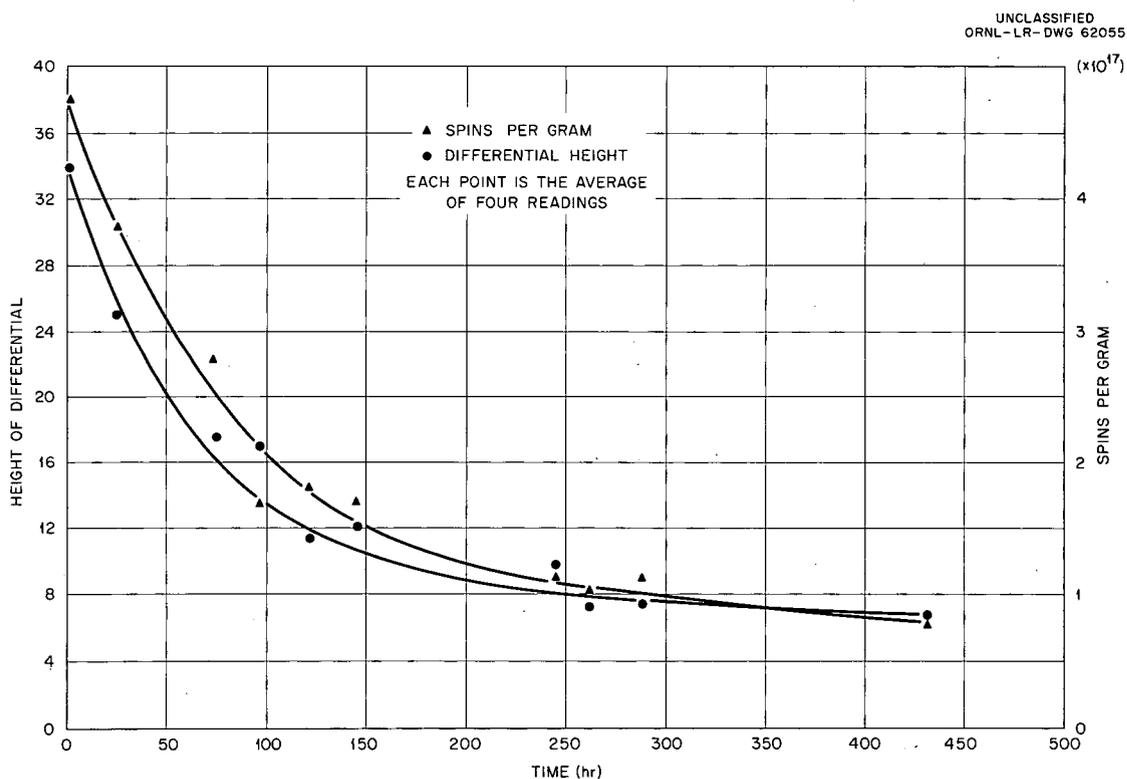


Fig. 19. Spins per Gram in Neutral Red, as a Function of Time After Induction.



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